The impact of tonsillectomy on chronic plaque psoriasis

A clinical, psychosocial and immunological study

Ragna Hlín Þorleifsdóttir

Thesis for the degree of Philosophiae Doctor

Supervisor:

Helgi Valdimarsson, MD, FRCPath, Professor Emeritus

Doctoral committee:

Andrew Johnston, PhD, Bárður Sigurgeirsson, MD, PhD,

Björn Rúnar Lúðvíksson, MD, PhD, Hannes Petersen, MD, PhD,

Jóhann Elí Guðjónsson, MD, PhD

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UNIVERSITY OF ICELAND
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Áhrif hálskirtlatöku á skellusóra

Klínísk, sálfélagsleg og ónæmisfræðileg rannsókn

Ragna Hlín Þorleifsdóttir

Ritgerð til doktorsgráðu

Umsjónarkennari:

Helgi Valdimarsson, MD, FRCPath, Professor Emeritus

Doktorsnefnd:

Andrew Johnston, PhD, Bárður Sigurgeirsson, MD, PhD,
Björn Rúnar Lúðvíksson, MD, PhD, Hannes Petersen, MD, PhD,
Jóhann Elí Guðjónsson, MD, PhD

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“Everything is theoretically impossible, until it is done”

− Robert A. Heinlein

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Ágrip

Sóri (e. psoriasis) er langvinnur ónæmismiðlaður bólgusjúkdómur í húð með sterkan erfðafræðilegan bakgrunn og veruleg neikvæð áhrif á lífgæði sjúklinga. Streptókokka hálsbólgur geta framkallað eða valdið versnun á einkennum hjá sumum sjúklingum, og nokkrar rannsóknir benda til þess að sóri geti batnað eftir hálskirtlatöku.

Markmið rannsóknarinnar var að meta áhrif hálskirtlatöku á skellusóra með tilliti til klínískra, sálfélagslegra og ónæmisfræðilegra þátta ásamt því að kanna nánar tengslin milli hálsbólgu og sóra.

Í upphafi var gerð slembiröðuð framskyggn samanburðarrannsókn þar sem 29 sambærilegum sjúklingum með skellusóra, og sögu um versnun í tengslum við hálsbólgur, var skipt í tvo hópa. Annar hópurinn fór í hálskirtlatöku (n = 15) en hinn var viðmiðunarhópur (n = 14). Sjúklingarnir voru metnir með reglulegu millibili í 24 mánuði af lækni sem var blindaður með tilliti til hópaskiptingar. Síðar var 13 sjúklingum bætt við hálskirtlatökuhópinn en eftirfylgni þeirra var ekki blinduð og því voru gögn þeirra aðskilin upprunalega hálskirtlatökuhópnum. Breytingar á útbreiðslu og virkni sóra voru klínískt metnar með PASI skori (Psoriasis Area and Severity Index) og heilsutengd lífsgæði voru metin með Psoriasis Disability Index (PDI skali) og Psoriasis Life Stress Inventory (PLSI skali). Jafnframt var tíðni húðsækinna T eitilfrumna sem virkjast bæði af peptíðum streptókokka, og samsvarandi peptíðum sem eru yfirtjáð í sóraskellum (víxlvirkar T eitilfrumur) metin í blóði og hálskirtlum. *HLA-Cw*0602* arfgerð var greind hjá öllum sjúklingum. Að lokum svöruðu 275 sórasjúklingar afturskyggnum spurningalista, en markmið hans var að meta hlutfall sórasjúklinga sem finna fyrir versnun á húðeinkennum við hálsbólgur.

Hálskirtlatökuhópurinn sýndi marktækan viðvarandi bata samanborið við viðmiðunarhóp ($p < 0.001$). Meðal PASI skor hjá 13 af 15 (87%) sjúklinga lækkaði um 30% til 90% og nálægt 60% af sjúklingum náðu PASI 50 (50% lækkun á PASI skori). Sjúklingar greindu frá auknum lífsgæðum ($p = 0.037$) og minni sóra tengdri streitu ($p < 0.002$) eftir aðgerðina og marktæk fylgni var milli klínísks bata og aukinna lífgæða ($r = 0.297$, $p = 0.008$) og minni streitu tengdri sóra ($r = 0.310$, $p = 0.005$). Auk þess var sterk marktæk fylgni milli klínísks bata og fækkunar á víxlvirkum húðsæknum Tc17 (CD8+IL-17+, $r = 0.560$, $p < 0.001$), Th17 (CD4+IL-17+, $r = 0.452$, $p < 0.003$) og Tc1 (CD8+IFN-γ+, $r = 0.594$, $p < 0.001$) frumum. Engar samsvarandi klínískar, sálfélagslegar eða ónæmisfræðilegar breytingar

sáust meðal sjúklinga viðmiðunarhóps. Arfhreinir *HLA-Cw*0602* sjúklingar sýndu marktækt meiri bata eftir hálskirtlatöku, bæði í formi klínískra einkenna ($p < 0.001$) og lífsgæða ($p < 0.001$), samanborið við arfblendna og neikvæða *HLA-Cw*0602* sjúklinga. Þannig höfðu allir arfhreinir *HLA-Cw*0602* sjúklingar náð PASI 75 eftir 6 mánuði og PASI 90 eftir 12 mánuði. Að auki höfðu arfhreinir sjúklingar marktækt oftar fengið streptókokka hálsbólgur um ævina ($p = 0.038$), og þeir röktu oftar upphaf sóraútbrota sinna til hálsbólgu ($p = 0.007$). Afturvirki spurningalistinn leiddi í ljós að 42% sjúklinga með skellusóra höfðu fundið fyrir versun á húðeinkennum tengdum hálsbólgum. Að auki höfðu 72% af sjúklingum með skellusóra, sem höfðu greinst með streptókokkasýkingu í hálsi, fundið fyrir versnun á sóra.

Þessar niðurstöður benda til náinna tengsla milli streptokokka sýkinga í hálsi og skellusóra, og að hálskirtlataka geti haft góð áhrif á bæði húðeinkenni og lífsgæði í undirhópi sórasjúklinga, sérstaklega hjá þeim sem eru *HLA-Cw*0602* arfhreinir.

Lykilorð: Sóri, hálskirtlataka, streptókokka sýking í hálskirtlum, heilsutengd lífsgæði, *HLA-Cw*0602*.

Abstract

Psoriasis is a chronic immune-mediated inflammatory skin disease with a strong genetic background and profound effects on psychosocial wellbeing. Streptococcal throat infections are known to trigger or exacerbate psoriasis in some patients, and several studies support the benefit of tonsillectomy.

The aim of the study was to evaluate the clinical, psychosocial and immunological effects tonsillectomy has on plaque psoriasis and to define further the association between throat infections and the onset or exacerbation of psoriasis.

Initially a prospective randomized controlled trial was conducted where 29 comparable patients with plaque psoriasis and a history of sore throat-associated psoriasis exacerbation were randomly selected to tonsillectomy (n = 15) or control (n = 14) groups. The patients were monitored for 24 months by the same observer, who was unaware of patients' tonsil status. Later, 13 additional patients with plaque psoriasis were assigned to the tonsillectomy group, but analysed separately as their follow-up was not observer-blinded. The patients were evaluated clinically using the Psoriasis Area and Severity Index (PASI) and health-related quality of life (HRQoL) and psoriasis-related stress were assessed with the Psoriasis Disability Index (PDI) and the Psoriasis Life Stress Inventory (PLSI). Immunological assessment consisted of enumeration of M/K peptide-reactive (responsive to homologous streptococcal M-proteins or skin keratin peptides) skin-homing T cells in tonsils and blood. All patients were genotyped for *HLA-Cw*0602*. Finally, a retrospective study-specific questionnaire, which sampled 275 patients with psoriasis, was used to estimate the proportion of patients with psoriasis with sore throat-associated psoriasis exacerbations.

The tonsillectomy group showed a significant clinical improvement compared with the controls ($p < 0.001$). Mean PASI scores decreased by 30% to 90% for 13 out of 15 (87%) tonsillectomized patients and up to 60% of patients reached a PASI 50 response (50% reduction in PASI score). HRQoL and stress associated with psoriasis also improved significantly in the tonsillectomy group compared with the control group ($p = 0.037$ and $p = 0.002$, respectively) with a significant positive correlation between clinical improvement and improved HRQoL ($r = 0.297$, $p = 0.008$) and psoriasis-related stress ($r = 0.310$, $p = 0.005$). Furthermore, there was a close correlation between the extent of clinical improvement and decrease in the frequency of circulating M/K peptide-reactive skin-homing Tc17 (CD8+IL-17+, $r = 0.560$, $p < 0.001$), Th17 (CD4+IL-17+, $r = 0.452$, $p < 0.003$) and Tc1 (CD8+IFNγ+,

$r = 0.594$, $p < 0.001$) cells. No corresponding clinical, psychosocial or immunologic changes were observed among the controls. Patients who were homozygous for the *HLA-Cw*0602* allele improved significantly more after tonsillectomy, both clinically ($p < 0.001$) and in terms of improved HRQoL ($p < 0.001$) compared with heterozygous and *HLA-Cw*0602*-negative patients. Thus, all *HLA-Cw*0602* homozygotes achieved PASI 75 by month 6 and PASI 90 by month 12. Moreover, the homozygous patients had significantly more frequent streptococcal throat infections per lifetime ($p = 0.038$), and could more often trace the onset of their psoriasis to a sore throat ($p = 0.007$) than the heterozygous and *HLA-Cw*0602*-negative patients. The retrospective study revealed that 42% of patients with plaque psoriasis have sore throat-associated psoriasis aggravations, and 72% of patients with plaque psoriasis with a confirmed streptococcal throat infection reported associated psoriasis exacerbation.

These findings collectively suggest a closer association between streptococcal throat infections and plaque psoriasis than previously reported and that tonsillectomy can have a beneficial effect on both skin lesions and quality of life in a subset of patients with psoriasis, especially those that are homozygous *HLA-Cw*0602* carriers.

Keywords: Psoriasis, tonsillectomy, streptococcal throat infection, health-related quality of life, *HLA-Cw*0602*.

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This thesis is the result of many joyful but difficult years of collaboration across oceans involving people in Reykjavik, Ann Arbor and Uppsala. Many people have contributed and I would like to express my sincere gratitude to all those who have supported me. In particular, I would like to thank the following:

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This work is dedicated to the loving memory of my father,
Þorleifur Björnsson

Contents

List of abbreviations

α	Alpha
AMPs	Antimicrobial peptides
ANOVA	Analysis of variance
APCs	Antigen-presenting cells
ASO	Anti-streptolysin O
β	Beta
B cells	B lymphocytes
BMI	Body mass index
CCL	Cysteine-Cysteine chemokine ligand
CCR	Cysteine-Cysteine chemokine receptor
CD	Cluster of differentiation
CI	Confidence interval
CLA	Cutaneous lymphocyte-associated antigen
CXCL	Cysteine-X-Cysteine chemokine ligand
CXCR	Cysteine-X-Cysteine chemokine receptor
DCs	Dendritic cells
DDc	Dermal dendritic cells
DLQI	Dermatology Life Quality Index
ELISA	Enzyme linked immunosorbent assay
ERAP	Endoplasmic reticulum aminopeptidase
FACS	Fluorescence activated cell sorting
GAS	Group A β-haemolytic streptococci
GCS	Group C β-haemolytic streptococci
GGS	Group G β-haemolytic streptococci
GPP	Generalized pustular psoriasis
GWAS	Genome-wide association studies
HBSS	Hank's balanced salt solution
HLA	Human leukocyte antigen
HRQoL	Health-related quality of life
IFN-γ	Interferon gamma
IL	Interleukin
IRF4	Interferon regulatory factor 4
KCs	Keratinocytes
K17	Keratin 17
LCE	Late cornified envelope
LL-37	Leucine-leucine 37
M6	Streptococcal M protein 6
MAIT	Mucosa-associated invariant T cells
MALT	Mucosa-associated lymphoid tissues
mDCs	Myeloid dendritic cells
MHC	Major histocompatibility complex
NFKBIA	Nuclear factor κBIA
NF-κB	Nuclear factor κB
NK cells	Natural killer cells
NKT cells	Natural killer T cells
NOD	Nucleotide oligomerization domains

OR	Odds ratios
PASI	Psoriasis Area and Severity Index
PASI 50	50% reduction in PASI score
PASI 75	75% reduction in PASI score
PASI 90	90% reduction in PASI score
PBS	Phosphate-buffered saline
PBMCs	Peripheral blood mononuclear cells
pDCs	Plasmacytoid dendritic cells
PDI	Psoriasis Disability Index
M/K peptide-reactive T cells	T cells that respond to amino acid sequences shared by streptococcal M proteins and skin keratins
PGN	Peptidoglycans
PLSI	Psoriasis Life Stress Inventory
PMN	Polymorphonuclear leukocytes
PPP	Palmoplantar pustulosis
PRR	Pattern-recognition receptor
PsA	Psoriatic arthritis
PSORS1	Psoriasis susceptibility locus 1
RCT	Randomized controlled trial
RPMI	Roswell Park Memorial Institute media 1640
RUNX3	Runt-related transcription factor 3
SAgs	Superantigens
SCID	Severe combined immunodeficiency
SD	Standard deviation
STAT3	Signal transducer and activator of transcription 3
T cells	T lymphocytes
T1	Type 1 T cells (both helper and cytotoxic)
T17	Type 17 T cells (both helper and cytotoxic)
T22	Type 22 T cells (both helper and cytotoxic)
Tc1	Type 1 cytotoxic T cell
Tc17	Type 17 cytotoxic T cell
Tc22	Type 22 cytotoxic T cell
TCR	T cell receptor
Th1	Type 1 helper T cell
Th17	Type 17 helper T cell
Th22	Type 22 helper T cell
TLR	Toll-like receptor
TMCs	Tonsil mononuclear cells
TNF-α	Tumor necrosis factor alpha
Trm	Tissue resident memory T cells
TX	Tonsillectomy
TYK2	Tyrosine kinase 2
Vβ	Variable region of the β chain of the TCR

List of figures

List of tables

List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-IV) as needed:

I. Thorleifsdottir RH, Sigurdardottir SL, Sigurgeirsson B, Olafsson JH, Sigurdsson MI, Petersen H, Arnadottir S, Gudjonsson JE, Johnston A, Valdimarsson H. Improvement of psoriasis after tonsillectomy is associated with a decrease in the frequency of circulating T cells that recognize streptococcal determinants and homologous skin determinants. *Journal of Immunology.* 2012 May 15;188(10):5160-65.

II. Thorleifsdottir RH, Sigurdardottir SL, Sigurgeirsson B, Olafsson JH, Sigurdsson MI, Petersen H, Gudjonsson JE, Johnston A, Valdimarsson H. Patient-reported outcomes and the association with clinical response in patients with moderate-to-severe plaque psoriasis treated with tonsillectomy: results from a randomised controlled trial. *Acta Dermato-Venereologica.* 2016. Nov 7. Epub ahead of print.

III. Thorleifsdottir RH, Sigurdardottir SL, Sigurgeirsson B, Olafsson JH, Petersen H, Sigurdsson MI, Gudjonsson JE, Johnston A, Valdimarsson H. HLA-Cw6 homozygosity in plaque psoriasis is associated with streptococcal throat infections and a pronounced improvement after tonsillectomy: A prospective case series. *Journal of the American Academy of Dermatology.* 2016 Nov;75(5):889-96.

IV. Thorleifsdottir RH, Eysteinsdottir JH, Olafsson JH, et al. Throat Infections are Associated with Exacerbation in a Substantial Proportion of Patients with Chronic Plaque Psoriasis. *Acta Dermato-Venereologica.* 2016 Aug 23;96(6):788-91.

Declaration of contribution

I participated in design of the study and formulation of the research questions together with Helgi Valdimarsson, Andrew Johnston, Bárður Sigurgeirsson, Jón Hjaltalín Ólafsson, Sigrún Laufey Sigurðardóttir and Jóhann Elí Guðjónsson.

Together with my supervisor, I was responsible for communicating with the Icelandic authorities, the National Bioethics committee, Data Protection Authority and the Ethics Committee of Landspitali-The National University Hospital of Iceland. I had a leading role in recruitment and screening of the study subjects, with the assistance of dermatologists Bárður Sigurgeirsson and Jón Hjaltalín Ólafsson. Jenna Huld Eysteinsdottir and I equally recruited patients for the cohort in paper IV. I was responsible for all the follow-up visits. I did the clinical examinations and Psoriasis Area and Severity Index (PASI) evaluations with essential guidance from Bárður Sigurgeirsson and Jón Hjaltalín Ólafsson. I prepared and presented the questionnaires used in this thesis, health-related quality of life, psoriasis-related stress, end-of-study questionnaire and the study-specific questionnaire used in paper IV with the help of Bárður Sigurgeirsson and Jón Hjaltalín Ólafsson.

My supervisor Helgi Valdimarsson did patient randomization and encryption. Hannes Petersen at the department of Otolaryngology-Head and Neck Surgery, Landspitali performed all the tonsillectomies. I drew all the blood samples, took skin biopsies and throat swabs. Sigrún Laufey Sigurðardóttir and I equally processed the tonsils. We also shared equally all the workload of isolation of lymphocytes, *in vitro* cell culturing and stimulation of peptide specific T cells in blood and tonsils and fluorescence activated cell-sorting (FACS) analysis with the assistance of Sigurlaug Árnadóttir. Paper I was also part of Sigrún Laufey Sigurðardóttir thesis, University of Iceland 2014. Serum cytokine measurement and *HLA-Cw*0602* genotyping were done by Andrew Johnston, University of Michigan Medical School, Ann Arbor, USA. Martin Ingi Sigurðsson did all major statistical analysis.

I prepared and interpreted the results presented in this thesis with the help of my supervisor and co-authors. I prepared all the manuscripts on which the thesis is based with critical revision of the final versions of the manuscripts from all authors. I have submitted abstracts and prepared and presented 6 posters and 4 oral presentations at international meetings.

1 Introduction

Psoriasis is a chronic immune-mediated inflammatory skin disease and probably the most prevalent autoimmune disease in humans.[1,2] Psoriasis was first recognized as a specific cutaneous entity in 1808 by the English dermatologist Robert Willan,[3] although its first description appeared in the 1st century AD, in a book by the roman scholar Celsus.[4] In recent years, breakthrough advances have been made in understanding psoriasis, in terms of genetics, immunological mechanisms and environmental factors, of which all play a role in the pathogenesis of psoriasis. Although these advances have generated highly effective treatment options, a permanent cure for psoriasis is not yet available. Many fundamental questions remain unanswered about the aetiology and pathogenesis of psoriasis. The disease is a major burden on health care systems and the society in general and is a significant health burden to patients with a high degree of morbidity and reduced quality of life.[5]

This thesis focuses on the effect of tonsillectomy on chronic plaque psoriasis and subsequent clinical, psychosocial and immunological changes. The association between psoriasis and β-haemolytic streptococci is also explored. The thesis summarizes the current literature on this subject and describes research performed from 2007-2012 in Reykjavik, Iceland resulting in new insights into psoriasis.

1.1 Psoriasis

1.1.1 Epidemiology

Psoriasis is a common disease with a 2-3% worldwide prevalence.[6,7] There is considerable geographic variation and prevalence in different populations is shown to vary from 0% to 8.5%.[7] Caucasians have a higher prevalence than other ethnic groups, and there is a certain trend of increased frequency of psoriasis in populations located farther from the equator.[8-10] It is likely that both genetic as well as environmental factors cause these variations. Hence, the prevalence of psoriasis in Sweden is 2%, 4.8-8.5% in Norway and 2.8% in the Faroe Islands[7,9] but is currently unknown in Iceland.

Psoriasis may appear at any age and has an equal distribution in both sexes.[11] Studies have shown a bimodal distribution of age of onset, between 15 and 25 years and 50 and 60 years.[12]

1.1.2 Clinical features

Psoriasis varies highly in morphology, distribution, course and severity. As a papulosquamous disease, it is generally characterized by scaling papules and plaques. Psoriasis is usually divided into several phenotypes.

1.1.2.1 Chronic plaque psoriasis

Plaque psoriasis or *psoriasis vulgaris* is the most prevalent clinical phenotype and accounts for about 90% of all cases.[13] It is characterized by sharply demarcated erythematous raised plaques with overlying silvery scale. The plaques are usually symmetrically distributed and commonly located on the scalp, lower back and extensor surfaces of the elbows and knees but any part of the skin can be involved. Plaque psoriasis is a heterogeneous phenotype and suggestions have been made for further subdivision. However, consensus is lacking on how subtyping should be performed. Based on the location of the plaques, plaque psoriasis can be divided into the following clinical subtypes (Figure 1): (1) Scalp psoriasis; (2) Nail psoriasis, occurring in 40-50% of plaque psoriasis, especially in patients with coexisting psoriasis joint disease.[14] These include pitting, red spots in the lunula, leukonychia (white spots), onycholysis (separation of the nail from the nail bed) and subungal hyperkeratosis (thickening of the nail due to scaling);[13] (3) Inverse psoriasis, involving the intertriginous areas such as the inguinal, genital, intergluteal, axillary and inframammary folds. The plaques are thin, well demarcated, shiny and usually without scaling; (4) Palmar and/or plantar psoriasis in weight-bearing areas of the soles and the center of the palms; (5) Sebopsoriasis, which is similar to seborrhoeic dermatitis and has a predilection for sebum-rich areas of the body, scalp, eyebrows, nasolabial creases and chest. Plaque psoriasis can also be divided according to size or thickness of lesions (small vs. large, thin vs. thick plaque psoriasis) or stability of lesions (stable vs. unstable, dynamic or eruptive plaque psoriasis).[13] In 1985 Henseler and Christophers[15] classified plaque psoriasis into 2 distinct subtypes: (1) Type I, characterized by an early-onset (age at onset 40 years or less), frequently a positive family history of psoriasis, and carriage of the human leukocyte antigen (HLA)-*Cw*0602* allele. Type I is more often associated with upper respiratory infections,[16] and accounts for about 75% of patients; (2) Type II plaque psoriasis, a late-onset disease (>40 years), with a distinct peak at 50-60 years, less often associated with carriage of the *HLA-Cw*0602* allele and less frequently a family history of psoriasis.[15]

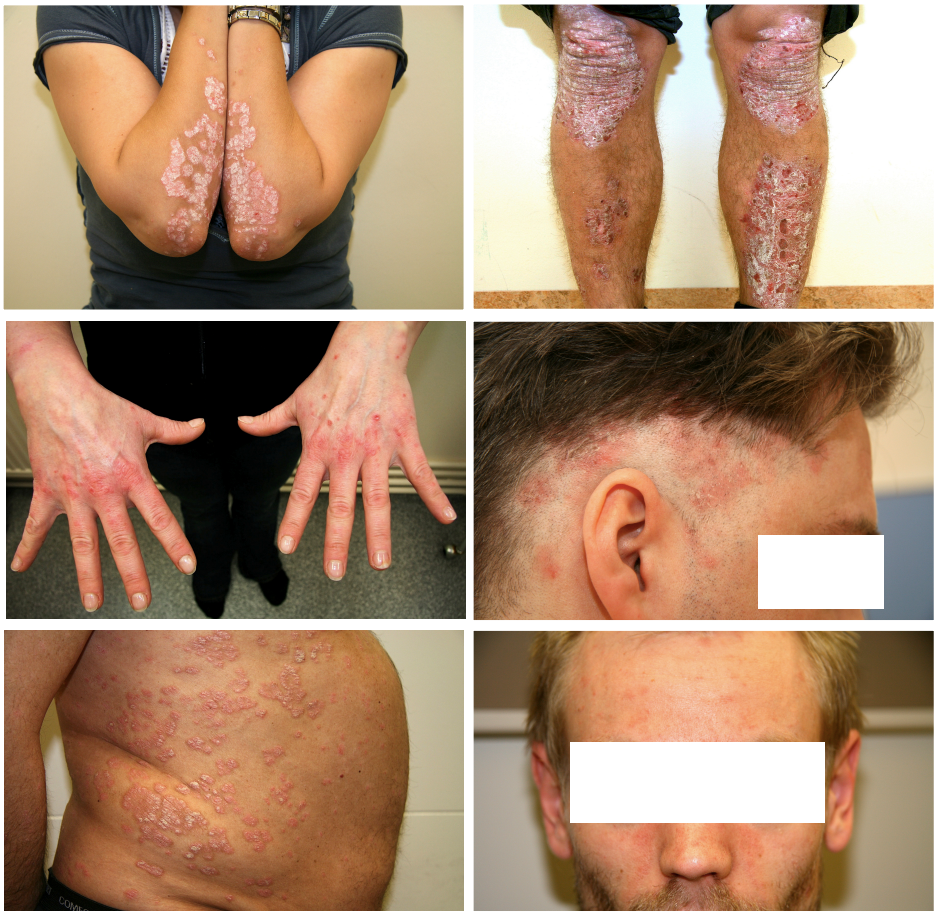

Figure 1. Chronic plaque psoriasis
Photographs were obtained from subjects of this study, and reproduced with permission.

1.1.1.1 Guttate psoriasis

Guttate psoriasis is the second most common phenotype.[17] Guttate derives from the Latin word *gutta* (a drop), but the clinical presentation is most often an acute eruption of numerous small (2-10 mm) drop-like erythematous scaly papules. Lesions are usually distributed on the trunk and proximal extremities and are more common in children and young adults. Guttate psoriasis is generally self-limiting within 3-4 months of onset, may intermittently recur, and in 33-68% of cases progress to plaque psoriasis.[18,19] Patients with plaque psoriasis can also get guttate psoriasis flares.[18]

A throat infection with beta (β)-haemolytic streptococci often precedes guttate psoriasis by 2-3 weeks.[20-23] Streptococcal perianal infections have also been linked to the onset of guttate psoriasis.[24] Case reports have linked the onset of guttate psoriasis with varicella,[25] and treatment with tumor necrosis factor-alpha (TNF-α) inhibitor therapy.[26] In 75-100% of guttate psoriasis cases, patients carry *HLA-Cw*0602*.[27,28]

1.1.1.2 Erythrodermic and pustular psoriasis

Erythrodermic and generalized pustular psoriasis (GPP) are severe and rare variants of

psoriasis. Erythrodermic psoriasis presents with generalized erythema and fine scaling, covering the entire body, and may be precipitated by infections, drugs, withdrawal of oral corticosteroids and excessive alcohol intake. GPP often presents acute or subacute with widespread erythema and small superficial pustules. Both these psoriasis phenotypes are potentially life threatening.[29,30] GPP may be a distinct entity from psoriasis but can occur concomitantly.[31]

1.1.1.3 Palmoplantar pustulosis

Palmoplantar pustulosis (PPP) is a painful chronic inflammatory condition that presents with sterile pustules on a scaly erythematous background, restricted to the palms and/or soles. It predominantly affects females in their 5th to 6th decade of life. Smoking as well as the use of TNF-α inhibitors is linked to the onset of PPP.[32,33] PPP belongs to the pustular dermatoses,[34,35] and does not share the same epidemiological, clinical and genetic profile as psoriasis.[13,34,36] However, PPP coexists with plaque psoriasis in 20% of cases. PPP and plaque psoriasis possibly share common trigger factors. Streptococcal throat infections can cause exacerbation of both entities and studies indicate improvement of PPP after tonsillectomy.[37-39]

1.1.2 Comorbidities

Psoriasis is primarily a disease of the skin but accumulating data have indicated that patients with psoriasis have an increased risk of various other chronic and serious health conditions, known as comorbidities.[40] Psoriasis can therefore be regarded as a multisystem chronic inflammatory disease associated with rheumatologic, cardiovascular, autoimmune, as well as psychiatric problems.[41] Patients with early onset severe psoriasis have a significantly reduced life expectancy compared with the general population and patients with mild psoriasis, due to a higher risk for multiple comorbid diseses.[42] Psoriasis comorbidities can be associated with psoriasis by shared genetic variants, activation of common inflammatory pathomechanisms or simply related to the persistence and chronic inflammation of the disease.[43-45] Psoriatic arthritis (PsA) is, like psoriasis, considered a T cell driven inflammatory disease,[6] present in 6-48% of patients with psoriasis.[46,47] Psoriatic arthritis usually develops several years after onset of skin lesions but up to 20% of patients get arthritis symptoms before any skin disease.[48] Comorbidities also linked to psoriasis include depression,[49] malignancy,[50] autoimmune disorders,[51] type 2 diabetes,[52] metabolic syndrome[44,52] and cardiovascular diseases.[53]

1.1.3 Assessing psoriasis outcomes and quality of life

The severity of psoriasis skin lesions can be assessed by several validated approaches, the Psoriasis Area and Severity Index (PASI), Physicians Global Assessment and the Body Surface Area.[54] The commonly used PASI score combines the affected body surface area with severity of lesions into a single score, ranging from 0 (no psoriasis) to 72 (maximal).[55] The PASI score is quite reliable, especially when the same researcher does the scoring. However, routine clinical assessments such as the PASI do not address the psychosocial impact that psoriasis has on patients' lives.

Health-related quality of life (HRQoL) is an important patient-reported outcome used to assess psoriasis. HRQoL measures patients well-being with respect to health, in terms of physical, emotional, social and functional aspects.[5] Although clinical improvements tend to correlate with improved HRQoL, studies indicate no direct correlation between HRQoL and PASI scores,[56] and improvement of HRQoL is a major goal of psoriasis management. Several methods are available to measure HRQoL in psoriasis.[57] These include generic questionnaires, disease-specific questionnaires such as the Dermatology Life Quality Index (DLQI),[58] and psoriasis-specific questionnaires like the Psoriasis Disability Index (PDI)[59] and the Psoriasis Life Stress Inventory (PLSI).[60]

Psoriasis is a chronic disease that has a significant negative impact on patients HRQoL.[61] The visible nature of psoriasis can be strongly disabling and often associated with embarrassment, low self-esteem, psychological distress, stigmatization and limitation in skin-exposing activities.[5,62] This may affect patients with psoriasis to the extent comparable to the effects of major medical diseases such as heart diseases, diabetes, depression and cancer.[63] The National Psoriasis Foundation reported that 75% of patients with psoriasis, experience a moderate to large negative impact on quality of life.[64] Despite major advances, psoriasis is still incurable and remains a major health burden to patients and the society.

1.1.4 Histology

Although psoriasis is primarily a clinical diagnosis, there are distinct histopathological features that can help diagnostically, especially when the clinical presentation is atypical. There is a rapid turn-over of the epidermis in psoriasis skin, as a result of increased proliferation and altered differentiation of keratinocytes.[65] This causes thickening (*acanthosis*) of the skin and elongation of the epidermal rete ridges (downward projections of the epidermis which interlock with the papillary dermis), in combination with an inflammatory infiltrate in the dermis. The abnormal differentiation of keratinocytes in the upper epidermis leads to retention of nuclei in the stratum corneum (*parakeratosis*) and the

end result is scaling at the surface of the psoriatic plaques.[66] Psoriatic keratinocytes express markers of proliferation such as keratins 6, 16 and 17 in the suprabasal layers of the epidermis in both involved and uninvolved skin.[67,68] Inflammatory infiltrates are present in both dermis and epidermis. The dermal infiltrates consist of cluster of differentiation (CD) 4+ and CD8+ T lymphocytes (T cells), antigen-presenting cells (APCs), including plasmacytoid dendritic cells (pDC) and dermal dendritic cells (DDc), macrophages, neutrophils and mast cells. In the epidermis CD8+ T cells predominate with Langerhans cells and neutrophils may form microabcesses in the *stratum corneum* (Munro microabcess) or the *stratum spinosum* (Kogoj microabcess).[69] The redness of a plaque is due to increased formation, elongation and dilation of capillaries in the papillary dermis (angiogenesis), leading to a tortuous and fragile capillary network, close to the surface of the skin.[66]

1.1.5 Genetics

Psoriasis is a multifactorial complex genetic disease,[70] indicating that multiple genetic determinants may be needed to generate the disease phenotypes in the presence of specific environmental conditions. Family and twin studies have demonstrated that psoriasis has a strong genetic basis and that a positive family history predisposes to a higher risk of psoriasis. Thus, the risk of developing psoriasis is 50% if both parents are affected and 16% if one of the parents has psoriasis.[71] Twin studies in the USA and Denmark have shown a concordance rate of up to 70% for monozygotic twins and 20% for dizygotic twins.[72,73] However, in an Australian study the concordance rate of identical twins was 35% and 12% for dizygotic twins.[74] This range of discordance in the monozygotic twins with psoriasis further suggests that the psoriasis phenotype is a complex interplay between genetic and environmental factors. Numerous pedigree studies have been carried out to elucidate further the genetic transmission of psoriasis.[75-77] For instance, the unique segregation analysis by Lomholt on the prevalence of psoriasis in all 10,000 inhabitants of the Faroe Islands highlighted a multifactorial inheritance with a complex polygenic-environmental interplay.[76] In recent years, substantial progress has been made to identify the genetic components of psoriasis and genome-wide association studies (GWAS) have identified over 60 susceptibility loci associated with psoriasis.[78-82] However, the identities of many of the candidate genes remain unclear. Most genes implicated in psoriasis are involved in the adaptive or innate immune system as well as skin barrier function.[83]

The psoriasis susceptibility locus 1 (*PSORS1*) is the most consistently reported genetic region with the strongest association with psoriasis.[84] *PSORS1* lies within a segment of the

major histocompatibility complex (MHC) on chromosome 6.[84] The MHC is a chromosomal region that encodes for human leukocyte antigens (HLA), which are complexes expressed on the surface of all nucleated human cells with the function of presenting peptide antigens to T cells. In the MHC region are 3 different gene clusters, class I, II and III, and the class I genes encode for HLA-A, HLA-B and HLA-C. Class I HLA molecules form complexes with β_2-microglobulin and intracellular-derived peptides (antigens) from self-proteins or viruses and are recognized by CD8+ T cells or natural killer (NK) cells. Class II molecules present extracellular peptides. The likely causal gene within *PSORS1* is *HLA-Cw*0602,* which encodes for the HLA class I molecule HLA-Cw6. *HLA-Cw*0602* is associated with psoriasis in different populations, making it the prime psoriasis susceptibility allele.[85-87] Interestingly, over 60% of patients with psoriasis carry 1 or 2 *HLA-Cw*0602* alleles, whereas the frequency in general population is 10-15%.[85,88] *HLA-Cw*0602* accounts for 50% of psoriasis heritability,[89-91] and heterozygous individuals (carrying 1 *HLA-Cw*0602* allele) have a 10-fold increased risk of developing psoriasis,[28,92] whereas *HLA-Cw*0602* homozygosity has a 20-fold risk of psoriasis.[92] *Endoplasmic reticulum aminopeptidase (ERAP) 1 and 2* are genes associated with psoriasis that code for enzymes that trim peptides before they are loaded onto MHC I molecules and presented at the cells surface. The ERAP genes have been shown to interact with *HLA-Cw*0602* and *ERAP1* only confers susceptibility to psoriasis in individuals carrying the *HLA-Cw*0602*.[90] This further reinforces the role of antigen presentation and subsequent T cell activation in the pathogenesis of psoriasis.

Studies indicate that clinical subtypes of psoriasis might have different genetic backgrounds.[93] *HLA-Cw*0602* has been associated with guttate and eruptive plaque psoriasis,[27,94-97] early onset psoriasis,[15,27,96-99] familial psoriasis[97] and female gender[100] (Table 1). Furthermore, frequent streptococcal throat infections or carriage[96,101] and streptococcal-associated psoriasis exacerbation[27,96] have been linked to *HLA-Cw*0602*. *HLA-Cw*0602* has also been associated with a risk for a higher incidence of Koebner phenomenon,[27,96,97] and a more severe disease.[27,96,97] Response to psoriasis treatment is also associated with different genotypes. Thus has *HLA-Cw*0602* been linked to a more favourable response to UV light,[27] and 2 recent trials have shown that *HLA-Cw*0602* predicts a better clinical response to the interleukin-12/23 inhibitor ustekinumab.[102,103] Moreover, patients with early onset psoriasis respond better to etanercept than late onset psoriasis.[104] Late onset psoriasis, usually *HLA-Cw*0602*-negative, seems to be a milder and more stable disease and respond more often to topical agents.[105] To a large extent, have *HLA-Cw*0602*-negative patients been shown to have more frequent psoriasis nail changes and a higher prevalence of PsA.[23,96]

Table 1. Some features of *HLA-Cw*0602*-positive and negative patients

	***HLA-Cw*0602*-positive**	***HLA-Cw*0602*-negative**
Psoriasis phenotype	Guttate and eruptive plaque	Plaque
Disease onset	Early (≤ 40 years)	Late (>40 years)
Clinical symptoms	Koebner phenomenon	Psoriasis nail changes
Clinical course	More severe, often requiring 2nd and 3rd line treatments	Less severe, responds more often to topical treatment
Streptococcal association	More often streptococcal carriage, throat infections and streptococcal-associated psoriasis exacerbation	Currently unknown
Familial psoriasis	Higher frequency within families	More often sporadic
Response to treatment	Better response to UV light, ustekinumab and etanercept	Currently unknown

A number of non-MHC genes have been implicated in psoriasis, some modify innate and adaptive immune responses, and others are involved in skin barrier function. A detailed discussion of psoriasis genetics is out of the scope of this thesis, however, many of these genes highlight the importance of abnormal T cell activation in psoriasis pathogenesis. Among these genes are those that involve regulatory cytokines and cytokine receptors in the interleukin (IL)-23/IL-17 axis such as the *IL12B, IL23A and IL23R* genes,[91,106] as are genes involved in cytokine signal transduction pathways like the *tyrosine kinase 2* (*TYK2), TRAF3IP2* and *nuclear factor kappa BIA (NFKBIA)* genes. Other reported psoriasis susceptibility genes are involved in regulation of transcriptional factors such as *runt-related transcription factor 3* (*RUNX3*) and *signal transducer and activator of transcription 3* (*STAT3*).[107] Genes involved in skin barrier dysfunction include the *late cornified envelope* (*LCE*) genes,[108] which incidentally have epistatic interactions with *HLA-Cw*0602 and ERAP1.*[109]

1.1.6 Environmental risk factors

Given that the highest reported concordance rate of psoriasis in monozygotic twins is 70%,[72,73] one can reasonably conclude that psoriasis is the result of interplay between genetic and environmental factors. A number of environmental stimuli have been identified that can both trigger the onset and cause aggravation of psoriasis (Figure 2). How these exogenous factors trigger or exacerbate psoriasis is not fully understood.

Throat infection with β-haemolytic streptococci is the most common infectious trigger for psoriasis (discussed in chapter 1.4). However, various other infections have been associated with psoriasis,[110] These include *Staphylococcus aureus,*[111] *Helicobacter pylori,*[112] *Malassezia furfur,*[110] Hepatitis C virus[113] and human immunodeficiency virus.[110]

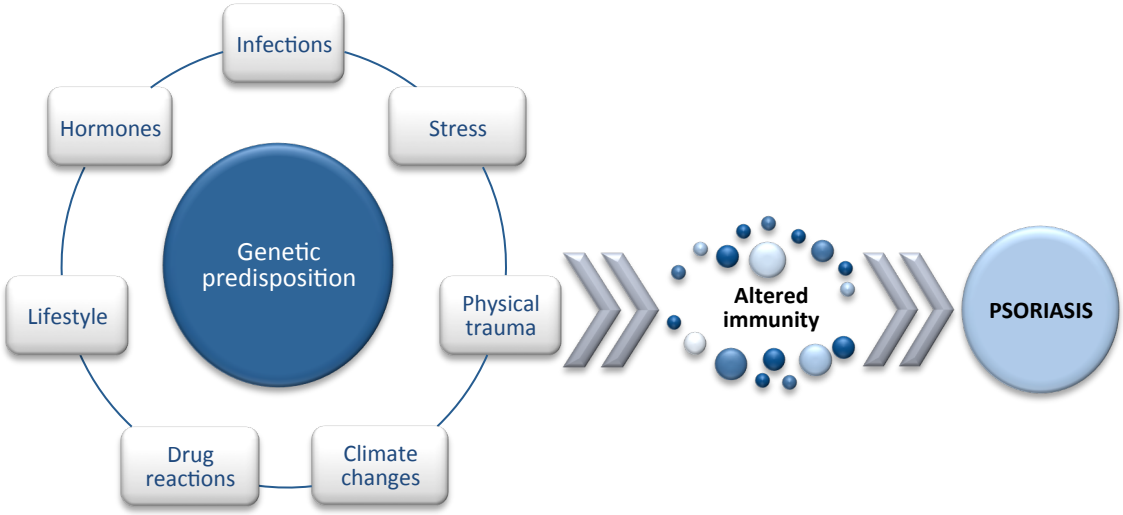

Figure 2. Psoriasis environmental risk factors

Stress is a common psoriasis trigger and exacerbation factor.[23,114] Often implicated are major stressful life events such as loss of a loved one, loss of a job or a divorce. However, even microstressors (daily hassle) can promote worsening of psoriasis.[115]

Psoriasis may appear as a consequence of injury to unaffected skin, including physical, chemical, surgical or infective lesions. This is referred to clinically as the Koebner phenomenon and was first described by the German dermatologist Heinrich Köbner, in the 18th century.[116] With Koebner phenomenon the morphology of the psoriasis lesion is identical to the initiating injury and can be seen for example following mild skin scratches, venepuncture, tattoos, piercing and surgical scars.

Climate changes can affect psoriasis and generally, there is a higher prevalence of psoriasis in colder and less humid parts of the world.[8] Many patients with psoriasis report that cold weather aggravates their psoriasis. Whether this is because of lack of sunlight and humidity has been debated. However, Lomholt reported that prolonged periods of low humidity could contribute to psoriasis exacerbation.[76] Although sunlight and therapeutic phototherapy is beneficial to psoriasis, 5-20% of patients have photosensitive psoriasis,[117] where sun exposure and especially sunburn can cause exacerbation in their condition. This is more frequent in patients with a fair skin type, a history of photosensitivity, advanced age and psoriasis affecting hands.[118]

A number of pharmacological agents have been linked with psoriasis aggravation, including beta-blockers, lithium, interferons and antimalarial drugs.[119] Many other drugs have been implicated, such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and terbinafin.[120]

Lifestyle can cause exacerbation of psoriasis. Smoking is a well-established trigger factor for PPP,[121] but can also cause aggravation of plaque psoriasis.[122] Both smokers and former smokers have a greater risk of developing psoriasis than non-smokers,[123] and cigarette smoking correlates with increased disease severity.[124] Alcohol is another lifestyle factor that is implicated in the exacerbation of psoriasis.[122] Patients who consume large quantities of alcohol tend to have a more severe psoriasis.[124] Furthermore, patients with psoriasis have higher alcohol consumption and alcoholism is more prevalent.[125,126]

It is postulated that hormones might trigger the onset of psoriasis, with peaks around puberty, postpartum and menopause. However psoriasis symptoms generally improve during pregnancy.[12,127]

1.1.7 Brief summary of the immune system involved in psoriasis

The immune system is traditionally divided into innate and adaptive arms. The innate immune system produces a fast acting, non-antigen specific response, which serves as a first line of defence against pathogens. The main components of the innate immune system are as follows: (1) Physical (epithelial surface) and chemical barriers (skin acidity, sebum); (2) Pattern-recognition receptors (PRRs) (toll-like receptors (TLR), nucleotide oligomerization domains (NOD)-like receptors and complement receptors); (3) Signaling molecules (cytokines, chemokines, antimicrobial peptides (AMPs)); (4) Epithelial cells lining the skin and mucosa; (5) Immune cells (mucosa-associated invariant T cells (MAIT), NK cells, dendritic cells (DCs), neutrophils and macrophages).[128-131] PRRs are expressed by the cells of the innate system and detect conserved primitive molecular patterns (molecules) associated with bacteria and viruses. PRRs distinguish microbes from host cells, which can initiate an inflammatory cascade with the release of pro-inflammatory cytokines, chemokines and antimicrobial peptides. This is mediated by transcription factors such as nuclear factor (NF)-κB, and facilitates killing of microbes.[132] If the innate immune system is unable to overcome the pathogen, or if the pathogen has been encountered before, the adaptive immune system is activated.

The adaptive immune system is an antigen specific, second line defence system whereby protective immunological memory is generated. Adaptive immunity includes humoral immune response (antibodies) mediated by B lymphocytes (B cells) and cellular immune response mediated by T lymphocytes. DCs are crucial for activation of the adaptive immune system and thereby, link these 2 systems together. Thus, DCs in peripheral tissues are continuously sampling the microenvironment with their PRRs. Microbial proteins are

phagocytized and broken down inside the cell, before being expressed as short peptides bound to class II HLA molecules on the DC surface. The activated DCs migrate to the nearest draining lymph node, reside in the T cell area and present their peptide antigens to naïve T cells that can thereby be activated to become effector and memory T cells.[133]

Following a first response to antigen, T cells expand clonally in the lymphoid tissues and become fully activated after 4-5 days. The activation involves: (1) signal 1, the T cell receptor (TCR) engagement; (2) signal 2, activation by co-stimulatory molecules and (3) signal 3, cytokine production, CD40L-CD40 or PRR binding. The resulting phenotype of T cells depends on the chemokines and cytokines that are present in the microenvironment during this priming event to direct an appropriate response. Microenvironmental factors also determine which tissue homing receptors the T cells express as activated T effectors cells. T1 cells (type 1 helper (Th1) and cytotoxic (Tc1) T cells) secrete interferon gamma (IFN-γ) and TNF-α and usually express the cysteine-cysteine chemokine receptor (CCR) 5 and cysteine-X-cysteine chemokine receptor (CXCR) 3. T17 cells (type 17 helper (Th17) and cytotoxic (Tc17) T cells) secrete IL-17 and express CCR6 and CCR4, and T22 cells (Th22 and Tc22)

Table 2. Major cells involved in the pathogenesis of psoriasis
A simplified summary of the major cells involved in psoriasis and their role

Cells	Role
Myeloid dendritic cells (mDCs)	Present antigens to T cells. Release TNF-α, iNOS, IL-12, IL-23, driving Th1 and T17 differentiation
Plasmacytoid dendritic cells (pDCs)	Release IFN-α and -β that stimulate mDCs
T cells	Key cells in adaptive immune response. Recognize the antigen-MHC complex. TCR, CD3+
T helper cells	CD4+, interact with MHC II molecules. Involved in signal transduction and activation of other T-cells
Th1	Induced by IL-12 and release IFN-γ and TNF-α. Involved in immune response against intracellular pathogens and autoimmunity
Th17	Induced by IL-23, TGF-β, IL-6 and IL-21 and release IL-17 and IL-22. Involved in inflammation, defence against extracellular pathogens and autoimmunity
Th22	Induced by IL-23, IL-6 and TGF-β, release IL-22. Involved in inflammation, induce keratinocyte acanthosis
T cytotoxic cells, Tc1-Tc17-Tc22	CD8+, interact with MHC I molecules. Induced by and secrete the same cytokines as Th1, Th17, Th22. Cytotoxic activity against intracellular pathogens
Keratinocytes	Key players in innate immunity, orchestrate innate and adaptive immune response. Recruit T cells to the skin and release CCL20, CXCL8, anti-microbial peptides, and pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, IL-23)
Polymorphonuclear leukocytes (PMN)	Effector cells of innate immunity. Show phagocytic properties against invading pathogens. Chemoattract other immune cells, release AMPs, proteases and IL-17
Other cells involved	Natural killer T (NKT) cells, T regulatory cells, γδ T cells, mast cells, innate lymphoid cell etc. Function in psoriasis not fully understood

secrete IL-22 and express CCR4, CCR6 and CCR10.[134,135] Expression of these chemokine receptors promotes homing of the cells to specific areas of inflammation. While primary responses and the generation of different subpopulations of effector and memory T cells mainly takes place in lymph nodes and other lymphoid tissues, lymphocyte homing is further nuanced by the expression of tissue-specific molecules. Thus, T cells that are destined to home to the skin express the cutaneous lymphocyte-associated antigen (CLA).[136]

Once in the tissues, effector T cells, and tissue macrophages orchestrate an elimination of the pathogen and promote healing through complex interactions. Then most of the T cells are eliminated by apoptosis, but a few survive to become long-lived recirculating memory T cells or tissue resident memory T cells (Trm), that are thought to create a tissue-localized immunological memory. These cells have been suggested to play a role in the chronicity of immune-mediated inflammatory diseases and repeated viral infections of the skin such as herpes simplex virus.[137]

1.1.8 Immunopathogenic mechanisms in psoriasis

The pathogenesis of psoriasis is multifactorial, complex and not yet fully understood. It involves multiple cell types, their receptors and signaling molecules (short summary in Table 2). Until the early 1980s, psoriasis was thought to be mostly an epidermal disease with aberrant proliferation of keratinocytes.[138] However, the finding that cyclosporin A, an immunosuppressant drug, proved to be highly effective in psoriasis, revealed an important role for T cells in psoriasis.[139,140]

For the past 3 decades, psoriasis has been regarded as an autoimmune disease where the T cells are key players.[141-143] This is supported by several lines of research: (1) The presence of activated T cells in psoriasis lesions;[144,145] (2) The efficacy of T cell targeted therapeutics in treating psoriasis;[146,147] (3) Decrease in T cells with effective treatment;[148,149] (4) Decrease in cytokines and cytokine receptors of T cells such as TNF-α, IFN-γ, IL-12/23, IL-17, IL-22, IL-23 with effective treatment;[150,151] (5) Psoriasis has been induced[152] or cured[153] in patients with bone marrow transplants; (6) Severe combined immunodeficiency (SCID, without T and B cells) mice transplanted with uninvolved skin from patients with psoriasis, developed psoriasis after intradermal injections of activated T cells;[154] (7) Inhibiting the entry of T cells into mouse epidermis prevents epidermal hyperplasia and psoriasis.[155]

There is increasing evidence that the development of psoriasis involves dysregulated interactions between the epidermal keratinocytes and the innate and adaptive immune systems, impacted by environmental and genetic factors.[156,157] Research in the last decade

has lead us to model of psoriasis pathogenesis that is described as the IL-23/Th17/IL-22 axis of psoriasis (Figure 3).[158] In genetically predisposed individuals, the immune system can become activated by various triggers. The exact mechanisms in this induction phase are not yet understood for many environmental factors, such as streptococcal infections and it can be difficult to pinpoint the specific triggers in each and every individual.[156] However, other triggers such as physical trauma might initiate the cascade by causing injured or stressed keratinocytes, which in turn release various antimicrobial peptides. Lande and Gilliet suggested a model of psoriasis initiation phase with the antimicrobial peptide leucine-leucine 37 (LL-37) cathelicidin that forms complexes with self-DNA released from necrotic cells.[159-161] In this view, self-DNA is turned into a pro-inflammatory stimulus that breaks immunologic tolerance. The LL-37/self-DNA complexes bind to intracellular TLR9 on pDCs and LL-37/self-RNA complexes activate pDCs through TLR7 and thereby initiate the inflammatory cascade.[162-164] Injured keratinocytes also produce high levels of cysteine-cysteine chemokine ligand (CCL) 20, which attracts mDCs, neutrophils and T17 cells into psoriasis skin.[165,166] Subsequently, mDCs are activated by the local inflammatory settings created in the skin by secretion of cytokines like IL-1β, IL-6 and TNF-α from stressed keratinocytes and by IFN-α and -β from the pDCs.[166] In addition, the LL-37/RNA complex can activate mDCs via TLR8.[156] The mDCs then migrate to the nearest draining lymph node where they drive the differentiation of naïve T cells to effector T cells; Th1 and Tc1 stimulated by IL-12, Th17, Tc17, Th22 and Tc22 stimulated by IL-23. Despite years of research it still remains debatable what antigen is presented during the activation of these T cells in the lymph node. It has been proposed that LL-37, amongst a number of candidates,[142,167] is an autoantigen in psoriasis, which drives both the activation of innate and adaptive inflammatory responses in psoriasis. The effector T cells migrate to inflamed skin, as they have become skin-homing T cells, expressing CLA. Activated mDCs secrete a plethora of cytokines including IL-12, which further stimulates Th1 and Tc1 cells to produce IFN-γ and TNF-α, and IL-1 and IL-23 which drive the expansion of Th17 and Tc17 cells, producing IL-17.

Many of the T cell-derived cytokines work co-operatively driving keratinocyte activation: TNF-α and IL-17 strongly induce CCL20, recruiting T cells and induce neutrophil recruitment by stimulating cysteine-X-cysteine chemokine ligand (CXCL) 1, CXCL2 and CXCL8 release. Activated mDCs and to a limited extent keratinocytes secrete IL-23, which

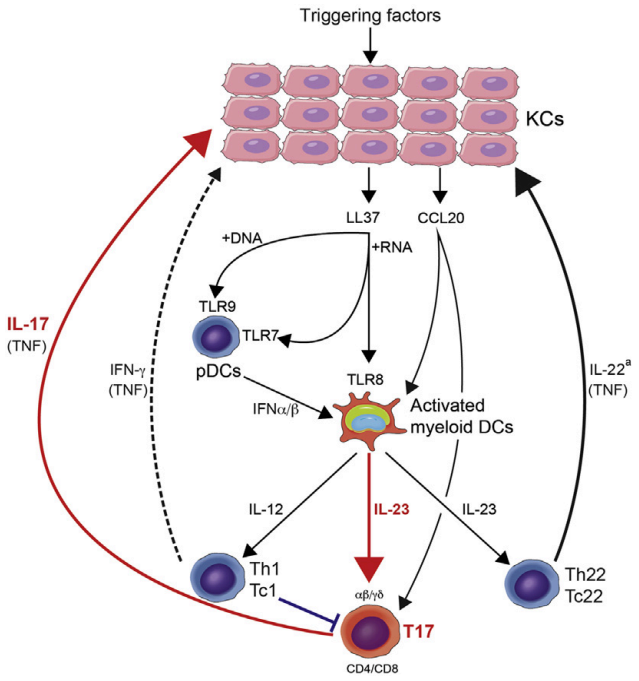

Figure 3. The IL-23/Th17/IL-22 axis
Trigger factors can cause injured keratinocytes (KCs) in the epidermis, which in turn activate plasmacytoid dendritic cells (pDCs) and myeloid dendritic cells through their Toll-like receptors (TLRs) and antimicrobial peptide LL37. CCL20 attracts mDCs and T17 cells. The activated mDCs stimulate expansion of T cells in the psoriatic lesion with cytokines; IL-12 (Th1, Tc1), IL-23 (T17, Th22, Tc22). The T cells produce cytokines that further stimulate the keratinocytes, IL-17 (T17), IFN-γ (Th1, Tc1), IL-22 (Th22, Tc22), thus a vicious inflammatory cycle is created. Reproduced with permission.[156] *Copyright Elsevier Inc.*

maintains the T17 and T22 cells and in return these cells produce a series of pro-inflammatory cytokines, IL-17 and IL-22. This leads to synthesis of antimicrobial peptides and hyperproliferation of the keratinocytes that respond by producing more cytokines and chemokines.[168,169] Thus, a vicious cycle of chronic inflammation is established. Trm remain in skin after resolution of psoriasis lesions with treatment, waiting to be reactivated. These cells produce IL-17 and IL-22 upon activation and might explain the reoccurrence of psoriasis at the same anatomic site.[170]

1.2 The palatine tonsils

The paired palatine tonsils are located in the lateral wall of the oropharynx, at the entrance to the gastrointestinal and upper respiratory tract. They are the first major barrier protecting us from invading pathogens, both airborne and alimentary.[171] The tonsils are part of the mucosa-associated lymphoid tissues (MALT) and form a protective immunological ring (Waldeyer´s ring) with the nasopharyngeal tonsil (adenoids), the paired tubal tonsils and the lingual tonsils, although the function of the tonsils is similar to lymph nodes[171,172] The palatine tonsils are covered with a stratified squamous epithelium that extends into deep

branched crypts, thereby giving rise to an enlarged surface area.[172] The surface of the crypts is lined by reticulated epithelium, often only one cell thick.[172] The reticulated crypt epithelium is ideal for productive antigen sampling with its unique cellular composition of epithelial cells, stromal cells, intraepithelial lymphocytes, dendritic cells, macrophages and neutrophils situated in close proximity. Under the epithelium are multiple lymphoid follicles with germinal centers surrounded by a connective tissue framework. The follicles are rich of B cells in different developmental stages, but the peripheral mantle zone, surrounding the germinal centers, is densely populated by T cells, with a relatively high CD4:CD8 T cell ratio.[173] Recurrent infections with or without enlargement (hypertrophy) are the most common tonsil diseases, which can lead to removal of the tonsils by tonsillectomy.

1.3 Streptococci

The *Streptococcus* is a spherical Gram-positive bacterium. Some *Streptococcus* species are highly virulent and can cause infections. However, many are not pathogenic and form part of the healthy human microbiota of the skin, mouth, respiratory tract and intestine. Species of streptococci are classified based on their haemolytic properties: α-haemolytic streptococci cause partial haemolysis on blood agar by secreting hydrogen peroxide, whereas β-haemolytic streptococci cause complete haemolysis by secreting streptolysin. The β-haemolytic streptococci are further classified to 20 known Lancefield serotypes, groups A to V, according to their carbohydrate surface antigens. Group A β-haemolytic streptococci (GAS), especially *Streptococcus pyogenes,* are responsible for a wide range of invasive and non-invasive infections, and post-infectious immune-mediated disorders. GAS are the most common bacterial cause of acute pharyngitis, accounting for 5-10% of cases in adults and 15-30% in children,[174] but group C and G streptococci (GCS and GGS) can also cause pharyngitis.[175] Autoimmune syndromes following GAS infections include acute rheumatic fever, rheumatic heart disease and post-streptococcal glomerulonephritis.[176] The *Streptococcus anginosus* group belongs mostly to the non-β-haemolytic streptococci group but some anginosus strains carry a typable Lancefield group antigen, A, C, G or F.[177] *Streptococcus anginosus* often resides commensally in the human oral cavity but can give rise to pharyngitis.[178]

Although traditionally an extracellular pathogen, the β-haemolytic streptococci are able to survive within macrophages[179] and epithelial cells,[180,181] which has been associated with persistent streptococcal carriage and recurrent infections.[182,183] Streptococci possess virulence factors that help them penetrate host tissues and counteract the host's immune

system, including M proteins, streptolysins and superantigens. The M proteins are a major streptococcal virulence factor and highly immunogenic. They are expressed on the surface of group A, C and G streptococci and are involved in adhesion to surfaces, cell invasion and interruption of host phagocytosis.[180,184,185] Over 200 different M proteins types exist, and these are used to classify group A, C and G streptococci.[186] Streptolysin O is an exotoxin released by β-haemolytic streptococci and antibodies to streptolysin O (ASO titres) can be measured to indicate a recent streptococcal infection. Streptococcal throat infections are most common in children between 5 and 15 years, often around puberty.[175,187] Interestingly, the onset of psoriasis is often around puberty, especially guttate psoriasis.

1.4 The involvement of streptococci in psoriasis

The association between streptococcal throat infections and the onset of guttate psoriasis was first reported about 100 years ago,[188] and has since then been confirmed in many studies. Patients with psoriasis have higher ASO titers than both controls and the general population, with patients with guttate psoriasis having the highest titers.[20,21,189,190] Streptococcal throat infections precede guttate lesions by 1-3 weeks in 56%-97% of guttate cases,[191] and guttate patients have a higher frequency of throat β-haemolytic streptococci compared with controls.[192] Mallbris *et al.* reported that 84% of guttate patients had disease onset associated with a recent infection, of which 63% had a confirmed streptococcal throat infection. However, only 7% of patients with newly diagnosed plaque psoriasis had streptococcal pharyngitis. This difference can partly be explained by earlier medical attention for guttate psoriasis, due to the sudden onset and extensiveness, compared with a slower onset of plaque psoriasis.[23] Recently it was reported that streptococcal extracts induce circulating memory CLA+ T cells, in guttate patients, to show a Th17 dominant response when co-cultured with epidermal cells. The response was especially prominent in *HLA-Cw*0602*-positive patients with a history of streptococcal-associated psoriasis exacerbation.[193]

The association between streptococci and chronic plaque psoriasis is less well examined. Tervaert *et al.* reported that 20-30% of plaque patients had positive streptococcal throat cultures and raised ASO titers,[194] and increased serum IgG levels against *Streptococcus pyogenes* have been reported in plaque patients.[195] Furthermore, recurrent sore throats have been shown to be 3 times more common in patients with plaque psoriasis than age and sex matched controls.[196] In line with this, Gudjonsson *et al.* followed over 200 patients with plaques psoriasis and matched household controls over the course of a year. Almost 30% of the patients got sore throats compared with only 2.6% of the controls and β-

haemolytic streptococci were 10 times more often cultured from patients' tonsils.[197] We have reported a 44% combined carrier rate for groups A, C and G streptococci in patients with plaque psoriasis and a history of sore throat-associated psoriasis exacerbation, which was significantly more compared with recurrently infected tonsils from patients without psoriasis.[198]

It has been suggested that patients with psoriasis are more susceptible to streptococcal throat infections because of their genetic background. Carriage of *HLA-Cw*0602* is associated with increased frequency of streptococcal throat carriage/infections[96,101] and streptococcal-associated psoriasis exacerbations.[27,101] Streptococci can induce the production of key psoriasis cytokines (IL-17, IL-22 and IFN-γ) in circulating CLA+ memory T cells cultured together with both lesional and nonlesional epidermal cell from psoriasis patients.[199] These observations indicate that streptococci are involved in pathological mechanisms of psoriasis, such as keratinocyte activation and IL-17 production.

Long-term antibiotic treatment for psoriasis has been reported. Two recent studies have shown the benefits of both intra muscular penicillin and oral azithromycin.[200,201] However, other studies have not supported a beneficial effect of antibiotics on psoriasis.[202-204] Antibiotics can reduce the bacterial load in the tonsils, but there are reservoirs of intracellular streptococci in the tonsillar epithelial cells and macrophages,[182] which can reactivate, recolonize and cause symptoms again. Tonsillectomy, on the other hand, should remove the entire pool of tonsil streptococci. Streptococcal throat infections have also been linked to exacerbation of PPP, especially α-haemolytic streptococci,[205,206] and studies have shown that PPP can improve after tonsillectomy.[37-39]

Proposed mechanisms for the association between psoriasis and streptococci include the following 3 hypotheses: (1) Superantigen induced immune response; (2) Molecular mimicry triggered T cell mediated autoimmune response; (3) Innate immune-mediated inflammatory response caused by peptidoglycans. Each of them will be explained in more detail in the next 3 subchapters.

1.4.1 Superantigen induced T cell activation

Streptococcal superantigens (SAgs) are extracellular protein toxins that bypass conventional antigen processing. They bind directly to the MHC class II molecule and the TCRs variable region of the β chain (Vβ).[207] Moreover, the superantigens can bind to more than 1 Vβ region. This leads to non-specific polyclonal T cell activation of a relatively large number of T cells, with massive release of pro-inflammatory cytokines, which can circumvent the

formation of an antigen-specific immune response.[208] Superantigens and inflammatory mediators such as IL-12 released during a streptococcal pharyngitis can induce the expression of the skin-homing molecule CLA on effector T cells, facilitating their homing to the skin.[209,210] The hypothesis of superantigen induced psoriasis is based on reports of Vβ restricted T cell proliferation in psoriasis skin, consistent with superantigen stimulation,[211,212] and selective expansion of Vβ2+ T cells, induced by the superantigen speC in guttate patients with a streptococcal throat infection.[213] However, Vβ analysis of T cells in the blood of patients with plaque psoriasis has not supported a role of superantigens.[214] Furthermore, numerous studies have demonstrated an oligoclonal T cell expansion in plaque lesions, indicating that the process is driven by specific antigen(s), rather than a superantigen-mediated process (reviewed in[142]).

1.4.2 Molecular mimicry

Molecular mimicry is created when amino acid sequences of self-peptides are sufficiently similar to pathogen-derived peptides to give rise to cross-activation of autoreactive T cells. Once activated, the cross-reactive T cells can initiate an autoimmune reaction, leading to inflammation and tissue damage.[215]

A number of studies have consistently indicated oligoclonal T cell expansion in psoriasis skin,[216-221] suggesting a major role for antigen-specific T cells in psoriasis.[142] Valdimarsson *et al.* proposed over 20 years ago that psoriasis might be initiated by a streptococcal tonsillitis and maintained by means of molecular mimicry between streptococcal and self-antigens.[141] A structural sequence homology between streptococcal M proteins and human keratins had then already been reported.[222] Tonsils from patients with psoriasis have an increased number of skin-homing T cells compared with patients without psoriasis.[198] T cells showing increased responses against streptococcal protein extracts and M proteins have been detected in the blood of patients with psoriasis but not in patients with atopic dermatitis or healthy controls.[223-228] Furthermore, T cells from patients with psoriasis show increased responses to homologous peptides from M proteins and epidermal keratins 16 and 17,[224,229,230] that are highly upregulated in psoriasis skin.[67] Moreover, when peripheral blood lymphocytes were stimulated with keratin peptides that share amino acid sequences with streptococcal M proteins, Johnston *et al.* found that the skin-homing CD8+ T cells and not the CD4+ T cells responded to these peptides. This was most pronounced in *HLA-Cw*0602*-positive patients.[224] In line with this, Prinz *et al.* isolated T cells with the same clonal T cell receptor Vβ gene rearrangements from tonsils and skin, indicating a mutual origin. These

patients had streptococcal-associated psoriasis exacerbations, and all experienced long-term remission after tonsillectomy.[231] Collectively these findings indicate that psoriasis may be a consequence of cross-reactive T cells reacting with epitopes from streptococcal M-proteins in the tonsils and epidermal keratins in the skin, and thus be a molecular mimicry based autoimmune disease.[141,142,232]

Homologies between human keratins and the streptococcal peptides RopA, RecF and FcR have been reported,[233] and keratin peptides that react with antibodies against *Streptococcus pyogenes*, including keratin 6, ezrin, maspin, peroxiredoxin 2 and HSP27 have been identified.[233]

1.4.3 Peptidoglycans

The third hypothesis involves peptidoglycans (PGN), which are a major constituent of the cell wall of Gram-positive bacteria, including streptococci. They enable bacteria to resist osmosis[234] and are recognized by peptidoglycan receptor proteins in tonsils, skin and gut, facilitating the killing of bacteria (reviewed by[235,236]). PGN are also recognized by TLRs and thereby initiate an immune response. It has been suggested that the innate immune system in psoriasis is continually stimulated by PGN within the skin and PGN containing cells (mostly macrophages) are found more frequently in close proximity to CD4+ T cells in lesional skin compared with normal or non-affected skin. PGN originating from tonsils have also been shown to trigger Th1 mediated response in psoriasis skin, and CD4+ T cells respond in an HLA-restricted way to peptides associated with PGN by producing IFN-γ.[237,238]

1.5 Psoriasis and tonsillectomy

Tonsillectomy is defined as the surgical excision of the palatine tonsils. The major indications for tonsillectomy are recurrent infections or obstruction caused by hypertrophic tonsils.[239,240] Most patients have an uncomplicated post-operative course, but potential adverse events include postoperative bleeding, infection, laryngospasm and broncho-spasm.[241]

The question of whether tonsillectomy is beneficial for psoriasis has long been disputed. Several studies have reported considerable clinical improvement of psoriasis after tonsillectomy and 3 reviews have recently summarized these studies.[242-244] Rachakonda *et al.* concluded that 290 out of 410 (70%) patients with psoriasis improved after the procedure and that tonsillectomy may be beneficial in selected cases of therapy-resistant psoriasis with a tendency to chronic tonsillitis and associated psoriasis exacerbations.[242] In the past 53 years, 23 studies have been published on the effect of tonsillectomy on psoriasis (Table 3).

One of those is a prospective randomized controlled trial,[245] which details are elaborated in the results and discussion sections of this thesis and in papers I and II.

Three prospective controlled studies have been published on the effect of tonsillectomy on psoriasis.[246-248] Ponomareva[246] (1965) reported improvement in 8 out of 9 tonsillectomized patients and Cepicka and Tielsch[247] (1967) showed complete clearance in 34 of 56 (61%) of tonsillectomized patients for 2-5 years, compared with 8 of 36 (22%) of controls. Nesterenko *et al.*[248] (1972) reported improvement of 34 of 66 (52%) tonsillectomized patients, compared with 3 of 35 (9%) of controls. The authors of these 3 studies did not provide information on clinical subtypes of psoriasis.

Three prospective uncontrolled observational studies have been published.[249-251] Lukovskii *et al.*[251] (1970) studied 57 patients with psoriasis and chronic tonsillitis where 51 (89%) cleared or improved after tonsillectomy. Furthermore, 44% were still completely clear of psoriasis after 6-10 years. Kataura and Tsubota[249] (1996) studied 35 patients with plaque psoriasis and reported that 49% of them improved following tonsillectomy. Interestingly, the procedure was more effective in women, where 84% of the women improved, compared with only 31% of the men. Furthermore, the tonsillectomy proved to be more effective in younger patients. The same year, Hone *et al.*[250] (1996) studied a mixed group of patients with recalcitrant guttate (n=6) and plaque (n=7) psoriasis. All patients with guttate psoriasis improved, and 5 cleared completely. Four out of 7 patients with plaque psoriasis improved after the tonsillectomy, thereof 2 that became completely cleared of psoriasis.

Three published studies are retrospective.[252-254] Nyfors *et al.*[252] (1976) reported improvement of in 72% of patients with psoriasis vulgaris refractory to topical treatments, with complete clearance in 24 out of 74 (32%) and a considerable improvement in 29 (39%). Interestingly, the authors also reported an improvement in patients without a history of sore throat-associated psoriasis exacerbation. However, the study was based on a retrospective questionnaire and thus subjected to recall bias. Ozawa *et al.*[253] (1999) conducted a study on patients with generalized pustular psoriasis where 2 out of 12 tonsillectomized patients improved. Bucolo *et al.*[254] (2013) recently published improvement of 5 plaque and 8 guttate patients with sore throat-associated psoriasis exacerbation. Six out of 8 patients with guttate psoriasis clinically improved with a PASI reduction ranging from 50-90%, lasting 6 months to 5 years. In 3 out of 5 of the patients with plaque psoriasis the PASI scores improved from 50-100% and the improvement lasted 6 months to 12 months.

Five case series have been published.[255-259] Tytar *et al.*[255] (1968) reported an improvement of 22 out of 29 (76%) patients with psoriasis, of which 13 (45%) completely

cleared. Bukharovich *et al.*[256] (1971) reported a complete clearance of 14 patients with psoriasis and chronic tonsillitis. Fukunaga[257] (1974) reported an improvement in 4 out of 5 tonsillectomized patients, of which 3 completely cleared. Rosenberg *et al.*[258] (1998) reported improvement of 14 patients, colonized by throat streptococci unresponsive of antibiotics, whereas 9 (65%) had complete clearance. Takahara *et al.*[259] (2001) followed up 7 tonsillectomized patients with psoriasis for 2–9 years. Three patients completely cleared and 2 patients were 80% clear of psoriasis.

Eight case reports are reported in the literature.[21,231,260-265] Vovk and Testemitsanu[260] (1963) reported an improvement of psoriasis and psoriatic arthritis, aggravated by tonsillitis. Saita *et al.*[262] (1979) described the complete clearance of 2 young sisters that developed guttate psoriasis 1 week after a sore throat. McMillin *et al.*[264] (1999) reported a complete remission of 2 children with psoriasis and a history streptococcal throat infection. The group of Prinz[231] (2006) reported 3 patients with therapy-resistant plaque psoriasis with guttate flares that were clear of psoriasis for 3 years after tonsillectomy. Interestingly, they reported the same T-cell receptor beta-chain variable region gene rearrangements within the skin and tonsils of these patients, suggesting a recirculation to the skin after activation in the tonsils. Simoes *et al.*[265] (2015) recently reported a case of severe therapy-resistant plaque psoriasis with recurrent acute pharyngitis and associated psoriasis exacerbation. Lesions almost completely cleared within 4 months with PASI decreasing from 26.8 to 1, lasting at least 2 years.

All of the above studies suggest a beneficial effect of tonsillectomy on psoriasis, at least in selected patients. In summary, the 23 studies included 436 cases; 141 plaque, 20 guttate, 12 GPP, and 263 unspecified psoriasis. Of those 436 cases, were 323 (74%) patients that improved after tonsillectomy; 99 (70%) with plaque psoriasis, 18 (90%) with guttate psoriasis, 2 (17%) with GPP and 192 (73%) of the unspecified psoriasis group. However, most of the data are based on case reports, case series and retrospective studies, without randomization and control groups, making it more difficult to draw concrete conclusions. Furthermore, many of the reports lack specification of psoriasis phenotype studied and in the older studies outcome measures are not standardized in terms of PASI scores or other psoriasis severity measures. Thus, a well-conducted randomized controlled trial on the effect of tonsillectomy on psoriasis was needed.

Table 3. Published studies on the effect of tonsillectomy on psoriasis

Study	Year	Country	Study design	Disease	Number in study	Gender	Age range (mean)	Outcome	Follow-up period
Simoes *et al.*	2015	Portugal	Case report	Plaque psoriasis	n = 1	M	39 y	Only residual plaques after 4 months, PASI reduction: 26.8 to 1	2 years
Bucolo *et al.*	2013	Italy	Retrospective case series	Plaque and guttate psoriasis with chronic tonsillitis	n = 13, 5 CPP, 8 GP	6 M, 7 F	9-46 y (18.2)	Plaque: 60% improved short term, 40% were unchanged Guttate: 25% improved long term, 50% short term and 25% unchanged	2-5 years
Thorleifsdottir *et al.*	2012	Iceland	Single-blinded, randomized controlled trial	Plaque psoriasis with history of exacerbation after sore throat	n = 29, Tx = 15, controls = 14	TX = 3 M, 12 F, controls = 6 M, 8 F	19–54 y (35.5)	86% showed 30–90% improvement in PASI, 13% were unchanged. No changes in control group	2 years
Diluvio *et al.*	2006	Germany	Case report	Recalcitrant nonpustular plaque psoriasis with guttate flares	n = 3	NR	21 y, 29 y, 33 y	Complete remission all 3 patients	> 3 years
Takahara *et al.*	2001	Japan	Case series	Psoriasis	n = 7	3 M, 4 F	9–46 y (23)	43% all cleared, 29% with 80% cleared lesions and 29% unchanged	2–9 years
Ozawa *et al.*	1999	Japan	Retrospective case series	Generalized pustular psoriasis	n = 12	NR	NR	17% effective, 50% of cases showed decrease in pustular lesions	NR
McMillin *et al.*	1999	U.S.A.	Case report	Guttate and plaque psoriasis	n = 2, 1 CPP, 1 GP	1 M, 1 F	5 and 11 y,	All lesions cleared	16 months
Rosenberg *et al.*	1998	U.S.A.	Case series	Psoriasis	n = 14	NR	NR	65% all cleared, 36% improved	NR
Kataura and Tsubota	1996	Japan	Prospective case series	Plaque psoriasis	n = 35	16 M, 19 F	NR	Remarkably effective in 29%, effective in 20%, partially effective in 11%, unchanged in 31% and worsened in 9%	3 months
Teranishi *et al.*	1995	Japan	Case report	Psoriasis	n = 4	NR	NR	75% improved, 25% unchanged	NR
Hone *et al.*	1996	Ireland	Prospective case series	Guttate and plaque psoriasis with history of exacerbation after sore throat	n = 13, 7 CPP, 6 GP	1 M, 12 F	6–28 y (17)	Plaque: 29% cleared, 29% improved, 43% were unchanged Guttate: 83% cleared, 17% improved	6–52 (26) months
Saita *et al.*	1979	Japan	Case report	Guttate psoriasis	n = 2	2 F	7 y, 11 y	Both were almost completely cleared 2 months after tonsillectomy	NR
Nyfors *et al.*	1976	Denmark	Retrospective case series	Plaque psoriasis	n = 74	18 M, 56 F	4–33 y (14.2)	32% cleared, 39% improved, 22% were unchanged and 7% worsened	4.5 years (7–204 months)
Fukunaga	1974	Japan	Case series	Psoriasis	n = 5	NR	NR	60% cleared, 20% improved, 20% unchanged	1-4 y
Nesterenko *et al.*	1972	U.S.S.R	Prospective case-control	Psoriasis with a history of exacerbation after sore throat	n = 101, Tx = 66, controls = 35	NR	NR	52% significantly improved after tonsillectomy vs. 9% of controls	up to 10 years
Bukharovich *et al.*	1971	U.S.S.R	Case series	Psoriasis and chronic tonsillitis	n = 14	NR	NR	100% all cleared after the tonsillectomy, after 1 year 79% still all clear	1 year
Lukovskii *et al.*	1970	U.S.S.R	Prospective case series	Psoriasis and chronic tonsillitis	n = 57	40 M, 17 F	<14 y: 9 pat, 15-21 y: 12 pats, 21-40 y: 21 pats, >40 y: 5 pats	37% all cleared, 53% improved, 11% unchanged. 9 patients followed 6-10 y: 44% still cleared, 44.4% treatable exacerbations, 11% unchanged	7-15 days after tonsillectomy. 9 patients: 6-10 years
Tytar and Bashmakov	1968	U.S.S.R	Case series	Psoriasis and chronic tonsillitis	n = 29	NR	NR	45% all cleared, 31% improved, 24% unchanged	NR
Stukalenko	1967	U.S.S.R	Case report	Psoriasis and chronic tonsillitis	n = 1	1 M	24 y	Improvement of psoriasis	1 Year
Cepicka and Tielsch	1967	Germany	Prospective case-control	Psoriasis associated with infection	n = 92, Tx = 56, controls = 36	NR	NR	61% all cleared after tonsillectomy vs. 22% of the controls	2–5 years
Ponomareva	1965	U.S.S.R	Prospective case-control	Psoriasis and chronic tonsillitis	n = 42, Tx = 9, controls = 33	NR	NR	89% improved, 11% unchanged after tonsillectomy	1.5 years
Whyte and Baughman	1964	U.S.A.	Case report	Guttate psoriasis with chronic tonsillitis	n = 3	2 M, 1 F	15–23 y (20)	Occasional plaques after tonsillectomy	1 year
Vovk and Testemitsanu	1963	U.S.S.R	Case report	Psoriasis and psoriasis arthritis aggravated by tonsillitis	n = 1	1 M	32 y	Skin and arthritis symptoms improved and the patient could walk independently again after 11 days	8 months

NR, not reported, CPP: chronic plaque psoriasis, GP: Guttate psoriasis, M: male, F. female, y: year

2 Aims

The association between streptococcal throat infection and psoriasis prompted the following working hypothesis: As the palatine tonsils have the components required for sampling and presenting antigens to naïve lymphocytes, they might prime streptococcal antigen-specific T cells that subsequently migrate from the tonsils into the skin where they may recognize keratin antigens that share amino acid sequences with streptococcal peptides, resulting in eruption of guttate or worsening of plaque psoriasis.

The overall aim of this thesis was to test this hypothesis, and to evaluate tonsillectomy as a treatment option for patients with chronic plaque psoriasis.

First, we aimed to evaluate, in a prospective randomized controlled trial the clinical and immunological effect of tonsillectomy on plaque psoriasis, by assessing disease severity (PASI score), and frequency of circulating T cells that recognize antigen entities that are shared by streptococcal M proteins and skin (paper I).

Second, we aimed to evaluate the psychological and social impact of tonsillectomy on plaque psoriasis by measuring health-related quality of life (paper II).

Third, we aimed to estimate the proportion of patients who would most likely have the greatest benefit from tonsillectomy, by evaluating the frequency of sore throat and/or streptococcal-associated exacerbations in patients with plaque psoriasis and establishing whether HLA genotype can aid this identification (paper III and IV).

3 Materials and methods

3.1 Study approval

The National Bioethics Committee of Iceland (VSNb2006090015/03-15), the Data Protection Authority of Iceland and the Ethics Committee of Landspitali-The National University Hospital of Iceland approved the study. It was performed in compliance with the 1964 Declaration of Helsinki and its later amendments.

3.2 Study design

A single center, 24-month, prospective, observer-blind, parallel, randomized controlled trial (RCT) was carried out to examine the clinical, psychosocial and immunological impact of tonsillectomy on patients with chronic plaque psoriasis (Paper I and II). A prospective case series was performed to explore if *HLA-Cw*0602* carriage was associated with a favourable outcome after tonsillectomy (Paper III). Data were collected between November 2007 and January 2011, within the departments of Dermatology, Immunology and Otolaryngology-Head and Neck Surgery at Landspitali-The National University Hospital of Iceland. A large retrospective case series was carried out from January 2011 to April 2011 to estimate the proportion of patients with psoriasis who have experienced exacerbation in association with sore throat, and might therefore be more likely to benefit from tonsillectomy (Paper IV). Data were collected at the dermatology outpatient center at Landspitali-The National University Hospital of Iceland, Hudlaeknastodin dermatology clinic, Kopavogur and the Blue Lagoon geothermal clinic, Grindavik.

3.3 Participants and randomization

Recruitment and screening of patients for the RCT took place from November 2007 to November 2008. Each patient gave written informed consent before initiation of study participation and 54 patients were screened. Most patients were referred by dermatologists (44%) or responded to advertisement (46%). A few patients heard about the study by other means (6%) and otolaryngologists referred 2 patients (4%). Key inclusion criteria are listed in Table 4. Before the patient recruitment began, the study supervisor created a sealed numerical allocation sequence, done with a simple randomization in a 1:1 ratio. Twenty-nine patients met all inclusion criteria and were randomly allocated into tonsillectomy (TX) and control groups by the allocation sequence. A numerical code was used to identify patients

and their specimens. All investigators except the study's supervisor were unaware of the treatment allocation, which was concealed until the end of the study in order to reduce study bias. Moreover, the patients received strict instructions not to reveal their tonsil status during follow-up. Thirteen patients with plaque psoriasis and a history of sore throat-associated psoriasis aggravation were later added to the TX group, 8 new patients and 5 patients who had previously been controls in the RCT, but opted for tonsillectomy after the 24-month follow-up (paper III). Additional 374 patients with psoriasis were invited to participate in a retrospective case series study. After a 4-month study period, 275 patients were included, yielding a 73.5% response rate (paper IV).

Table 4. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Moderate to severe chronic plaque psoriasis diagnosed by a dermatologist	Other psoriasis phenotypes (e.g. guttate, pustular, erythrodermic)
Age ≥ 18 years	Previous tonsillectomy
History of psoriasis exacerbation in association with sore throat and/or streptococcal throat infection	Unwillingness to stop psoriasis treatment at least 4 weeks prior to study entry
Psoriasis onset before 40 years of age	Alcohol or drug abuse
Signed informed consent	Pregnancy or planned pregnancy within a year
Consent to have tonsillectomy	Participation in another clinical trial concurrently
	Significant underlying medical conditions increasing patients risk for anaesthesia and surgery, such as: Chronic heart diseases Chronic lung diseases Chronic kidney diseases Bleeding disorders

3.4 Visit schedule

Patients in the RCT were followed up at regular intervals for 24 months by the same investigator, who was unaware of patient's tonsil status. The additional 13 patients were also followed up for 24 month, however unblinded. Detailed data was accumulated during screening: demographics, psoriasis features, association to various factors reported to exacerbate psoriasis and prior and concomitant psoriasis treatments (Figure 4). Health and medical history as well as alcohol and smoking habits were also noted. The severity of psoriasis was assessed and a brief physical examination, mainly heart and lung function, was performed as well as measurement of vital signs, height and weight. Patients included in the RCT then proceeded to a 4-week washout period, which included no antibiotics or psoriasis treatments other than moisturizers, before the baseline visit. The baseline visit included assessment of psoriasis clinical severity, health-related quality of life and psoriasis–related stress, heparinized venous blood sample and skin biopsy. Patients who had given permission

for photographic documentation were photographed and a bacterial throat swab was collected. Thereafter, the patients were asked to remain off all psoriasis treatments for 8 weeks or until the first follow-up visit and subsequently they could be treated according to what they and their dermatologists felt indicated. The clinical follow-up was scheduled 2, 6, 12, 18 and 24 months after the baseline visit and venous blood samples for immunological measurements were collected after 2, 12 and 24 months.

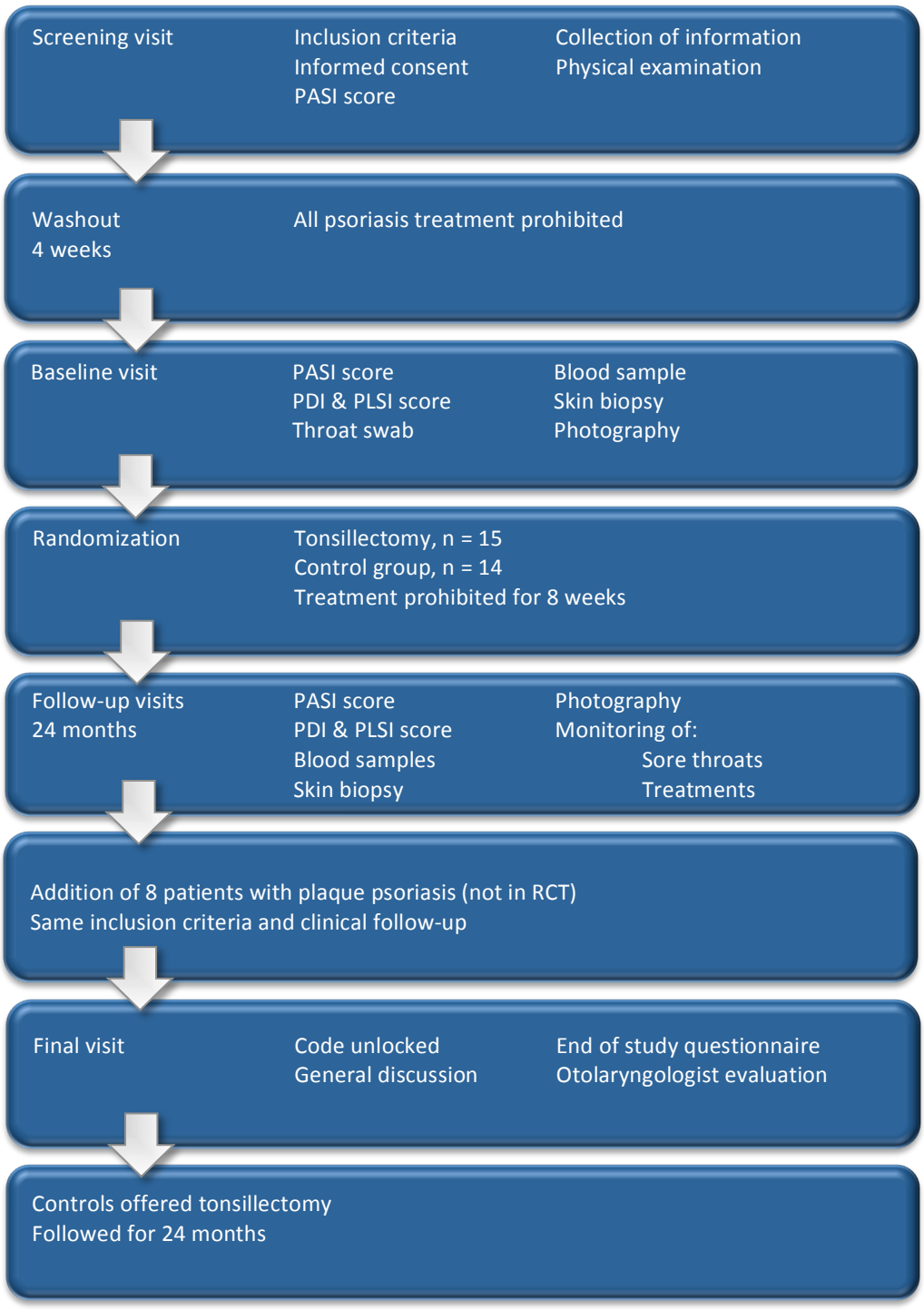

Figure 4. Flow chart of the study design
PASI, Psoriasis Area and Severity Index; PDI, Psoriasis Disability Index; PLSI, Psoriasis Life Stress Inventory.

Follow-up questionnaires for assessment of health-related quality of life and psoriasis–related stress were collected after 12 and 24 months. Patients were photographed at regular intervals and a skin biopsy was taken 6 months after the baseline visit. The use of psoriasis treatments during the follow-up period was closely monitored as well as the frequency of sore throats or streptococcal throat infections. At the end of the study, all tonsillectomized patients were examined for tonsillar remnants by an otolaryngologist and answered a study-specific end-of-study questionnaire. Furthermore, patients who had been allocated to control group were offered tonsillectomy.

3.5 Study end points and safety assessments

The primary endpoints were as following: (1) Significant clinical improvements and a coinciding decrease in the frequency of blood T cells that recognize autoantigens in the skin after tonsillectomy; (2) Significant improvements in patient-reported outcomes (health-related quality of life) after tonsillectomy; (3) Significant improvements in tonsillectomy outcomes of patients carrying *HLA-Cw*0602*. The key secondary endpoints included: (1) Correlation of T cells that respond to homologous keratin and streptococcal peptides in tonsils and blood at the time of tonsillectomy; (2) Proportion of patients who achieve PASI 50 (≥ 50% reduction in PASI from baseline), PASI 75 and PASI 90 responses; (3) Correlation between the change in clinical status (PASI scores) and health-related quality of life (PDI and PLSI scores).

Post operative safety assessment following tonsillectomy, including evaluation of complications, was in the hands of the otolaryngologist who performed the procedures.

3.6 Assessment of clinical symptoms

The severity of psoriatic skin lesions was evaluated with the Psoriasis Area and Severity Index (PASI), which is a widely validated score for assessing the extent and severity of psoriasis.[55] The score is based on 3 variables, erythema (E), infiltration (I) and desquamation (D), graded from 0 to 4 (none, slight, moderate, severe and very severe). The body is divided to 4 regions; head and neck (H), trunk (T), upper extremities (U) and lower extremities (L) and the area (A) covered by psoriasis in each region is calculated as a percentage of the total regional area. Each region is then weighted in the total score according to its percentage of the whole body; head and neck 10%, upper extremities 20%, trunk 30% and lower extremities 40%. Thus, the PASI score is calculated:

$$PASI = 0.1(E_H+I_H+D_H)A_H+0.2(E_U+I_U+D_U)A_U+0.3(E_T+I_T+D_T)A_T+0.4(E_L+I_L+D_L)A_L$$

The PASI score may range from 0 to 72. However, scores over 10 generally represent severe psoriasis and scores under 10 a mild to moderate disease.

3.7 Questionnaires

3.7.1 Evaluation of health-related quality of life

Health-related quality of life (HRQoL) was determined at study entry and at 12 and 24 months with 2 patient-reported outcomes, the Psoriasis Disability Index (PDI)[59] and the Psoriasis Life Stress Inventory (PLSI).[60] The PDI is a self-administered, psoriasis-specific quality of life questionnaire, which has been validated and used in many studies. It grades functional disability due to psoriasis in the preceding 4 weeks before its completion. The PDI consist of 15 questions that can further be subgrouped to 5 issues: daily activities, work/school, personal relationships, leisure, and treatments. With a 4-point scale, rating from "not at all" to "very much", the grades are summed to yield a total score, from 0 to 45.

The PLSI evaluates patients' stress related to having to cope with psoriasis on an every day bases. The PLSI is also composed of 15 questions, which are rated on a 4-point scale from "not at all" to "very much" experienced stress in the previous 4 weeks. The score is calculated by summing the grades for each question and can range from 0 to 45. Both the PDI and PLSI have been used in English for evaluation of psoriasis. Icelandic versions of the questionnaires were used, previously translated and validated by the Nordic Quality of Life study.[266]

3.7.2 End of study questionnaire

At the end of the RCTs 24-month study period, all patients answered a study-specific questionnaire composed of 20 multiple-choice and 3 short-answer questions. The main objective of this questionnaire was to rate the overall experience of having tonsillectomy as a treatment for psoriasis. The answers were rated on a scale ranging from "not at all" to "very much". Included in the questionnaire were issues concerning the difficulty of the procedure, experience of complications, recovery time and if the patient felt that the operation was worthwhile. Patients were also asked if their clinical symptoms had improved by the surgery and if their use of psoriasis treatments had changed. Furthermore, the questionnaire contained questions about the quality of life and frequency of sore throat and/or streptococcal throat infections and subsequent exacerbation of psoriasis after removal of their tonsils.

3.7.3 Retrospective study questionnaire

A study-specific anonymous questionnaire containing 15 short-answer and multiple-choice questions was designed. The main objective of this questionnaire was to evaluate the ratio of patients with psoriasis exacerbations associated with sore throats to assess the usefulness of tonsillectomy as a treatment option.

The questionnaire was divided into 5 main topics: (1) General demographics; (2) Psoriasis phenotypes, age at onset and onset associated with a sore throat or streptococcal throat infection; (3) History of sore throats and previous streptococcal throat infections, diagnosed by a throat culture, rapid antigen detection test (strep test) or a physician. Further, whether psoriasis exacerbations were experienced within 3 weeks of a sore throat/streptococcal throat infection; (4) Other known psoriasis aggravating factors, including general malaise, stressful life events, cold climate, alcohol and drugs; (5) History of tonsillectomy after psoriasis onset, the age at the time of the surgery and the tonsillectomy's impact on psoriasis.

3.8 Preparation of tonsil tissue

After surgical removal from the pharynx, the tonsils were immersed in cold sterile saline and transported to the department of Immunology. There the tonsils were prepared for utilisation (Figure 5). This part of the research was done in collaboration with Sigrún Laufey Sigurðardóttir who conducted her PhD project at the same time.[198,244,267]

3.8.1 Bacterial culture and typing

Bacterial throat swabs were taken from all included patients at study entry. After tonsil excision, swabs were taken from the surface epithelia and tonsil crypts. Typing of the bacteria was performed by culture on sheep blood agar. Subspecies of *Streptococcus* were identified with a Streptex kit (Thermo Fisher Scientific, Remel, Lenexa, USA).

3.8.2 Segregation of tonsil cells

Tonsils were minced into small pieces, passed through a tea strainer and washed with Hank's balanced salt solution (HBSS, Gibco, Invitrogen Ltd, Paisley, UK). Tonsil mononuclear cells (TMCs) were isolated using a Ficoll density gradient centrifugation (Sigma-Aldrich, St. Louis, MO, USA) by collection at the interphase fraction,[268] and washed with HBSS. TMCs were then set up for cell culture by resuspending them in Roswell Park Memorial Institute media 1640 (RPMI, Gibco) at a density of 1.0×10^6 cells/ml, with 10% heat-inactivated fetal calf serum (Gibco), 100 μg/mL streptomycin (Sigma-Aldrich) and 100 U/mL penicillin (Sigma-Aldrich).

3.9 Isolation of blood and skin cells

Venous blood samples were taken at months 2, 12 and 24. Peripheral blood mononuclear cells (PBMCs) were collected the same way as the TMCs or at the interphase fraction formed by Ficoll density gradient centrifugation. The cells were resuspended in supplemented RPMI-1640 medium for cell culturing (Figure 5).

Four mm skin biopsies were taken from lesional psoriasis skin and stored in saline until transported to the department of Immunology. Statamatrix-TM matrices (Cell Sciences, Canton, USA) were prepared as previously described.[269] In short, subcutaneous fat was removed from the skin biopsies and the tissue minced to 2 mm pieces before being placed on the surface of the matrix. The matrix with skin explants was then placed into one well of a 24-well cell culture plate (Nunc, Thermo Fisher Scientific, Roskilde, Denmark). The culture was maintained with 2 ml/well Iscove's Modified Dulbecco's Medium (Gibco) with 10% heat-inactivated fetal calf serum, 100 µg/mL streptomycin, 100 U/mL penicillin (Sigma-Aldrich) and 3.5 µl/L 2-mercaptoethanol. Culture medium was replaced 3 times per week and treated with IL-2 (100 U/ml) and IL-15 (20 ng/ml). T cells were expected to spill from the matrices into the wells from day 7 to day 21. However, T cells yielded were too low and thus this analysis was aborted.

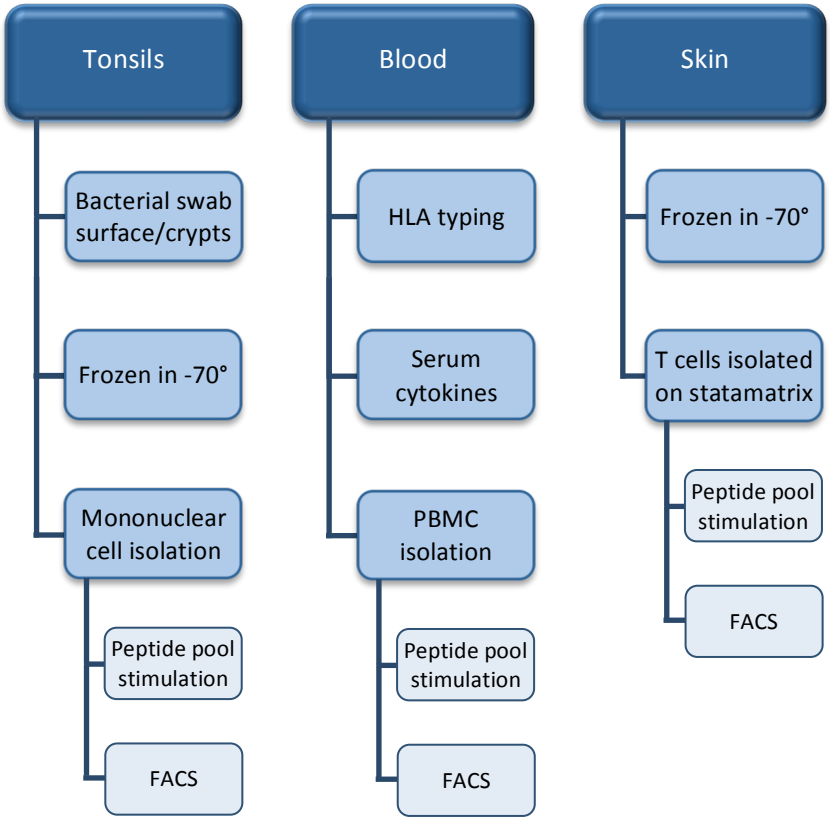

Figure 5. Processing of tonsil tissue, blood samples and skin biopsies

3.10 Peptide stimulation

3.10.1 Homologous streptococcal and keratin peptides

Arranged preparations of homologous peptides derived from human keratin 17 (K17) and streptococcal M protein 6 (M6) were used to stimulate T cells. These had previously been prepared as described by Johnston *et al*.[224] In summary, the amino acid sequence of K17 was split into a complete set of 9 overlapping residue peptides, and compared with the sequence of the M6 protein using the FASTA algorithm. The FASTA algorithm is a software package used to identify similar protein and nucleic acid sequences.[270] Sequence homologous peptides were confined to the conserved C-terminal of the M6 protein whereas the homologous K17 peptides were located in the coil-forming part of the keratin. The K17 peptides that shared 4-6 amino acids with the M6 peptides, were then selected according to predicted binding to *HLA-Cw*0602*. These homologous K17 and M6 peptides were then blended into 16 peptide pools, each containing 8 peptides. Thus, in total 64 short peptides were used (Table 5).

3.10.2 Determination of M/K peptide-reactive T cells

After isolation of PBMCs and TMCs, cells were cultured in complete RPMI in cell culture tubes (Nunc) at a density of $1x10^6$ cells/ml. The cells were stimulated with the 16-peptide pools (2 µg/ml total peptide each) for 16 hours, with the addition of anti-CD28 and anti-CD49d monoclonal antibodies (1µg/ml each, Serotec Scandinavia, Oslo, Norway) as co-stimulation. After the first 2 hours of stimulation, brefeldin A (10µg/ml, Sigma-Aldrich) was added to the cell cultures to inhibit cytokine secretion and thus promote an intracellular

Table 5. Composition of peptide pools
The table shows the size and location of the homologous keratin17 and M6 peptides.

	1	2	3	4	5	6	7	8
9	31K17-12	130-K17-9	206-K17-9	236K17-12	372-K17-9	261-M6-9	262M6-12	355-M6-9
10	101-K17-12	134K17-12	212K17-12	238-K17-9	379K17-12	282-M6-9	285M6-12	368M6-16
11	125-K17-9	135-K17-9	217-K17-9	239K17-12	379-K17-9	299M6-12	146M49	382M6-12
12	127-K17-9	139-K17-9	220-K17-9	309-K17-9	396-K17-9	299-M6-9	338M6-20	384-M6-9
13	128-K17-9	162-K17-12	220K17-12	334-K17-12	404-K17-9	306-M6-9	338M6-12	464M6-12
14	128-K17-10	165-K17-12	145K10	338-K17-12	407-K17-9	319M6-12	344M6-12	468M6-16
15	146K17	168-K17-9	145K17	351K17-12	415-K17-12	324-M6-9	354M6-12	GAGE[a]
16	146K9	179-K17-9	231-K17-9	366-K17-12	255-M6-12	327-M6-9	345-M6-9	BMRF-1[b]

Blue boxes: human keratin peptides, yellow boxes: streptococcal peptides, grey boxes: control peptides. [a]*Peptide used as a negative control for Cw6 binding.*[271] [b]*Peptide from Epstein-Barr Virus Protein BMRF-1 as a positive control.*[272] *Each peptide is situated in 2 different pools.*

cytokine build up. The cultures were then further incubated for 14 hours at a 5° slant at 37°C in a humidified 5% CO_2 atmosphere. Anti-CD3 antibodies (1µg/ml, Serotec Scandinavia) and streptokinase (200U/ml, Hoechst Marion Roussel AB, Stockholm, Sweden) were used as positive controls for T cell activation. As >90% of the T cells that respond to the homologous peptides express the skin-homing molecule CLA,[224] anti-CLA as well as anti-CD4 and anti-CD8 were used to characterize cells of interest. The cells were stained on ice in the dark for 20 minutes, washed twice with phosphate-buffered saline (PBS) before being fixed with 500µl cold (4°C) 2% paraformaldehyde for 10 min and further washed with PBS. Finally the cells were treated with permeabilizing buffer (0.5% BSA, 0.1% saponin, 0.1% sodium azide, Sigma-Aldrich) for 10 min at room temperature, followed by a wash and resuspension with the same buffer. The cells were then stained with anti-IL-17A or anti-IFN-γ in the dark for 20 min at 4°C, washed with permeabilizing buffer (1.5 ml) and centrifuged at 500x*g* for 5 min. Lastly, the cells were resuspended in 250µl of a fixing buffer composed of permeabilizing buffer and 1% paraformaldehyde. Fluorescence activated cell-sorting (FACS) analysis was carried out on a FACScalibur flow cytometer (BD Biosciences), gated on light scatter, CD4, CD8 and CLA expression, capturing a minimum of 200,000 events guided by appropriate isotype control antibodies. Data were analysed with CellQuest (BD) software.

Table 6. Antibodies for cell surface receptors and intracellular cytokines

Antibody	Conjugate	Clone	Provider
CD3	APC	UCHT-1	Biolegend[a]
CD4	PE	Clone 3.9	Biolegend
CD4	Percp-cy5.5	RPA-T4	Biolegend
CD8	Percp-cy5.5	RPA-T8	Biolegend
CLA	FITC	HECA-452	Biolegend
CD45RO	PE	UCHL1	Biolegend
CD45RA	PE	HI100	Biolegend
CCR4	PE	205410	R&D Systems[c]
CCR6	APC	53103	R&D Systems
CCR7	APC	150503	R&D Systems
CCR8	APC	191704	R&D Systems
CCR10	APC	314305	R&D Systems
CXCR3	PE	173	Biolegend
CXCR6	PE	56811	R&D Systems
IL-10	PE	JES3-7D9	Biolegend
IL-17A	AF 647	eBio64CAP17	eBioscience[d]
IL-22	PE	142928	R&D Systems
IFN-γ	PE	4S.B3	Biolegend

[a]Biolegend, San Diego, CA, USA, [b]Becton Dickinson, Franklin Lakes, NJ, USA, [c]R&D Systems, Minneapolis, MN, USA, [d]eBioscience, San Diego, CA, USA

3.11 Cytokine measurements

Enzyme linked immunosorbent assay (ELISA, R&D Systems, Minneapolis, MN) measured serum cytokines before and after tonsillectomy with 50µl of serum or standard, measured in duplicates. The optical density was identified at 450 nm and cytokine concentrations calculated from the standard curve.

3.12 *HLA-Cw*0602* typing

All participating patients were genotyped for *HLA-Cw*0602* after being followed up for 24-months. The DNA was prepared from PBMCs and the *HLA-Cw*0602* status determined by allele specific PCR amplification by genotyping 7 SNPs in exons 2 and 3 of the *HLA-C* gene, as previously described.[85]

3.13 Expression of the data and statistics

Data analyses were performed using R software, version 2.10 (The R foundation, Austria) and P values of less than or equal to 0.05 were considered significant. Patient demographics were summarised descriptively and data were tested for normality using the Kolmogorov-Smimov test. In general, categorical variables were compared with Chi-Square or Fisher's exact test as appropriate and continuous variables with unpaired Student's *t*-test. Data were analysed on an intention-to-treat basis and missing values replaced with the last observed value (last observation carried forward). Correlation data were determined with Spearman's rank correlation test.

Intracellular flow cytometric responses were determined as followed: if more than 0.05% of the CLA+CD8+ or CD4+ T cells expressed IFN-γ or IL-17 compared with the unstimulated T cell culture or fluorescence minus 1 control the response was interpreted positive. After stimulation with the 16-peptide pools, the frequency of T cells that responded to each pool was interpreted as a percentage of CLA+ cells in each of the T cell subpopulations, normalized using square root normalization, and finally expressed as an average for each patient.

Analysis of variance (ANOVA) test for repeated measurements was used to compare peptide responses, PASI, PDI and PLSI scores between the groups and different time points. The square root of the peptide responses, PDI and PLSI scores was used to better approximate normality in the ANOVA model. For the retrospective case study, multivariable analyses were performed with a logistic regression and a model of sore throat aggravation was pursued. Variables with a $p < 0.1$ were entered into the logistic regression model and associations in the multivariate logistic models were presented as odds ratios (OR) with 95%

confidence interval (CI). Linear regression was used to evaluate the effects of *HLA-Cw*0602* carriage on improvement after tonsillectomy (change in PASI, PDI and PLSI over 24 months). Of the 3 different models that were tested, dominant (phenotype effected by 1 or 2 *HLA-Cw*0602* alleles), recessive (both *HLA-Cw*0602* alleles required to effect phenotype) and additive (phenotype changes sequentially with each *HLA-Cw*0602* allele), the recessive model was used. The linear model assumptions were checked by visual analysis of residual plot.

4 Results

Recruitment for the randomized controlled trial took place from November 2007 to November 2008. Of the 54 screened patients, were 25 excluded. Sixteen did not meet the inclusion criteria and 3 eligible patients declined participation after being screened. Six patients were excluded for reasons of chronic diseases, pregnancy and ongoing systemic treatment for psoriasis (Figure 6). The remaining 29 patients fulfilled all the inclusion criteria and were enrolled in the study. All enrolled patients had plaque psoriasis and a history of psoriasis exacerbation during or after a sore throat. No significant differences were between groups, and although the TX group had slightly higher baseline PASI, PDI and PLSI scores compared with the controls, these differences were not statistically significant (Table 7).

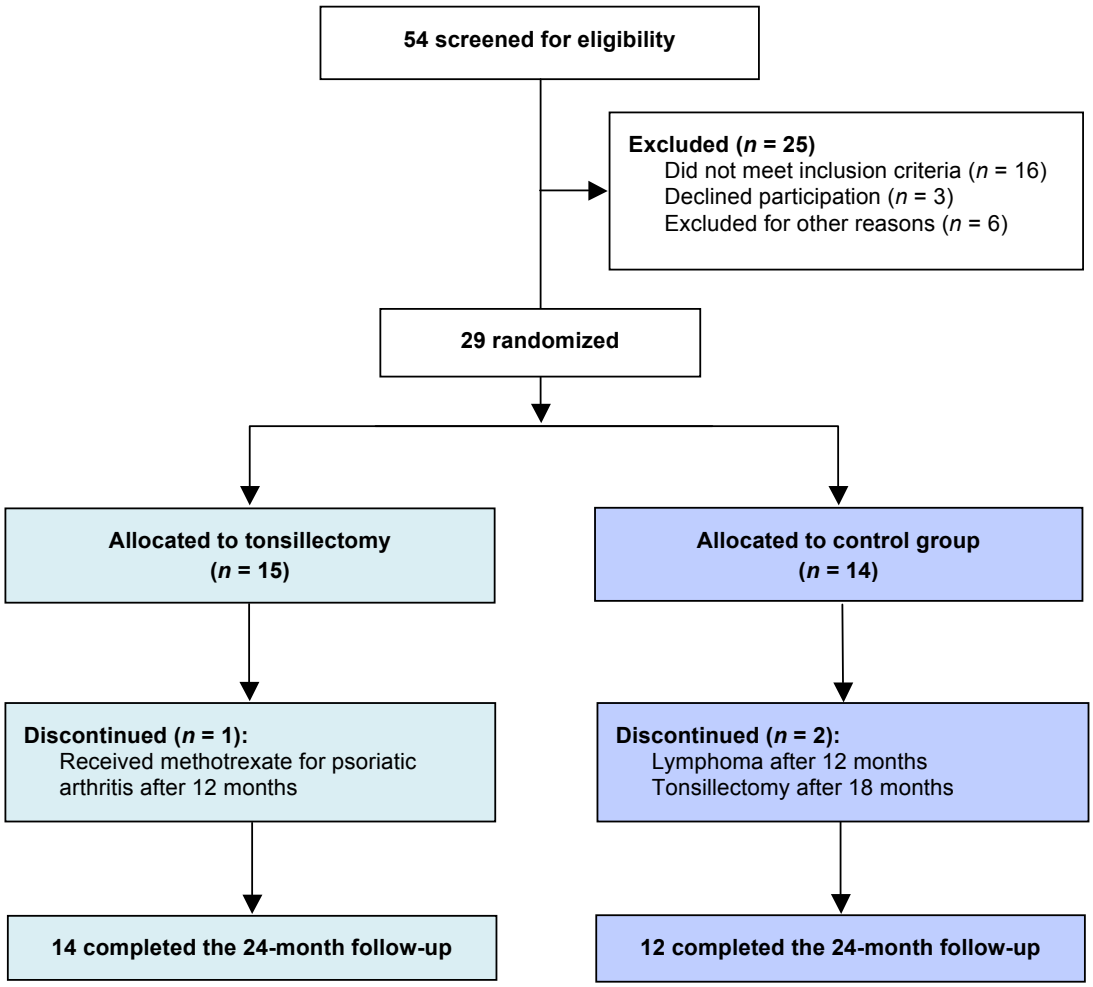

Figure 6. Study flow diagram

Of the 29 included patients, 14 patients in the TX group and 12 controls completed the 24-month follow-up period. One patient in the TX group discontinued the study after 12 months for reasons of psoriatic arthritis where his rheumatologist initiated methotrexate treatment. Two controls were discontinued, 1 because of a lymphoma diagnosis after 12 months of follow-up and the other violated the protocol and had tonsillectomy after 18 months of follow-up.

Table 7. Baseline demographic information of the patients in the RCT

	TX group (n = 15)	Control group (n = 14)
Men, *n* (%)	3 (20)	6 (43)
Age, mean yr ± SD	35.3 ± 9.9	35.9 ± 9.8
BMI (kg/m^2) mean ± SD	25.2 ± 5.3	25.4 ± 3.6
Smokers, *n* (%)	4 (27)	6 (43)
Age at psoriasis onset, mean yr ± SD	15 ± 7.9	15 ± 7.1
Sore throat induced psoriasis onset, *n* (%)	3 (20)	2 (14)
Duration of psoriasis, mean yr ± SD	19.9 ± 9.5	20.5 ± 11.7
Psoriasis family history, *n* (%)	12 (80)	12 (86)
Psoriatic arthritis, *n* (%)	4 (27)	1 (7)
PASI score, mean ± SD	11.0 ± 5.7	9.3 ± 3.7
PDI score, mean ± SD	10.4 ± 7.1	9.3 ± 7.3
PLSI score, mean ± SD	12.0 ± 6.1	10.0 ± 7.0
Treatments, *n* (%)		
Topical agents	8 (53)	8 (57)
Phototherapy	5 (33)	4 (29)
Systemic therapy	1 (7)	0

TX, tonsillectomy; SD, standard deviation; BMI, body mass index; PDI, Psoriasis Disability Index; PLSI, Psoriasis Life Stress Inventory; PASI, Psoriasis Area and Severity Index.

4.1 Tonsillectomy improves plaque psoriasis

The TX group showed clinical improvement with a significant reduction in the mean PASI score, both with time and compared with the control group (Figure 7A, $p < 0.001$). Up to 60% of the TX group reached a PASI 50 response (50% reduction in PASI score) (Figure 7B), and the PASI reduction ranged from 30-90% for 13 of 15 tonsillectomized patients (Figure 7C). The improvement was observed already at the first follow-up visit 2 months after the surgery and was generally maintained throughout the study period (Figure 7A and 7C). The controls showed no corresponding improvements (Figure 7D). Two tonsillectomized patients did not clinically improve (patient 4 and 6, Figure 7C).

4.2 Tonsillectomy reduces M/K peptide-reactive T cells

In order to investigate the effect of tonsillectomy on M/K peptide-reactive T cells (T cells that respond to both streptococcal M proteins and skin keratins), PBMCs isolated from tonsils and blood were stimulated with homologous M proteins and keratin peptides

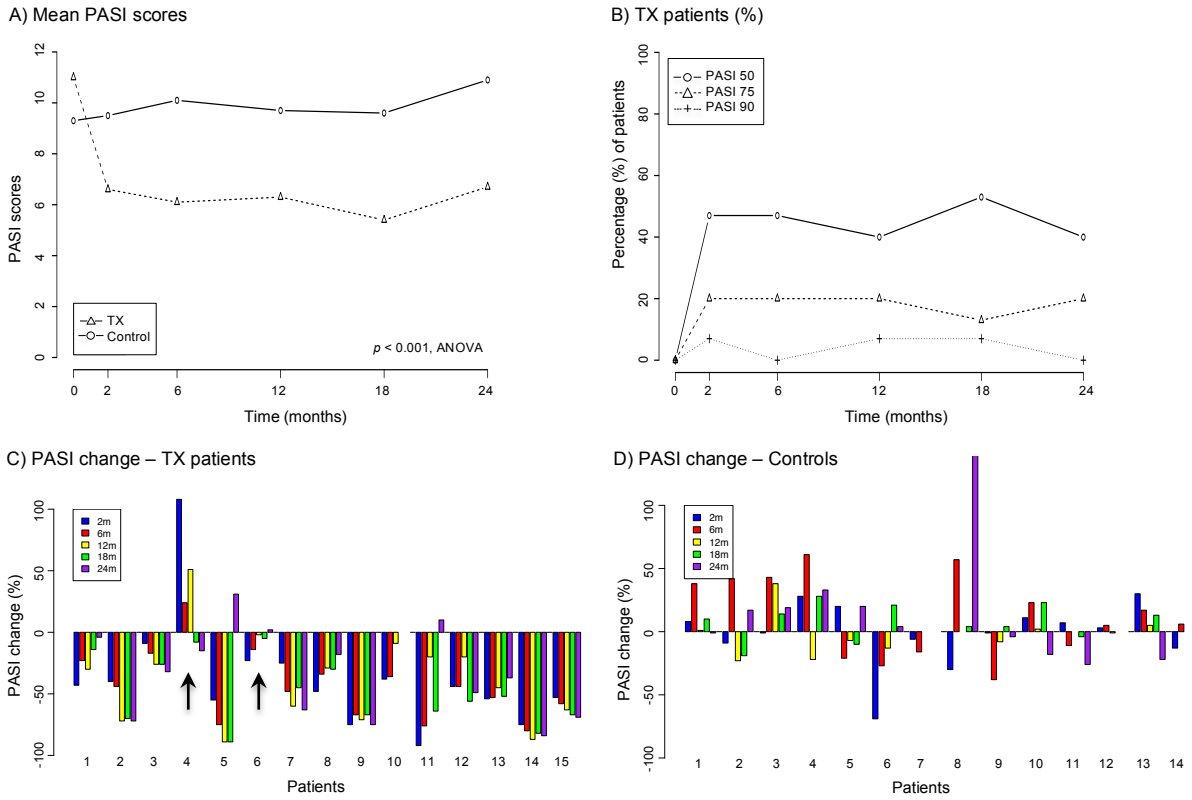

Figure 7. Clinical improvement throughout the 24-month study period
(A). The tonsillectomized patients had a significantly lower mean PASI score compared with the controls ($p < 0.001$). (B). Percentage of patients in the tonsillectomy (TX) group that reached 50% improvement (PASI 50), 75% improvement (PASI 75), and 90% improvement (PASI 90) at each time point measured. (C). Individual percentage PASI changes for the TX group during the 24-month study period. PASI score at study entry is set as 0. Patients 4 and 6 (arrows) did neither clinically improve nor show significant changes in M/K peptide-reactive skin-homing T cells in blood. (D). The controls showed no corresponding clinical improvement.

(Table 5) and then stained for CLA, CD8, CD4 and intracellular expressed IL-17 (T17 cells) and IFN-γ (T1 cells). For each patient the responses to all 16 peptide pools were determined at study entry and at 2, 12 and 24 months after square root normalization and expressed as an average percentage of CLA+ T cells in each T cell subpopulation. Tonsillectomy was generally associated with a marked reduction in the frequency of skin-homing T cells responding to the 16-peptide pools by producing cytokines highly relevant to the pathogenesis of psoriasis (Figure 3A, paper I). Similar results were not observed for the control group (Figure 3B, paper I).

4.2.1 The frequency of M/K peptide-reactive T cells in tonsils and the blood correlate

The frequency of M/K peptide-reactive skin-homing T cells in tonsils and blood at the time of tonsillectomy showed a strong positive correlation. This applied to IL-17 producing Tc17 cells (Figure 8A, $r = 0.644$, $p = 0.015$), IFN-γ producing Tc1 (Figure 8B, $r = 0.788$, $p < 0.001$) and Th1 ($r = 0.679$, $p = 0.001$, data not shown) cells. However, significant correlation was not observed for Th17 ($r = 0.315$, $p = 0.273$, data not shown).

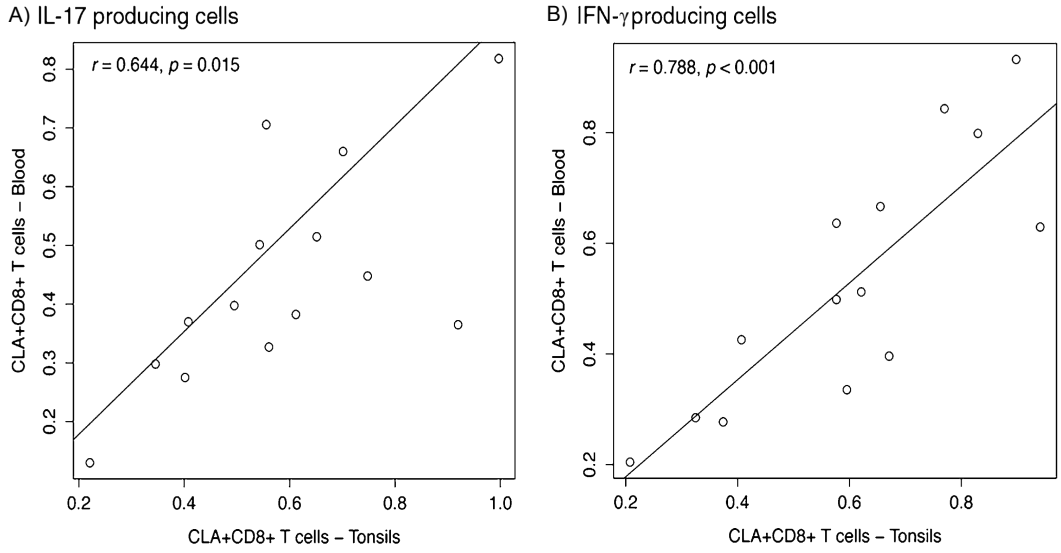

Figure 8. Correlation of M/K peptide-reactive T cells in tonsils and blood
A significant correlation was observed between the frequency of M/K peptide-reactive skin-homing CD8+ T cells in blood and tonsils at the time of tonsillectomy. (A). M/K peptide-reactive CLA+CD8+ T cells producing IL-17 (r = 0.644, p = 0.015) (B). M/K peptide-reactive CLA+CD8+ T cells producing IFN-γ (r = 0.788, p > 0.001).

4.2.2 M/K peptide-reactive T cells decrease after tonsillectomy

The frequency of M/K peptide-reactive skin-homing T cells that produce IL-17 in the blood decreased significantly after tonsillectomy compared with the controls. This applied both to CD8+ Tc17 cells (Figure 9A, p = 0.009) and CD4+ Th17 cells (p = 0.003). Similar frequency reduction was observed for M/K peptide-reactive skin-homing IFN-γ–producing CD8+ Tc1 cells (Figure 5A, paper I, p = 0.003). No significant reduction in the frequency of M/K peptide-reactive skin-homing CD4+ Th1 cells was seen (p = 0.137). The frequency of T cells responding to anti-CD3 antibodies or streptokinase antigen did not decrease after tonsillectomy, indicating the specificity of the response. Interestingly, the 2 patients who did not clinically improve after tonsillectomy (patient 4 and 6, Figure 7C) did not show significant changes in the frequency of M/K peptide-reactive skin-homing T cells, further supporting the association between clinical improvement and reduction in the frequency of M/K peptide-reactive cells.

4.2.3 A positive correlation exists between PASI and the frequency of M/K peptide-reactive T cells

A significant positive correlation was observed between the degree of clinical improvement and reduction in the frequency of circulating M/K peptide-reactive skin-homing IL-17 producing T cells, both Tc17 (Figure 9B, r = 0.565, p < 0.001) and Th17 (r = 0.452, p = 0.003, data not shown). A significant positive correlation was also seen between clinical improvement and reduction in the frequency of M/K peptide-reactive skin-homing Tc1 cells

(Figure 5B, paper I, $r = 0.594$, $p < 0.001$). No correlation was observed for Th1 cells ($r = 0.236$, $p = 0.137$) or controls (Figure 5C and 9C, paper I).

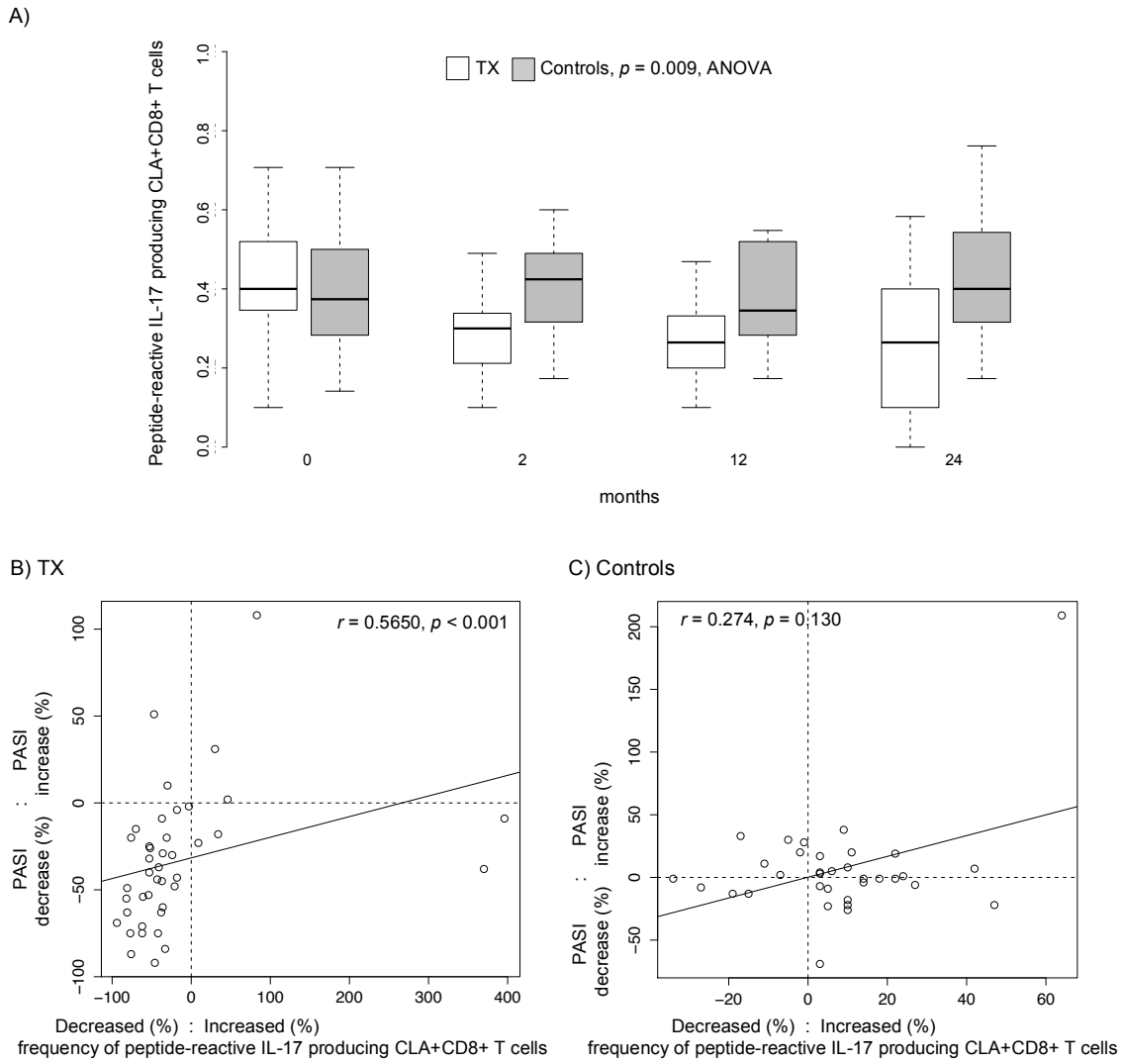

Figure 9. Frequency of circulating M/K peptide-reactive CD8+IL-17+ T cells
(A). Tonsillectomized patients showed a significant reduction in the frequency of circulating M/K peptide-reactive skin-homing CD8+ T cells that produce IL-17 compared with the controls (p = 0.009). (B). A strong correlation between percentage reduction of PASI (clinical improvement) and percentage decrease of circulating M/K peptide-reactive CLA+CD8+IL-17+ T cells (r = 0.5650, p < 0.001) was seen throughout the 24-month study period. (C). No correlation was observed for the controls (r = 0.274, p = 0.130). (B) and (C). T cell frequency and PASI score at study entry were set as 0 for each participant. The vertical axis displays the percentage changes in PASI and horizontal axis percentage changes in the frequency of M/K peptide-reactive CD8+ T cells (3 data points/patient).

4.3 Tonsillectomy improves health-related quality of life

The Psoriasis Disability Index (PDI) was used to evaluate changes in HRQoL after tonsillectomy, and was applied at study entry and after 12 and 24 months. Tonsillectomized patients reported a significant improvement in HRQoL both with time ($p = 0.026$) and compared with the control group (Figure 10, $p = 0.037$, 95% confidence interval (CI), 1.43 to 3.58) with an average 50% improvement of quality of life after tonsillectomy.

Furthermore, a significant positive correlation between clinical improvement (PASI score) and HRQoL (PDI score) was seen (Figure 3A, paper II, $r = 0.297$, $p = 0.008$). The HRQoL domains most markedly influenced were work and/or school activity, relationships and the use of psoriasis treatment (Table II, paper II). No corresponding improvement was observed in the control group.

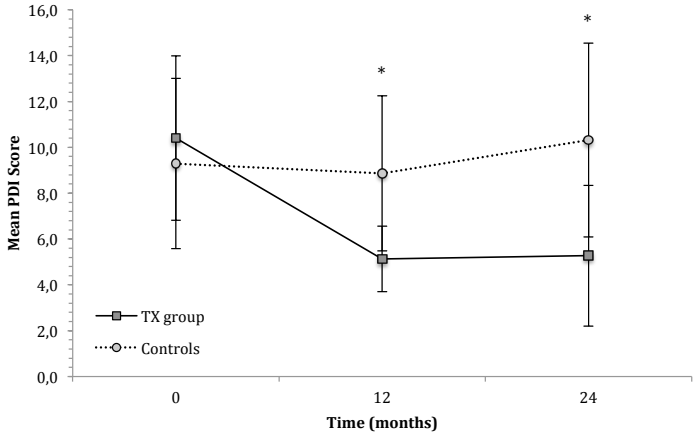

Figure 10. Changes in health-related quality of life
*The mean Psoriasis Disability Index (PDI) score decreased significantly in the tonsillectomy group both with time (p = 0.026) and compared with the control group (p = 0.037). No corresponding changes were seen for the controls. *Denotes statistical significance.*

The Psoriasis Life Stress Inventory (PLSI) was also completed at study entry and at 12 and 24 months. Tonsillectomized patients observed significantly less stress associated with psoriasis both with time ($p < 0.001$) and compared with the controls (Figure 11, $p < 0.002$, 95% CI, 1.39 to 3.10). The PLSI score improved on average by 59% for the TX group. The control group did not report corresponding changes. Furthermore, a positive correlation was observed between clinical improvement and stress due to psoriasis (PLSI) (Figure 3B, paper II, $r = 0.310$, $p = 0.005$).

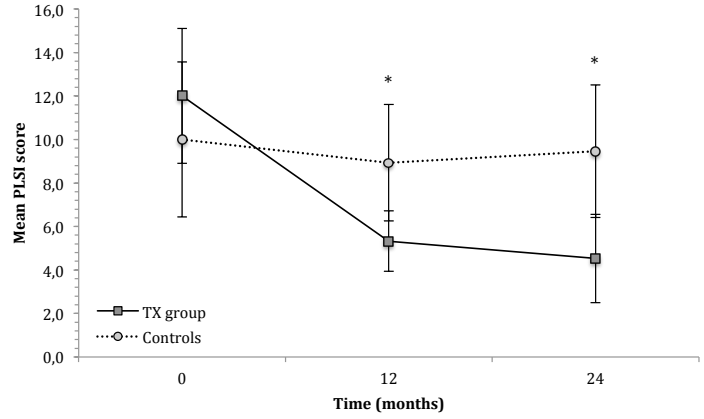

Figure 11. Changes in psoriasis-related stress
*The Psoriasis Life Stress Inventory (PLSI) score decreased significantly in the TX group, both with time and compared with the control group (p < 0.001 and p = 0.002 respectively). No corresponding changes were reported by the controls. *Denotes statistical significance.*

4.4 Tonsillectomized patients require less treatment

No reported differences in psoriasis treatments were seen between the TX and control group prior to tonsillectomy. The first 8 weeks after randomization patients were required to be off all psoriasis treatments. Thereafter, treatment requirements were monitored closely throughout the 24-month study period. The TX group required significantly less topical treatment after tonsillectomy compared with the control group (27% vs 86%, $p = 0.003$). No difference in phototherapy was seen between the groups. Despite a marked clinical improvement, one patient in the TX group was started on methotrexate by his rheumatologist due to psoriatic arthritis, 12 months after tonsillectomy (Table II, paper I).

4.5 Tonsillectomy safety assessment

The otolaryngologist who performed the tonsillectomies followed patients after the surgery. No major post-operative complications were reported. One patient required a visit to the hospital the day after tonsillectomy because of minor post-tonsillectomy bleeding. Tonsil tissue remnants could not be detected in tonsillectomized patients, evaluated at the end of the 24-month follow-up.

4.6 End of study questionnaire

At the end of the RCT, after the patient code was broken, the tonsillectomized patients completed a short study-specific questionnaire that was aimed at summing up their experience. Majority (80%) reported that the procedure was quite difficult and that the recovery time after the surgery was longer than they had anticipated in the beginning. There were no major post-operative complications reported, only one minor bleeding the day after the tonsillectomy. Nevertheless, 13 out of 15 (87%) tonsillectomized patients deemed the whole experience worthwhile and 12 out of 15 (80%) concluded that they had observed a marked improvement of their psoriasis lesions after tonsillectomy. One patient was unsure about the clinical improvement and 2 patients reported no or minimal changes in psoriasis symptoms. This is in concordance with the objective evaluations of the study, where 13 out of 15 (87%) tonsillectomized patients improved significantly compared to the controls. Twelve (80%) of the tonsillectomized patients reported a noticeable reduction in the requirement of psoriasis treatments, mostly moisturizers and topical treatments. Furthermore, one of the 4 patients, who had concomitant psoriatic arthritis, reported an improvement of arthritis after the tonsillectomy.

4.7 *HLA-Cw*0602* carriage is a predictor of tonsillectomy outcomes

Thirteen patients with plaque psoriasis that met all inclusion criteria were added to the original RCT tonsillectomy group, 8 new patients and 5 controls from the RCT that underwent tonsillectomy after the initial 24-month follow-up (demographics, Table I, paper III). These patients were followed up for 24-months with regular assessments of PASI, PDI and PLSI scores. Hence, a total of 28 tonsillectomized patients were included in the cohort presented in paper III. All patients were genotyped for *HLA-Cw*0602*. Four patients (14%) were homozygous carriers of *HLA-Cw*0602*, 17 (61%) were heterozygous and 7 (25%) were *HLA-Cw*0602*-negative (Table II, paper III). Twenty-five patients completed the 24-month study period. One *HLA-Cw*0602*-negative patient discontinued after 18 months and 2 heterozygous patients after 12 and 18 months.

Homozygous carriers of *HLA-Cw*0602* improved significantly more after tonsillectomy, with a mean 82% improvement in PASI, compared with a mean 42 and 31% PASI improvement of the heterozygous and *HLA-Cw*0602*-negative patients respectively ($p < 0.001$) (Figure 12 and Table III, paper III). Thus, all homozygous patients reached PASI 75 at month 6 and almost PASI 90 by month 12 after the tonsillectomy. In accordance with the marked clinical improvement, the homozygous *HLA-Cw*0602* patients also reported a significantly better HRQoL after the surgery, with a mean 87% reduction in PDI scores compared with 38% and 41% for the heterozygous and *HLA-Cw*0602*-negative patients, respectively ($p < 0.001$). A significant difference was also seen in psoriasis-related stress, with the homozygous patients reporting a mean 82% decrease in PLSI scores compared with 60% for the heterozygous patients and 54% for the *HLA-Cw*0602*-negative patients ($p < 0.001$). The homozygous *HLA-Cw*0602* patients had a closer association to streptococcal throat infections and they significantly more often reported that their psoriasis onset was triggered by a throat infection compared with the heterozygous and *HLA-Cw*0602*-negative patients (100% vs. 29% and 29% respectively, $p = 0.007$). Moreover the homozygous patients reported a significantly higher frequency of streptococcal throat infections per lifetime than the heterozygous and *HLA-Cw*0602*-negative carriers (3.5 vs 1.4 and 1.1 times, respectively, $p = 0.038$) (Table II, paper III). Tonsil throat swabs, taken from the tonsil crypts after surgical removal, showed that 3 out of 4 homozygous patients carried group A, C, G streptococci or *Streptococcus anginosus* compared with 11 out of 17 and 3 out of 7 of the heterozygous and *HLA-Cw*0602*-negative patients respectively. Cigarette smoking was significantly more common in the homozygous patient group compared with the heterozygous and negative patients (100% vs. 29% and 43%, $p = 0.013$).

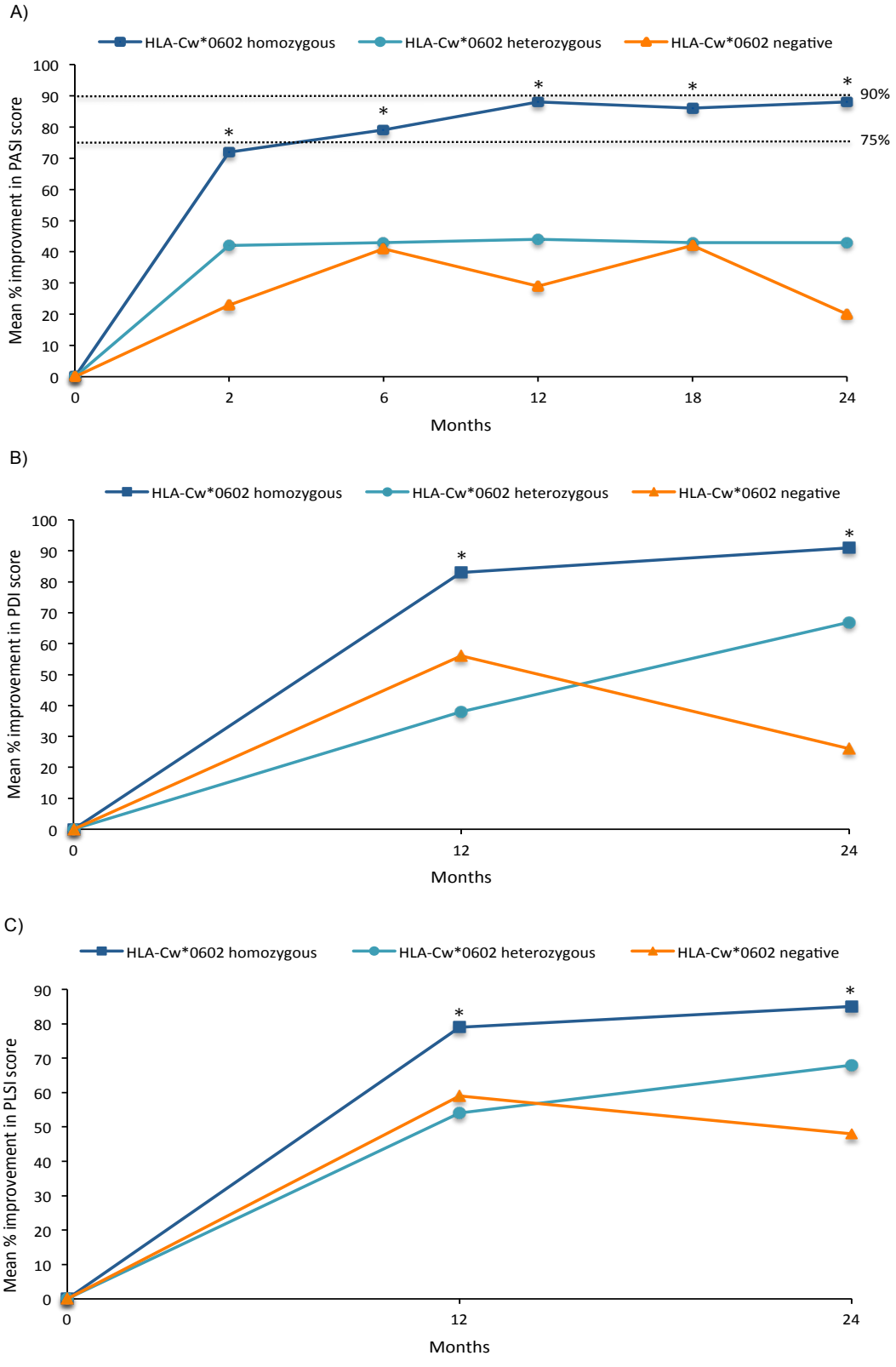

Figure 12. Tonsillectomy efficacy outcomes and *HLA-Cw*0602* status
*Homozygous HLA-Cw*0602 patients showed a significant mean percentage reduction of (A) Psoriasis Area Severity Index (PASI), (B) Psoriasis Disability Index (PDI) and (C) Psoriasis Life Stress Inventory (PLSI) after tonsillectomy at all time points measured, compared with heterozygous and HLA-Cw*0602-negative* patients. **Statistical significance.*

4.8 Streptococcal throat infections cause exacerbation of plaque psoriasis

A study-specific questionnaire was designed to estimate of the proportion of patients with plaque psoriasis who have disease exacerbations after sore throat and/or streptococcal throat infection and might therefore benefit from tonsillectomy. A total of 275 patients with psoriasis were recruited, 127 males and 148 females (demographics Table I, paper IV).

Of the 207 patients with plaque psoriasis (75% of participants), 42% reported exacerbation associated with a sore throat. Furthermore, of the 96 patients with plaque psoriasis and a history of confirmed streptococcal throat infection, 72% reported streptococcal-associated psoriasis exacerbation (Table II, paper IV). Patients, who reported aggravation during streptococcal throat infections, were more likely to report psoriasis exacerbation associated with sore throat (92% vs 8%, $p < 0.001$). Moreover, patients with early-onset psoriasis reported sore throat-associated psoriasis exacerbation more frequently than late-onset patients with psoriasis (51% vs. 30%, $p = 0.046$). Women reported an association between sore throat and aggravation of psoriasis more frequently than men (61% vs. 32%, $p < 0.001$), and this difference remained significant after adjustment for age, psoriasis subtypes (see Table I, paper IV), and other aggravating factors (OR = 2.5, 95%CI, 1.37 - 4.58, $p = 0.003$).

Other environmental factors reported to cause psoriasis exacerbation such as general malaise, stress, cold climate and alcohol intake were significantly more often reported by the patients who had psoriasis exacerbation associated with sore throat (Table III, paper IV). After adjustment for gender, age, and psoriasis subtypes, this difference was still significant for general malaise (OR = 3.0, 95%CI, 1.58 - 5.74, $p < 0{\cdot}001$) and cold climate (OR = 9.2, 95%CI, 3.83 - 22.3, $p < 0{\cdot}001$).

Twenty percent (56 out of 275) of included patients had been tonsillectomized after the onset their psoriasis. Of the 37 tonsillectomized patients with plaque psoriasis, 49% reported a marked improvement after the tonsillectomy. Moreover, 3 out of 4 of patients with guttate psoriasis and 6 out of 11 patients with both guttate and plaque psoriasis reported improvement after tonsillectomy (Table IV, paper IV). Furthermore, all patients in this sub-cohort who reported improvement after tonsillectomy had early onset psoriasis and they also more often reported sore throat-associated psoriasis exacerbation compared with patients who did not improve after the tonsillectomy ($p = 0.015$).

5 Discussion

Psoriasis is a complex multifactorial autoimmune disease that has a profound effect on patients' quality of life.[5] The disease is caused by a combination of multiple genetic polymorphisms[78-82] and environmental factors,[110,118] manifesting as a range of disease phenotypes, which differ in course, severity, and response to treatment.[13,273]

Streptococcal throat infections are associated with both the initiation and exacerbation of psoriasis,[23,196,197] and tonsillectomy has been reported to induce long-term remissions.[242,244] T cells that respond to amino acid sequences shared by streptococcal M proteins and skin keratins (M/K peptide-reactive T cells) have been detected in the blood of patients with psoriasis.[224] These T cells are CD8+ and recognize the M/K peptides most likely in the context of MHC class I molecules, such as HLA-Cw6, which is the product of the strongest psoriasis susceptibility allele.[85] Furthermore, T cells clones with the same T cell receptor Vβ gene rearrangements have been isolated from the skin and the palatine tonsils of the same patients with psoriasis,[231] suggesting that these cells are responding to one or a few dominant closely-related antigen determinants. Based on these and other findings, the investigations described in this thesis were devised to test the hypothesis that immune responses against streptococcal throat infections may induce streptococcal antigen-specific T cells in the tonsils in patients with psoriasis and promote their migration into the skin. In the skin they are able to recognize homologous keratin antigens resulting in eruption of guttate or exacerbation of plaque psoriasis. If true, removal of the tonsils might be expected to be associated with reduced frequency of these cells in the periphery and subsequent clinical improvement of psoriasis. The overall aim of this thesis was to test this hypothesis and assess the clinical, psychosocial and immunological effects of tonsillectomy on patients with chronic plaque psoriasis.

The results show that patients with plaque psoriasis and a history of sore throat-associated psoriasis exacerbation significantly improve after tonsillectomy, and that tonsillectomy leads to significant decrease in the frequency of circulating M/K peptide-reactive T cells compared with matched controls. Clinical improvement after tonsillectomy was accompanied by significantly improved health-related quality of life. Furthermore, patients with plaque psoriasis that were homozygous *HLA-Cw*0602* carriers had significantly greater benefit from tonsillectomy than heterozygous and non-carriers and had

stronger association with streptococcal throat infections. Finally, the findings indicate that 42% of Icelandic patients with plaque psoriasis experience disease exacerbation associated with sore throat and 72% report worsening of psoriasis associated with confirmed streptococcal throat infections.

5.1 Tonsillectomy is beneficial for selected patients with plaque psoriasis

Although previous studies have reported the beneficial effect of tonsillectomy (reviewed in[242-244]), this is to our knowledge, the first prospective randomized controlled study on the effect of tonsillectomy on psoriasis. Thirteen out of 15 tonsillectomized patients clinically improved, with PASI reductions ranging from 30-90% and up to 60% of patients reached a PASI 50 response. The improvement was observed already 2 months after surgery and was generally maintained throughout the 24-month follow-up period. Moreover, tonsillectomized patients required significantly less topical treatment than controls, indicating that tonsillectomy may be used as an adjunct to existing treatment, to reduce the use of time consuming therapy or allow the use of less potent drugs to control the disease. Patients in the RCT recently completed a 5-year follow-up after tonsillectomy indicating long-lasting beneficial effects of tonsillectomy.

The ultimate aim of psoriasis treatment is achieving clear skin. A PASI 75 response is still considered the standard of response assessment, used in most clinical trials of psoriasis,[274] although PASI 90 and even PASI 100 response rates are becoming important secondary endpoints. To that end, a 60% PASI 50 response of tonsillectomized patients does not seem impressive. However, patients on methotrexate have also been shown to have about 60% PASI 50 response after 16 weeks of treatment,[275,276] and methotrexate is usually the first choice systemic agent for patients with moderate to severe psoriasis.

Patients in the RCT did not show any clear association between the degree of improvement and carriage of the *HLA-Cw*0602* allele. *HLA-Cw*0602* is the major psoriasis susceptibility allele,[85] and we predicted that *HLA-Cw*0602*-positive patients would show more improvement than non-carriers, since carriage of *HLA-Cw*0602* has been linked to a higher frequency of streptococcal throat carriage/infections[96,101] and streptococcal-associated psoriasis exacerbations.[27,101] After including 8 additional patients with plaque psoriasis and a history of sore-throat associated psoriasis exacerbation and 5 controls from the RCT to the tonsillectomy group, we found that patients who were homozygous for *HLA-Cw*0602* showed significantly more improvement compared with heterozygous *HLA-Cw*0602* patients and non-carriers. Strikingly, all 4 homozygous patients reached PASI 75

by months 6 and PASI 90 by month 12. These results are similar to those seen with recently introduced biologics.[277] Why should patients carrying two copies of the *HLA-Cw*0602* allele respond better to tonsillectomy than heterozygotes? Possibly, they show a stronger response to streptococcal proteins since homozygotes have twice as many *HLA-Cw*0602* molecules on the surface of their cells than heterozygotes. It has been reported that homozygous *HLA-Cw*0602* individuals are 2.5 fold more likely to get psoriasis and have an earlier disease onset compared with heterozygotes.[92] While over 60% of patients with psoriasis are *HLA-Cw*0602*-positive, the frequency in the general population is only 10-15%.[85,88] However, 7-26% of patients with plaque psoriasis have been shown to be homozygous *HLA-Cw*0602* carriers.[92,100] Patients with psoriasis might also be carrying other risk alleles such as ERAP1, or LCE genes that possibly magnify the effects of *HLA-Cw*0602* carriage.[90,109] Increased risk for psoriasis has been seen in *HLA-Cw*0602*-positive smokers compared to *HLA-Cw*0602*-positive non-smokers,[278] suggesting an additive effect of smoking in triggering psoriasis in some genetically predisposed individuals. Interestingly, smoking was significantly more common in the homozygous group compared to the heterozygous and non-carriers, with all 4 homozygous patients being smokers. Smoking is a known risk factor for psoriasis,[279] that increases oxidative damage, promotes an inflammatory state, and may even modify gene expression of psoriasis-associated genes, including the HLA genes.[280]

These observations are interesting because accumulating evidence shows that different psoriasis genotypes can be useful in predicting treatment reponses.[281] Thus, patients carrying *HLA-Cw*0602* respond better to the interleukin-12/23 inhibitor ustekinumab[102,103] and etanercept has also been shown to be more effective in early-onset psoriasis (typically associated with *HLA-Cw*0602* carriage) compared with late-onset psoriasis.[104] The clinical findings of this thesis suggest that *HLA-Cw*0602* homozygosity may be a relatively strong predictor of favourable outcomes after tonsillectomy of patients with plaque psoriasis and a history of streptococcal-associated psoriasis exacerbation.

5.2 CD8+ T cells might be the ultimate effector cells in psoriasis

The palatine tonsils are in many ways unique; They are the only secondary lymphoid tissue that is encapsulated by a multilayer squamous epithelium.[172] Furthermore, they are ideally situated for antigen sampling of the large amount of material that is inhaled or ingested every day. We found that tonsillectomy was associated with a significant reduction in the frequency of M/K peptide-reactive skin-homing T cells, i.e, those CLA+ T cells that

respond to homologous peptides present in both streptococcal M proteins and keratins upregulated in psoriatic skin, by producing cytokines highly relevant to the pathogenesis of psoriasis. Thus, the frequency of M/K peptide-reactive skin-homing Tc17 and Tc1 cells not only decreased significantly after tonsillectomy compared with the controls, but the cell reduction correlated strongly with clinical improvement. Moreover, the frequency of M/K peptide-reactive skin-homing Tc17 and Tc1 was strongly correlated in the tonsils and blood. These findings suggest that effector CD8+ T cells originating in the tonsils may be involved in psoriasis pathogenesis, at least in a subset of patients. Although both T17 and T1 cells are thought to be important in psoriasis pathogenesis,[156] increasing evidence supports IL-17 producing T cells as the main effector pathogenic cells.[282] T17 cytokines are crucial for sustaining the chronic inflammation in psoriasis and blockade of IL-23 or IL-17 has been shown to completely clear psoriasis.[283-285] Furthermore, recent studies have confirmed an important role of CD8+ T cells in psoriasis, possibly making Tc17 cells key effector cells in psoriasis pathogenesis.[143,155,286,287] This is in line with the findings of this thesis since the M/K peptide-reactive CD4+ T cells did not shown as much consistency as the CD8+ IL-17- and IFN-γ-producing T cells. CD8+ T cells produce a similar profile of proinflammatory cytokines as CD4+ T cells,[288] and are abundant in the psoriatic epidermis close to keratinocytes, inducing characteristic histological changes such as parakeratosis and acanthosis.[143,289] Moreover, using a human-mouse xenograft model it has been shown that blocking α1β1-intregrin, which is critical for the entry of T cells into the transplanted human epidermis,[155] or neutralization of CD8+ T cells, prevents the development of psoriasis.[287] CD8+ T cells have also been shown to migrate early in the marginal zone of evolving psoriatic plaque.[290] Furthermore and importantly, CD8+ T cells recognize peptide antigens presented in the context of MHC class I molecules, such as HLA-Cw6,[167] that is the protein product of the strongest psoriasis susceptibility allele.[85] However, it is important to note, that the immunological part of the RCT was designed to detect maximal numbers of T cells that responded to determinants that streptococcal M proteins share with human type 1 keratins. Thus, the study does not distinguish between T cells that might respond exclusively to either keratin or M protein determinants. Moreover, it is not possible to evaluate if the responding T cells are specific for dominant autoepitopes or determinants that reflect epitope spreading.

Psoriasis is an autoimmune disease, likely mediated by clonally expanded T cells,[141,143,291,292] and T cells clones with the same TCR Vβ gene rearrangements in both tonsils and psoriasis skin have been reported, suggesting a common origin.[231] Despite great

advances in characterization of the molecular mechanisms operating in psoriasis, the putative autoantigen(s), responsible for activating and sustaining the immune reaction seen in psoriasis, is still unknown. A spectrum of possible autoantigens have been suggested, many of which are keratinocyte-derived.[142,233,293] Our findings do not exclude that other antigens may be involved in the pathogenesis such as maspin, ezrin, PRDX2, HSP27[233] or melanocyte-derived peptides, also presented in the context of *HLA-Cw*0602*.[167] Indeed, there maybe several autoantigens involved in psoriasis. However, cross-reaction between streptococcal M proteins and human keratins has repeatedly been suggested,[195,222-224,229,294,295] and increased frequency of circulating M/K peptide-reactive skin-homing CD8+ T cells has been observed in patients with psoriasis, compared with non-psoriatic controls.[224] Furthermore, streptococci can induce the production of key psoriasis cytokines (IL-17, IL-22 and IFN-γ) in circulating CLA+ memory T cells cultured together with both lesional and nonlesional epidermal cell from psoriasis patients.[199] Nevertheless, we do not exclude the possibility that other streptococcal antigens, including peptidoglycans[237] might play a role in the immunopathogenesis of psoriasis.

Of the 15 patients in the RCT, 2 patients did not show clinical improvement after tonsillectomy. Interestingly, these 2 patients did not exhibit any change in the frequency of circulating M/K peptide-reactive T cells either. Furthermore, one of these admitted during the final study visit that he never observed any association between his psoriasis exacerbations and sore throat and/or streptococcal throat infections. The second patient was also uncertain about this association. These observations support the notion that only patients with a history of skin disease exacerbation associated with sore throat may benefit from tonsillectomy.

Collectively the results indicate that streptococcal throat infections in the tonsils might induce a T cell-mediated autoimmune response against skin proteins, preferentially presented by HLA-Cw6, in some patients with chronic plaque psoriasis. The initial priming phase might involve streptococcal superantigens, which stimulate the upregulation of skin-homing molecules on T cells. This streptococcal-induced immune response could then extend to the skin by molecular mimicry.

5.3 Tonsillectomy improves patient reported outcomes

The second aim of the thesis was to assess the psychosocial effects tonsillectomy has on plaque psoriasis by using patient reported outcomes. The tonsillectomy had a significant positive impact on the quality of life of patients with chronic plaque psoriasis compared with

the matched control group. Thus, HRQoL improved by 50% and psoriasis-related stress was almost 60% lower after the surgery. As expected, the improvements in quality of life and stress associated with psoriasis correlated positively with clinical improvement. The quality of life and stress indices are patient reported measures or the "real-world" impact of treatment and extend the clinical findings. Furthermore, the end-of-study questionnaire, which was applied to all tonsillectomized patients at the end of the 24-month follow-up, gave important feedback and evaluation of the whole experience of treating plaque psoriasis with tonsillectomy. Tonsillectomy involves risks inherent in both anaesthesia and surgery. Studies on tonsillectomy have shown that majority of patients have an uncomplicated post-operative course, although there is a minor risk of laryngospasm, bronchospasm, infections and post-operative bleeding.[241] Furthermore, recovery time for adults tends to be longer than for children. As such, 80% of the patients in the study concluded that the surgery had been quite difficult and that the recovery time was longer than they had anticipated. However, despite these risks and a prolonged recovery time, 13 out of 15 (87%) patients in the RCT reported that the procedure was worth all the effort. Moreover, 80% of the tonsillectomized patients noted a general decreased need for psoriasis treatments after the surgery, which is in accord with the finding that tonsillectomized patients with plaque psoriasis used fewer symptomatic treatments than controls.

5.4 Streptococci cause exacerbation of chronic plaque psoriasis

The third aim of the thesis was to assess the frequency of patients reporting psoriasis exacerbation associated with symptomatic sore throat, as to better estimate the potential of tonsillectomy as a treatment option. The retrospective questionnaire reported that 42% of the participating Icelandic patients associate exacerbation of plaque psoriasis with sore throats. Moreover, 72% reported an aggravation of plaque psoriasis associated with a confirmed streptococcal pharyngitis. This strong association could in part be explained recall bias, but is nevertheless noteworthy and higher than previously reported.[196] Furthermore, our cohort only included Icelandic patients with psoriasis and Iceland's geographical isolation through the centuries might have resulted in the emergence of patients that are more influenced by streptococcal throat infections and other psoriasis trigger factors. However, we are not aware of an increased carriage of *HLA-Cw*0602* among Icelandic patients with psoriasis or increased frequency of streptococcal throat infections in Iceland, compared with other countries.

Patients with early onset psoriasis significantly more often reported sore throat-associated psoriasis aggravation, which is in line with previous studies.[16,105] Furthermore, patients that reported sore throat-associated aggravation of psoriasis significantly more often reported psoriasis exacerbation due to various other environmental factors. These findings are consistent with emerging data that plaque psoriasis is indeed a synonym for different phenotypes such as early and late onset psoriasis[16] and stable and dynamic psoriasis.[143] Our results show that patients prone to psoriasis exacerbations linked with sore throat and/or streptococcal throat infections have a dynamic plaque phenotype, which is characterized by a fluctuating disease course and increased sensitivity to numerous environmental triggers. Furthermore, patients with dynamic psoriasis are more often carriers of *HLA-Cw*0602*.[27] One might consider this plaque phenotype somewhat similar to guttate psoriasis,[96] or a phenotype that lies between typical chronic plaque and guttate psoriasis, sharing some characteristics from both.

Interestingly, females reported significantly more frequent sore throat-associated psoriasis exacerbation than men. The reason for this is currently unclear and although female sex hormones might be involved, there are no reports available. Women tend to have an earlier psoriasis onset than men,[27,100,296,297] and they have been reported to be more frequently carriers of *HLA-Cw*0602*,[100,298,299] although there is no logical explanation to why women should be *HLA-Cw*0602*-positive more often than men. One study has observed that women are more frequently affected with recurrent tonsillitis than men.[300]

The tonsils are a major site for streptococcal carriage and infections. In the cohort from paper IV, the combined asymptomatic carrier frequency of Lancefield groups A, C, G streptococci and Streptococcus anginosus was 67% (14 out of 21) in the *HLA-Cw*0602* patient groups and 43% (3 out of 7) in the *HLA-Cw*0602*-negative group. This is in line with previous studies showing increased prevalence of positive streptococcal throat swabs in *HLA-Cw*0602*-positive patients compared to *HLA-Cw*0602*-negative patients,[101] and suggests that *HLA-Cw*0602* carriage may impact the bacterial colonization of the tonsils. Although a streptococcal carrier frequency of 43-67% is very high, especially compared to the frequency among the general population,[175,301] it should be noted that included patients were selected on the basis of their history of sore throat-associated psoriasis exacerbation. Streptococci are known to invade epithelial cells and macrophages efficiently,[180-182,184] making antibiotics unable to eradicate group A streptococci in up to 30% of patients with pharyngotonsillitis.[302] Although commonly used for treatment of streptococci, penicillin does not reach high intracellular concentration,[303] probably because the intracellular niche is

protected from treatment. Moreover, even treatment with antibiotics that are active intracellularly is likely to have a temporary benefit since up to 20% of the general populations are carriers of throat streptococci.[197] Our results do not exclude the possibility that the microbiota of the skin has a role in psoriasis pathogenesis. However, streptococci have not been reported to be significantly more common in psoriatic skin compared to controls.[304]

The homozygous patients also reported significantly more streptococcal throat infections per lifetime, and more often that their psoriasis onset was triggered by a sore throat, compared to the heterozygous and *HLA-Cw*0602*-negative patients. Proteins from β-haemolytic streptococci have been shown to induce the expression of the skin-homing molecule CLA on T cells,[209,210] thus providing an immunological link between the tonsils and the skin. CLA+ T cells are more frequent in the tonsils and blood of patients with psoriasis compared with healthy individuals.[305] The immunological development of the tonsils of genetically predisposed individuals may become dysregulated because of chronic streptococcal throat infections. The dysregulation might even cause a further vulnerability to streptococcal infections. This is supported by the notation that psoriasis tonsils are histologically different from hypertrophic tonsils,[198] and patients with psoriasis do not have increased susceptibility to other throat infections.[198] Furthermore, *HLA-Cw*0602*-positive healthy individuals do not have increased risk for streptococcal pharyngitis,[306] which points to the possibility of involvement of other psoriasis susceptibility alleles. Thus, neither *HLA-Cw*0602*, nor streptococcal infections *per se* are necessary for the development of psoriasis. The mechanism whereby *HLA-Cw*0602* predisposes to psoriasis has yet to be explained. Our data, and those of others[167,199,224,228,231] are consistent with the hypothesis that autoantigens presented in the binding pockets of *HLA-Cw*0602* on epidermal cells are recognized by CD8+ T lymphocytes infiltrating lesional epidermis.[142]

5.5 Hypothetical scenarios

It has been suggested that several mechanisms might induce pathogenic T cells that drive psoriasis skin inflammation, since psoriasis is both genetically and clinically heterogeneous. Two of the best-defined psoriasis pathomechanisms hitherto proposed are the molecular-mimicry based pathogenesis that generates potentially auto-reactive T cells in the tonsils and a mechanism that produces pathogenic T cells by expansion of T cells within the skin or local skin lymph nodes. Possibly both of these pathomechanisms are operating in patients in different ratios. Thus, it is likely that the bulk of pathogenic T cells in patients with guttate

psoriasis and some patients with plaque psoriasis are produced in the tonsils while in other patients, effector T cells are produced mainly in other secondary lymphoid tissues. This might explain why guttate patients often go into spontaneous remission or clear completely after tonsillectomy while plaque psoriasis is more chronic and seldom heals completely after tonsillectomy. Plaque psoriasis usually evolves during a much longer time compared to the acute onset of guttate psoriasis and often it is difficult for plaque patients to pinpoint the exact time they started noticing their lesions and thus if psoriasis debut was associated with a sore throat. Furthermore, many streptococcal species colonize the throat without giving symptoms. We propose therefore that when genetically predisposed individuals get acute, subacute or asymptomatic streptococcal infection in the throat, a certain scenario can be initiated.

First, streptococci infect or colonise the tonsils causing a bacterial-driven immune reaction (Figure 13A). This includes invasion of tonsil cells both epithelial cells, macrophages and APCs. Streptococcal antigens, such as M proteins, are presented in the context of MHC to naïve T cells recirculating through the tonsils, resulting in clonal expansion of streptococcal-specific effector T cells, both CD4+ and CD8+ T cells. Activation of CD8+ T cells might involve both a direct activation by a streptococcal infected dendritic cell or cross-presentation of extracellular streptococcal antigens through MHC class I. Streptococcal superantigens may aid by mass activation of T cells and inducing skin-homing properties on T cells.[209,210] The activated T cells take part in fighting the ongoing bacterial infection along with humoral immune responses. After resolution, most of the clones die through apoptosis but some become memory T cells, ready to respond upon a second antigen encounter. Some streptococcal antigens may also enter the bloodstream where they become internalized by monocytes that present them as APCs in other secondary lymphoid tissues such as skin draining lymph nodes. Some of the streptococcal-reactive memory T cells, which include (M protein) peptide-reactive T cells stay in the tonsils as tissue-resident memory T cells, others, recirculate going from lymph node to lymph node, and those memory T cells that express CLA also home to skin.

In the skin, the CD8+ T cells migrate to the epidermis where they cross-recognise keratin peptides, some of which are homologous to streptococcal M peptides, presented by APCs or in HLA-Cw6 molecules on keratinocytes. The CD8+ T cells might also become activated in local draining lymph nodes by APCs that have migrated from lesional epidermis. CD4+ T cells become activated in local draining lymph nodes, but to what antigen they are responding is yet to be shown. It has been suggested that CD4+ T cells

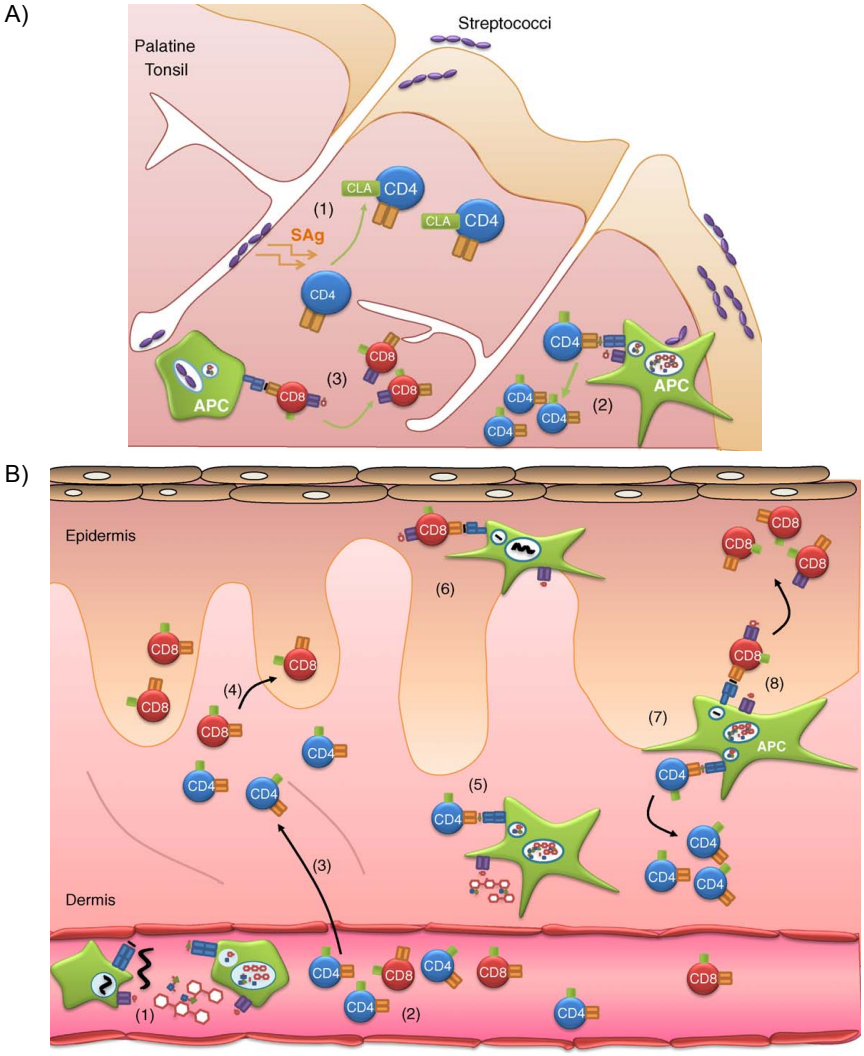

Figure 13. Activation of M/K peptide-active T cells in tonsils and skin
(A) An infection or colonization of the tonsils by streptococci causes activation of effector CD4+ (blue) and CD8+ (red) T cells. Streptococcal superantigens induce the expression of skin-homing cutaneous lymphocyte-associated antigen (CLA) on effector T cells. (B) Some streptococcal components, M-proteins, enter the bloodstream and are taken up by monocytes that migrate as APCs into skin. Circulating CLA+ effector CD4+ T cells migrate into the dermis and CLA+ effector CD8+ T cells migrate mostly into the epidermis where they might promote the characteristic keratinocyte proliferation of psoriasis by cross-recognizing keratin peptides that share sequences with streptococcal M peptides. APCs at the dermal–epidermal junction can present PG to CD4+ T cells via HLA class II molecules and keratin determinants to CD8+ T cells via HLA class I. Reproduced with permission.[142] Copyright Elsevier Inc.

respond to peptidoglycans presented by APCs.[237] Recognition of self-peptides causes a pro-inflammatory stimulus that breaks immunologic tolerance, causing activation of pDCs, mDCs, neutrophils, T cells and more cells relevant in the pathogenesis of psoriasis. This causes characteristic proliferation of keratinocytes and the release of key cytokines and chemokines. In the end, a vicious chronic psoriasis inflammatory loop, sustained by the IL-23/IL-17 axis is established. It is therefore possible that the CD8+ T cells are key players in the initiation of the plaque formation but require a complex interplay between various cell types such as the CD4+ T cells, within the skin for maintenance of the chronic

inflammation. Hypothetically, an effective way to break the cycle would be to shut down the production of effector T cells in the tonsils and kill self-reactive T cells already in the skin. This might be achieved with tonsillectomy, perhaps followed by UVB phototherapy.

5.6 Strengths and limitations of the study

This thesis adds important knowledge to the psoriasis literature on the association of streptococcal throat infections with plaque psoriasis and the effect of tonsillectomy. One of the major strengths of this study is its design as a prospective randomized controlled trial with observer-blinded assessments. Furthermore, there were no significant demographic differences between the tonsillectomy group and the controls, which further strengthen the study. Nevertheless, it is essential to critically evaluate the overall study design and acknowledge its various weaknesses.

The study's small cohort may limit the study. Iceland is a small country and has proportionally fewer patients with psoriasis to choose from, and thus not easy to recruit patients who are willing to undergo surgery and be followed-up with interviews, biopsies and blood samples for 24 months. The study cohort is furthermore limited to Icelandic patients with psoriasis. Possibly, Icelanders are more influenced by psoriasis environmental trigger factors such as streptococcal throat infections, although we are not aware of any such increased influence.

Observer bias was addressed, by having the same person performing all the interviews and assessments. Furthermore this person was observer-blinded in regards to tonsils status, which further reduced bias. However, patients were not blinded to tonsillectomy treatment and this might have influenced patient reported outcomes, HRQoL and psoriasis-related stress questionnaires.

Retrospective data were used in the retrospective questionnaire (paper IV) and some of the data collected for papers I and III. This may have caused recall bias because of inaccurate recall of past events and inflated the findings. Checking against medical records could have been undertaken, to minimize this form of bias, although many streptococcal throat infections are diagnosed with an instant strep-test or by physicians, most often at the primary healthcare level. Additionally, physicians working at hospitals in Iceland cannot easily access primary health care records, and it would have been time-consuming to contact these facilities for better information.

Response bias (selection bias) could have affected the results in paper IV. It could be argued that patients who agreed to answer the questionnaire differ with regards to disease

characteristics from those who declined participation and hence, produce biased results. Furthermore, that study might reflect patients with a more severe form of psoriasis, since dermatologists were treating the patients recruited.

6 Conclusions

The key aim of this thesis was to investigate the effect tonsillectomy has on plaque psoriasis and to define further the association between throat infections and the onset or exacerbation of psoriasis. The data herein demonstrate significant clinical, psychosocial and immunological improvement after tonsillectomy of patients with plaque psoriasis and history of sore throat-associated psoriasis exacerbation, compared with controls. In particular, it was demonstrated that sore throat and streptococcal throat infection causes exacerbation of psoriasis in more patients with plaque psoriasis than previously reported. Furthermore, removing the tonsils from patients with plaque psoriasis causes clinical improvement, which correlates closely with a decreased frequency of circulating T cells that recognize auto-epitopes that are over-expressed in psoriatic skin lesions. This was especially apparent for patients carrying both *HLA-Cw*0602* alleles. These findings are in concordance with the hypothesis that the palatine tonsils may play a crucial role in the pathogenesis of psoriasis by producing pathogenic T cells, which migrate to the skin, react to autoantigens and thereby take part in creating the vicious cycle of chronic inflammation representative of psoriasis.

Possible future avenues of research would include a larger, randomized controlled tonsillectomy trial to confirm the findings of the current study, where emphasis should be placed on a much larger group of homozygous *HLA-Cw*0602* patients. In particular, it would be interesting to explore if M/K peptide-reactive skin-homing T cells in homozygous patients decrease significantly more compared to the non-carriers. Furthermore, a larger cohort opens up possibilities for further stratification of patients that could benefit from tonsillectomy. This might be done with respect to disease history, severity, gender and genotype. The next steps would also include determination of peptide-reactive skin-homing T cells in the skin. In this respect, tonsil tissue, blood and skin samples could be collected and compared for skin-homing markers and TCR rearrangements, determined using DNA and RNA sequencing.

The heterogeneity of psoriasis emphasises the importance of tailoring individual therapies for patients (personalized medicine). Stratification of psoriasis phenotypes is central in personalized psoriasis treatments and in the near future we will likely be able to assess patients from both a clinical and genetic perspective and create genomic and

transcriptomic patient profiles.[307] In conclusion, the observations in this thesis add important information that can aid in the stratification of patients with psoriasis and they elucidate further the pathogenic relationship between streptococcal throat infections and autoimmune sequelae in psoriasis.

References

1. Foundation NP. https://www.psoriasis.org/about-psoriasis. 2015.
2. Duffin KC, Chandran V, Gladman DD, Krueger GG, Elder JT, Rahman P. Genetics of psoriasis and psoriatic arthritis: update and future direction. *J Rheumatol.* 2008;35(7):1449-1453.
3. Willan R. *On Cutaneous Diseases.* London1808.
4. Bechet PE. Psoriasis: A brief historical review. *Archives of Dermatology and Syphilology.* 1936;33(2):327-334.
5. Augustin M, Radtke MA. Quality of life in psoriasis patients. *Expert Rev Pharmacoecon Outcomes Res.* 2014;14(4):559-568.
6. Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med.* 2009;361(5):496-509.
7. Parisi R, Symmons DP, Griffiths CE, et al. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. *J Invest Dermatol.* 2013;133(2):377-385.
8. Raychaudhuri S, Farber E. The prevalence of psoriasis in the world. *J Eur Acad Dermatol Venereol.* 2001;15(1):16-17.
9. Gudjonsson JE, Elder JT. Psoriasis: epidemiology. *Clin Dermatol.* 2007;25(6):535-546.
10. Springate DA, Parisi R, Kontopantelis E, Reeves D, Griffiths CE, Ashcroft DM. Incidence, prevalence and mortality of patients with psoriasis: a UK population-based cohort study. *Br J Dermatol.* 2016.
11. Fry L. Psoriasis. *Br J Dermatol.* 1988;119(4):445-461.
12. Smith AE, Kassab JY, Rowland Payne CM, Beer WE. Bimodality in age of onset of psoriasis, in both patients and their relatives. *Dermatology.* 1993;186(3):181-186.
13. Griffiths CE, Christophers E, Barker JN, et al. A classification of psoriasis vulgaris according to phenotype. *Br J Dermatol.* 2007;156(2):258-262.
14. Jiaravuthisan MM, Sasseville D, Vender RB, Murphy F, Muhn CY. Psoriasis of the nail: anatomy, pathology, clinical presentation, and a review of the literature on therapy. *J Am Acad Dermatol.* 2007;57(1):1-27.
15. Henseler T, Christophers E. Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris. *J Am Acad Dermatol.* 1985;13(3):450-456.
16. Theodorakopoulou E, Yiu ZZ, Bundy C, et al. Early- and late-onset psoriasis: a cross-sectional clinical and immunocytochemical investigation. *Br J Dermatol.* 2016.
17. Langley RG, Krueger GG, Griffiths CE. Psoriasis: epidemiology, clinical features, and quality of life. *Ann Rheum Dis.* 2005;64 Suppl 2:ii18-23; discussion ii24-15.
18. Martin BA, Chalmers RJ, Telfer NR. How great is the risk of further psoriasis following a single episode of acute guttate psoriasis? *Arch Dermatol.* 1996;132(6):717-718.
19. Ko HC, Jwa SW, Song M, Kim MB, Kwon KS. Clinical course of guttate psoriasis: long-term follow-up study. *J Dermatol.* 2010;37(10):894-899.
20. Telfer NR, Chalmers RJ, Whale K, Colman G. The role of streptococcal infection in the initiation of guttate psoriasis. *Arch Dermatol.* 1992;128(1):39-42.
21. Whyte HJ, Baughman RD. Acute Guttate Psoriasis and Streptococcal Infection. *Arch Dermatol.* 1964;89:350-356.

22. Norrlind R. Psoriasis following infections with hemolytic streptococci. *Acta Derm Venereol.* 1950;30(1):64-72.
23. Mallbris L, Larsson P, Bergqvist S, Vingard E, Granath F, Stahle M. Psoriasis phenotype at disease onset: clinical characterization of 400 adult cases. *J Invest Dermatol.* 2005;124(3):499-504.
24. Ulger Z, Gelenava T, Kosay Y, Darcan S. Acute guttate psoriasis associated with streptococcal perianal dermatitis. *Clin Pediatr (Phila).* 2007;46(1):70-72.
25. Veraldi S, Lunardon L, Dassoni F. Guttate psoriasis triggered by chickenpox. *G Ital Dermatol Venereol.* 2009;144(4):501-502.
26. Guerra I, Algaba A, Perez-Calle JL, et al. Induction of psoriasis with anti-TNF agents in patients with inflammatory bowel disease: a report of 21 cases. *J Crohns Colitis.* 2012;6(5):518-523.
27. Gudjonsson JE, Karason A, Antonsdottir AA, et al. HLA-Cw6-positive and HLA-Cw6-negative patients with Psoriasis vulgaris have distinct clinical features. *J Invest Dermatol.* 2002;118(2):362-365.
28. Mallon E, Newson R, Bunker CB. HLA-Cw6 and the genetic predisposition to psoriasis: a meta-analysis of published serologic studies. *J Invest Dermatol.* 1999;113(4):693-695.
29. Borges-Costa J, Silva R, Goncalves L, Filipe P, Soares de Almeida L, Marques Gomes M. Clinical and laboratory features in acute generalized pustular psoriasis: a retrospective study of 34 patients. *Am J Clin Dermatol.* 2011;12(4):271-276.
30. Boyd AS, Menter A. Erythrodermic psoriasis. Precipitating factors, course, and prognosis in 50 patients. *J Am Acad Dermatol.* 1989;21(5 Pt 1):985-991.
31. Marrakchi S, Guigue P, Renshaw BR, et al. Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis. *N Engl J Med.* 2011;365(7):620-628.
32. O'Doherty CJ, MacIntyre C. Palmoplantar pustulosis and smoking. *Br Med J (Clin Res Ed).* 1985;291(6499):861-864.
33. Mossner R, Thaci D, Mohr J, et al. Manifestation of palmoplantar pustulosis during or after infliximab therapy for plaque-type psoriasis: report on five cases. *Arch Dermatol Res.* 2008;300(3):101-105.
34. Brunasso AM, Puntoni M, Aberer W, Delfino C, Fancelli L, Massone C. Clinical and epidemiological comparison of patients affected by palmoplantar plaque psoriasis and palmoplantar pustulosis: a case series study. *Br J Dermatol.* 2013;168(6):1243-1251.
35. de Waal AC, van de Kerkhof PC. Pustulosis palmoplantaris is a disease distinct from psoriasis. *J Dermatolog Treat.* 2011;22(2):102-105.
36. Mrowietz U, van de Kerkhof PC. Management of palmoplantar pustulosis: do we need to change? *Br J Dermatol.* 2011;164(5):942-946.
37. Ono T, Jono M, Kito M, et al. Evaluation of tonsillectomy as a treatment for pustulosis palmaris et plantaris. *Acta Otolaryngol Suppl.* 1983;401:12-16.
38. Takahara M. Clinical outcome of tonsillectomy for palmoplantar pustulosis and etiological relationship between palmoplantar pustulosis and tonsils. *Adv Otorhinolaryngol.* 2011;72:86-88.
39. Yokoyama M, Hashigucci K, Yamasaki Y. Effect of tonsillectomy in patients with pustulosis palmaris et plantaris. *Acta Otolaryngol.* 2004;124(9):1109-1110.
40. Augustin M, Reich K, Glaeske G, Schaefer I, Radtke M. Co-morbidity and age-related prevalence of psoriasis: Analysis of health insurance data in Germany. *Acta Derm Venereol.* 2010;90(2):147-151.
41. Christophers E. Comorbidities in psoriasis. *Clin Dermatol.* 2007;25(6):529-534.

42. Gelfand JM, Troxel AB, Lewis JD, et al. The risk of mortality in patients with psoriasis: results from a population-based study. *Arch Dermatol.* 2007;143(12):1493-1499.
43. Davidovici BB, Sattar N, Prinz J, et al. Psoriasis and systemic inflammatory diseases: potential mechanistic links between skin disease and co-morbid conditions. *J Invest Dermatol.* 2010;130(7):1785-1796.
44. Lu Y, Chen H, Nikamo P, et al. Association of cardiovascular and metabolic disease genes with psoriasis. *J Invest Dermatol.* 2013;133(3):836-839.
45. Martinez-Borra J, Gonzalez S, Santos-Juanes J, et al. Psoriasis vulgaris and psoriatic arthritis share a 100 kb susceptibility region telomeric to HLA-C. *Rheumatology (Oxford).* 2003;42(9):1089-1092.
46. Gladman DD. Psoriatic arthritis. *Dermatol Ther.* 2009;22(1):40-55.
47. Reich K, Kruger K, Mossner R, Augustin M. Epidemiology and clinical pattern of psoriatic arthritis in Germany: a prospective interdisciplinary epidemiological study of 1511 patients with plaque-type psoriasis. *Br J Dermatol.* 2009;160(5):1040-1047.
48. Olivieri I, Padula A, D'Angelo S, Cutro MS. Psoriatic arthritis sine psoriasis. *J Rheumatol Suppl.* 2009;83:28-29.
49. Esposito M, Saraceno R, Giunta A, Maccarone M, Chimenti S. An Italian study on psoriasis and depression. *Dermatology.* 2006;212(2):123-127.
50. Brauchli YB, Jick SS, Miret M, Meier CR. Psoriasis and risk of incident cancer: an inception cohort study with a nested case-control analysis. *J Invest Dermatol.* 2009;129(11):2604-2612.
51. Wu JJ, Nguyen TU, Poon KY, Herrinton LJ. The association of psoriasis with autoimmune diseases. *J Am Acad Dermatol.* 2012;67(5):924-930.
52. Mallbris L, Ritchlin CT, Stahle M. Metabolic disorders in patients with psoriasis and psoriatic arthritis. *Curr Rheumatol Rep.* 2006;8(5):355-363.
53. Mehta NN, Azfar RS, Shin DB, Neimann AL, Troxel AB, Gelfand JM. Patients with severe psoriasis are at increased risk of cardiovascular mortality: cohort study using the General Practice Research Database. *Eur Heart J.* 2010;31(8):1000-1006.
54. Feldman SR, Krueger GG. Psoriasis assessment tools in clinical trials. *Ann Rheum Dis.* 2005;64 Suppl 2:ii65-68; discussion ii69-73.
55. Fredriksson T, Pettersson U. Severe psoriasis--oral therapy with a new retinoid. *Dermatologica.* 1978;157(4):238-244.
56. Reich K, Griffiths CE. The relationship between quality of life and skin clearance in moderate-to-severe psoriasis: lessons learnt from clinical trials with infliximab. *Arch Dermatol Res.* 2008;300(10):537-544.
57. Kitchen H, Cordingley L, Young H, Griffiths CE, Bundy C. Patient-reported outcome measures in psoriasis: the good, the bad and the missing! *Br J Dermatol.* 2015;172(5):1210-1221.
58. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)--a simple practical measure for routine clinical use. *Clin Exp Dermatol.* 1994;19(3):210-216.
59. Finlay AY, Kelly SE. Psoriasis--an index of disability. *Clin Exp Dermatol.* 1987;12(1):8-11.
60. Gupta MA, Gupta AK. The Psoriasis Life Stress Inventory: a preliminary index of psoriasis-related stress. *Acta Derm Venereol.* 1995;75(3):240-243.
61. Krueger G, Koo J, Lebwohl M, Menter A, Stern R, Rolstad T. The impact of psoriasis on quality of life: results of a 1998 National Psoriasis Foundation patient-membership survey. *Arch Dermatol.* 2001;137(3):280-284.
62. Nelson PA, Barker Z, Griffiths CE, Cordingley L, Chew-Graham CA, Team I. 'On the surface': a qualitative study of GPs' and patients' perspectives on psoriasis. *BMC Fam Pract.* 2013;14:158.

63. Rapp SR, Feldman SR, Exum ML, Fleischer AB, Jr., Reboussin DM. Psoriasis causes as much disability as other major medical diseases. *J Am Acad Dermatol.* 1999;41(3 Pt 1):401-407.
64. Armstrong AW, Schupp C, Wu J, Bebo B. Quality of life and work productivity impairment among psoriasis patients: findings from the National Psoriasis Foundation survey data 2003-2011. *PLoS One.* 2012;7(12):e52935.
65. McKay IA, Leigh IM. Altered keratinocyte growth and differentiation in psoriasis. *Clin Dermatol.* 1995;13(2):105-114.
66. Krengel S, Schaumburg-Lever, G., Geilen, C. & Orfanos, C. Histopathology and Electron microscopy of psoriasis. In: Roenigk HM, H, ed. *Psoriasis*. 3 ed ed. New York: Marcel Dekker; 1998.
67. Leigh IM, Navsaria H, Purkis PE, McKay IA, Bowden PE, Riddle PN. Keratins (K16 and K17) as markers of keratinocyte hyperproliferation in psoriasis in vivo and in vitro. *Br J Dermatol.* 1995;133(4):501-511.
68. Bhawan J, Bansal C, Whren K, Schwertschlag U, Group ILPS. K16 expression in uninvolved psoriatic skin: a possible marker of pre-clinical psoriasis. *J Cutan Pathol.* 2004;31(7):471-476.
69. Schon MP, Boehncke WH. Psoriasis. *N Engl J Med.* 2005;352(18):1899-1912.
70. Elder JT, Bruce AT, Gudjonsson JE, et al. Molecular dissection of psoriasis: integrating genetics and biology. *J Invest Dermatol.* 2010;130(5):1213-1226.
71. Watson W, Cann HM, Farber EM, Nall ML. The genetics of psoriasis. *Arch Dermatol.* 1972;105(2):197-207.
72. Farber EM, Nall ML, Watson W. Natural history of psoriasis in 61 twin pairs. *Arch Dermatol.* 1974;109(2):207-211.
73. Brandrup F, Holm N, Grunnet N, Henningsen K, Hansen HE. Psoriasis in monozygotic twins: variations in expression in individuals with identical genetic constitution. *Acta Derm Venereol.* 1982;62(3):229-236.
74. Duffy D, Spelman L, Martin N. Psoriasis in Australian twins. *J Am Acad Dermatol.* 1993;29(3):428-434.
75. Burden AD, Javed S, Bailey M, Hodgins M, Connor M, Tillman D. Genetics of psoriasis: paternal inheritance and a locus on chromosome 6p. *J Invest Dermatol.* 1998;110(6):958-960.
76. Lomholt G. Prevalence of skin diseases in a population; A census Study from the Faroe Islands. *Danish medical bulletin.* 1964;11:1-7.
77. Swanbeck G, Inerot A, Martinsson T, Wahlstrom J. A population genetic study of psoriasis. *Acta Derm Venereol Suppl (Stockh).* 1994;186:7-8.
78. Tsoi LC, Spain SL, Ellinghaus E, et al. Enhanced meta-analysis and replication studies identify five new psoriasis susceptibility loci. *Nat Commun.* 2015;6:7001.
79. Barker J. Psoriasis heritability: 125 years and counting. *Br J Dermatol.* 2014;171(1):3-5.
80. Das S, Stuart PE, Ding J, et al. Fine mapping of eight psoriasis susceptibility loci. *Eur J Hum Genet.* 2015;23(6):844-853.
81. Okada Y, Han B, Tsoi LC, et al. Fine mapping major histocompatibility complex associations in psoriasis and its clinical subtypes. *Am J Hum Genet.* 2014;95(2):162-172.
82. Sheng Y, Jin X, Xu J, et al. Sequencing-based approach identified three new susceptibility loci for psoriasis. *Nat Commun.* 2014;5:4331.
83. Mahil SK, Capon F, Barker JN. Update on psoriasis immunopathogenesis and targeted immunotherapy. *Semin Immunopathol.* 2015.

84. Nair RP, Stuart P, Henseler T, et al. Localization of psoriasis-susceptibility locus PSORS1 to a 60-kb interval telomeric to HLA-C. *Am J Hum Genet.* 2000;66(6):1833-1844.
85. Nair RP, Stuart PE, Nistor I, et al. Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. *Am J Hum Genet.* 2006;78(5):827-851.
86. Christophers E. Genotyping psoriasis. *J Invest Dermatol.* 2003;120(4):xvii.
87. Zhang XJ, Zhang AP, Yang S, et al. Association of HLA class I alleles with psoriasis vulgaris in southeastern Chinese Hans. *J Dermatol Sci.* 2003;33(1):1-6.
88. Elder JT, Nair RP, Henseler T, et al. The genetics of psoriasis 2001: the odyssey continues. *Arch Dermatol.* 2001;137(11):1447-1454.
89. Veal CD, Capon F, Allen MH, et al. Family-based analysis using a dense single-nucleotide polymorphism-based map defines genetic variation at PSORS1, the major psoriasis-susceptibility locus. *Am J Hum Genet.* 2002;71(3):554-564.
90. Strange A, Capon F, Spencer CC, et al. A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nat Genet.* 2010;42(11):985-990.
91. Nair R, Duffin K, Helms C, et al. Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet.* 2009;41(2):199-204.
92. Gudjonsson JE, Karason A, Antonsdottir A, et al. Psoriasis patients who are homozygous for the HLA-Cw*0602 allele have a 2.5-fold increased risk of developing psoriasis compared with Cw6 heterozygotes. *Br J Dermatol.* 2003;148(2):233-235.
93. Allen MH, Ameen H, Veal C, et al. The major psoriasis susceptibility locus PSORS1 is not a risk factor for late-onset psoriasis. *J Invest Dermatol.* 2005;124(1):103-106.
94. Holm SJ, Sakuraba K, Mallbris L, Wolk K, Stahle M, Sanchez FO. Distinct HLA-C/KIR genotype profile associates with guttate psoriasis. *J Invest Dermatol.* 2005;125(4):721-730.
95. Mallon E, Bunce M, Savoie H, et al. HLA-C and guttate psoriasis. *Br J Dermatol.* 2000;143(6):1177-1182.
96. Gudjonsson JE, Karason A, Runarsdottir EH, et al. Distinct clinical differences between HLA-Cw*0602 positive and negative psoriasis patients--an analysis of 1019 HLA-C- and HLA-B-typed patients. *J Invest Dermatol.* 2006;126(4):740-745.
97. Fan X, Yang S, Sun LD, et al. Comparison of clinical features of HLA-Cw*0602-positive and -negative psoriasis patients in a Han Chinese population. *Acta Derm Venereol.* 2007;87(4):335-340.
98. Ikaheimo I, Tiilikainen A, Karvonen J, Silvennoinen-Kassinen S. HLA risk haplotype Cw6,DR7,DQA1*0201 and HLA-Cw6 with reference to the clinical picture of psoriasis vulgaris. *Arch Dermatol Res.* 1996;288(7):363-365.
99. Enerback C, Martinsson T, Inerot A, et al. Evidence that HLA-Cw6 determines early onset of psoriasis, obtained using sequence-specific primers (PCR-SSP). *Acta Derm Venereol.* 1997;77(4):273-276.
100. Indhumathi S, Rajappa M, Chandrashekar L, Ananthanarayanan PH, Thappa DM, Negi VS. The HLA-C*06 allele as a possible genetic predisposing factor to psoriasis in South Indian Tamils. *Arch Dermatol Res.* 2016;308(3):193-199.
101. Mallbris L, Wolk K, Sanchez F, Stahle M. HLA-Cw*0602 associates with a twofold higher prevalence of positive streptococcal throat swab at the onset of psoriasis: a case control study. *BMC Dermatol.* 2009;9:5.
102. Talamonti M, Botti E, Galluzzo M, et al. Pharmacogenetics of psoriasis: HLA-Cw6 but not LCE3B/3C deletion nor TNFAIP3 polymorphism predisposes to clinical

response to interleukin 12/23 blocker ustekinumab. *Br J Dermatol.* 2013;169(2):458-463.
103. Chiu HY, Wang TS, Chan CC, Cheng YP, Lin SJ, Tsai TF. Human leucocyte antigen-Cw6 as a predictor for clinical response to ustekinumab, an interleukin-12/23 blocker, in Chinese patients with psoriasis: a retrospective analysis. *Br J Dermatol.* 2014;171(5):1181-1188.
104. Griffiths CE, Christophers E, Szumski A, Jones H, Mallbris L. Impact of early vs. late disease onset on treatment response to etanercept in patients with psoriasis. *Br J Dermatol.* 2015.
105. Ferrandiz C, Pujol RM, Garcia-Patos V, Bordas X, Smandia JA. Psoriasis of early and late onset: a clinical and epidemiologic study from Spain. *J Am Acad Dermatol.* 2002;46(6):867-873.
106. Cargill M, Schrodi SJ, Chang M, et al. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet.* 2007;80(2):273-290.
107. Tsoi LC, Spain SL, Knight J, et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. *Nat Genet.* 2012;44(12):1341-1348.
108. Riveira-Munoz E, He SM, Escaramis G, et al. Meta-analysis confirms the LCE3C_LCE3B deletion as a risk factor for psoriasis in several ethnic groups and finds interaction with HLA-Cw6. *J Invest Dermatol.* 2011;131(5):1105-1109.
109. Bergboer JG, Zeeuwen PL, Schalkwijk J. Genetics of psoriasis: evidence for epistatic interaction between skin barrier abnormalities and immune deviation. *J Invest Dermatol.* 2012;132(10):2320-2331.
110. Fry L, Baker BS. Triggering psoriasis: the role of infections and medications. *Clin Dermatol.* 2007;25(6):606-615.
111. Pouessel G, Ythier H, Carpentier O, Vachee A, Etienne J, Catteau B. Childhood pustular psoriasis associated with Panton-Valentine leukocidin-producing Staphylococcus aureus. *Pediatr Dermatol.* 2007;24(4):401-404.
112. Qayoom S, Ahmad QM. Psoriasis and Helicobacter pylori. *Indian J Dermatol Venereol Leprol.* 2003;69(2):133-134.
113. Imafuku S, Naito R, Nakayama J. Possible association of hepatitis C virus infection with late-onset psoriasis: a hospital-based observational study. *J Dermatol.* 2013;40(10):813-818.
114. Davidsson S, Blomqvist K, Molin L, et al. Lifestyle of Nordic people with psoriasis. *Int J Dermatol.* 2005;44(5):378-383.
115. Mazzetti M, Mozzetta A, Soavi GC, et al. Psoriasis, stress and psychiatry: psychodynamic characteristics of stressors. *Acta Derm Venereol Suppl (Stockh).* 1994;186:62-64.
116. Köbner H. Zur Aetiologie der Psoriasis. *Vjschr Dermatol.* 1876;8:559-561.
117. Rutter KJ, Watson RE, Cotterell LF, Brenn T, Griffiths CE, Rhodes LE. Severely photosensitive psoriasis: a phenotypically defined patient subset. *J Invest Dermatol.* 2009;129(12):2861-2867.
118. Dika E, Bardazzi F, Balestri R, Maibach HI. Environmental factors and psoriasis. *Curr Probl Dermatol.* 2007;35:118-135.
119. Armstrong AW. Psoriasis provoked or exacerbated by medications: identifying culprit drugs. *JAMA Dermatol.* 2014;150(9):963.
120. Basavaraj KH, Ashok NM, Rashmi R, Praveen TK. The role of drugs in the induction and/or exacerbation of psoriasis. *Int J Dermatol.* 2010;49(12):1351-1361.
121. Michaelsson G, Gustafsson K, Hagforsen E. The psoriasis variant palmoplantar pustulosis can be improved after cessation of smoking. *J Am Acad Dermatol.* 2006;54(4):737-738.

122. Higgins E. Alcohol, smoking and psoriasis. *Clin Exp Dermatol.* 2000;25(2):107-110.
123. Armstrong AW, Harskamp CT, Dhillon JS, Armstrong EJ. Psoriasis and smoking: a systematic review and meta-analysis. *Br J Dermatol.* 2014;170(2):304-314.
124. Poikolainen K, Karvonen J, Pukkala E. Excess mortality related to alcohol and smoking among hospital-treated patients with psoriasis. *Arch Dermatol.* 1999;135(12):1490-1493.
125. Poikolainen K, Reunala T, Karvonen J, Lauharanta J, Karkkainen P. Alcohol intake: a risk factor for psoriasis in young and middle aged men? *BMJ.* 1990;300(6727):780-783.
126. Lindegard B. Diseases associated with psoriasis in a general population of 159,200 middle-aged, urban, native Swedes. *Dermatologica.* 1986;172(6):298-304.
127. Ceovic R, Mance M, Bukvic Mokos Z, Svetec M, Kostovic K, Stulhofer Buzina D. Psoriasis: female skin changes in various hormonal stages throughout life--puberty, pregnancy, and menopause. *Biomed Res Int.* 2013;2013:571912.
128. Sugita K, Kabashima K, Atarashi K, Shimauchi T, Kobayashi M, Tokura Y. Innate immunity mediated by epidermal keratinocytes promotes acquired immunity involving Langerhans cells and T cells in the skin. *Clin Exp Immunol.* 2007;147(1):176-183.
129. Hamerman JA, Ogasawara K, Lanier LL. NK cells in innate immunity. *Curr Opin Immunol.* 2005;17(1):29-35.
130. Kobayashi SD, DeLeo FR. Role of neutrophils in innate immunity: a systems biology-level approach. *Wiley interdisciplinary reviews Systems biology and medicine.* 2009;1(3):309-333.
131. Teunissen MB, Yeremenko NG, Baeten DL, et al. The IL-17A-producing CD8+ T-cell population in psoriatic lesional skin comprises mucosa-associated invariant T cells and conventional T cells. *J Invest Dermatol.* 2014;134(12):2898-2907.
132. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol.* 2010;11(5):373-384.
133. Steinman RM. Linking innate to adaptive immunity through dendritic cells. *Novartis Found Symp.* 2006;279:101-109; discussion 109-113, 216-109.
134. Trifari S, Kaplan CD, Tran EH, Crellin NK, Spits H. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. *Nat Immunol.* 2009;10(8):864-871.
135. Bromley SK, Mempel TR, Luster AD. Orchestrating the orchestrators: chemokines in control of T cell traffic. *Nat Immunol.* 2008;9(9):970-980.
136. Barker JN. Adhesion molecules in cutaneous inflammation. *Ciba Foundation symposium.* 1995;189:91-101; discussion 101-106.
137. Clark RA. Resident memory T cells in human health and disease. *Sci Transl Med.* 2015;7(269):269rv261.
138. Farber EM, Cox AJ. The biology of psoriasis. *J Invest Dermatol.* 1967;49(4):348-357.
139. Mueller W, Herrmann B. Cyclosporin A for psoriasis. *N Engl J Med.* 1979;301(10):555.
140. Valdimarsson H, Bake BS, Jonsdottir I, Fry L. Psoriasis: a disease of abnormal Keratinocyte proliferation induced by T lymphocytes. *Immunol Today.* 1986;7(9):256-259.
141. Valdimarsson H, Baker BS, Jonsdottir I, Powles A, Fry L. Psoriasis: a T-cell-mediated autoimmune disease induced by streptococcal superantigens? *Immunol Today.* 1995;16(3):145-149.

142. Valdimarsson H, Thorleifsdottir RH, Sigurdardottir SL, Gudjonsson JE, Johnston A. Psoriasis--as an autoimmune disease caused by molecular mimicry. *Trends Immunol.* 2009;30(10):494-501.
143. Gudjonsson JE, Johnston A, Sigmundsdottir H, Valdimarsson H. Immunopathogenic mechanisms in psoriasis. *Clin Exp Immunol.* 2004;135(1):1-8.
144. Baker BS, Swain AF, Valdimarsson H, Fry L. T-cell subpopulations in the blood and skin of patients with psoriasis. *Br J Dermatol.* 1984;110(1):37-44.
145. Prinz JC, Gross B, Vollmer S, et al. T cell clones from psoriasis skin lesions can promote keratinocyte proliferation in vitro via secreted products. *Eur J Immunol.* 1994;24(3):593-598.
146. Bos JD, Meinardi MM, van Joost T, Heule F, Powles AV, Fry L. Use of cyclosporin in psoriasis. *Lancet.* 1989;2(8678-8679):1500-1502.
147. Gottlieb SL, Gilleaudeau P, Johnson R, et al. Response of psoriasis to a lymphocyte-selective toxin (DAB389IL-2) suggests a primary immune, but not keratinocyte, pathogenic basis. *Nat Med.* 1995;1(5):442-447.
148. Piaserico S, Sandini E, Saldan A, Abate D. Effects of TNF-Alpha Inhibitors on Circulating Th17 Cells in Patients Affected by Severe Psoriasis. *Drug Development Research.* 2014;75:S73-S76.
149. Antiga E, Volpi W, Cardilicchia E, et al. Etanercept Downregulates the Th17 Pathway and Decreases the IL-17+/IL-10+ Cell Ratio in Patients with Psoriasis Vulgaris. *Journal of Clinical Immunology.* 2012;32(6):1221-1232.
150. Gaspari AA, Tyring S. New and emerging biologic therapies for moderate-to-severe plaque psoriasis: mechanistic rationales and recent clinical data for IL-17 and IL-23 inhibitors. *Dermatol Ther.* 2015;28(4):179-193.
151. Tan KW, Griffiths CE. Novel systemic therapies for the treatment of psoriasis. *Expert Opin Pharmacother.* 2015:1-14.
152. Snowden JA, Heaton DC. Development of psoriasis after syngeneic bone marrow transplant from psoriatic donor: further evidence for adoptive autoimmunity. *Br J Dermatol.* 1997;137(1):130-132.
153. Eedy DJ, Burrows D, Bridges JM, Jones FG. Clearance of severe psoriasis after allogenic bone marrow transplantation. *BMJ.* 1990;300(6729):908.
154. Wrone-Smith T, Nickoloff BJ. Dermal injection of immunocytes induces psoriasis. *J Clin Invest.* 1996;98(8):1878-1887.
155. Conrad C, Boyman O, Tonel G, et al. Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. *Nat Med.* 2007;13(7):836-842.
156. Kim J, Krueger JG. The immunopathogenesis of psoriasis. *Dermatol Clin.* 2015;33(1):13-23.
157. Krueger JG, Bowcock A. Psoriasis pathophysiology: current concepts of pathogenesis. *Ann Rheum Dis.* 2005;64 Suppl 2:ii30-36.
158. Di Cesare A, Di Meglio P, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. *J Invest Dermatol.* 2009;129(6):1339-1350.
159. Ganguly D, Chamilos G, Lande R, et al. Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. *J Exp Med.* 2009;206(9):1983-1994.
160. Gilliet M, Lande R. Antimicrobial peptides and self-DNA in autoimmune skin inflammation. *Curr Opin Immunol.* 2008;20(4):401-407.
161. Lande R, Gregorio J, Facchinetti V, et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature.* 2007;449(7162):564-569.
162. Nakagawa Y, Gallo RL. Endogenous intracellular cathelicidin enhances TLR9 activation in dendritic cells and macrophages. *J Immunol.* 2015;194(3):1274-1284.

163. Kahlenberg JM, Kaplan MJ. Little peptide, big effects: the role of LL-37 in inflammation and autoimmune disease. *J Immunol.* 2013;191(10):4895-4901.
164. Morizane S, Yamasaki K, Muhleisen B, et al. Cathelicidin antimicrobial peptide LL-37 in psoriasis enables keratinocyte reactivity against TLR9 ligands. *J Invest Dermatol.* 2012;132(1):135-143.
165. Kennedy-Crispin M, Billick E, Mitsui H, et al. Human keratinocytes' response to injury upregulates CCL20 and other genes linking innate and adaptive immunity. *J Invest Dermatol.* 2012;132(1):105-113.
166. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. *Annu Rev Immunol.* 2014;32:227-255.
167. Arakawa A, Siewert K, Stohr J, et al. Melanocyte antigen triggers autoimmunity in human psoriasis. *J Exp Med.* 2015;212(13):2203-2212.
168. Wolk K, Witte E, Warszawska K, et al. The Th17 cytokine IL-22 induces IL-20 production in keratinocytes: a novel immunological cascade with potential relevance in psoriasis. *Eur J Immunol.* 2009;39(12):3570-3581.
169. Zheng Y, Danilenko DM, Valdez P, et al. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature.* 2007;445(7128):648-651.
170. Cheuk S, Wiken M, Blomqvist L, et al. Epidermal Th22 and Tc17 cells form a localized disease memory in clinically healed psoriasis. *J Immunol.* 2014;192(7):3111-3120.
171. Brandtzaeg P. Immune functions of nasopharyngeal lymphoid tissue. *Adv Otorhinolaryngol.* 2011;72:20-24.
172. Perry M, Whyte A. Immunology of the tonsils. *Immunol Today.* 1998;19(9):414-421.
173. MacLennan IC, Gulbranson-Judge A, Toellner KM, et al. The changing preference of T and B cells for partners as T-dependent antibody responses develop. *Immunol Rev.* 1997;156:53-66.
174. Schroeder BM. Diagnosis and management of group A streptococcal pharyngitis. *Am Fam Physician.* 2003;67(4):880, 883-884.
175. Wessels MR. Clinical practice. Streptococcal pharyngitis. *N Engl J Med.* 2011;364(7):648-655.
176. Martin WJ, Steer AC, Smeesters PR, et al. Post-infectious group A streptococcal autoimmune syndromes and the heart. *Autoimmun Rev.* 2015;14(8):710-725.
177. Facklam R. What happened to the streptococci: overview of taxonomic and nomenclature changes. *Clin Microbiol Rev.* 2002;15(4):613-630.
178. Whiley RA, Hall LM, Hardie JM, Beighton D. A study of small-colony, beta-haemolytic, Lancefield group C streptococci within the anginosus group: description of Streptococcus constellatus subsp. pharyngis subsp. nov., associated with the human throat and pharyngitis. *Int J Syst Bacteriol.* 1999;49 Pt 4:1443-1449.
179. Thulin P, Johansson L, Low DE, et al. Viable group A streptococci in macrophages during acute soft tissue infection. *PLoS Med.* 2006;3(3):e53.
180. Dombek PE, Cue D, Sedgewick J, et al. High-frequency intracellular invasion of epithelial cells by serotype M1 group A streptococci: M1 protein-mediated invasion and cytoskeletal rearrangements. *Mol Microbiol.* 1999;31:859-870.
181. LaPenta D, Rubens C, Chi E, Cleary PP. Group A streptococci efficiently invade human respiratory epithelial cells. *Proc Natl Acad Sci U S A.* 1994;91:12115-12119.
182. Osterlund A, Popa R, Nikkila T, Scheynius A, Engstrand L. Intracellular reservoir of Streptococcus pyogenes in vivo: a possible explanation for recurrent pharyngotonsillitis. *Laryngoscope.* 1997;107:640-647.

183. Kaplan EL, Chhatwal GS, Rohde M. Reduced ability of penicillin to eradicate ingested group A streptococci from epithelial cells: clinical and pathogenetic implications. *Clin Infect Dis.* 2006;43(11):1398-1406.
184. Rohde M, Cleary PP. Adhesion and invasion of Streptococcus pyogenes into host cells and clinical relevance of intracellular streptococci. In: Ferretti JJ, Stevens DL, Fischetti VA, eds. *Streptococcus pyogenes: Basic Biology to Clinical Manifestations*. Oklahoma City (OK): University of Oklahoma Health Sciences Center(c) The University of Oklahoma Health Sciences Center.; 2016.
185. Oehmcke S, Shannon O, Morgelin M, Herwald H. Streptococcal M proteins and their role as virulence determinants. *Clin Chim Acta.* 2010;411(17-18):1172-1180.
186. Commons RJ, Smeesters PR, Proft T, Fraser JD, Robins-Browne R, Curtis N. Streptococcal superantigens: categorization and clinical associations. *Trends Mol Med.* 2014;20(1):48-62.
187. Hope-Simpson RE. Streptococcus pyogenes in the throat: a study in a small population, 1962-1975. *J Hyg (Lond).* 1981;87(1):109-129.
188. Winfield JM. Psoriasis as a sequel to acute inflammations of the tonsils: a clinical note. *J Cutan Dis.* 1916;34:441-443.
189. Norholm-Pedersen A. Infections and psoriasis; a preliminary communication. *Acta Derm Venereol.* 1952;32(2):159-167.
190. Norrlind R. The significance of infections in the origination of psoriasis. *Acta Rheumatol Scand.* 1955;1(2):135-144.
191. Prinz J. Psoriasis vulgaris--a sterile antibacterial skin reaction mediated by cross-reactive T cells? An immunological view of the pathophysiology of psoriasis. *Clin Exp Dermatol.* 2001;26(4):326-332.
192. Zhao G, Feng X, Na A, et al. Acute guttate psoriasis patients have positive streptococcus hemolyticus throat cultures and elevated antistreptococcal M6 protein titers. *J Dermatol.* 2005;32(2):91-96.
193. Ruiz-Romeu E, Ferran M, Sagrista M, et al. Streptococcus pyogenes-induced cutaneous lymphocyte antigen-positive T cell-dependent epidermal cell activation triggers TH17 responses in patients with guttate psoriasis. *J Allergy Clin Immunol.* 2016;138(2):491-499.e496.
194. Tervaert W, Esseveld H. A study of the incidence of haemolytic streptococci in the throat in patients with psoriasis vulgaris, with reference to their role in the pathogenesis of this disease. *Dermatologica.* 1970;140(5):282-290.
195. El-Rachkidy RG, Hales JM, Freestone PP, Young HS, Griffiths CE, Camp RD. Increased blood levels of IgG reactive with secreted Streptococcus pyogenes proteins in chronic plaque psoriasis. *J Invest Dermatol.* 2007;127(6):1337-1342.
196. Wardrop P, Weller R, Marais J, Kavanagh G. Tonsillitis and chronic psoriasis. *Clin Otolaryngol Allied Sci.* 1998;23(1):67-68.
197. Gudjonsson JE, Thorarinsson AM, Sigurgeirsson B, Kristinsson KG, Valdimarsson H. Streptococcal throat infections and exacerbation of chronic plaque psoriasis: a prospective study. *Br J Dermatol.* 2003;149(3):530-534.
198. Sigurdardottir SL, Thorleifsdottir RH, Valdimarsson H, Johnston A. The association of sore throat and psoriasis might be explained by histologically distinctive tonsils and increased expression of skin-homing molecules by tonsil T cells. *Clin Exp Immunol.* 2013;174(1):139-151.
199. Ferran M, Galvan AB, Rincon C, et al. Streptococcus induces circulating CLA(+) memory T-cell-dependent epidermal cell activation in psoriasis. *J Invest Dermatol.* 2013;133(4):999-1007.
200. Saxena VN, Dogra J. Long-term use of penicillin for the treatment of chronic plaque psoriasis. *Eur J Dermatol.* 2005;15(5):359-362.

201. Saxena VN, Dogra J. Long-term oral azithromycin in chronic plaque psoriasis: a controlled trial. *Eur J Dermatol.* 2010;20(3):329-333.
202. Dogan B, Karabudak O, Harmanyeri Y. Antistreptococcal treatment of guttate psoriasis: a controlled study. *Int J Dermatol.* 2008;47(9):950-952.
203. Vincent F, Ross JB, Dalton M, Wort AJ. A therapeutic trial of the use of penicillin V or erythromycin with or without rifampin in the treatment of psoriasis. *J Am Acad Dermatol.* 1992;26(3 Pt 2):458-461.
204. Owen CM, Chalmers RJ, O'Sullivan T, Griffiths CE. A systematic review of antistreptococcal interventions for guttate and chronic plaque psoriasis. *Br J Dermatol.* 2001;145(6):886-890.
205. Yoshizaki T, Bandoh N, Ueda S, et al. Up-regulation of CC chemokine receptor 6 on tonsillar T cells and its induction by in vitro stimulation with alpha-streptococci in patients with pustulosis palmaris et plantaris. *Clin Exp Immunol.* 2009;157(1):71-82.
206. Ueda S, Takahara M, Tohtani T, Yoshizaki T, Kishibe K, Harabuchi Y. Up-regulation of ß1 integrin on tonsillar T cells and its induction by in vitro stimulation with α-streptococci in patients with pustulosis Palmaris et Plantaris. *J Clin Immunol.* 2010;30(6):861-871.
207. Fraser JD, Proft T. The bacterial superantigen and superantigen-like proteins. *Immunol Rev.* 2008;225:226-243.
208. Proft T, Schrage B, Fraser JD. The cytokine response to streptococcal superantigens varies between individual toxins and between individuals: implications for the pathogenesis of group A streptococcal diseases. *J Interferon Cytokine Res.* 2007;27(7):553-557.
209. Sigmundsdottir H, Johnston A, Gudjonsson JE, Valdimarsson H. Differential effects of interleukin 12 and interleukin 10 on superantigen-induced expression of cutaneous lymphocyte-associated antigen (CLA) and alphaEbeta7 integrin (CD103) by CD8+ T cells. *Clin Immunol.* 2004;111(1):119-125.
210. Leung DY, Gately M, Trumble A, Ferguson-Darnell B, Schlievert PM, Picker LJ. Bacterial superantigens induce T cell expression of the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen, via stimulation of interleukin 12 production. *J Exp Med.* 1995;181(2):747-753.
211. Lewis HM, Baker BS, Bokth S, et al. Restricted T-cell receptor V beta gene usage in the skin of patients with guttate and chronic plaque psoriasis. *Br J Dermatol.* 1993;129(5):514-520.
212. Leung DY, Walsh P, Giorno R, Norris DA. A potential role for superantigens in the pathogenesis of psoriasis. *J Invest Dermatol.* 1993;100(3):225-228.
213. Leung DY, Travers JB, Giorno R, et al. Evidence for a streptococcal superantigen-driven process in acute guttate psoriasis. *J Clin Invest.* 1995;96(5):2106-2112.
214. Davison SC, Ballsdon A, Allen MH, Barker JN. Early migration of cutaneous lymphocyte-associated antigen (CLA) positive T cells into evolving psoriatic plaques. *Exp Dermatol.* 2001;10(4):280-285.
215. Kohm AP, Fuller KG, Miller SD. Mimicking the way to autoimmunity: an evolving theory of sequence and structural homology. *Trends Microbiol.* 2003;11(3):101-105.
216. Chang JC, Smith LR, Froning KJ, et al. CD8+ T cells in psoriatic lesions preferentially use T cell receptor V beta 3 and/or V beta 13.1 genes. *Proc Natl Acad Sci U S A.* 1994;91:9282-9289.
217. Menssen A, Trommler P, Vollmer S, et al. Evidence for an antigen-specific cellular immune response in skin lesions of patients with psoriasis vulgaris. *J Immunol.* 1995;155(8):4078-4083.

218. Bour H, Puisieux I, Even J, et al. T-cell repertoire analysis in chronic plaque psoriasis suggests an antigen-specific immune response. *Hum Immunol.* 1999;60(8):665-676.
219. Prinz JC, Vollmer S, Boehncke WH, Menssen A, Laisney I, Trommler P. Selection of conserved TCR VDJ rearrangements in chronic psoriatic plaques indicates a common antigen in psoriasis vulgaris. *Eur J Immunol.* 1999;29(10):3360-3368.
220. Lin W-J, Norris DA, Achziger M, Kotzin BL, Tomkinson B. Oligoclonal Expansion of Intraepidermal T Cells in Psoriasis Skin Lesions. *J Invest Dermatol.* 2001;117(6):1546-1553.
221. Hwang HY, Bahk YY, Kim TG, Kim TY. Identification of a commonly used CDR3 region of infiltrating T cells expressing Vbeta13 and Vbeta15 derived from psoriasis patients. *J Invest Dermatol.* 2003;120(3):359-364.
222. McFadden J, Valdimarsson H, Fry L. Cross-reactivity between streptococcal M surface antigen and human skin. *Br J Dermatol.* 1991;125(5):443-447.
223. Sigmundsdottir H, Sigurgeirsson B, Troye-Blomberg M, Good MF, Valdimarsson H, Jonsdottir I. Circulating T cells of patients with active psoriasis respond to streptococcal M-peptides sharing sequences with human epidermal keratins. *Scand J Immunol.* 1997;45(6):688-697.
224. Johnston A, Gudjonsson JE, Sigmundsdottir H, Love TJ, Valdimarsson H. Peripheral blood T cell responses to keratin peptides that share sequences with streptococcal M proteins are largely restricted to skin-homing CD8(+) T cells. *Clin Exp Immunol.* 2004;138(1):83-93.
225. Baker BS, Brown DW, Fischetti VA, et al. Skin T cell proliferative response to M protein and other cell wall and membrane proteins of group A streptococci in chronic plaque psoriasis. *Clin Exp Immunol.* 2001;124(3):516-521.
226. Baker BS, Bokth S, Powles A, et al. Group A streptococcal antigen-specific T lymphocytes in guttate psoriatic lesions. *Br J Dermatol.* 1993;128(5):493-499.
227. Brown DW, Baker BS, Ovigne JM, Hardman C, Powles AV, Fry L. Skin CD4+ T cells produce interferon-gamma in vitro in response to streptococcal antigens in chronic plaque psoriasis. *J Invest Dermatol.* 2000;114(3):576-580.
228. Ovigne J-M, Baker BS, Davison S. C, Powles AV, Fry L. Epidermal CD8+ T cells reactive with group A streptococcal antigens in chronic plaque psoriasis. *Exp Dermatol.* 2002;11(4):357-364.
229. Gudmundsdottir AS, Sigmundsdottir H, Sigurgeirsson B, Good MF, Valdimarsson H, Jonsdottir I. Is an epitope on keratin 17 a major target for autoreactive T lymphocytes in psoriasis? *Clin Exp Immunol.* 1999;117(3):580-586.
230. Shen Z, Wang G, Fan J-Y, Li W, Liu Y-F. HLA DR B1*04, *07-restricted epitopes on Keratin 17 for autoreactive T cells in psoriasis. *J Dermatol Sci.* 2005;38(1):25-39.
231. Diluvio L, Vollmer S, Besgen P, Ellwart JW, Chimenti S, Prinz JC. Identical TCR beta-chain rearrangements in streptococcal angina and skin lesions of patients with psoriasis vulgaris. *J Immunol.* 2006;176(11):7104-7111.
232. Prinz JC. Disease mimicry--a pathogenetic concept for T cell-mediated autoimmune disorders triggered by molecular mimicry? *Autoimmun Rev.* 2004;3(1):10-15.
233. Besgen P, Trommler P, Vollmer S, Prinz JC. Ezrin, maspin, peroxiredoxin 2, and heat shock protein 27: potential targets of a streptococcal-induced autoimmune response in psoriasis. *J Immunol.* 2010;184(9):5392-5402.
234. Boneca IG. The role of peptidoglycan in pathogenesis. *Curr Opin Microbiol.* 2005;8(1):46-53.
235. Royet J, Gupta D, Dziarski R. Peptidoglycan recognition proteins: modulators of the microbiome and inflammation. *Nat Rev Immunol.* 2011;11(12):837-851.

236. Liu C, Xu Z, Gupta D, Dziarski R. Peptidoglycan recognition proteins: a novel family of four human innate immunity pattern recognition molecules. *J Biol Chem.* 2001;276(37):34686-34694.
237. Baker B, Powles A, Fry L. Peptidoglycan: a major aetiological factor for psoriasis? *Trends Immunol.* 2006;27(12):545-551.
238. Baker BS, Laman JD, Powles A, et al. Peptidoglycan and peptidoglycan-specific Th1 cells in psoriatic skin lesions. *J Pathol.* 2006;209(2):174-181.
239. Hoddeson EK, Gourin CG. Adult tonsillectomy: current indications and outcomes. *Otolaryngol Head Neck Surg.* 2009;140(1):19-22.
240. Baugh RF, Archer SM, Mitchell RB, et al. Clinical practice guideline: tonsillectomy in children. *Otolaryngol Head Neck Surg.* 2011;144(1 Suppl):S1-30.
241. Ramos SD, Mukerji S, Pine HS. Tonsillectomy and adenoidectomy. *Pediatric clinics of North America.* 2013;60(4):793-807.
242. Rachakonda TD, Dhillon JS, Florek AG, Armstrong AW. Effect of tonsillectomy on psoriasis: a systematic review. *J Am Acad Dermatol.* 2015;72(2):261-275.
243. Wu W, Debbaneh M, Moslehi H, Koo J, Liao W. Tonsillectomy as a treatment for psoriasis: a review. *J Dermatolog Treat.* 2014;25(6):482-486.
244. Sigurdardottir SL, Thorleifsdottir RH, Valdimarsson H, Johnston A. The role of the palatine tonsils in the pathogenesis and treatment of psoriasis. *Br J Dermatol.* 2013;168(2):237-242.
245. Thorleifsdottir RH, Sigurdardottir SL, Sigurgeirsson B, et al. Improvement of psoriasis after tonsillectomy is associated with a decrease in the frequency of circulating T cells that recognize streptococcal determinants and homologous skin determinants. *J Immunol.* 2012;188(10):5160-5165.
246. Ponomareva LV. [Psoriasis and Chronic Tonsillitis]. *Vestn Dermatol Venerol.* 1965;39:26-30.
247. Cepicka W, Tielsch R. [Focal infections and Psoriasis vulgaris]. *Dermatol Wochenschr.* 1967;153(8):193-199.
248. Nesterenko GB, Lukovskii LA, Tytar GM, Bashmakov GV. [Pathogenetic relation of psoriasis and chronic tonsillitis]. *Vrach Delo.* 1972;7:118-120.
249. Kataura A, Tsubota H. Clinical analyses of focus tonsil and related diseases in Japan. *Acta Otolaryngol Suppl.* 1996;523:161-164.
250. Hone SW, Donnelly MJ, Powell F, Blayney AW. Clearance of recalcitrant psoriasis after tonsillectomy. *Clin Otolaryngol Allied Sci.* 1996;21(6):546-547.
251. Lukovskii LA, Nesterenko GB, Tytar GM, Bashmakov GV. [Immediate and remote results of tonsillectomy in chronic tonsillitis and psoriasis]. *Vestn Otorinolaringol.* 1970;32(6):23-26.
252. Nyfors A, Rasmussen PA, Lemholt K, Eriksen B. Improvement of recalcitrant psoriasis vulgaris after tonsillectomy. *J Laryngol Otol.* 1976;90(8):789-794.
253. Ozawa A, Ohkido M, Haruki Y, et al. Treatments of generalized pustular psoriasis: a multicenter study in Japan. *J Dermatol.* 1999;26(3):141-149.
254. Bucolo S, Torre V, Romano G, et al. Effects of Tonsillectomy on Psoriasis and Tonsil Histology-Ultrastructure. In: Lima H, ed. *Psoriasis - Types, Causes and Medication*: InTech; 2013:159-182.
255. Tytar' G, Bashmakov G. [The interrelationship between chronic tonsillitis and psoriasis]. *Zh Ushn Nos Gorl Bolezn.* 1968;28(6):68-70.
256. Bukharovich MN, Foderman VM, Tikhaia SK. [Therapeutic methods in psoriasis in patients with chronic tonsillitis]. *Zh Ushn Nos Gorl Bolezn.* 1971;31(2):62-64.
257. Fukunaga T. Studies on Skin Disease Due to Tonsillar Focal Infection. *Auris Nasus Larynx.* 1974;1(2):151-159.

258. Rosenberg EW, Skinner RB, Noah PW. Anti-infectious therapy in psoriasis. In: Roenigk HH, Maibach HI, eds. *Psoriasis*. 3rd ed. New York: Marcel Dekker; 1998:373-379.
259. Takahara M, Bandoh N, Imada M, Hayashi T, Nonaka S, Harabuchi Y. Efficacy of tonsillectomy on psoriasis and tonsil histology. *Nippon Jibiinkoka Gakkai Kaiho [Japan Ortorhinolaryngology Society Journal]*. 2001;104(11):1065-1070.
260. Vovk IF, Testemitsanu PA. [On the problem of tonsillectomy in arthropathic psoriasis.]. *Zdravookhranenie.* 1963;6:57-58.
261. Stukalenko AA. [Recovery from psoriasis after tonsillectomy]. *Vestn Otorinolaringol.* 1967;29(2):101-102.
262. Saita B, Ishii Y, Ogata K, Kikuchi I, Inoue S, Naritomi K. Two sisters with guttate psoriasis responsive to tonsillectomy: case reports with HLA studies. *J Dermatol.* 1979;6(3):185-189.
263. Teranishi Y, Mizutani H, Murata M, Shimizu M, Matsushima K. Increased spontaneous production of IL-8 in peripheral blood monocytes from the psoriatic patient: relation to focal infection and response to treatments. *J Dermatol Sci.* 1995;10(1):8-15.
264. McMillin BD, Maddern BR, Graham WR. A role for tonsillectomy in the treatment of psoriasis? *Ear Nose Throat J.* 1999;78(3):155-158.
265. Simoes JF, Ribeiro J, Ferreira BR, Paiva S. The role of tonsillectomy in psoriasis treatment. *BMJ Case Rep.* 2015;2015.
266. Zachariae R, Zachariae H, Blomqvist K, et al. Quality of life in 6497 Nordic patients with psoriasis. *Br J Dermatol.* 2002;146(6):1006-1016.
267. Sigurdardottir SL, Thorleifsdottir RH, Guzman AM, Gudmundsson GH, Valdimarsson H, Johnston A. The anti-microbial peptide LL-37 modulates immune responses in the palatine tonsils where it is exclusively expressed by neutrophils and a subset of dendritic cells. *Clin Immunol.* 2012;142(2):139-149.
268. Johnston A, Sigurdardottir SL, Ryon JJ. Isolation of mononuclear cells from tonsillar tissue. *Curr Protoc Immunol.* 2009;Chapter 7:Unit 7 8.
269. Clark RA, Chong B, Mirchandani N, et al. The vast majority of CLA+ T cells are resident in normal skin. *J Immunol.* 2006;176(7):4431-4439.
270. Pearson WR, Lipman DJ. Improved tools for biological sequence comparison. *Proc Natl Acad Sci U S A.* 1988;85(8):2444-2448.
271. Van den Eynde B, Peeters O, De Backer O, Gaugler B, Lucas S, Boon T. A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. *J Exp Med.* 1995;182(3):689-698.
272. Steven NM, Annels NE, Kumar A, Leese AM, Kurilla MG, Rickinson AB. Immediate early and early lytic cycle proteins are frequent targets of the Epstein-Barr virus-induced cytotoxic T cell response. *J Exp Med.* 1997;185(9):1605-1617.
273. Karczewski J, Poniedzialek B, Rzymski P, Adamski Z. Factors affecting response to biologic treatment in psoriasis. *Dermatol Ther.* 2014;27(6):323-330.
274. Mrowietz U, Kragballe K, Reich K, et al. Definition of treatment goals for moderate to severe psoriasis: a European consensus. *Arch Dermatol Res.* 2011;303(1):1-10.
275. Barker J, Hoffmann M, Wozel G, et al. Efficacy and safety of infliximab vs. methotrexate in patients with moderate-to-severe plaque psoriasis: results of an open-label, active-controlled, randomized trial (RESTORE1). *Br J Dermatol.* 2011;165(5):1109-1117.
276. Saurat JH, Stingl G, Dubertret L, et al. Efficacy and safety results from the randomized controlled comparative study of adalimumab vs. methotrexate vs. placebo in patients with psoriasis (CHAMPION). *Br J Dermatol.* 2008;158(3):558-566.

277. Thaci D, Blauvelt A, Reich K, et al. Secukinumab is superior to ustekinumab in clearing skin of subjects with moderate to severe plaque psoriasis: CLEAR, a randomized controlled trial. *J Am Acad Dermatol.* 2015;73(3):400-409.
278. Jin Y, Yang S, Zhang F, et al. Combined effects of HLA-Cw6 and cigarette smoking in psoriasis vulgaris: a hospital-based case-control study in China. *J Eur Acad Dermatol Venereol.* 2009;23(2):132-137.
279. Setty A, Curhan G, Choi H. Smoking and the risk of psoriasis in women: Nurses' Health Study II. *Am J Med.* 2007;120(11):953-959.
280. Armstrong AW, Armstrong EJ, Fuller EN, Sockolov ME, Voyles SV. Smoking and pathogenesis of psoriasis: a review of oxidative, inflammatory and genetic mechanisms. *Br J Dermatol.* 2011;165(6):1162-1168.
281. Griffiths CE, Barnes MR, Burden AD, et al. Establishing an Academic-Industrial Stratified Medicine Consortium: Psoriasis Stratification to Optimize Relevant Therapy. *J Invest Dermatol.* 2015;135(12):2903-2907.
282. Lowes MA, Russell CB, Martin DA, Towne JE, Krueger JG. The IL-23/T17 pathogenic axis in psoriasis is amplified by keratinocyte responses. *Trends Immunol.* 2013;34(4):174-181.
283. Sofen H, Smith S, Matheson RT, et al. Guselkumab (an IL-23-specific mAb) demonstrates clinical and molecular response in patients with moderate-to-severe psoriasis. *J Allergy Clin Immunol.* 2014;133(4):1032-1040.
284. Langley RG, Elewski BE, Lebwohl M, et al. Secukinumab in plaque psoriasis--results of two phase 3 trials. *N Engl J Med.* 2014;371(4):326-338.
285. Gordon KB, Blauvelt A, Papp KA, et al. Phase 3 Trials of Ixekizumab in Moderate-to-Severe Plaque Psoriasis. *N Engl J Med.* 2016;375(4):345-356.
286. Kryczek I, Bruce AT, Gudjonsson JE, et al. Induction of IL-17+ T cell trafficking and development by IFN-gamma: mechanism and pathological relevance in psoriasis. *J Immunol.* 2008;181(7):4733-4741.
287. Di Meglio P, Villanova F, Navarini AA, et al. Targeting CD8(+) T cells prevents psoriasis development. *J Allergy Clin Immunol.* 2016;138(1):274-276 e276.
288. Hijnen D, Knol EF, Gent YY, et al. CD8(+) T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-gamma, IL-13, IL-17, and IL-22. *J Invest Dermatol.* 2013;133(4):973-979.
289. Bovenschen HJ, Seyger MM, Van de Kerkhof PC. Plaque psoriasis vs. atopic dermatitis and lichen planus: a comparison for lesional T-cell subsets, epidermal proliferation and differentiation. *Br J Dermatol.* 2005;153(1):72-78.
290. Vissers WH, Arndtz CH, Muys L, Van Erp PE, de Jong EM, van de Kerkhof PC. Memory effector (CD45RO+) and cytotoxic (CD8+) T cells appear early in the margin zone of spreading psoriatic lesions in contrast to cells expressing natural killer receptors, which appear late. *Br J Dermatol.* 2004;150(5):852-859.
291. Ellis CN, Krueger GG. Treatment of chronic plaque psoriasis by selective targeting of memory effector T lymphocytes. *N Engl J Med.* 2001;345(4):248-255.
292. Vollmer S, Menssen A, Prinz JC. Dominant lesional T cell receptor rearrangements persist in relapsing psoriasis but are absent from nonlesional skin: evidence for a stable antigen-specific pathogenic T cell response in psoriasis vulgaris. *J Invest Dermatol.* 2001;117(5):1296-1301.
293. Lande R, Botti E, Jandus C, et al. The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis. *Nat Commun.* 2014;5:5621.
294. Valdimarsson H, Sigmundsdottir H, Jonsdottir I. Is psoriasis induced by streptococcal superantigens and maintained by M-protein-specific T cells that cross-react with keratin? *Clin Exp Immunol.* 1997;107 Suppl 1:21-24.

295. Pérez-Lorenzo R, Núñez-Oreza L, Garma-Quen P, López-Pacheco E, Bricaire-Bricaire G. Peripheral blood mononuclear cells proliferation and Th1/Th2 cytokine production in response to streptococcal M protein in psoriatic patients. *Int J Dermatol.* 2006;45(5):547-553.
296. Fan X, Yang S, Sun L, et al. Comparison of clinical features of HLA-Cw*0602-positive and -negative psoriasis patients in a Han Chinese population. *Acta Derm Venereol.* 2007;87(4):335-340.
297. Julia A, Tortosa R, Hernanz JM, et al. Risk variants for psoriasis vulgaris in a large case-control collection and association with clinical subphenotypes. *Hum Mol Genet.* 2012;21(20):4549-4557.
298. Mabuchi T, Ota T, Manabe Y, et al. HLA-C*12:02 is a susceptibility factor in late-onset type of psoriasis in Japanese. *J Dermatol.* 2014;41(8):697-704.
299. Suomela S, Kainu K, Onkamo P, et al. Clinical associations of the risk alleles of HLA-Cw6 and CCHCR1*WWCC in psoriasis. *Acta Derm Venereol.* 2007;87(2):127-134.
300. Kvestad E, Kvaerner KJ, Røysamb E, Tambs K, Harris JR, Magnus P. Heritability of recurrent tonsillitis. *Arch Otolaryngol Head Neck Surg.* 2005;131(5):383-387.
301. Roberts AL, Connolly KL, Kirse DJ, et al. Detection of group A Streptococcus in tonsils from pediatric patients reveals high rate of asymptomatic streptococcal carriage. *BMC Pediatr.* 2012;12:3.
302. Neeman R, Keller N, Barzilai A, Korenman Z, Sela S. Prevalence of internalisation-associated gene, prtF1, among persisting group-A streptococcus strains isolated from asymptomatic carriers. *Lancet.* 1998;352(9145):1974-1977.
303. Passali D, Lauriello M, Passali GC, Passali FM, Bellussi L. Group A streptococcus and its antibiotic resistance. *Acta Otorhinolaryngol Ital.* 2007;27(1):27-32.
304. Fahlen A, Engstrand L, Baker BS, Powles A, Fry L. Comparison of bacterial microbiota in skin biopsies from normal and psoriatic skin. *Arch Dermatol Res.* 2012;304(1):15-22.
305. Sigmundsdottir H, Gudjonsson JE, Jonsdottir I, Ludviksson BR, Valdimarsson H. The frequency of CLA+ CD8+ T cells in the blood of psoriasis patients correlates closely with the severity of their disease. *Clin Exp Immunol.* 2001;126(2):365-369.
306. Weisenseel P, Laumbacher B, Besgen P, et al. Streptococcal infection distinguishes different types of psoriasis. *J Med Genet.* 2002;39(10):767-768.
307. Swindell WR, Sarkar MK, Stuart PE, et al. Psoriasis drug development and GWAS interpretation through in silico analysis of transcription factor binding sites. *Clin Transl Med.* 2015;4:13.

Original publications

Paper I

Improvement of Psoriasis after Tonsillectomy Is Associated with a Decrease in the Frequency of Circulating T Cells That Recognize Streptococcal Determinants and Homologous Skin Determinants

Ragna Hlin Thorleifsdottir,[*,†,‡] Sigrun Laufey Sigurdardottir,[*,‡] Bardur Sigurgeirsson,[†] Jon Hjaltalin Olafsson,[†,‡] Martin Ingi Sigurdsson,[‡,§] Hannes Petersen,[‡,¶] Sigurlaug Arnadottir,[*,‡] Johann Eli Gudjonsson,[‖] Andrew Johnston,[‖] and Helgi Valdimarsson[*,‡]

Exacerbation of chronic psoriasis can be associated with streptococcal throat infections, and T cells that respond to peptide sequences common to streptococcal M proteins and skin keratins have been detected in patients' blood. To our knowledge, we have conducted the first blinded, prospective study to assess the impact of tonsillectomy on psoriasis. Twenty-nine patients with chronic psoriasis and history of exacerbation after sore throat were randomly assigned to tonsillectomy (n = 15) or control (n = 14) groups and monitored for 2 y clinically and by enumeration of circulating skin homing T cells that respond to short homologous M protein or keratin peptides. Thirteen patients (86%) showed sustained improvement after tonsillectomy ranging from 30 to 90% reduction in disease severity. Furthermore, there was a close correlation between the degree of clinical improvement in individual patients and reduction in the frequency of peptide-reactive skin-homing T cells in their circulation. No corresponding clinical or immunologic changes were observed among the controls. These findings indicate that tonsillectomy may have a beneficial effect on chronic psoriasis because the palatine tonsils generate effector T cells that recognize keratin determinants in the skin. ***The Journal of Immunology*****, 2012, 188: 000–000.**

Psoriasis is a common inflammatory skin disease that can be associated with arthritis and tends to have a fluctuating course. Several distinct but overlapping clinical variants have been identified, but chronic plaque-type lesions are most common. Psoriatic plaques are characterized by a marked keratinocyte hyperproliferation, altered differentiation, and keratin expression, and they are associated with dermal and epidermal infiltration of leukocytes. It is now generally accepted that these changes are triggered and maintained by oligoclonal T lymphocytes, indicating that the psoriatic process is driven by conventional Ags (1). The pathological epidermal hyperplasia coincides with epidermal influx of $\alpha_1\beta_1$ integrin-positive $CD8^+$ T cells and can be prevented by specific blocking of this integrin (2). Recent studies indicate that, in addition to Th1 cells, Th17 cells also have an important role in psoriasis (3), as well as IL-17–producing $CD8^+$ T cells (4), and that the keratinocyte hyperproliferation might be driven by the Th17 cytokine IL-22, either directly or indirectly (5, 6). IFN-γ is also a powerful inducer of the chemokine CCL20, a ligand for CCR6, which is expressed by T cells that can produce IL-17. In this way IFN-γ may play a major role in enhancing the IL-17 response (4). Psoriasis has a strong genetic component, with a 40–70% concordance in identical twins (7, 8). Several susceptibility alleles have now been identified and confirmed (9), and HLA-Cw*0602 has recently been implicated as the strongest susceptibility allele in psoriasis (10). On the basis of this and various other pathological features of psoriasis, we have argued that $CD8^+$ T cells are likely to be the ultimate effector cells that recognize autoepitopes presented in the context of HLA-Cw6 or other HLA class I molecules on the surface of APCs and keratinocytes (11). The pathogenic activity of these $CD8^+$ T cells is, however, likely to require a local interaction with $CD4^+$ T cells, involving cross-presenting dendritic cells (11).

A strong association between streptococcal throat infection and the acute guttate variant of psoriasis (an early onset form) has been demonstrated in many studies. Furthermore, it has been demonstrated in a prospective study that chronic plaque psoriasis may also exacerbate after such infections and, furthermore, the psoriasis patients had an ~10-fold higher frequency of streptococcal throat infections than age-matched household controls (12). Moreover, worsening was exclusively associated with throat infection by the three groups of β-hemolytic streptococci (A, C, and G) that express M protein on their surface (12), a major virulence factor composed of two polypeptide chains with α helical coiled-coil configuration. Interestingly, mAbs raised against group A streptococci cross-reacted with keratin defined as the α helical coiled-

*Department of Immunology, Landspitali University Hospital, 101 Reykjavik, Iceland; [†]Department of Dermatology, Landspitali University Hospital, 101 Reykjavik, Iceland; [‡]Faculty of Medicine, University of Iceland, 101 Reykjavik, Iceland; [§]Department of Medicine, Landspitali University Hospital, 101 Reykjavik, Iceland; [¶]Department of Ear, Nose, and Throat, Landspitali University Hospital, 101 Reykjavik, Iceland; and [‖]Department of Dermatology, University of Michigan, Ann Arbor, MI 48109

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Address correspondence and reprint requests to Prof. Helgi Valdimarsson, Department of Immunology, Landspitali University Hospital, Hringbraut, 101 Reykjavik, Iceland. E-mail address: helgiv@landspitali.is

The online version of this article contains supplemental material.

Abbreviations used in this article: CLA, cutaneous lymphocyte-associated Ag; PASI, Psoriasis Area and Severity Index; TX, tonsillectomy.

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coil autoantigen in human skin (13, 14). Although psoriasis is mediated by T cells and not by Abs, these findings potentially linked keratin to psoriasis.

An extensive homology between streptococcal M protein and keratin was first reported ~20 y ago (15). Of ~4200 mammalian proteins that were compared, human type 1 keratins that are upregulated in psoriasis (16) showed the strongest homology with the streptococcal M protein. On the basis of this and other findings, it was proposed in 1995 that psoriasis can be initiated by streptococcal superantigens and maintained by T cells that recognize streptococcal M protein determinants in the palatine tonsils and homologous keratin determinants in the skin (17). Subsequently, a markedly increased frequency of T cells that recognize such determinants was detected in the blood of patients with chronic psoriasis compared with allergic dermatitis patients and HLA-Cw*0602–positive healthy controls (18, 19). Notably, the great majority (>90%) of the circulating T cells that responded to the homologous K and M peptides expressed the skin-homing entity cutaneous lymphocyte-associated Ag (CLA) (19).

There are several reports of partial or complete remission of psoriasis following tonsillectomy (20, 21), including three patients who were shown to have identical T cell clones in their palatine tonsils and skin lesions (20). However, to our knowledge, no controlled prospective trial has been reported. As most psoriasis patients have a fluctuating disease activity and spontaneous remissions are not uncommon, matched patient controls are essential to determine whether tonsillectomy has any beneficial effect.

To our knowledge, we have conducted the first blinded, prospective study to assess the clinical and immunologic impact of tonsillectomy on chronic psoriasis. We argued that if T cells, primed against streptococcal M protein determinants in the palatine tonsils, play a pathogenic role in psoriasis, then the numbers of these cells should decrease in the circulation after tonsillectomy and this should be associated with reduced disease activity.

Materials and Methods

Study population and clinical follow-up

Twenty-nine patients with chronic plaque psoriasis were recruited and randomly allocated into tonsillectomy (TX) and control groups. The study was approved by the Icelandic National Bioethics Committee (VSNb2006090015/03-15). Written informed consent was obtained from each patient. Patients were eligible for the study if they were ≥18 y age, had been diagnosed by a dermatologist with chronic plaque psoriasis, and had a history of psoriasis exacerbation during or shortly after throat infections. Patients with heart and lung diseases were excluded. The patients were off treatment, including antibiotics, for at least 4 wk before they entered the study and for 2 mo thereafter. Subsequently, the participants were allowed to have treatment according to what they and their dermatologists thought indicated. Their disease course was followed for at least 2 y and their disease severity assessed by the Psoriasis Area and Severity Index (PASI) (22), which is the standard method for evaluating changes in the extent and activity of this disease. The clinical evaluation was observer-blinded with regard to tonsillectomy. The participants were evaluated clinically at study entry and after 2, 6, 12, 18, and 24 mo and blood samples were obtained at study entry and after 2, 12, and 24 mo. The patients' need for anti-psoriasis treatment during the follow-up was also monitored. The patients were all examined for tonsillar remnants at the end of the study.

Homologous peptide Ags

The amino acid sequence of keratin 17 was split into a complete set of overlapping residue peptides that were then used as a library to compare with the sequence of the M6 protein using the FASTA algorithm (23). The homologous M peptides were restricted to the conserved C-terminal half of the protein whereas the homologous keratin peptides were present in two coil-forming regions of keratin 17. Each homologous M peptide shared four to six amino acids with the corresponding K peptide, and they were further selected on the basis of predicted binding to HLA-Cw*0602 as previously described (19). Thus, 64 short, mostly 9- to 12-aa overlapping peptides derived from human cytokeratin 17 or streptococcal M6 protein (homologous K/M peptides) were selected on the basis of sequence homology and predicted binding to HLA-Cw*0602 as previously described (19). The peptides were blended into 16 peptide pools, each containing 8 peptides. Supplemental Table I shows the size and location of the overlapping homologous K/M peptides, and supplemental Table II shows their sequences and lists some relevant references.

Enumeration of peptide-reactive T cells

The frequency of T cells that respond to amino acid sequences common to keratin and M protein was determined as previously described (19). Briefly, PBMCs or tonsillar mononuclear cells were isolated from heparinized venous blood of psoriatic individuals or tonsillar tissue (24) by Ficoll (Sigma-Aldrich, St. Louis, MO) density gradient sedimentation. Single-cell suspensions of tonsillar mononuclear cells were prepared as described (24). The PBMCs or tonsillar mononuclear cells were cultured at a density of 1×10^6 cells/ml in complete RPMI 1640 in cell culture tubes (Nunc, Thermo Fisher Scientific, Roskilde, Denmark) and stimulated for 16 h with the 16 peptide pools (see Supplemental Table I) at a final concentration of 2 μg/ml, in the presence of the costimulatory Abs to CD28 and CD49d (1 μg/ml each; Serotec Scandinavia, Oslo, Norway). After the first 2 h the secretion inhibitor brefeldin A was added (10 μg/ml; Sigma-Aldrich) and the cultures incubated for a further 14 h at a 5° slant at 37°C in a humidified 5% CO_2 atmosphere. Anti-CD3 (1 μg/ml; Serotec Scandinavia) and streptokinase (200 U/ml; Hoechst Marion Roussel, Stockholm, Sweden) were used as positive controls. The great majority (>90%) of the T cells responding to the homologous K and M peptides express the skin-homing entity CLA (19). After the incubation, the mononuclear cells were therefore stained with anti–CLA-FITC (BioLegend, San Diego, CA) and anti-CD4 or CD8-PerCP mAbs (BioLegend) on ice, in the dark for 20 min. After two washes in PBS, the cells were fixed in 500 μl cold (4°C) 2% paraformaldehyde for 10 min at room temperature and after a further wash they were treated with permeabilizing buffer (0.5% BSA, 0.1% saponin, 0.1% sodium azide; Sigma-Aldrich) for 10 min at room temperature, followed by a wash and resuspension in the same buffer. The cells were then stained with anti–IFN-γ-PE (BioLegend) and anti–IL-17A-AF647 for 20 min at 4°C in the dark, washed in 1.5 ml permeabilizing buffer, centrifuged for 5 min at 500 × *g*, and resuspended in 250 μl permeabilizing buffer supplemented with 1% paraformaldehyde. Lymphocytes were analyzed using a FACSCalibur flow cytometer (BD Biosciences) gating on light scatter and CD4, CD8, and CLA expression, capturing a minimum of 200,000 events guided by appropriate isotype control Abs. The source and characteristics of the various Ab conjugates used for the flow cytometric analyses are shown in Supplemental Table III.

*HLA-Cw*0602 typing*

Genotyping of blood mononuclear cells for HLA-Cw*0602 alleles was performed as previously described (10).

Serum IL-8 ELISA

Serum IL-8 was used as a serological inflammatory marker and measured at study entry and after 24 mo by Quantikine ELISA (R&D Systems, Minneapolis, MN) as directed by the manufacturer.

Table I. Baseline demographic information and disease characteristics of the patients

	TX Group (*n* = 15)	Control Group (*n* = 14)
Males/females	3/12	6/8
Age, y (±SD)	35.3 ± 9.9	35.9 ± 9.8
Body mass index ± SD	25.2 ± 5.3	25.4 ± 3.6
Duration of psoriasis, y (±SD)	19.9 ± 9.5	20.5 ± 11.7
Age at onset, y (range)	15 (4–35)	15 (2–28)
Family history (*n*)	12	12
Psoriatic arthritis (*n*)	4	1
Psoriatic nails (*n*)	10	9
HLA-Cw*060-positive	11	13
PASI score ± SD	11.0 ± 5.7	9.3 ± 3.7
Prior treatments (*n*)		
Topical	8	8
UVB	5	4
Systemic	1	0

Values are means unless specified otherwise.

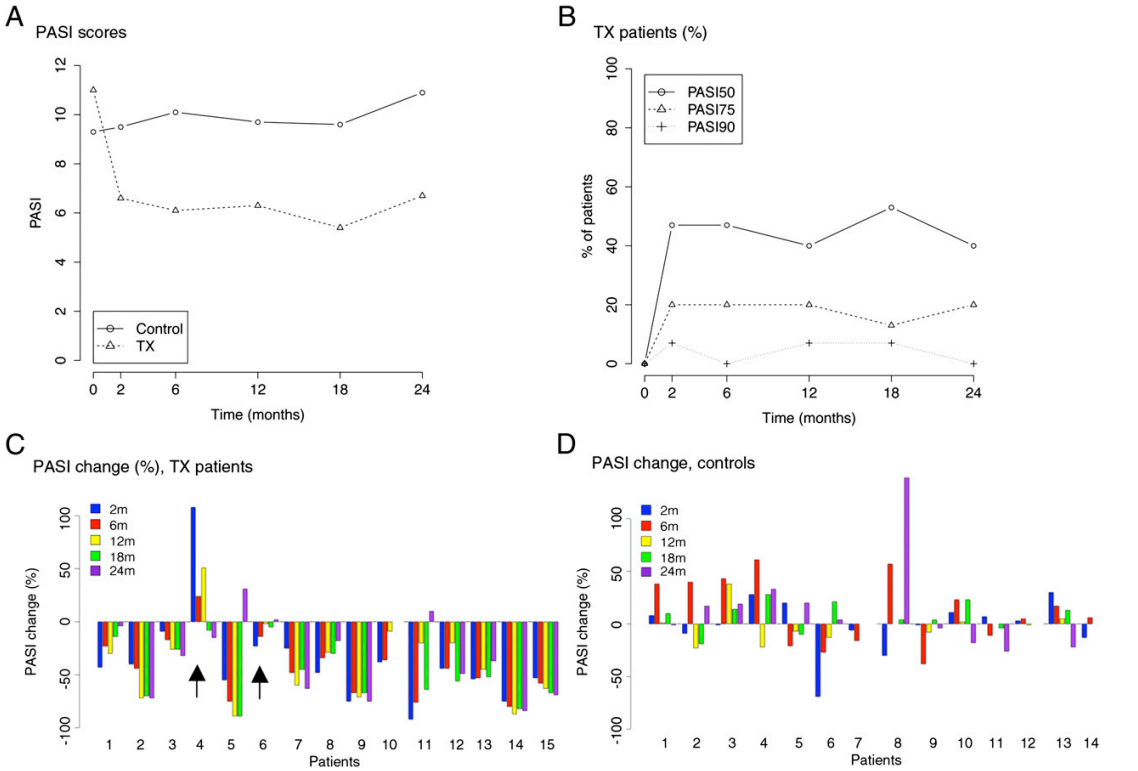

FIGURE 1. Clinical changes during the 2-y follow-up. (**A**) A significant decrease in the mean PASI score was observed in the tonsillectomized patients both with time and when compared with the controls ($p < 0.001$). (**B**) Percentage of tonsillectomized patients that reached 50% improvement (PASI50), 75% improvement (PASI75), and 90% improvement (PASI90) at each time point. (**C**) Percent changes in the PASI scores of individual participants throughout the 2-y of study, with the scores at study entry for each patient being set as 0. Thirteen of 15 participants in the TX group improved clinically (range, 30–90%) after tonsillectomy. Patients 4 and 6 (arrows) did not show significant changes in blood frequencies of peptide-reactive skin-homing T cells. No corresponding clinical improvement was observed in the controls (**D**).

Expression of the data and statistics

Flow cytometric responses were rated positive when >0.05% of the CLA^+ CD4 or $CD8^+$ T cell populations expressed IFN-γ or IL-17 brighter than the corresponding unstimulated culture or fluorescence minus one control.

The frequency of T cells that responded to each of the 16 peptide pools was determined as a percentage of CLA^+ cells in each of the T cell subpopulations and then expressed as an average for each patient after square root normalization.

Data were tested for normality using the Kolmogorov–Smimov test. PASI scores and peptide responses were compared between the groups and different time points using an ANOVA test for repeated measurements. For peptide response measurements, the square root of the peptide responses was used to better approximate normality. Correlation between peptides responses was performed using R, version 2.10 (The R Foundation, Vienna, Austria).

Results

Clinical findings

Twenty-nine patients with chronic plaque psoriasis were recruited and randomly allocated into TX and control patient groups. Demographic information about the participants at study entry and their disease characteristics are presented in Table I.

As depicted in Fig. 1A the mean PASI score decreased significantly in the TX group, both with time after tonsillectomy and compared with the controls ($p < 0.001$). Thus, 13 of 15 tonsillectomized patients showed an improvement ranging from 30 to 90% reduction of the PASI score (Fig. 1C), and up to 60% (9 of 15) reached 50% reduction in skin lesions at some stage during the study (Fig. 1B). The improvement was in most cases observed within 2 mo and was generally maintained throughout the 2-y follow-up (Fig. 1C). No consistent corresponding clinical changes were observed among the control patients (Fig. 1D). Furthermore, 12 (86%) of the controls used topical treatment at some time point during the study compared with only 4 (27%) in the TX group (Table II). However, three patients in each group had been given phototherapy and one patient in the TX group was started on methotrexate after 12 mo because of arthritis. There was no clear association between the degree of improvement and carriage of the HLA-Cw*0602 allele, but more patients need to be studied in this context.

The effect of tonsillectomy on serum concentration of IL-8

To objectively assess changes in inflammatory activity, serum levels of IL-8 were measured at study entry and after 24 mo, and, as shown in Fig. 2, a slight but significant decrease was observed in the tonsillectomized patients but not in the control patients despite their more frequent topical treatment (Table II).

Table II. Psoriasis treatments during the 2-y follow-up

Treatment 6–24 mo	TX Group (n = 15)	Control Group (n = 14)
Topical, n (%)[a]	4 (27)	12 (86)
Phototherapy, n (%)	3 (20)	3 (21)
Systemic, n (%)	1 (7)	

[a]Corticosteroid creams and vitamin D analog creams.

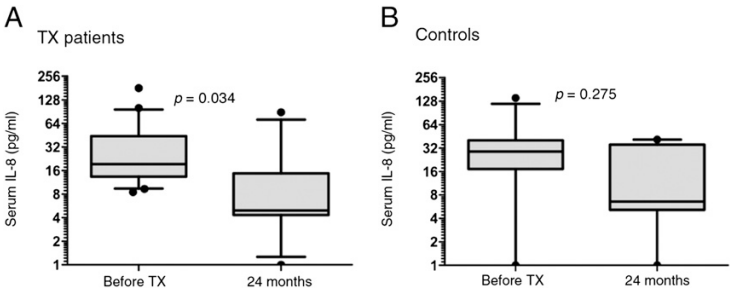

FIGURE 2. Serum concentrations of IL-8. Serum levels of IL-8 were measured at study entry and after 24 mo, and (**A**) a slight but significant decrease was observed in the tonsillectomized patients ($p = 0.034$) but not in the control patients (**B**) ($p = 0.275$) despite more topical treatment (Table II) (Wilcoxon matched pairs signed rank test).

The effect of tonsillectomy on the frequency of the circulating peptide-reactive T cells

The frequency of T cells responding to each of 16 pools of homologous M protein and keratin peptides (see Supplemental Table I) was determined at study entry and after 2, 12, and 24 mo by flow cytometry as a percentage of skin homing (CLA^+) cells in each T cell subpopulation. Fig. 3A shows the marked general decline usually observed in the frequency of circulating $CD8^+$ T cells responding to each of the 16 peptide pools in one representative patient before and 2 mo after tonsillectomy. Similar declines were observed in the tonsillectomized patients after 12 and 24 mo, whereas no consistent corresponding changes were detected in the control patients (Fig. 3B). The average responses of each patient to all 16 peptide pools were then calculated at each time point after square root normalization (Figs. 4, 5, Supplemental Fig. 1).

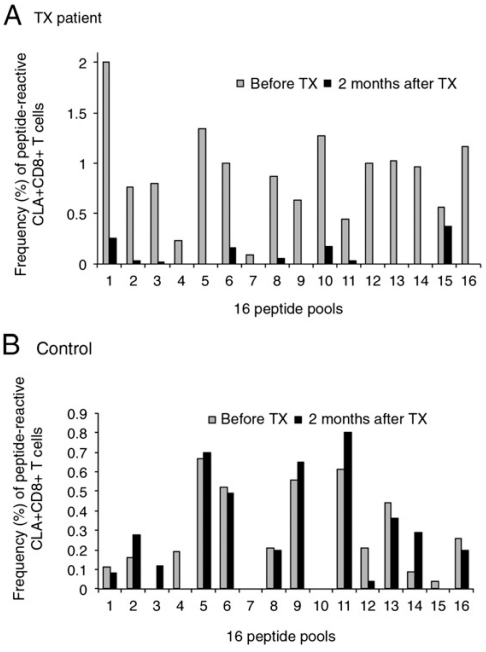

FIGURE 3. Tonsillectomy was associated with a striking reduction in the frequency (percentage) of circulating T cells that recognized the 16 homologous peptide pools. Frequency (percentage) of circulating CLA^+ $CD8^+$ T cells producing IFN-γ after stimulation with the homologous keratin and M protein peptide pools at study entry and after 2 mo: (**A**) representative tonsillectomized psoriasis patient; (**B**) representative control psoriasis patient. The responses of each patient to all 16 peptide pools were then calculated for each time point and expressed, after square root normalization, as an average for each patient (see Fig. 5, Supplemental Fig. 1).

As shown in Fig. 4, there was a very strong positive correlation between the frequency of peptide-reactive skin homing $CD8^+$ T cells in tonsils and blood at the time of the tonsillectomy. This applied to both IFN-γ– and IL-17–producing $CD8^+$ T cells ($r = 0.788$, $p < 0.001$ and $r = 0.644$, $p = 0.015$, respectively). A significant correlation was also observed between the frequency of IFN-γ–producing skin-homing $CD4^+$ T cells in tonsils and blood ($r = 0.679$, $p = 0.001$), but a corresponding correlation was not observed for IL-17–producing skin-homing $CD4^+$ T cells ($r = 0.315$, $p = 0.273$) (data not shown). After tonsillectomy, the frequency of circulating peptide-reactive IFN-γ–producing skin homing $CD8^+$ T cells decreased significantly compared with the controls ($p = 0.003$) (Fig. 5A). Furthermore, there was a highly significant correlation between the extent of clinical improvement (decreases in PASI scores) of individual patients and the degree of decline in the frequency of peptide-reactive skin homing IFN-γ^+ $CD8^+$ T cells in their blood ($r = 0.594$, $p < 0.001$). As depicted in Supplemental Fig. 1, similar associations were observed for peptide-reactive skin-homing IL-17–producing $CD8^+$ T cells in the tonsillectomized patients ($r = 0.560$, $p < 0.001$). A weaker association was found for skin-homing IL-17^+CD4^+ T cells ($p = 0.003$), but the association was not significant for IFN-γ^+ $CD4^+$ T cells ($p = 0.137$). No associations between changes in PASI scores and frequency of circulating peptide-reactive $CD8^+$ or $CD4^+$ T cells were found in the control patients (Fig. 5C, Supplemental Fig. 1C). No decreases were observed after tonsillectomy in the frequencies of circulating T cells that responded to anti-CD3 Ab stimulation or to the control Ag streptokinase (data not shown).

Discussion

It has previously been reported that psoriasis patients have in their circulation T cells that recognize determinants that streptococcal M protein share with some human keratins (18, 19) and the great majority of these T cells are CLA^+ (19). We now report that patients with chronic psoriasis and a history of disease exacerbation in association with sore throat generally improve after tonsillectomy, and concurrently the numbers of circulating T cells that recognize these shared determinants show a marked decline. These findings indicate that effector T cells originating from the palatine tonsils may be involved in the pathogenesis of psoriasis. First, there is a very close correlation between the frequency of such T cells in the tonsils and peripheral blood (Fig. 4), suggesting that the tonsillar T cells are recirculating. Second, the extent of the decline in the numbers of these T cells in the circulation correlates fairly closely with the degree of clinical improvement of individual patients (Fig. 5B).

Note that this study was designed to detect maximal numbers of T cells that are specific for determinants that streptococcal M proteins share with human type 1 keratins. Thus, the study does not distinguish between T cells that recognize primary, dominant

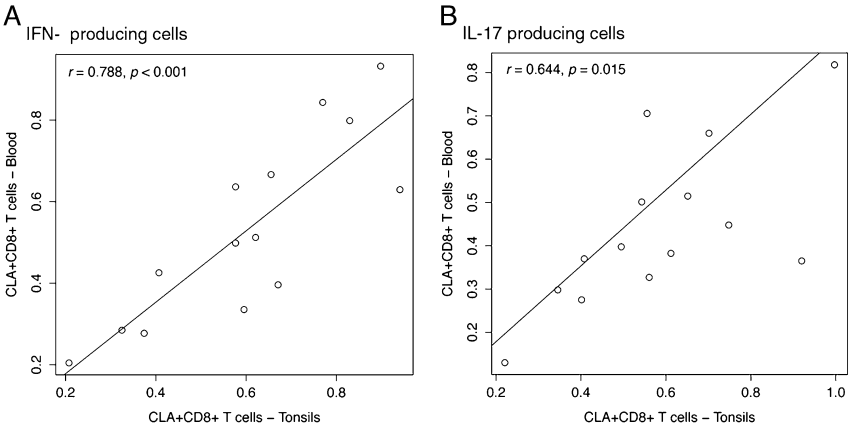

FIGURE 4. There was a significant correlation between the frequency of peptide-reactive skin-homing $CD8^+$ T cells in the blood and tonsils at the time of the tonsillectomy. (**A**) IFN-γ–producing peptide-specific $CD8^+$ T cells ($r = 0.788$, $p < 0.001$). (**B**) IL-17–producing peptide-specific $CD8^+$ T cells ($r = 0.644$, $p = 0.015$). Data expressed as a square root normalized average of peptide-reactive skin-homing T cells (Spearman rank correlation test).

autoepitopes and determinants that reflect epitope spreading or T cells that may respond exclusively to either keratin or M protein determinants.

Furthermore, our observations do not exclude the possibility that other Ags may be involved in the pathogenesis of psoriasis, including other streptococcal (25) or peptidoglycan components (26). Note in this context that we selected M protein and keratin peptides that were predicted to bind relatively strongly with HLA-Cw*0602, which is carried by <50% of patients with chronic psoriasis (27), although this may vary between populations (28, 29). Second, we selected for this study patients who reported aggravation of their disease in association with sore throat, which only applies to ~40% of patients with chronic psoriasis in Iceland (R.H. Thorleifsdottir, J.H. Eysteinsdottir, J.H. Olafsson, B. Sigurgeirsson, M.I. Sigurdsson, and H. Valdimarsson, manuscript in preparation). It remains to be investigated whether patients who have not noticed worsening in association with sore throat also improve after tonsillectomy. Furthermore, two of the patients in our study did not improve after tonsillectomy (see arrows in Fig. 1C), although no tonsillar remnants could be detected in these patients after the operation. Thus, indications for tonsillectomy of patients with chronic psoriasis remain to be pre-

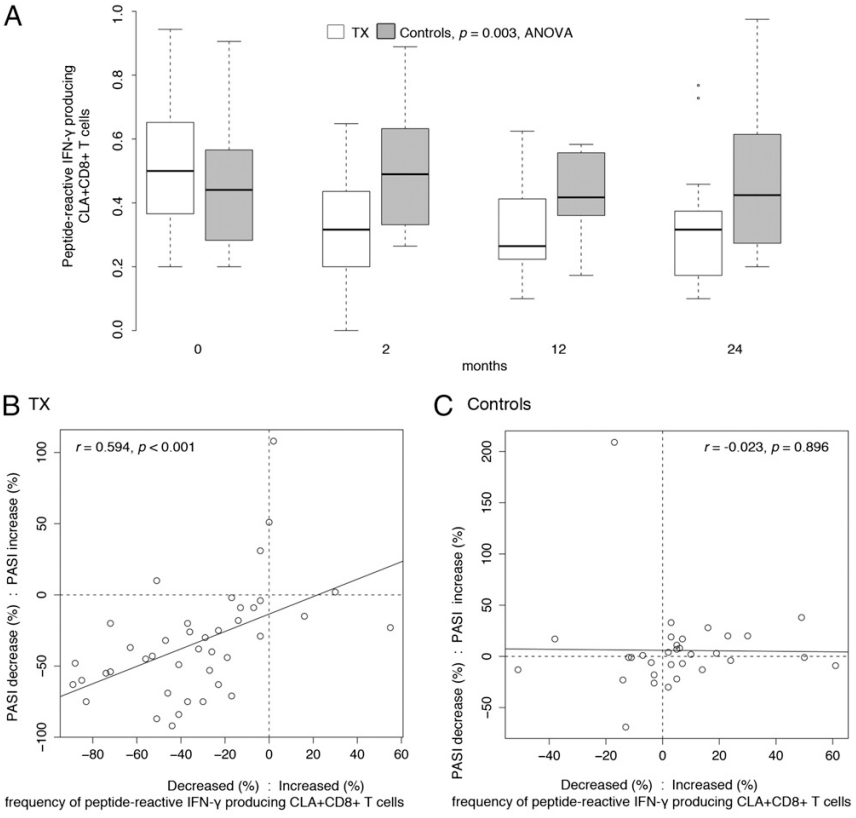

FIGURE 5. Changes in the blood frequencies of IFN-γ–producing peptide-specific $CD8^+$ T cells in the tonsillectomized and control patients. (**A**) Box plot shows a significant decrease in the average frequency of peptide-reactive IFN-γ–producing skin-homing (CLA^+) $CD8^+$ T cells in the tonsillectomized compared with the controls ($p = 0.003$, ANOVA). (**B**) Close correlation throughout the 2-y study period (three data points per patient) between clinical improvement (percentage reduction of PASI) and percentage reduction in the blood frequency of skin-homing IFN-γ producing peptide-reactive $CD8^+$ T cells ($r = 0.594$, $p < 0.001$). No such correlation was observed in the controls ($r = -0.023$, $p = 0.896$). The T cell frequency data are expressed as a square root normalized average of peptide-reactive skin-homing T cells. PASI and T cell frequency at study entry were set as 0 for each participant. In (B) and (**C**), the vertical axis shows percentage changes in the PASI scores and the horizontal axis percentage changes in the frequency of peptide-reactive CLA^+CD8^+ T cells during the study period (Spearman rank correlation test).

cisely defined. It is therefore not possible at this stage to estimate how large a proportion of patients with chronic psoriasis might benefit from tonsillectomy, and information is also lacking about how long such improvement may last beyond the 2-y follow-up in the present study. Note that the tonsillectomized patients not only benefited in terms of reduction of skin lesions but also required less symptomatic treatment than the control patients, and longer term follow-up studies should therefore also focus on that issue.

Recent studies have indicated that $CD8^+$ T cells may play a more direct role than $CD4^+$ T cells in the pathogenesis of psoriasis (2, 4, 11). Thus, the great majority of T cells in lesional epidermis are $CD8^+$ and, furthermore, psoriasis lesions do not develop when $CD8^+$ T cells are prevented to migrate from dermis into epidermis (2). Our data support this, as there is a stronger correlation between clinical improvement and reduction in the frequency of cross-reactive $CD8^+$ than $CD4^+$ T cells.

Our findings may help to identify some of the autoepitopes that are recognized by T cells in psoriatic lesions. It has been reported by many groups that these lesions are infiltrated by oligoclonal T cells (1), and although most of these clones are transient (1) and probably reflect autoepitope spreading (30), others are dominant and persist or even reappear in lesional skin after treatment-induced remission (31, 32).

Only symptomatic treatments are currently available for psoriasis, and symptoms usually relapse when treatment is discontinued. It is still not clear if and to what extent Ag-specific immunotherapeutic measures, which are curative in some animal models of autoimmunity, are directly applicable to human autoimmune diseases. This is probably partly because epitope spreading makes it difficult to identify primary and dominant autoepitopes in humans (30, 33). However, identification of circulating T cells that respond to homologous M protein and keratin determinants in patients with treatment-induced remission (32) may help to identify primary autoepitopes that might be targeted for highly specific immunotherapy for psoriasis.

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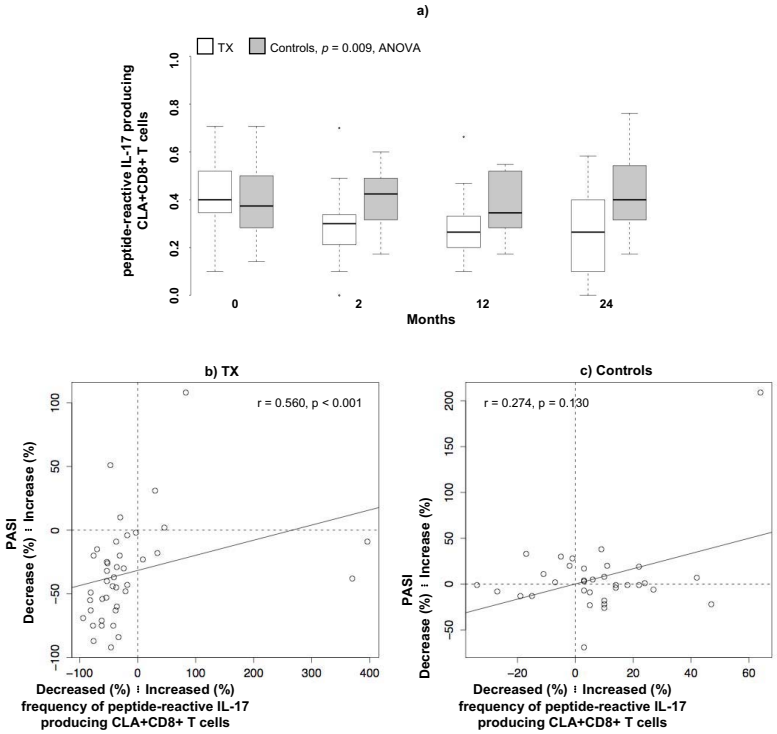
Disclosures

The authors have no financial conflicts of interest.

References

1. Valdimarsson, H., R. H. Thorleifsdottir, S. L. Sigurdardottir, J. E. Gudjonsson, and A. Johnston. 2009. Psoriasis: as an autoimmune disease caused by molecular mimicry. *Trends Immunol.* 30: 494–501.
2. Conrad, C., O. Boyman, G. Tonel, A. Tun-Kyi, U. Laggner, A. de Fougerolles, V. Kotelianski, H. Gardner, and F. O. Nestle. 2007. $\alpha_1\beta_1$ integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. *Nat. Med.* 13: 836–842.
3. Lowes, M. A., T. Kikuchi, J. Fuentes-Duculan, I. Cardinale, L. C. Zaba, A. S. Haider, E. P. Bowman, and J. G. Krueger. 2008. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *J. Invest. Dermatol.* 128: 1207–1211.
4. Kryczek, I., A. T. Bruce, J. E. Gudjonsson, A. Johnston, A. Aphale, L. Vatan, W. Szeliga, Y. Wang, Y. Liu, T. H. Welling, et al. 2008. Induction of IL-17^+ T cell trafficking and development by IFN-γ: mechanism and pathological relevance in psoriasis. *J. Immunol.* 181: 4733–4741.
5. Ma, H. L., S. Liang, J. Li, L. Napierata, T. Brown, S. Benoit, M. Senices, D. Gill, K. Dunussi-Joannopoulos, M. Collins, et al. 2008. IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation. *J. Clin. Invest.* 118: 597–607.
6. Sa, S. M., P. A. Valdez, J. Wu, K. Jung, F. Zhong, L. Hall, I. Kasman, J. Winer, Z. Modrusan, D. M. Danilenko, and W. Ouyang. 2007. The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. *J. Immunol.* 178: 2229–2240.
7. Brandrup, F., N. Holm, N. Grunnet, K. Henningsen, and H. E. Hansen. 1982. Psoriasis in monozygotic twins: variations in expression in individuals with identical genetic constitution. *Acta Derm. Venereol.* 62: 229–236.
8. Duffy, D. L., L. S. Spelman, and N. G. Martin. 1993. Psoriasis in Australian twins. *J. Am. Acad. Dermatol.* 29: 428–434.
9. Elder, J. T., A. T. Bruce, J. E. Gudjonsson, A. Johnston, P. E. Stuart, T. Tejasvi, J. J. Voorhees, G. R. Abecasis, and R. P. Nair. 2010. Molecular dissection of psoriasis: integrating genetics and biology. *J. Invest. Dermatol.* 130: 1213–1226.
10. Nair, R. P., P. E. Stuart, I. Nistor, R. Hiremagalore, N. V. Chia, S. Jenisch, M. Weichenthal, G. R. Abecasis, H. W. Lim, E. Christophers, et al. 2006. Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. *Am. J. Hum. Genet.* 78: 827–851.
11. Gudjonsson, J. E., A. Johnston, H. Sigmundsdottir, and H. Valdimarsson. 2004. Immunopathogenic mechanisms in psoriasis. *Clin. Exp. Immunol.* 135: 1–8.
12. Gudjonsson, J. E., A. M. Thorarinsson, B. Sigurgeirsson, K. G. Kristinsson, and H. Valdimarsson. 2003. Streptococcal throat infections and exacerbation of chronic plaque psoriasis: a prospective study. *Br. J. Dermatol.* 149: 530–534.
13. Shikhman, A. R., and M. W. Cunningham. 1994. Immunological mimicry between *N*-acetyl-beta-D-glucosamine and cytokeratin peptides: evidence for a microbially driven anti-keratin antibody response. *J. Immunol.* 152: 4375–4387.
14. Swerlick, R. A., M. W. Cunningham, and N. K. Hall. 1986. Monoclonal antibodies cross-reactive with group A streptococci and normal and psoriatic human skin. *J. Invest. Dermatol.* 87: 367–371.
15. McFadden, J., H. Valdimarsson, and L. Fry. 1991. Cross-reactivity between streptococcal M surface antigen and human skin. *Br. J. Dermatol.* 125: 443–447.
16. Leigh, I. M., H. Navsaria, P. E. Purkis, I. A. McKay, P. E. Bowden, and P. N. Riddle. 1995. Keratins (K16 and K17) as markers of keratinocyte hyperproliferation in psoriasis in vivo and in vitro. *Br. J. Dermatol.* 133: 501–511.
17. Valdimarsson, H., B. S. Baker, I. Jónsdóttir, A. Powles, and L. Fry. 1995. Psoriasis: a T-cell-mediated autoimmune disease induced by streptococcal superantigens? *Immunol. Today* 16: 145–149.
18. Gudmundsdottir, A. S., H. Sigmundsdottir, B. Sigurgeirsson, M. F. Good, H. Valdimarsson, and I. Jonsdottir. 1999. Is an epitope on keratin 17 a major target for autoreactive T lymphocytes in psoriasis? *Clin. Exp. Immunol.* 117: 580–586.
19. Johnston, A., J. E. Gudjonsson, H. Sigmundsdottir, T. J. Love, and H. Valdimarsson. 2004. Peripheral blood T cell responses to keratin peptides that share sequences with streptococcal M proteins are largely restricted to skin-homing $CD8^+$ T cells. *Clin. Exp. Immunol.* 138: 83–93.
20. Diluvio, L., S. Vollmer, P. Besgen, J. W. Ellwart, S. Chimenti, and J. C. Prinz. 2006. Identical TCR β-chain rearrangements in streptococcal angina and skin lesions of patients with psoriasis vulgaris. *J. Immunol.* 176: 7104–7111.
21. Nyfors, A., P. A. Rasmussen, K. Lemholt, and B. Eriksen. 1975. Improvement of refractory psoriasis vulgaris after tonsillectomy. *Dermatologica* 151: 216–222.
22. Fredriksson, T., and U. Pettersson. 1978. Severe psoriasis: oral therapy with a new retinoid. *Dermatologica* 157: 238–244.
23. Pearson, W. R., and D. J. Lipman. 1988. Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci. USA* 85: 2444–2448.
24. Johnston, A., S. Sigurdardottir, and J. Ryon. 2009. Isolation of mononuclear cells from tonsillar tissue. *Curr. Protoc. Immunol.* Chapter 7: Unit 7.8.
25. Besgen, P., P. Trommler, S. Vollmer, and J. C. Prinz. 2010. Ezrin, maspin, peroxiredoxin 2, and heat shock protein 27: potential targets of a streptococcal-induced autoimmune response in psoriasis. *J. Immunol.* 184: 5392–5402.
26. Baker, B. S., A. Powles, and L. Fry. 2006. Peptidoglycan: a major aetiological factor for psoriasis? *Trends Immunol.* 27: 545–551.
27. Mallbris, L., K. Wolk, F. Sánchez, and M. Ståhle. 2009. HLA-Cw*0602 associates with a twofold higher prevalence of positive streptococcal throat swab at the onset of psoriasis: a case control study. *BMC Dermatol.* 9: 5.
28. Fry, L., A. V. Powles, S. Corcoran, S. Rogers, J. Ward, and D. J. Unsworth. 2006. HLA Cw*06 is not essential for streptococcal-induced psoriasis. *Br. J. Dermatol.* 154: 850–853.
29. Gudjonsson, J. E., A. Karason, E. H. Runarsdottir, A. A. Antonsdottir, V. B. Hauksson, H. H. Jónsson, J. Gulcher, K. Stefansson, and H. Valdimarsson. 2006. Distinct clinical differences between HLA-Cw*0602 positive and negative psoriasis patients: an analysis of 1019 HLA-C- and HLA-B-typed patients. *J. Invest. Dermatol.* 126: 740–745.
30. Vanderlugt, C. L., and S. D. Miller. 2002. Epitope spreading in immune-mediated diseases: implications for immunotherapy. *Nat. Rev. Immunol.* 2: 85–95.
31. Curran, S. A., O. M. FitzGerald, P. J. Costello, J. M. Selby, D. J. Kane, B. Bresnihan, and R. Winchester. 2004. Nucleotide sequencing of psoriatic arthritis tissue before and during methotrexate administration reveals a complex inflammatory T cell infiltrate with very few clones exhibiting features that suggest they drive the inflammatory process by recognizing autoantigens. *J. Immunol.* 172: 1935–1944.
32. Vollmer, S., A. Menssen, and J. C. Prinz. 2001. Dominant lesional T cell receptor rearrangements persist in relapsing psoriasis but are absent from nonlesional skin: evidence for a stable antigen-specific pathogenic T cell response in psoriasis vulgaris. *J. Invest. Dermatol.* 117: 1296–1301.
33. Steinman, L. 2004. Immune therapy for autoimmune diseases. *Science* 305: 212–216.



SUPPLEMENTARY FIGURE 1. Changes in the blood frequencies of IL-17 producing peptide-reactive CD8+ T cells the tonsillectomized and control patients. Panel a) shows by a box plot the average frequency of peptide-reactive IL-17 producing skin homing CD8+ T cells in the tonsillectomized and control patients (p = 0.009, ANOVA). Panel b) shows a close correlation between clinical improvement (percent reduction of PASI) and percent reduction in the blood frequency of skin homing IL-17 producing peptide-reactive CD8+ T cells (r = 0.594, p < 0.001). No such correlation was observed in the controls (panel c, r = 0.274, p = 0.130). Data expressed as a square root normalized average peptide-reactive skin homing T cells. PASI and T cell frequency at study entry was set as zero for each participant. Spearman's rank correlation test.

Supplemental Table I. *Composition of peptide pools*[1]

	1	2	3	4	5	6	7	8
9	31-K17-12	130-K17-9	206-K17-9	236K17-12	372-K17-9	261-M6-9	262-M6-12	355-M6-9
10	101-K17-12	134K17-12	212K17-12	238-K17-9	379-K17-12	282-M6-9	285-M6-12	368-M6-16
11	125-K17-9	135-K17-9	217-K17-9	239K17-12	379-K17-9	299-M6-12	146M49	382-M6-12
12	127-K17-9	139-K17-9	220-K17-9	309-K17-9	396-K17-9	299-M6-9	M146	384-M6-9
13	128-K17-9	162-K17-12	220-K17-12	334-K17-12	404-K17-9	306-M6-9	338M6-12	464-M6-12
14	128-K17-10	165-K17-12	145K10	338-K17-12	407-K17-9	319-M6-12	344-M6-12	M159
15	146K17	168-K17-9	145K17	351-K17-12	415-K17-12	324-M6-9	354-M6-12	GAGE
16	146K9	179-K17-9	231-K17-9	366-K17-12	255-M6-12	327-M6-9	345-M6-9	BMRF-1

[1] White boxes are human keratin peptides, light gray boxes are streptococcal peptides and dark grey boxes are controls.

Supplemental Table II. *Peptide sequences derived from keratin and M6 protein.*

Peptide name [1]	Sequence [2]	Peptide name	Sequence
31-K17-12	ISS**V**L**A**G**A**SCP**A**	464-M6-12	ALT**V**M**A**T**A**GVA**A**[3]
101-K17-12	GGGFAGGDGLLV[4]		
125-K17-9	R**L**ASY**LDKV**	306-M6-9	N**L**TAE**LDKV**
127-K17-9	ASYLDKVRA		
128-K17-10	SYLDKVRALE		
146K17	SYLDKVR**ALEEAN**AD**L**EVKI[5]	M146	AKKQVEK**ALEEAN**SK**L**AALE[5,6]
146K9	SYLDKVQ**ALEEAN**ND**L**ENKI[5]	146M49	AKKKVEAD**LAEAN**SK**L**QALE[5]
128-K17-9	SYLDKVRAL		
130-K17-9	LDKVRALEE	344-M6-12	K**ALEEAN**SK**L**AA
134-K17-12	R**ALEEAN**AD**L**EV	354-M6-12	A**ALE**KL**N**KE**LE**E
135-K17-9	**ALEEAN**AD**L**	345-M6-9	**ALEEAN**SK**L**
309-K17-9	KT**EE**L**NKE**V	355-M6-9	**ALE**KL**N**KE**L**
139-K17-9	A**N**AD**L**EVKI		
162-K17-12	YSPYFKTIEDLR[4]		
165-K17-12	YFKTIEDLRNKI[4]		
168-K17-9	TIEDLRNKI		
179-K17-9	ATIENAHAL		
206-K17-9	ART**GLR**QTV	324-M6-9	SRQ**GLRR**D**L**
217-K17-9	DVN**GLRR**V**L**	282-M6-9	SRK**GLRR**D**L**
372-K17-9	QIQGLIGSV		
212-K17-12	**Q**TVE**A**DVN**GLRR**	319-M6-12	**Q**ISD**A**SRQ**GLRR**
220-K17-12	**GLRR**V**LD**ELTL**A**	285-M6-12	**GLRR**D**LD**ASRE**A**
220-K17-9	**GLRR**V**LD**EL	327-M6-9	**GLRR**D**LD**AS
145K17	LRRVLDELTLARTDLEMQIE[5]		
145K10	LRRVLDELTLTKADLEMQIE[5]	382-M6-12	**E**AEAKA**LKE**Q**LA**
231-K17-9	ARTDLEMQI	261-M6-9	D**I**GA**LK**Q**EL**
236-K17-12	**E**MQIEG**LKE**E**LA**	384-M6-9	EAKA**LKE**Q**L**
238-K17-9	Q**I**EG**LKEEL**	262-M6-12	**I**GA**LK**Q**ELA**KKD
239-K17-12	**I**EG**LKE****ELA**YLR	368-M6-16	KLTEKEKAELQAKLEA
334-K17-12	LRRVLQGLEIIL[7]	M149	KLTEKEKAELQAKLEAEAKA[5,6]
338-K17-12	LQGLEIILQSQL[7]	255-M6-12	EQKSKQDIGALK[4]
351-K17-12	M**K**ASL**E**NS**LEE**T	338-M6-12	A**K**KQVEK**ALEEA**[6]
366-K17-12	YCMQLSQIQGLI[4,7]		
379-K17-12	S**VE**EQ**LA**QL**R**CE	299-M6-12	Q**VE**KD**LA**N**L**TAE
379-K17-9	S**VE**EQ**LA**Q**L**	299-M6-9	Q**VE**KD**LA**N**L**
396-K17-9	QEYQILLDV		
404-K17-9	VKTRLEQEI		
407-K17-9	RLEQEIATY	GAGE	YRPRPRRY[8]
415-K17-12	YRRLLEGEDAHL[4]	BMRF1	YRSGIIAVV[9]

[1] Peptide name indicates the start position in the protein sequence, protein name and sequence length.
[2] Sequences used in Johnston A. *et al*. Clin Exp Immunol 2004; 138:83-93, except where indicated.
[3] Examples of sequence homologies shared by K and M peptides in bold face.
[4] Sequence or partial sequence used in Dionne S.O. *et al*. Immunogenetics 2004; 56:391-8.
[5] Sequence used in Gudmundsdottir A.S. *et al*. Clin Exp Immunol 1999; 117:580-6.
[6] Sequence used in Sigmundsdottir H. *et al*. Scand J Immunol 1997; 45:688-97.
[7] Sequence used in Shen Z. *et al*. J Dermatol Sci 2005; 38:25-39.
[8] A peptide used as a negative control for Cw6 binding (van den Eynde B. *et al*. J. Exp. Med. 1995 182 689-698).
[9] A peptide from Epstein-Barr Virus Protein BMRF1 as a positive control (Steven N.M. *et al.*, J. Exp. Med. 1997 185 1605-1617).

Supplemental Table III. *Antibodies used for flow cytometric analysis.*

Target	Conjugate	Source	Clone	Isotype
CD4	PerCP-Cy5.5	BD Biosci	SK3	G1
CD8	PerCP-Cy5.5	BD Biosci	SK1	G1
IgG1 control	PerCP-Cy5.5	BD Biosci	X40	
CLA	FITC	BioLegend	HECA-452	Rat IgM
Rat IgM control	FITC	BioLegend		
IFN-γ	PE	BioLegend	4S.B3	G1
IL-17A	A647	eBioscience	eBio64CAP17	G1

Paper II

ActaDV Acta Dermato-Venereologica ActaDV Advances in dermatology and venereology

CLINICAL REPORT

Patient-reported Outcomes and Clinical Response in Patients with Moderate-to-severe Plaque Psoriasis Treated with Tonsillectomy: A Randomized Controlled Trial

Ragna Hlin THORLEIFSDOTTIR[1,2], Sigrun Laufey SIGURDARDOTTIR[3], Bardur SIGURGEIRSSON[1], Jon Hjaltalin OLAFSSON[1], Martin Ingi SIGURDSSON[4], Hannes PETERSEN[5], Johann Eli GUDJONSSON[6], Andrew JOHNSTON[6] and Helgi VALDIMARSSON[3]

[1]Faculty of Medicine, Section of Dermatology, University of Iceland, Reykjavik, Iceland, [2]Department of Medical Sciences, Dermatology, Uppsala University, Uppsala, Sweden, [3]Department of Immunology, [4]Department of Anesthesiology and Critical Care, [5]Department of Otolaryngology-Head and Neck Surgery, Landspitali – The National University Hospital of Iceland, Reykjavik, Iceland, and [6]Department of Dermatology, University of Michigan Medical School, Ann Arbor, MI, USA

Psoriasis is a chronic inflammatory skin disease with profound effects on patients' health-related quality of life (HRQoL). Twenty-nine patients with plaque psoriasis and a history of streptococcal-associated psoriasis exacerbations were randomly assigned to tonsillectomy ($n=15$) or control ($n=14$) groups and followed for 24 months. Patients were evaluated with the Psoriasis Disability Index, Psoriasis Life Stress Inventory and Psoriasis Area and Severity Index. HRQoL and psoriasis-related stress improved significantly in the tonsillectomy group compared with the control group ($p=0.037$ and $p=0.002$, respectively), with a mean 50% improvement in HRQoL and a mean 59% improvement in psoriasis-induced stress. Clinical improvement correlated significantly with improved HRQoL ($r=0.297$, $p=0.008$) and psoriasis-related stress ($r=0.310$, $p=0.005$). Of the tonsillectomized patients, 87% concluded that the procedure was worthwhile. Tonsillectomy may improve quality of life for selected patients with plaque psoriasis.

Key words: chronic plaque psoriasis; streptococcal throat infection; tonsillectomy; health-related quality of life; Psoriasis Disability Index; Psoriasis Life Stress Inventory.

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Corr: Ragna Hlin Thorleifsdottir, Department of Medical Sciences, Dermatology, Uppsala University, Akademiska University Hospital, SE-751 85 Uppsala, Sweden. E-mail: ragnahlin@gmail.com

Psoriasis is a complex multifactorial disease caused by a combination of genetic and environmental factors (1), affecting approximately 2–3% of the world's population (2). Psoriasis has a significant negative impact on many areas of health-related quality of life (HRQoL), including physical, occupational, social, psychological, and sexual wellbeing (3–5). Patients with psoriasis have reduced HRQoL, similar to that caused by major chronic illnesses such as cancer, myocardial infarction and diabetes mellitus (6). Many patients have low self-esteem and feel embarrassed, helpless, and stigmatized due to the visible nature of psoriasis (7, 8), which causes significant daily stress for patients (9). Clinical assessments, such as the Psoriasis Area and Severity Index (PASI) (10), do not adequately reflect the general impact that the disease has on the lives of patients with psoriasis, but instead give a static score of clinical disease severity (11).

Many environmental factors have been implicated in psoriasis; in particular, throat infection with β-haemolytic streptococci has been associated with both the initiation and exacerbation of psoriasis (12–15). T cells primed by streptococcal antigens in the tonsils may react with homologous antigens in the skin (16–18), and there are several reports of partial or complete remission of psoriasis after tonsillectomy (reviewed by (19–21)). We reported previously on a randomized controlled trial examining the clinical efficacy and immunological impact of tonsillectomy on plaque psoriasis. The results indicated that tonsillectomy can lead to a significant clinical improvement in plaque psoriasis, with a significant reduction in the frequency of skin-homing T cells that recognize homologous streptococcal M-protein and skin keratins (22). We report here findings related to patient HRQoL and psoriasis-induced stress and their association with clinical improvement after tonsillectomy.

METHODS

Patients

Patient eligibility criteria have been detailed previously (22). Briefly, eligible patients were: ≥18 years of age; had moderate-to-severe chronic plaque psoriasis diagnosed by a dermatologist; had a history of sore throat-associated psoriasis exacerbation; and were willing to undergo tonsillectomy. Exclusion criteria were: underlying medical conditions, such as heart and lung diseases and bleeding disorders; alcohol or drug abuse; pregnancy; and previous tonsillectomy. Before study initiation patients were required to discontinue all psoriasis treatment except moisturizers within the previous 4 weeks.

Fifty-four patients were screened for the study, the majority of whom were referred by a dermatologist (44%) or responded to an advertisement (46%). A few patients had heard about the study by other means (6%) or were referred by an otolaryngologist (4%). Written informed consent was obtained from each patient before initiation of study participation. A total of 29 patients met the inclusion criteria.

Study design

This was a single-centre, 24-month, parallel, assessor-blind, randomized controlled trial. Data were collected within the de-

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partments of Dermatology and Otolaryngology-Head and Neck Surgery in Landspitali, The National University Hospital of Iceland, Reykjavik, Iceland, from November 2007 to January 2011. The study was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments and approved by the National Bioethics Committee of Iceland (VSNb2006090015/03-15) and the Data Protection Authority of Iceland.

A sealed allocation sequence with a numerical code was created by the study supervisor before recruitment began, with a simple randomization in a 1:1 ratio. The 29 patients who met the inclusion criteria were allocated into tonsillectomy and control groups (Fig. 1). The allocation was concealed until the end of the study in order to reduce study bias. Recruitment and follow-up of all patients was performed by the same investigator throughout the study, who was unaware of the allocation system and thus the tonsil status of the patients. Detailed data were collected on patients' demographics and psoriasis features. Patient-reported outcomes were measured at baseline and at months 12 and 24.

Study end-points

Primary end-points were defined as clinically significant changes in HRQoL, assessed by the Psoriasis Disability Index (PDI) and Psoriasis Life Stress Inventory (PLSI) at 12 and 24 months. These parameters, along with secondary end-points, were compared with baseline scores and between the 2 groups. Secondary end-points included the correlations between the change in clinical disease severity (Psoriasis Area and Severity Index, PASI score) and HRQoL scores (PDI and PLSI) as well as evaluation of a study-specific questionnaire, applied at the end of the study.

Questionnaires

HRQoL was assessed with a psoriasis-specific questionnaire, the Psoriasis Disability Index (PDI) (23). The PDI is a validated self-administered questionnaire covering aspects of functional disability owing to psoriasis in the preceding 4 weeks. The PDI addresses 15 issues that can group to 5 domains: daily activities, work/school, personal relationships, leisure, and treatment. The PDI score is rated on a 4-point scale, with grades from "not at all" to "very much" and the total score, ranging from 0 to 45, is calculated by summing the scores given to each question. The total score can therefore range from none to maximal impairment of quality of life.

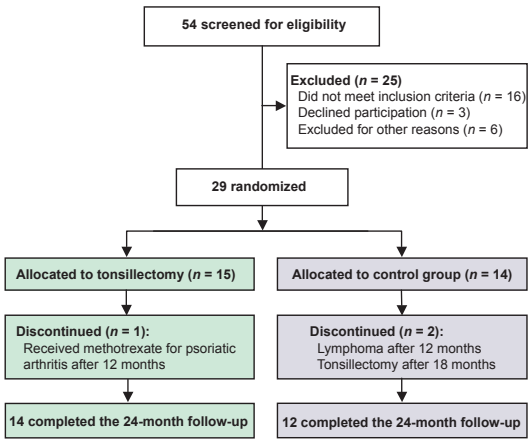

Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) flow diagram.

Psoriasis-related stress was assessed with the Psoriasis Life Stress Inventory (PLSI) (24). The PLSI is a 15-item questionnaire that estimates psychosocial stress due to psoriasis. For each question the patients must rate the level of stress experienced over the previous 4 weeks on a 4-point scale, ranging from "not at all" to "very much." The PLSI score, ranging from 0 to 45, is calculated by summing the scores for each question. Both the PDI and PLSI have been used in the English version for evaluation of psoriasis. The questions were translated into Icelandic using the translation-back-translation procedure and validated by the Nordic Quality of Life study (25). The patients completed both questionnaire at study entry and at 12 and 24 months.

After the 24-month study period, participants answered a study-specific questionnaire with the aim of rating the overall experience of having tonsillectomy as a treatment for psoriasis. The questionnaire was composed of 20 multiple-choice questions, rated on a scale ranging from "not at all" to "very much" and 3 short-answer questions. The questions addressed difficulties associated with the tonsillectomy, recovery time, complications, and whether the operation had been worthwhile. Also, questions about self-perceived improvement in psoriasis, psoriasis nails and/or psoriatic arthritis and regarding HRQoL and psoriasis stress. Moreover, participants were asked about the use of psoriasis treatments, such as moisturizers, topical treatments or systemic therapy, and if they had experienced sore throat or streptococcal throat infections with subsequent exacerbation of psoriasis after removal of their tonsils.

Clinical evaluation

Clinical follow-up has been detailed previously (22). Briefly, clinical severity was assessed by PASI score (10) at study entry and at 2, 6, 12, 18 and 24 months. The use of psoriasis treatment agents during the follow-up period was monitored closely.

Statistical analysis

Patient demographics were summarized descriptively and data were tested for normality using the Kolmogorov-Smirnov test. PDI and PLSI scores at months 12 and 24 were compared between the groups and with baseline scores using analysis of variance (ANOVA) test for repeated measurements. Data for months 12 and 24 were analysed with the intent-to-treat method, where any missing data were replaced using the last observation carried forward (LOCF) method. Square root transformation of the results

Table I. Demographic data for the 29 participating patients with psoriasis

	Tonsillectomy group ($n = 15$)	Control group ($n = 14$)
Men, *n* (%)	3 (20)	6 (43)
Age, years, mean±SD	35.3±9.9	35.9±9.8
BMI (kg/m²) mean±SD	25.2±5.3	25.4±3.6
Smokers, *n* (%)	4 (27)	6 (43)
Age at psoriasis onset, years, mean±SD	15±7.9	15±7.1
Sore throat psoriasis onset, *n* (%)	3 (20)	2 (14)
Duration of psoriasis, years, mean±SD	19.9±9.5	20.5±11.7
Psoriasis family history, *n* (%)	12 (80)	12 (86)
Psoriatic arthritis, *n* (%)	4 (27)	1 (7)
PASI score, mean±SD	11.0±5.7	9.3±3.7
PDI score, mean±SD	10.4±7.1	9.3±7.3
PLSI score, mean±SD	12.0±6.1	10.0±7.0
Previous treatments, *n* (%)		
Topical agents	8 (53)	8 (57)
Phototherapy	5 (33)	4 (29)
Systemic therapy	1 (7)	0

SD: standard deviation; BMI: body mass index, PASI: Psoriasis Area and Severity Index, PDI: Psoriasis Disability Index, PLSI: Psoriasis Life Stress Inventory.

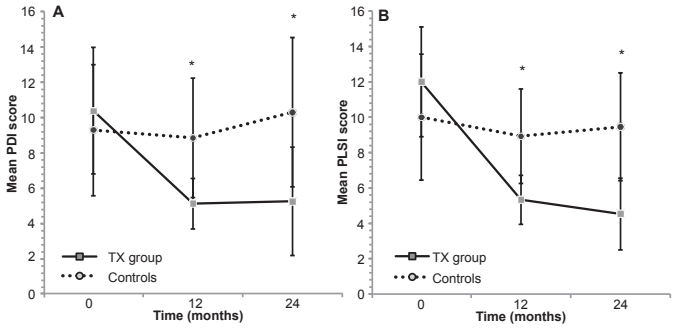

Fig. 2. Changes in health-related quality of life (HRQoL) and psoriasis-induced stress of the 29 participating psoriasis patients during the 24-month follow-up. Panel A: Tonsillectomized patients reported an improvement in HRQoL with a significant decrease in the mean Psoriasis Disability Index (PDI) score, both with time (p=0.026) and compared with the controls ($p=0.037$, 95% confidence interval (CI) 1.43–3.58). No corresponding changes were observed for the control group. Panel B: Tonsillectomized patients reported less daily stress associated with their psoriasis, which was reflected in significantly decreased Psoriasis Life Stress Inventory (PLSI) score, both with time ($p<0.001$) and compared with the controls ($p=0.002$, 95% CI 1.39–3.10). The control group observed no corresponding changes. TX: tonsillectomy group. *Statistical significance.

was used to better approximate normality in the ANOVA model. Statistical significance was defined by $p<0.05$. Spearman's rank correlation analysis was performed to evaluate the relationships between clinical improvement and improvement in HRQoL (PDI and PLSI scores) from baseline to month 24. Data analyses were performed using R software, version 2.10 (The R foundation, Austria).

RESULTS

Of the 54 patients with psoriasis who were screened, 38 met all the inclusion criteria. Six eligible patients were excluded due to chronic disease, ongoing systemic psoriasis treatment and pregnancy, and 3 declined to participate after screening. Thus, a total of 29 patients with plaque psoriasis and a history of psoriasis exacerbation during or after a sore throat were enrolled in the study (Fig. 1). Fourteen patients in the tonsillectomy group and 12 in the control group completed the 24-month follow-up, which took place from November 2008 to January 2011. One patient in the tonsillectomy group was started on methotrexate to treat his psoriatic arthritis after 12 months of participation. Two patients in the control group did not complete the study, one was diagnosed with lymphoma after 12 months, and the other violated the protocol by having tonsillectomy after 18 months of follow-up. There were no clinically meaningful differences between the groups and, although the tonsillectomy group had slightly higher baseline PASI, PDI and PLSI scores compared with the control group, these differences were not statistically significant (Table I).

There was a significant improvement in HRQoL after tonsillectomy (Fig. 2A). The mean PDI score decreased significantly in the tonsillectomized group, both with time ($p=0.026$) and compared with the controls ($p=0.037$, 95% confidence interval (CI), 1.43–3.58). The patients' quality of life improved in mean by 50%. In accordance with a report (26), we divided the PDI data into 5 domains, to identify the areas that were most influenced by the tonsillectomy (Table II). There were significant changes in HRQoL associated with work and/or school after 12 and 24 months ($p=0.02$ and $p=0.022$, respectively) compared with the control group. The change in HRQoL associated with relationships was significant after 12 months ($p=0.04$) and the change in HRQoL linked to the treatments was significant at 24 months ($p=0.04$). No corresponding improvement in PDI scores ($p=0.803$, 95% CI 3.38–4.23) or PDI domains was observed in the control group.

The tonsillectomized patients reported that their stress associated with having psoriasis decreased considerably after the tonsillectomy (Fig. 2B). Their PLSI score decreased significantly both with time ($p<0.001$) and compared with the controls ($p=0.002$, 95% CI 1.39–3.10), and improved, in mean by 59%. The control group did not exhibit any significant improvement in psychosocial stress related to coping with psoriasis ($p=0.654$, 95% CI 2.67–3.56).

A significant positive correlation was observed between clinical improvement and increased HRQoL ($r=0.297$, $p=0.008$) (Fig. 3A). Likewise, there was a

Table II. Mean changes in domains of the Psoriasis Disability Index (PDI) before and after tonsillectomy of the 29 participating patients with psoriasis

	PDI change at 12 months			PDI change at 24 months		
	Tonsillectomy Mean±SD	Controls Mean±SD	*p*-value	Tonsillectomy Mean±SD	Controls Mean±SD	*p*-value
Daily activity	1.53±3.18	0.29±1.20	ns	1.79±2.91	0.18±2.89	ns
Work/school	1.00±1.56	–0.07±0.47	0.02	0.86±1.66	–0.64±1.29	0.02
Relationships	1.13±1.96	0.07±0.27	0.04	0.71±1.98	–0.27±1.10	ns
Leisure	1.07±1.91	0.00±0.88	ns	1.07±2.13	0.27±1.19	ns
Treatment	0.60±1.06	0.07±0.92	ns	1.00±1.11	0.09±0.94	0.04

ns: non-significant; SD: standard deviation.

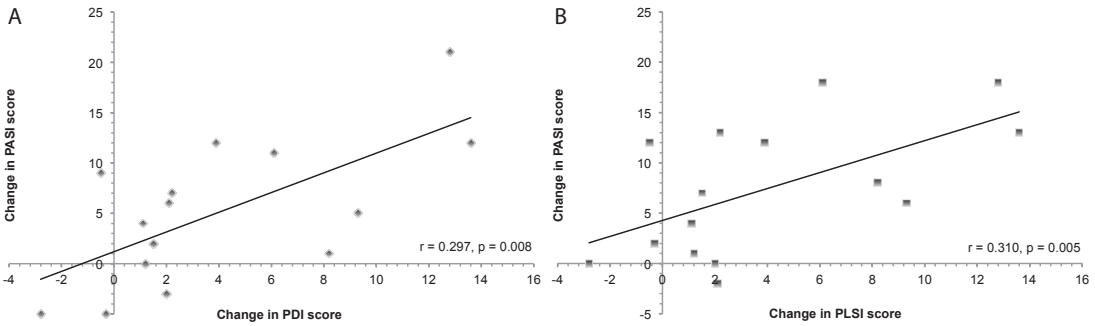

Fig. 3. Correlation between clinical improvement, improved health-related quality of life (HRQoL) and psoriasis-related stress of the 15 tonsillectomized patients with psoriasis during the 24-month follow-up. Panel A: A significant positive correlation was noted between the change in clinical status (PASI score) and change in health-related quality of life (PDI score) ($r = 0.297$, $p = 0.008$). Panel B: Likewise, there was a significant positive correlation between the change in clinical status and the change in psoriasis-related stress (PLSI score) ($r = 0.310$, $p = 0.005$). Spearman's rank correlation test. PASI: Psoriasis Area and Severity Index, PDI: Psoriasis Disability Index, PLSI: Psoriasis Life Stress Inventory.

significant positive correlation between improvement in PASI score and improved psoriasis-related stress ($r = 0.310$, $p = 0.005$) (Fig. 3B).

At the end of the study, all the patients who had tonsillectomy answered a short, study-specific, questionnaire. Twelve (80%) patients felt that the surgery had been quite difficult and that the recovery took more time than they had expected. No major post-operative complications were reported, but one patient had minor bleeding the day after the procedure. Nevertheless, 13 (87%) of the patients thought that the surgery was worthwhile and 12 (80%) concluded that their psoriasis had improved markedly after the surgery. Two patients did not think that there was any clinical difference regarding psoriasis symptoms and one was unsure. When asked about the use of moisturizers, topical treatments and other psoriasis treatments during the 2 years after the tonsillectomy 12 (80%) patients reported less or fewer psoriasis treatments after the surgery. One out of 4 patients who had concomitant psoriasis arthritis reported an improvement in arthritis after the tonsillectomy.

DISCUSSION

We and others (22, 27, 28) have previously reported that tonsillectomy can have marked clinical benefits for selected patients with psoriasis. We now extend these findings to demonstrate that the improvement in clinical activity of psoriasis achieved through tonsillectomy, despite being an overall difficult procedure to go through, leads to a significant positive impact on the activities of daily life and psychosocial wellbeing of patients. Thus, the HRQoL improved by 50% and tonsillectomized patients reported almost 60% lower psoriasis-related stress after the surgery. Furthermore, we found that the increased HRQoL and improved psoriasis-related stress correlated positively with the observed clinical improvement, as assessed by PASI scores.

Patients with psoriasis have a 10-fold higher frequency of symptomatic streptococcal throat infections than matched household controls (14), and the asymptomatic carrier rate for group A, C and G Streptococci has been reported to be as high as 44% in patients with plaque psoriasis and a known history of psoriasis exacerbation associated with sore throat (29). Furthermore, up to 70% of Icelandic patients with plaque psoriasis report an exacerbation of psoriasis symptoms during streptococcal throat infections (30). Thus, the genetic background of patients with psoriasis appears to be permissive for both streptococcal carriage and symptomatic streptococcal throat infections, and the latter has long been associated with flares of guttate psoriasis (12, 15, 31) as well as worsening of chronic plaque psoriasis (13, 14). It would be of interest to measure antistreptolysin O (ASO) titres (32) in patients who have tonsillectomy and correlate titres with both clinical improvement and increased HRQoL. We have recently reported that patients who benefit most from tonsillectomy, both clinically and in terms of quality of life, significantly more often have psoriasis onset associated with a throat infection. Furthermore, these patients reported an increased frequency of streptococcal throat infections per lifetime and were carriers of both copies of *HLA-Cw*0602* (33). The mechanism whereby *HLA-Cw*0602* predisposes to psoriasis is currently unknown. Our data, and those of others (17, 34–37), are consistent with the hypothesis that autoantigens presented in the binding pockets of HLA-Cw*0602 on epidermal cells are recognized by CD8+ T lymphocytes infiltrating lesional epidermis (18).

Although not yet validated, our end-of-study questionnaire gave an insight to the experience of having tonsillectomy to treat plaque psoriasis. Despite the risk of having the surgery, risk of post-operative complications and prolonged recovery time, 87% of the patients thought that the procedure was worthwhile, and 80% reported a marked improvement, which is concordant with the

recorded reduction in PASI scores, ranging from 30% to 90%, in 87% of patients (22). Furthermore, 80% of the patients reported that their need for psoriasis treatment in the form of moisturizers, topical treatments, ultraviolet light treatment or other treatments was noticeably less after the tonsillectomy. This is in accordance with our previous finding that patients with plaque psoriasis needed less symptomatic treatment after tonsillectomy compared with controls (22).

One out of the 4 patients with concomitant plaque psoriasis and psoriatic arthritis (PsA) reported at the end of the study that his arthritis had improved after the tonsillectomy. Interestingly, this patient was homozygous for *HLA-Cw*0602*, which is strongly associated with cutaneous psoriasis (38, 39), early onset psoriasis (40–42), and psoriasis exacerbations after streptococcal throat infection (43, 44). There are very few studies that have explored a possible link between PsA, the tonsils and streptococcal throat infections. DNA encoding the 16S ribosomal RNA gene of group A streptococci has been found in the blood and synovial fluid of patients with PsA (45), and synovial T cells from patients with PsA have been reported to respond to streptococcal superantigens, but not to conventional streptococcal antigens (46).

There is currently no cure for psoriasis, and available treatments only offer symptomatic relief, as psoriasis typically relapses when treatments are discontinued. Our results suggest that selected patients with plaque psoriasis and a history of sore throat-associated exacerbation could benefit from tonsillectomy, both with respect to clinical measures of disease severity (PASI) as well as improved quality of life and reduced disease-related stress. Although our patient cohort was followed for only 2 years, we have observed that the improvement remains at least 5 years post-tonsillectomy (unpublished data). We therefore conclude that tonsillectomy may be a significant addition to the current psoriasis treatment for a selected patient group, and offer a long-lasting improvement. However, more robust trials and long-term follow-up of tonsillectomized patients with plaque psoriasis are needed.

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The authors declare no conflicts of interest.

REFERENCES

1. Elder J, Bruce A, Gudjonsson J, Johnston A, Stuart P, Tejasvi T, et al. Molecular dissection of psoriasis: integrating genetics and biology. J Invest Dermatol 2010; 130: 1213–1226.
2. Gudjonsson JE, Elder JT. Psoriasis: epidemiology. Clin Dermatol 2007; 25: 535–546.
3. Krueger G, Koo J, Lebwohl M, Menter A, Stern R, Rolstad T. The impact of psoriasis on quality of life: results of a 1998 National Psoriasis Foundation patient-membership survey. Arch Dermatol 2001; 137: 280–284.
4. Langley RG, Krueger GG, Griffiths CE. Psoriasis: epidemiology, clinical features, and quality of life. Ann Rheum Dis 2005; 64 Suppl 2: ii18–23; discussion ii24–15.
5. Dalgard FJ, Gieler U, Tomas-Aragones L, Lien L, Poot F, Jemec GB, et al. The psychological burden of skin diseases: a cross-sectional multicenter study among dermatological out-patients in 13 European countries. J Invest Dermatol 2015; 135: 984–991.
6. Rapp SR, Feldman SR, Exum ML, Fleischer AB, Jr., Reboussin DM. Psoriasis causes as much disability as other major medical diseases. J Am Acad Dermatol 1999; 41: 401–407.
7. Fortune DG, Richards HL, Main CJ, Griffiths CE. What patients with psoriasis believe about their condition. J Am Acad Dermatol 1998; 39: 196–201.
8. Nelson PA, Barker Z, Griffiths CE, Cordingley L, Chew-Graham CA, Team I. 'On the surface': a qualitative study of GPs' and patients' perspectives on psoriasis. BMC Fam Pract 2013; 14: 158.
9. Fortune D, Main C, O'Sullivan T, Griffiths C. Quality of life in patients with psoriasis: the contribution of clinical variables and psoriasis-specific stress. Br J Dermatol 1997; 137: 755–760.
10. Fredriksson T, Pettersson U. Severe psoriasis – oral therapy with a new retinoid. Dermatologica 1978; 157: 238–244.
11. Sampogna F, Sera F, Abeni D, Investigators IMPRoVEI. Measures of clinical severity, quality of life, and psychological distress in patients with psoriasis: a cluster analysis. J Invest Dermatol 2004; 122: 602–607.
12. Telfer NR, Chalmers RJ, Whale K, Colman G. The role of streptococcal infection in the initiation of guttate psoriasis. Arch Dermatol 1992; 128: 39–42.
13. Wardrop P, Weller R, Marais J, Kavanagh G. Tonsillitis and chronic psoriasis. Clin Otolaryngol Allied Sci 1998; 23: 67–68.
14. Gudjonsson JE, Thorarinsson AM, Sigurgeirsson B, Kristinsson KG, Valdimarsson H. Streptococcal throat infections and exacerbation of chronic plaque psoriasis: a prospective study. Br J Dermatol 2003; 149: 530–534.
15. Mallbris L, Larsson P, Bergqvist S, Vingard E, Granath F, Stahle M. Psoriasis phenotype at disease onset: clinical characterization of 400 adult cases. J Invest Dermatol 2005; 124: 499–504.
16. Valdimarsson H, Baker BS, Jonsdottir I, Powles A, Fry L. Psoriasis: a T-cell-mediated autoimmune disease induced by streptococcal superantigens? Immunol Today 1995; 16: 145–149.
17. Diluvio L, Vollmer S, Besgen P, Ellwart JW, Chimenti S, Prinz JC. Identical TCR beta-chain rearrangements in streptococcal angina and skin lesions of patients with psoriasis vulgaris. J Immunol 2006; 176: 7104–7111.
18. Valdimarsson H, Thorleifsdottir RH, Sigurdardottir SL, Gudjonsson JE, Johnston A. Psoriasis – as an autoimmune disease caused by molecular mimicry. Trends Immunol 2009; 30: 494–501.
19. Sigurdardottir SL, Thorleifsdottir RH, Valdimarsson H, Johnston A. The role of the palatine tonsils in the pathogenesis and treatment of psoriasis. Br J Dermatol 2013; 168: 237–242.
20. Wu W, Debbaneh M, Moslehi H, Koo J, Liao W. Tonsillectomy as a treatment for psoriasis: a review. J Dermatolog Treat 2014; 25: 482–486.
21. Rachakonda TD, Dhillon JS, Florek AG, Armstrong AW. Effect

of tonsillectomy on psoriasis: a systematic review. J Am Acad Dermatol 2015; 72: 261–275.
22. Thorleifsdottir RH, Sigurdardottir SL, Sigurgeirsson B, Olafsson JH, Sigurdsson MI, Petersen H, et al. Improvement of psoriasis after tonsillectomy is associated with a decrease in the frequency of circulating T cells that recognize streptococcal determinants and homologous skin determinants. J Immunol 2012; 188: 5160–5165.
23. Finlay AY, Kelly SE. Psoriasis – an index of disability. Clin Exp Dermatol 1987; 12: 8–11.
24. Gupta MA, Gupta AK. The Psoriasis Life Stress Inventory: a preliminary index of psoriasis-related stress. Acta Derm Venereol 1995; 75: 240–243.
25. Zachariae R, Zachariae H, Blomqvist K, Davidsson S, Molin L, Mork C, et al. Quality of life in 6497 Nordic patients with psoriasis. Br J Dermatol 2002; 146: 1006–1016.
26. Chern E, Yau D, Ho JC, Wu WM, Wang CY, Chang HW, et al. Positive effect of modified Goeckerman regimen on quality of life and psychosocial distress in moderate and severe psoriasis. Acta Derm Venereol 2011; 91: 447–451.
27. Hone SW, Donnelly MJ, Powell F, Blayney AW. Clearance of recalcitrant psoriasis after tonsillectomy. Clin Otolaryngol Allied Sci 1996; 21: 546–547.
28. Nyfors A, Rasmussen PA, Lemholt K, Eriksen B. Improvement of recalcitrant psoriasis vulgaris after tonsillectomy. J Laryngol Otol 1976; 90: 789–794.
29. Sigurdardottir SL, Thorleifsdottir RH, Valdimarsson H, Johnston A. The association of sore throat and psoriasis might be explained by histologically distinctive tonsils and increased expression of skin-homing molecules by tonsil T cells. Clin Exp Immunol 2013; 174: 139–151.
30. Thorleifsdottir RH, Eysteinsdottir JH, Olafsson JH, Sigurdsson MI, Johnston A, Valdimarsson H, et al. Throat infections are associated with exacerbation in a substantial proportion of patients with chronic plaque psoriasis. Acta Derm Venereol 2016; 96: 788–791.
31. Whyte HJ, Baughman RD. Acute guttate psoriasis and streptococcal infection. Arch Dermatol 1964; 89: 350–356.
32. Cunningham MW. Pathogenesis of group A streptococcal infections. Clin Microbiol Rev 2000; 13: 470–511.
33. Thorleifsdottir RH, Sigurdardottir SL, Sigurgeirsson B, Olafsson JH, Petersen H, Sigurdsson MI, et al. HLA-Cw6 homozygosity in plaque psoriasis is associated with streptococcal throat infections and pronounced improvement after tonsillectomy: A prospective case series. J Am Acad Dermatol 2016; 75: 889–896.
34. Ovigne J-M, Baker BS, Davison SC, Powles AV, Fry L. Epidermal CD8+ T cells reactive with group A streptococcal antigens in chronic plaque psoriasis. Exp Dermatol 2002; 11: 357–364.
35. Johnston A, Gudjonsson JE, Sigmundsdottir H, Love TJ, Valdimarsson H. Peripheral blood T cell responses to keratin peptides that share sequences with streptococcal M proteins are largely restricted to skin-homing CD8(+) T cells. Clin Exp Immunol 2004; 138: 83–93.
36. Ferran M, Galvan AB, Rincon C, Romeu ER, Sacrista M, Barboza E, et al. Streptococcus induces circulating CLA(+) memory T-cell-dependent epidermal cell activation in psoriasis. J Invest Dermatol 2013; 133: 999–1007.
37. Arakawa A, Siewert K, Stohr J, Besgen P, Kim SM, Ruhl G, et al. Melanocyte antigen triggers autoimmunity in human psoriasis. J Exp Med 2015; 212: 2203–2212.
38. Nair RP, Stuart PE, Nistor I, Hiremagalore R, Chia NV, Jenisch S, et al. Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. Am J Hum Genet 2006; 78: 827–851.
39. Gudjonsson JE, Karason A, Antonsdottir A, Runarsdottir EH, Hauksson VB, Upmanyu R, et al. Psoriasis patients who are homozygous for the HLA-Cw*0602 allele have a 2.5-fold increased risk of developing psoriasis compared with Cw6 heterozygotes. Br J Dermatol 2003; 148: 233–235.
40. Henseler T, Christophers E. Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris. J Am Acad Dermatol 1985; 13: 450–456.
41. Mallon E, Bunce M, Wojnarowska F, Welsh K. HLA-CW*0602 is a susceptibility factor in type I psoriasis, and evidence Ala-73 is increased in male type I psoriatics. J Invest Dermatol 1997; 109: 183–186.
42. Enerbäck C, Martinsson T, Inerot A, Wahlström J, Enlund F, Yhr M, et al. Evidence that HLA-Cw6 determines early onset of psoriasis, obtained using sequence-specific primers (PCR-SSP). Acta Derm Venereol 1997; 77: 273–276.
43. Gudjonsson JE, Karason A, Antonsdottir AA, Runarsdottir EH, Gulcher JR, Stefansson K, et al. HLA-Cw6-positive and HLA-Cw6-negative patients with Psoriasis vulgaris have distinct clinical features. J Invest Dermatol 2002; 118: 362–365.
44. Mallbris L, Wolk K, Sanchez F, Stahle M. HLA-Cw*0602 associates with a twofold higher prevalence of positive streptococcal throat swab at the onset of psoriasis: a case control study. BMC Dermatol 2009; 9: 5.
45. Wang Q, Vasey FB, Mahfood JP, Valeriano J, Kanik KS, Anderson BE, et al. V2 regions of 16S ribosomal RNA used as a molecular marker for the species identification of streptococci in peripheral blood and synovial fluid from patients with psoriatic arthritis. Arthritis Rheum 1999; 42: 2055–2059.
46. Thomssen H, Hoffmann B, Schank M, Elewaut D, Meyer zum Buschenfelde KH, Marker-Hermann E. There is no disease-specific role for streptococci-responsive synovial T lymphocytes in the pathogenesis of psoriatic arthritis. Med Microbiol Immunol 2000; 188: 203–207.

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Paper III

HLA-Cw6 homozygosity in plaque psoriasis is associated with streptococcal throat infections and pronounced improvement after tonsillectomy: A prospective case series

Ragna H. Thorleifsdottir, MD,[a,b,e] Sigrun L. Sigurdardottir, PhD,[b] Bardur Sigurgeirsson, MD, PhD,[a] Jon H. Olafsson, MD, PhD,[a] Hannes Petersen, MD, PhD,[c] Martin I. Sigurdsson, MD, PhD,[d] Johann E. Gudjonsson, MD, PhD,[f] Andrew Johnston, PhD,[f] and Helgi Valdimarsson, MD, FRCP[b]
Reykjavik, Iceland; Uppsala, Sweden; and Ann Arbor, Michigan

Background: Carriage of the HLA-Cw*0602 allele is associated with a particular set of clinical features and treatment responses in psoriasis. Tonsillectomy can improve psoriasis.

Objectives: We sought to evaluate whether HLA-Cw*0602 predicts a favorable outcome after tonsillectomy of patients with psoriasis.

Methods: This prospective case series followed up 28 tonsillectomized patients with plaque psoriasis for 24 months. The Psoriasis Area and Severity Index, Psoriasis Disability Index, and Psoriasis Life Stress Inventory were used for assessment. Tonsils were swabbed for bacteria and patients genotyped for HLA-Cw*0602.

Results: After tonsillectomy, HLA-Cw*0602 homozygotes showed significantly more improvement, compared with heterozygous and HLA-Cw*0602−negative patients. Thus, Psoriasis Area and Severity Index score was reduced by 82% in the homozygous patients compared with 42% and 31%, respectively ($P < .001$), Psoriasis Disability Index score improved by 87% compared with 38% and 41%, respectively ($P < .001$), and Psoriasis Life Stress Inventory score was 82% reduced compared with 60% and 54%, respectively ($P < .001$). The homozygotes more often had psoriasis onset associated with a throat infection ($P = .007$) and an increased frequency of streptococcal throat infections per lifetime ($P = .038$).

Limitations: Few patients were included and some data were retrospective.

Conclusions: Homozygous HLA-Cw*0602 carriage in plaque psoriasis may predict a favorable outcome after tonsillectomy. (J Am Acad Dermatol 2016;75:889-96.)

Key words: chronic plaque psoriasis; HLA-Cw*0602; Psoriasis Disability Index; Psoriasis Life Stress Inventory; sore throat; streptococcal throat infection; tonsillectomy.

From the Section of Dermatology, Faculty of Medicine, University of Iceland[a]; Departments of Immunology,[b] Otolaryngology—Head and Neck Surgery,[c] and Anesthesiology and Critical Care,[d] Landspitali—National University Hospital of Iceland; Department of Dermatology, Akademiska University Hospital, Uppsala[e]; and Department of Dermatology, University of Michigan Medical School.[f]

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Reprint requests: Ragna H. Thorleifsdottir, MD, Department of Dermatology, Akademiska University Hospital, 75185 Uppsala, Sweden. E-mail: ragnahlin@gmail.com.

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Psoriasis is a common T-lymphocyte-mediated skin disease caused by a combination of genetic and environmental factors[1] with over 60 genetic susceptibility loci now reported to be associated with psoriasis.[2,3] HLA-Cw*0602 is the major psoriasis susceptibility allele[4] and over 60% of patients with psoriasis carry 1 or 2 copies of HLA-Cw*0602, whereas the frequency in the general population is only 10% to 15%.[4] Although various environmental factors have been reported to influence psoriasis, throat infections with β-hemolytic streptococci have most convincingly been associated with both initiation and exacerbation of psoriasis.[5-7] It was proposed that T cells, primed by streptococcal antigens in the palatine tonsils, may migrate to the skin where they may react to antigens that share sequence homology with streptococcal proteins.[8-10] A number of studies have shown partial or complete remission of psoriasis after tonsillectomy,[11-13] and we have recently conducted a prospective, randomized, and controlled study indicating that tonsillectomy can lead to a marked clinical improvement of chronic plaque psoriasis.[14]

CAPSULE SUMMARY

- Tonsillectomy can improve psoriasis, yet the patient group likely to benefit the most is poorly defined.
- We show that homozygous HLA-Cw*0602 carriage is associated with a particularly favorable outcome after tonsillectomy in patients with streptococcal-associated psoriasis exacerbation.
- Profiling patients with psoriasis can identify those who could benefit most from tonsillectomy.

Carriage of the HLA-Cw*0602 allele has been associated with a particular set of clinical features in psoriasis.[15] HLA-Cw*0602–positive patients usually have a younger onset age,[16,17] more severe psoriasis course,[18] guttate or eruptive plaque psoriasis phenotypes,[15,19] more frequent streptococcal throat carriage or infections,[18,20] and streptococcal-associated psoriasis exacerbation.[18] We therefore wanted to evaluate whether patients with psoriasis carrying the HLA-Cw*0602 allele, with a history of sore throat–induced onset or exacerbation of psoriasis, responded more favorably to tonsillectomy than HLA-Cw*0602–negative patients.

METHODS

Study design

This was a prospective case series study with a 24-month follow-up period. The research was approved by the National Bioethics Committee of Iceland (VSNb2006090015/03-15) and the Data Protection Authority of Iceland, and conducted in accordance with the 1964 Declaration of Helsinki and its later amendments. Data were collected within the Departments of Otolaryngology-Head and Neck Surgery, Dermatology, and Immunology at Landspitali-National University Hospital of Iceland, Reykjavik, Iceland, from November 2007 to January 2011.

Subjects and follow-up

In all, 28 patients with chronic plaque psoriasis were included in the study and signed informed consent was obtained from each participant before initiation. Fifteen patients participated in our previous study,[14] and an additional 13 patients who fulfilled the inclusions criteria were recruited. Inclusion criteria included: (1) age at least 18 years; (2) dermatologist-diagnosed moderate to severe plaque psoriasis; (3) history of psoriasis exacerbation in association with sore throats and/or streptococcal throat infections, as recalled by the patients; (4) no other health issues that could pose a risk for patients undergoing tonsillectomy and anesthesia, including heart and lung diseases, alcohol and substance abuse, and bleeding disorders; and (5) consent to have tonsillectomy.

Demographic data and psoriasis features were collected at study entry. Included patients were followed up for 24 months, starting 2 months after tonsillectomy. Psoriasis severity was assessed by the Psoriasis Area and Severity Index (PASI)[21] at study entry and at 2, 6, 12, 18, and 24 months. The participants were evaluated by the same observer throughout the study period and the clinical evaluation was carried out before patients were typed for HLA-Cw*0602 carriage.

The Psoriasis Disability Index (PDI)[22] was used to assess health-related quality of life. The PDI is a validated psoriasis-specific questionnaire that includes 15 questions concerning functional disability as a result of psoriasis in the preceding month. The score is rated on a 4-point scale and the total score, which can range from 0 to 45, is calculated by summing the scores to each question. For assessment of stress related to having to cope with psoriasis on a daily bases the Psoriasis Life Stress Inventory (PLSI)[23] was used. The PLSI is a 15-item scale that rates the level of stress experienced over the previous month. The PLSI score is calculated from a 4-point scale, ranging from 0 to 45, by summation. The higher the PDI and PLSI scores, the greater impairment in

Abbreviations used:

PASI: Psoriasis Area and Severity Index
PDI: Psoriasis Disability Index
PLSI: Psoriasis Life Stress Inventory

quality of life. Both these questionnaires were completed by the patients at study entry and at 12 and 24 months and both were previously translated into Icelandic using the translation-back-translation procedure and validated by the Nordic Quality of Life study.[24]

HLA-Cw*0602 genotyping

All participating patients were genotyped for HLA-Cw*0602 after the 24-month clinical follow-up period. DNA was prepared from peripheral blood mononuclear cells and HLA-Cw*0602 was determined by polymerase chain reaction amplification by genotyping 7 single nucleotide polymorphisms in exons 2 and 3 of the *HLA-C* gene, as previously described.[4]

Bacterial culture and typing

After removal of the tonsils, bacterial throat swabs were taken, both from the tonsil surface as from deep within the tonsil crypts. Typing of the bacteria was performed by culture on sheep blood agar. Subspecies of *Streptococcus* were identified with a Streptex kit (Thermo Fisher Scientific/Remel, Lenexa, KS).

Statistical analysis

Data were tested for normality using the Kolmogorov-Smirnov test. Categorical variables were compared with Fisher exact test and statistical significance was defined by P less than or equal to .05 on 2-tailed tests. The effects of HLA-Cw*0602 genotype and improvement after tonsillectomy (determined by change in PASI, PDI, and PLSI scores over 24 months) were modelled using linear regression and analyzed on an intention-to-treat basis with missing values being replaced with the last non-missing assessment (last observation carried forward). Three different models were tested, dominant (either 1 or 2 HLA-Cw*0602 alleles effects phenotype), recessive (2 HLA-Cw*0602 alleles required to effect phenotype), and additive (phenotype changes sequentially with each HLA-Cw*0602 allele), where the recessive model was used for calculations. The linear model assumptions were checked by visual analysis of residual plot. Data analyses were performed using software (R, Version 2.10, R Foundation, Vienna, Austria).

RESULTS

The study cohort consisted of 28 patients (6 men and 22 women) with chronic plaque psoriasis and a history of psoriasis exacerbation associated with sore throat and/or streptococcal throat infection (Table I). All patients reported an early onset of psoriasis (type I psoriasis)[16] and the onset was attributed to streptococcal pharyngitis in 11 (39%) patients. There were no differences between the groups at baseline, although the homozygous HLA-Cw*0602 carriers had slightly higher baseline PASI, PDI, and PLSI scores compared with the heterozygous and HLA-Cw*0602−negative groups. Four (14%) patients were HLA-Cw*0602 homozygotes, 17 (61%) were heterozygotes, and 7 (25%) were HLA-Cw*0602 negative. In all, 25 patients finished the 24-month follow-up. Two patients in the heterozygous group discontinued the study after 12 and 18 months of follow-up and 1 patient in the HLA-Cw*0602−negative group discontinued after 18 months.

HLA-Cw*0602 homozygosity is associated with streptococcal throat infections

The HLA-Cw*0602 homozygous patients significantly more often reported that their psoriasis onset was triggered by a throat infection, compared with HLA-Cw*0602 heterozygotes and noncarriers (100% vs 29%, P = .007) (Table II). In concordance with this, cultures from tonsil swabs, which were taken after removal of the tonsils, revealed that 75% of the homozygous patients were carriers of group A, C, or G streptococci or *S anginosus* at the time of the surgery, compared with 65% of the heterozygous patients and 43% of the HLA-Cw*0602−negative patients. Furthermore, HLA-Cw*0602 homozygotes reported a significantly higher frequency of streptococcal throat infections per lifetime than the heterozygotes and noncarriers (3.5 vs 1.4 and 1.1 times, respectively, P = .038). The homozygous patients were all smokers, and they all had a family history of psoriasis (Table II). There were no significant differences between homozygous, heterozygous, and HLA-Cw*0602−negative patients concerning age at psoriasis onset, body mass index, psoriasis nail changes, arthritis involvement, or stress- or alcohol-associated psoriasis exacerbation (data not shown).

HLA-Cw*0602 homozygosity is associated with pronounced improvement after tonsillectomy

There was an association between the degree of clinical improvement after tonsillectomy and carriage of HLA-Cw*0602 (Fig 1). Thus, patients who were homozygous HLA-Cw*0602 carriers showed significantly more clinical improvement than the

Table I. Demographic and clinical characteristics of the 28 participating patients with psoriasis

	HLA-Cw*0602		
Demographics	**Homozygous, n = 4**	**Heterozygous, n = 17**	**Negative, n = 7**
Men, n (%)	0	5 (29)	1 (14)
Age, y, mean ± SD	32.5 ± 5.9	33.2 ± 11.8	35.1 ± 6.0
Body mass index, kg/m^2 mean ± SD	24.0 ± 1.5	25.6 ± 4.9	23.6 ± 3.4
Age at psoriasis onset, y, mean ± SD	14.3 ± 6.9	13.9 ± 6.5	16.0 ± 7.3
Psoriasis duration, y, mean ± SD	18.3 ± 10.1	19.3 ± 9.3	19.1 ± 8.6
Psoriatic arthritis, n (%)	1 (25)	4 (24)	1 (14)
Initial PASI score, mean ± SD	13.5 ± 1.7	12.3 ± 4.9	9.9 ± 3.2
Initial PDI score, mean ± SD	13.3 ± 2.8	12.4 ± 7.2	10.4 ± 6.4
Initial PLSI score, mean ± SD	14.0 ± 1.9	12.0 ± 5.1	12.3 ± 5.5

PASI, Psoriasis Area and Severity Index; *PDI*, Psoriasis Disability Index; *PLSI*, Psoriasis Life Stress Inventory.

Table II. HLA-C genotypes and disease characteristics of the 28 participating patients with psoriasis

	HLA-Cw*0602			
Disease characteristics	**Homozygous**	**Heterozygous**	**Negative**	***P* value***
n (%)	4 (14)	17 (61)	7 (25)	
Sore throat–induced psoriasis onset,† n (%)	4 (100)	5 (29)	2 (29)	.007‡
Sore throat/y,† mean ± SD	5.3 ± 3.5	3.9 ± 2.9	6.9 ± 3.6	.768
Strep throat/lifetime,§ mean ± SD	3.5 ± 2.1	1.4 ± 1.5	1.1 ± 0.6	.038‡
Streptococcal carriage,‖ n (%)	3 (75)	11 (65)	3 (43)	.527
Cigarette smoking, n (%)	4 (100)	5 (29)	3 (43)	.013‡
Psoriasis family history, n (%)	4 (100)	14 (82)	5 (71)	.314

*HLA-Cw*0602 homozygous patients compared with heterozygous and HLA-Cw*0602–negative patients.
†As recalled by the patients.
‡Statistically significant.
§Streptococcal throat infections diagnosed by a physician, throat culture, or strep test (rapid antigen detection test).
‖Cultured at the time of tonsillectomy.

heterozygous and the HLA-Cw*0602–negative patients at all time points (Table III). The homozygous patients had a mean 82% PASI reduction during the 24-month follow-up, compared with 42% for the heterozygous group and 31% for the HLA-Cw*0602–negative group ($P < .001$ for overall change by linear regression). All 4 homozygotes achieved at least 75% improvement in PASI score by month 6 and almost 90% improvement in PASI score by month 12. The clinical improvement was accompanied by markedly improved health-related quality of life (PDI) and psoriasis-related stress (PLSI). Again, the homozygous patients fared best, reporting mean PDI and PLSI score reductions of 87% and 82%, compared with 38% and 60% for the heterozygous patients and 41% and 54% for the HLA-Cw*0602–negative patients ($P < .001$ for overall change by linear regression).

The use of psoriasis treatments was monitored throughout the 24-month follow-up period (Table IV). There was a significant decrease in the use of psoriasis treatments after tonsillectomy. Before tonsillectomy, 75% (n = 21) of patients used some form of treatment for psoriasis (topicals, phototherapy, or systemic therapy), but after tonsillectomy only 32% (n = 9) of patients required treatment ($P = .003$).

DISCUSSION

HLA-Cw*0602 is the major psoriasis susceptibility allele, located in the psoriasis susceptibility locus 1.[4] Given that HLA-Cw*0602 carriage has been associated with a higher frequency of streptococcal throat carriage/infections[18,20] and streptococcal-associated psoriasis exacerbation,[18] we typed our study cohort for HLA-Cw*0602 and found an association between the magnitude of improvement after tonsillectomy and HLA-Cw*0602 carriage status. Patients who were homozygotes showed significantly more improvement than the HLA-Cw*0602 heterozygous and noncarriers. Thus, all HLA-Cw*0602 homozygotes reached at least 75% improvement in PASI score by month 6 and approached 90% improvement in PASI score by month 12. Their health-related quality of life also improved markedly (almost 90% reduction in PDI score) after the surgery and they experienced 82% less stress related to having psoriasis. These improvements are comparable with results seen by recently introduced biologics.[25]

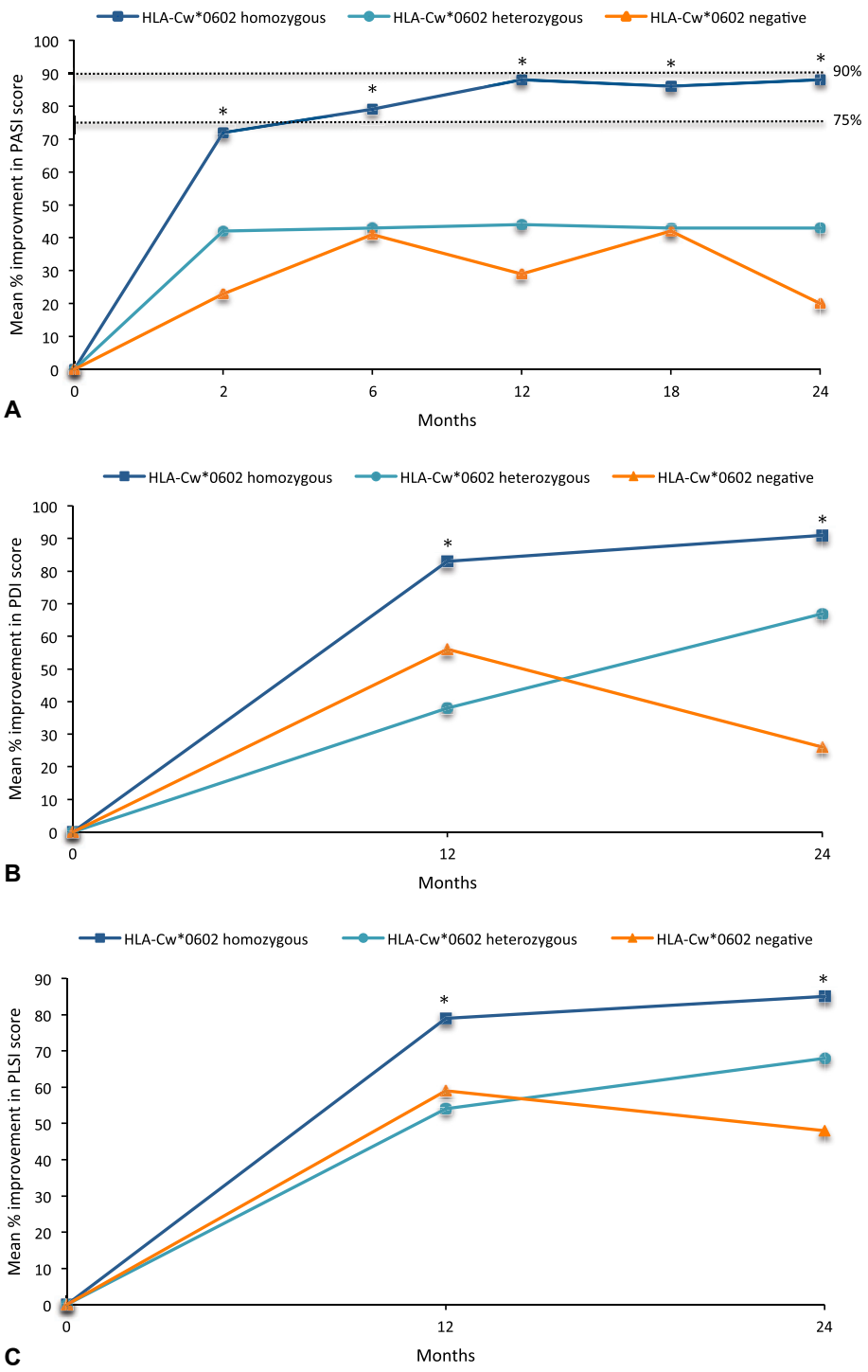

Fig 1. Tonsillectomy efficacy outcomes through month 24. **A**, Mean percentage reduction of Psoriasis Area Severity Index (*PASI*) score; point lines denote 75% and 90% reduction from baseline. **B**, Mean percentage improvement in Psoriasis Disability Index (*PDI*) score. **C**, Mean percentage improvement in Psoriasis Life Stress Inventory score. *Statistical significance, where HLA-Cw*0602 homozygous patients are compared with heterozygous and HLA-Cw*0602–negative patients.

Table III. Tonsillectomy outcomes in relation to HLA-Cw*0602 carriage during the 24-month follow-up

Parameter	HLA-Cw*0602			*P* value*
	Homozygous, n = 4	Heterozygous, n = 17	Negative, n = 7	
Mean PASI score improvement, %	82	42	31	<.001
Month 2	72	42	23	<.001
Month 6	79	43	41	<.001
Month 12	88	44	29	<.001
Month 18	86	43	42	<.001
Month 24	88	43	20	<.001
Mean PDI score improvement, %	87	38	41	<.001
Month 12	83	38	56	.004
Month 24	91	67	26	.005
Mean PLSI score improvement, %	82	60	54	<.001
Month 12	79	54	59	.03
Month 24	85	68	48	.04

PASI, Psoriasis Area and Severity Index; *PDI*, Psoriasis Disability Index; *PLSI*, Psoriasis Life Stress Inventory.
*HLA-Cw*0602 homozygous patients compared with heterozygous and HLA-Cw*0602–negative patients.

Table IV. Use of psoriasis treatments before and after tonsillectomy of the 28 participating patients

Treatment, n (%)	HLA-Cw*0602		
	Homozygous, n = 4	Heterozygous, n = 17	Negative, n = 7
Before tonsillectomy			
Topical therapy*	0	7 (41)	4 (57)
Phototherapy†	2 (50)	5 (29)	2 (29)
Systemic therapy	1‡ (25)	0	0
After tonsillectomy			
Topical therapy*	0	3 (18)	2 (29)
Phototherapy†	0	3 (18)	0
Systemic therapy	1‡ (25)	0	0

*Corticosteroid creams, vitamin-D analog creams, or combination of both.
†With or without topical psoriasis treatment.
‡This patient was treated with methotrexate before tonsillectomy. His rheumatologist initiated methotrexate treatment again for psoriatic arthritis after 12 mo of study follow-up.

The carriage of HLA-Cw*0602 has been associated with a particular set of clinical features in patients with psoriasis,[15] such as guttate or eruptive plaque psoriasis phenotypes,[15,19] and more frequent streptococcal throat carriage or infections.[18,20] Furthermore, an increasing body of evidence has emerged suggesting that different psoriasis genotypes might predict different treatment responses.[26] Thus, 2 recent studies showed that carriage of HLA-Cw*0602 predicts a better response to the interleukin-12/23 inhibitor ustekinumab[27,28] and etanercept was also shown to be more effective in early-onset psoriasis compared with late-onset psoriasis.[29]

The mechanism whereby HLA-Cw*0602 predisposes to psoriasis remains to be elucidated. However, our data and those of others[9,30-33] are consistent with the hypothesis that $CD8^+$ T lymphocytes infiltrating lesional epidermis recognize autoantigens presented in the context of HLA-Cw6 expressed on the surface of epidermal cells.[10] Thus HLA-Cw*0602 may play a direct role in the pathogenesis of psoriasis.[34] Our data and those of an earlier study[20] indicate that HLA-Cw*0602 carriage may impact the bacterial colonization of the tonsils. The tonsils are a major site for streptococcal carriage, and streptococcal throat infections are associated with onset and exacerbation of psoriasis.[6,7,10,11] Throat swabs taken from both the surface and from deep within tonsil crypts revealed a high level of streptococcal throat carriage in our patients. Combined carrier frequency of Lancefield groups A, C, or G *Streptococcus* or *S anginosus* was almost 70% in homozygous and heterozygous HLA-Cw*0602 patients compared with 43% of noncarriers. *S anginosus* can cause pharyngitis, is sometimes β-hemolytic, and carries a typeable Lancefield group antigen, A, C, G, or F.[35] The genetic background of patients with psoriasis is thus associated with both asymptomatic and symptomatic streptococcal throat infections compared with age- and sex-matched control subjects,[7,36] and streptococcal tonsillitis has long been associated with flares of guttate psoriasis[5,37,38] and exacerbation of plaque psoriasis.[6,7,39] It should be noted that the participants included in the current study all had a history of psoriasis exacerbation in association with sore throat, which applies to approximately 40% of patients with plaque psoriasis in Iceland.[40]

Smoking is an important environmental risk factor for many chronic diseases, including several autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus.[41,42] Although there is a strong link between palmoplantar pustulosis and smoking, with up to 80% of patients being smokers or ex-smokers,[43] smoking is also a risk factor for the development of psoriasis.[44] Interestingly, smoking

was significantly more common in our homozygous HLA-Cw*0602 patients compared with the heterozygous and HLA-Cw*0602−negative patients (P = .013). Smoking increases oxidative damage and promotes heightened inflammatory state and may modify expression of several psoriasis-associated genes, including the HLA genes.[45] Homozygous HLA-Cw*0602 individuals have about a 2.5-fold increased risk for psoriasis compared with HLA-Cw*0602 heterozygotes[46] and a recent study reported that HLA-Cw*0602−positive smokers have a further increased risk of developing psoriasis compared with nonsmoking HLA-Cw*0602 carriers, suggesting that smoking might trigger psoriasis in some genetically predisposed individuals.[47]

In this study, the need for psoriasis treatment decreased significantly after tonsillectomy. Although all groups required less treatment after the procedure, this was not significant for the homozygous HLA-Cw*0602 group. However, because the homozygous group only included 4 patients, it is difficult to draw concrete conclusions. The only patient who received systemic treatment before tonsillectomy was homozygous for HLA-Cw*0602. He improved considerably after the tonsillectomy but systemic therapy was restarted after 12 months of follow-up because of psoriatic arthritis symptoms. The other 3 homozygotes did not require any psoriasis treatment during the 24-month follow-up. Thus, available data do not indicate that improvement of psoriasis after tonsillectomy can be attributed to additional psoriasis treatment. Furthermore, we have previously shown that tonsillectomized patients with plaque psoriasis use less treatment and improve significantly more compared with matched control subjects.[14]

Taken together, our findings indicate that HLA-Cw*0602 homozygosity can be regarded as a predictor of favorable outcomes after tonsillectomy of patients with plaque psoriasis and a history of streptococcal-associated psoriasis exacerbation. Profiling patients who have psoriasis with respect to disease history and genotype can help identify patients with psoriasis who could benefit the most from tonsillectomy. Although our patients were only followed up for 24 months, we observed that improvement after tonsillectomy remains largely unchanged for at least 5 years (unpublished data). However, in view of the relatively few patients genotyped for HLA-Cw*0602, our findings need to be expanded with more HLA-C-typed patients.

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REFERENCES

1. Elder JT, Bruce AT, Gudjonsson JE, et al. Molecular dissection of psoriasis: integrating genetics and biology. *J Invest Dermatol*. 2010;130(5):1213-1226.
2. Tsoi LC, Spain SL, Ellinghaus E, et al. Enhanced meta-analysis and replication studies identify five new psoriasis susceptibility loci. *Nat Commun*. 2015;6:7001.
3. Mahil SK, Capon F, Barker JN. Genetics of psoriasis. *Dermatol Clin*. 2015;33(1):1-11.
4. Nair RP, Stuart PE, Nistor I, et al. Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. *Am J Hum Genet*. 2006;78(5):827-851.
5. Telfer NR, Chalmers RJ, Whale K, Colman G. The role of streptococcal infection in the initiation of guttate psoriasis. *Arch Dermatol*. 1992;128(1):39-42.
6. Wardrop P, Weller R, Marais J, Kavanagh G. Tonsillitis and chronic psoriasis. *Clin Otolaryngol Allied Sci*. 1998;23(1):67-68.
7. Gudjonsson JE, Thorarinsson AM, Sigurgeirsson B, Kristinsson KG, Valdimarsson H. Streptococcal throat infections and exacerbation of chronic plaque psoriasis: a prospective study. *Br J Dermatol*. 2003;149(3):530-534.
8. Valdimarsson H, Baker BS, Jonsdottir I, Powles A, Fry L. Psoriasis: a T-cell-mediated autoimmune disease induced by streptococcal superantigens? *Immunol Today*. 1995;16(3):145-149.
9. Diluvio L, Vollmer S, Besgen P, Ellwart JW, Chimenti S, Prinz JC. Identical TCR beta-chain rearrangements in streptococcal angina and skin lesions of patients with psoriasis vulgaris. *J Immunol*. 2006;176(11):7104-7111.
10. Valdimarsson H, Thorleifsdottir RH, Sigurdardottir SL, Gudjonsson JE, Johnston A. Psoriasis—as an autoimmune disease caused by molecular mimicry. *Trends Immunol*. 2009;30(10):494-501.
11. Sigurdardottir SL, Thorleifsdottir RH, Valdimarsson H, Johnston A. The role of the palatine tonsils in the pathogenesis and treatment of psoriasis. *Br J Dermatol*. 2013;168(2):237-242.
12. Wu W, Debbaneh M, Moslehi H, Koo J, Liao W. Tonsillectomy as a treatment for psoriasis: a review. *J Dermatolog Treat*. 2014;25(6):482-486.
13. Rachakonda TD, Dhillon JS, Florek AG, Armstrong AW. Effect of tonsillectomy on psoriasis: a systematic review. *J Am Acad Dermatol*. 2015;72(2):261-275.
14. Thorleifsdottir RH, Sigurdardottir SL, Sigurgeirsson B, et al. Improvement of psoriasis after tonsillectomy is associated with a decrease in the frequency of circulating T cells that recognize streptococcal determinants and homologous skin determinants. *J Immunol*. 2012;188(10):5160-5165.
15. Gudjonsson JE, Karason A, Antonsdottir AA, et al. HLA-Cw6--positive and HLA-Cw6-negative patients with psoriasis vulgaris have distinct clinical features. *J Invest Dermatol*. 2002;118(2):362-365.
16. Henseler T, Christophers E. Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris. *J Am Acad Dermatol*. 1985;13(3):450-456.
17. Enerback C, Martinsson T, Inerot A, et al. Significantly earlier age at onset for the HLA-Cw6-positive than for the Cw6-negative psoriatic sibling. *J Invest Dermatol*. 1997;109(5):695-696.

18. Gudjonsson JE, Karason A, Runarsdottir EH, et al. Distinct clinical differences between HLA-Cw*0602 positive and negative psoriasis patients—an analysis of 1019 HLA-C- and HLA-B-typed patients. *J Invest Dermatol*. 2006;126(4):740-745.
19. Mallon E, Bunce M, Savoie H, et al. HLA-C and guttate psoriasis. *Br J Dermatol*. 2000;143(6):1177-1182.
20. Mallbris L, Wolk K, Sanchez F, Stahle M. HLA-Cw*0602 associates with a twofold higher prevalence of positive streptococcal throat swab at the onset of psoriasis: a case control study. *BMC Dermatol*. 2009;9:5.
21. Fredriksson T, Pettersson U. Severe psoriasis—oral therapy with a new retinoid. *Dermatologica*. 1978;157(4):238-244.
22. Finlay AY, Kelly SE. Psoriasis—an index of disability. *Clin Exp Dermatol*. 1987;12(1):8-11.
23. Gupta MA, Gupta AK. The Psoriasis Life Stress Inventory: a preliminary index of psoriasis-related stress. *Acta Derm Venereol*. 1995;75(3):240-243.
24. Zachariae R, Zachariae H, Blomqvist K, et al. Quality of life in 6497 Nordic patients with psoriasis. *Br J Dermatol*. 2002;146(6):1006-1016.
25. Thaci D, Blauvelt A, Reich K, et al. Secukinumab is superior to ustekinumab in clearing skin of subjects with moderate to severe plaque psoriasis: CLEAR, a randomized controlled trial. *J Am Acad Dermatol*. 2015;73(3):400-409.
26. Griffiths CE, Barnes MR, Burden AD, et al. Establishing an academic-industrial stratified medicine consortium: psoriasis stratification to optimize relevant therapy. *J Invest Dermatol*. 2015;135(12):2903-2907.
27. Talamonti M, Botti E, Galluzzo M, et al. Pharmacogenetics of psoriasis: HLA-Cw6 but not LCE3B/3C deletion nor TNFAIP3 polymorphism predisposes to clinical response to interleukin 12/23 blocker ustekinumab. *Br J Dermatol*. 2013;169(2):458-463.
28. Chiu HY, Wang TS, Chan CC, Cheng YP, Lin SJ, Tsai TF. Human leucocyte antigen-Cw6 as a predictor for clinical response to ustekinumab, an interleukin-12/23 blocker, in Chinese patients with psoriasis: a retrospective analysis. *Br J Dermatol*. 2014;171(5):1181-1188.
29. Griffiths CE, Christophers E, Szumski A, Jones H, Mallbris L. Impact of early vs. late disease onset on treatment response to etanercept in patients with psoriasis. *Br J Dermatol*. 2015;173(5):1271-1273.
30. Ovigne J-M, Baker BS, Davison SC, Powles AV, Fry L. Epidermal $CD8^+$ T cells reactive with group A streptococcal antigens in chronic plaque psoriasis. *Exp Dermatol*. 2002;11(4):357-364.
31. Johnston A, Gudjonsson JE, Sigmundsdottir H, Love TJ, Valdimarsson H. Peripheral blood T cell responses to keratin peptides that share sequences with streptococcal M proteins are largely restricted to skin-homing $CD8(^+)$ T cells. *Clin Exp Immunol*. 2004;138(1):83-93.
32. Ferran M, Galvan AB, Rincon C, et al. Streptococcus induces circulating $CLA(^+)$ memory T-cell-dependent epidermal cell activation in psoriasis. *J Invest Dermatol*. 2013;133(4):999-1007.
33. Arakawa A, Siewert K, Stohr J, et al. Melanocyte antigen triggers autoimmunity in human psoriasis. *J Exp Med*. 2015;212(13):2203-2212.
34. Valdimarsson H. The genetic basis of psoriasis. *Clin Dermatol*. 2007;25(6):563-567.
35. Facklam R. What happened to the streptococci: overview of taxonomic and nomenclature changes. *Clin Microbiol Rev*. 2002;15(4):613-630.
36. Sigurdardottir SL, Thorleifsdottir RH, Valdimarsson H, Johnston A. The association of sore throat and psoriasis might be explained by histologically distinctive tonsils and increased expression of skin-homing molecules by tonsil T cells. *Clin Exp Immunol*. 2013;174(1):139-151.
37. Whyte HJ, Baughman RD. Acute guttate psoriasis and streptococcal infection. *Arch Dermatol*. 1964;89:350-356.
38. Mallbris L, Larsson P, Bergqvist S, Vingard E, Granath F, Stahle M. Psoriasis phenotype at disease onset: clinical characterization of 400 adult cases. *J Invest Dermatol*. 2005;124(3):499-504.
39. Bartenjev I, Rogl Butina M, Potocnik M. Subclinical microbial infection in patients with chronic plaque psoriasis. *Acta Derm Venereol Suppl (Stockh)*. 2000;(211):17-18.
40. Thorleifsdottir RH, Eysteinsdottir JH, Olafsson JH, et al. Throat infections are associated with exacerbation in a substantial proportion of patients with chronic plaque psoriasis. *Acta Derm Venereol*. doi: 10.2340/00015555-2408. Published online March 17, 2016.
41. Costenbader KH, Kim DJ, Peerzada J, et al. Cigarette smoking and the risk of systemic lupus erythematosus: a meta-analysis. *Arthritis Rheum*. 2004;50(3):849-857.
42. Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. *Arthritis Rheum*. 2004;50(10):3085-3092.
43. O'Doherty CJ, MacIntyre C. Palmoplantar pustulosis and smoking. *Br Med J (Clin Res Ed)*. 1985;291(6499):861-864.
44. Setty A, Curhan G, Choi H. Smoking and the risk of psoriasis in women: Nurses' Health Study II. *Am J Med*. 2007;120(11):953-959.
45. Armstrong AW, Armstrong EJ, Fuller EN, Sockolov ME, Voyles SV. Smoking and pathogenesis of psoriasis: a review of oxidative, inflammatory and genetic mechanisms. *Br J Dermatol*. 2011;165(6):1162-1168.
46. Gudjonsson JE, Karason A, Antonsdottir A, et al. Psoriasis patients who are homozygous for the HLA-Cw*0602 allele have a 2.5-fold increased risk of developing psoriasis compared with Cw6 heterozygotes. *Br J Dermatol*. 2003;148(2):233-235.
47. Jin Y, Yang S, Zhang F, et al. Combined effects of HLA-Cw6 and cigarette smoking in psoriasis vulgaris: a hospital-based case-control study in China. *J Eur Acad Dermatol Venereol*. 2009;23(2):132-137.

Paper IV

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CLINICAL REPORT

Throat Infections are Associated with Exacerbation in a Substantial Proportion of Patients with Chronic Plaque Psoriasis

Ragna H. THORLEIFSDOTTIR[1–3], Jenna H. EYSTEINSDOTTIR[1,2], Jon H. OLAFSSON[1], Martin I. SIGURDSSON[4], Andrew JOHNSTON[5], Helgi VALDIMARSSON[2] and Bardur SIGURGEIRSSON[1]

[1]Faculty of Medicine, Section of Dermatology, University of Iceland, Departments of [2]Immunology and [4]Medicine, Landspitali-The National University Hospital of Iceland, Reykjavik, Iceland, Departments of Dermatology, [3]Akademiska University Hospital, Uppsala University, Uppsala, Sweden, and [5]University of Michigan Medical School, Ann Arbor, MI, USA

Streptococcal throat infections are known to trigger or exacerbate psoriasis, and several studies support the benefit of tonsillectomy. To evaluate the potential of tonsillectomy as a treatment, we used a retrospective study-specific questionnaire to assess the proportion of psoriasis patients with sore throat-associated psoriasis exacerbations. Our survey sampled 275 psoriasis patients. Of patients with plaque psoriasis, 42% reported sore throat-associated psoriasis exacerbations, and of patients with confirmed streptococcal infections, 72% reported aggravation. Notably, women and patients with early onset psoriasis were more likely to report psoriasis exacerbation after a sore throat ($p<0.001$, $p=0.046$, respectively). Other psoriasis aggravation factors were more common in patients with sore throat-associated exacerbations ($p<0.01$). Of tonsillectomized patients, 49% reported subsequent improvement and had more frequent sore throat-associated aggravation of psoriasis than patients who did not improve after tonsillectomy ($p=0.015$). These findings suggest a closer association between sore throats, streptococcal throat infections and plaque psoriasis than reported previously. ***Key words: chronic plaque psoriasis; sore throat; streptococcal throat infections; tonsillectomy.***

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Ragna Hlin Thorleifsdottir, Department of Dermatology, Akademiska University Hospital, SE-751 85 Uppsala, Sweden. E-mail: ragnahlin@gmail.com

Psoriasis is a multifactorial disease caused by a combination of genetic and environmental factors. The disease has a strong genetic basis; more than 60 susceptibility loci have been identified (1, 2), including HLA-Cw6, carriage of which is associated with an approximate 10-fold increased risk of developing psoriasis (3). Numerous environmental agents have been reported to trigger and/or exacerbate psoriasis, including psychological stressors, physical trauma, cold climate, cigarette smoking, alcohol intake, and certain drugs (4–7). Similarly, various microorganisms have been implicated, including fungi (*Malassezia, Candida albicans*) and viruses (papillomaviruses, retroviruses) (7). However, throat infections with β-haemolytic streptococci have most convincingly been linked with the initiation and exacerbation of psoriasis.

The association between guttate psoriasis and streptococcal infections has been recognized for 100 years (6, 8–10). However, only a few retrospective (11, 12) and one prospective study (13) have linked exacerbation of plaque psoriasis with streptococcal throat infections. Moreover, such infections are approximately 10 times more frequent in patients with plaque psoriasis compared with age- and sex-matched household controls (13). It has been reported that tonsils from patie nts with psoriasis are more frequently infected with β-haemolytic streptococci, especially group C streptococci, than are recurrently infected tonsils from patients without psoriasis (14). Nevertheless, the immunological basis for the association of psoriasis and streptococcal throat infections is still under investigation.

Palmoplantar pustulosis (PPP) is a painful chronic inflammatory condition, restricted to the palms and/or soles, which was previously regarded as a variant of pustular psoriasis, but is now categorized with acropustular diseases (15). Up to 20% of patients with PPP have concomitant plaque psoriasis (16), and although PPP and plaque psoriasis have different sites of predilection and pathomechanisms (16), they may share common triggering mechanisms. Streptococcal throat infections have been linked to PPP and studies have indicated that PPP may improve after tonsillectomy (17–19).

We have recently reported that patients with plaque psoriasis and a history of sore throat-associated psoriasis exacerbation improve after tonsillectomy (20). The main aim of the current study was to estimate the proportion of patients with plaque psoriasis who experienced disease aggravation after sore throats or streptococcal throat infections, and therefore might be more likely to benefit from tonsillectomy.

METHODS

Study design and cohort

This study is a retrospective large case series that took place from January 2011 to April 2011 at the following dermatology

outpatient units in Iceland: the dermatology outpatient centre at Landspitali – The National University Hospital of Iceland, Reykjavik; Hudlaeknastodin dermatology clinic, Kopavogur; and the Blue Lagoon geothermal clinic, Grindavik (21). A total of 374 patients with psoriasis visiting these clinics were invited to participate; 275 (127 men and 148 women) agreed (73.5% response rate). All participants were over 18 years of age and had been diagnosed with psoriasis by a dermatologist. The study was approved by the National Bioethics Committee of Iceland, the Data Protection Authority of Iceland and performed in compliance with the 1964 Declaration of Helsinki and its later amendments.

A self-report anonymous questionnaire, composed of 15 multiple-choice and short-answer questions (Appendix S1[1]), was designed. Participants were asked to answer as accurately as possible. The questionnaire addressed 5 main topics: (*i*) general demographics; (*ii*) psoriasis subtype, age at onset and whether psoriasis onset had been associated with a sore throat or streptococcal throat infection; (*iii*) frequency of sore throats, defined by a painful inflammation/infection of the mucus membranes in the pharynx, and the frequency of streptococcal throat infections diagnosed by a throat culture, rapid antigen detection test (strep test) or by a physician. Further questions covered: exacerbation of psoriasis during or within 3 weeks of a sore throat or streptococcal throat infection; (*iv*) psoriasis aggravating factors other than sore throat, including general malaise, cold climate, stressful life events, alcohol intake, diet and drugs; (*v*) whether the participant had been subjected to tonsillectomy after the onset of psoriasis; age at time of surgery; and whether the tonsillectomy was associated with changes in the activity of their skin disease.

Statistical analysis

Patient demographics were summarized descriptively. Categorical variables were compared with χ^2 and Fisher's exact test. Level of statistical significance was set at $p \leq 0.05$. A logistic regression model of sore throat aggravation was pursued. All variables with a $p < 0.1$ were entered into the logistic regression model. Odds ratio and 95% confidence intervals were then estimated. All statistics were performed in R, version 2.10 (The R Foundation, Austria).

RESULTS

All 275 recruited participants, 127 men and 148 women, completed the study questionnaire. See Table I for demographic information. The majority of responders (75%) had been diagnosed with plaque psoriasis, 14% with both guttate and plaque psoriasis and 8% with guttate psoriasis. Four patients reported PPP, but 5 of the 275 study participants did not belong to any of the above categories. Early-onset psoriasis, defined as age at onset of 40 years or less (22), was reported by the majority of study participants (87%).

Psoriasis exacerbation associated with a sore throat was reported by 42% of patients with plaque psoriasis, 67% of patients with guttate psoriasis, and 70% of patients with a history of both guttate and plaque psoriasis. This was also the case for 2 out of 4 patients with PPP. Moreover, of the 140 participants with a history of confirmed streptococcal throat infections (Table II), 75% reported streptococcal-associated psoriasis exacerbation. This applied to 72% of patients with plaque psoriasis, 94% of patients with guttate psoriasis, and 79% of patients with both guttate and plaque psoriasis. Furthermore, patients who reported sore throat-associated aggravation were more likely to report streptococcal-associated psoriasis exacerbation (93% vs. 7%, $p < 0.001$). This also applied to subgroups of psoriasis patients: plaque psoriasis (92% vs. 8%, $p < 0.001$), guttate psoriasis (94% vs. 6%, $p = 0.01$) and patients with both guttate and chronic plaque psoriasis (100% vs. 0%, $p = 0.005$). A significantly higher ratio of patients with early-onset psoriasis reported psoriasis exacerbation associated with a sore throat, compared with patients with late-onset psoriasis (51% vs. 30%, $p = 0.046$).

Sore throat-associated aggravation was notably more common among women than men (61% vs. 32%, $p < 0.001$). Even after adjustment for the influence of age, psoriasis subtypes (see Table I), and other psoriasis exacerbation factors, females still had a significantly higher risk of sore throat-associated psoriasis aggravation (odds ratio (OR) = 2.5, 95% confidence interval (CI) 1.37–4.58, $p = 0.003$). Psoriasis exacerbation associated with general malaise, cold climate, stress, consumption of alcohol or various diets were reported significantly more often by patients who also reported sore throat-associated psoriasis aggravation (Table III), and this difference was still significant for cold climate and general malaise, after adjustment for age, gender and psoriasis subtypes (OR = 9.2 and 3.0, 95% CI 3.83–22.3 and 1.58–5.74, $p < 0.001$, respectively). There were no differences between men and women in this respect.

Table I. *Baseline characteristics of the 275 participating patients with psoriasis*

Characteristics	
Men, % (*n*)	47 (127)
Age, years, mean ± SD	42.3 ± 14.2
Age at psoriasis onset, years, mean ± SD	22.3 ± 13.4
Early onset psoriasis (onset before or at 40 years), % (*n*)	87 (240)
Late onset psoriasis (onset after the age of 40 years), % (*n*)	10 (27)
Psoriasis subtype, % (*n*)	
Plaque psoriasis	75 (207)
Guttate psoriasis	8 (21)
Guttate and plaque psoriasis	14 (38)
Psoriasis nail changes	42 (116)
Psoriatic arthritis	20 (56)
Palmoplantar pustulosis, % (*n*)	1 (4)

SD: standard deviation.

Table II. *Streptococcal-associated psoriasis exacerbations among participants with confirmed[a] streptococcal infections*

	% (*n*)
Participants with confirmed streptococcal throat infections[a]	51 (140)
Streptococcal-associated psoriasis exacerbation	75 (105)
Plaque psoriasis	72 (69/96)
Guttate psoriasis	94 (15/16)
Guttate and plaque psoriasis	79 (19/24)
Palmoplantar pustulosis	50 (1/2)

[a]Confirmed by throat culture, rapid antigen detection test or a physician.

[1]http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2408

Table III. *Associations between sore throat-associated exacerbations and other factors reported to aggravate psoriasis*

Psoriasis exacerbating factors	*n*	Patients with sore throat-induced exacerbation % (*n*)	Patients without sore throat-induced exacerbation % (*n*)	*p*-value
General malaise	65	88 (57)	12 (8)	<0.001
Cold climate	162	64 (103)	36 (59)	<0.001
Stress	173	64 (110)	36 (63)	<0.001
Alcohol	68	71 (48)	29 (20)	0.005
Diet[a]	36	75 (27)	25 (9)	0.01
Drugs[b]	9	78 (7)	22 (1)	n.s.

[a]Not specified. [b]Including lithium, beta-blockers, penicillin and methotrexate.

Of the 275 participants, 56 (20%) had been tonsillectomized after the onset of psoriasis (Table IV), and 48% of these reported that tonsillectomy was associated with improvement in their psoriasis. Interestingly, 18/37 (49%) of patients with plaque psoriasis and 3/4 (75%) of guttate patients noted an improvement in psoriasis after tonsillectomy. This also applied to 6/11 (55%) of patients with both guttate and plaque psoriasis (Table IV). Patients who noted improvement after tonsillectomy more frequently reported sore throat-associated aggravation ($p = 0.015$). All patients who reported improvement after tonsillectomy also reported early onset of psoriasis.

DISCUSSION

Psoriasis is a heterogeneous disease with respect to both genetic (1, 2) and pathological components (23, 24) and several external factors have been reported to contribute to the onset and exacerbation of psoriasis (4–6). However, streptococcal throat infection is the only environmental factor that has convincingly been connected to the immunological mechanisms thought to operate in psoriasis (25, 26), especially in patients carrying the HLA-Cw6 allele. With the HLA-Cw6 as the major psoriasis susceptibility allele, the $CD8^+$ T cells are thought to be the major effector cells in psoriasis, as they may respond to peptide antigens presented in the context of HLA-Cw6. Furthermore, chronic stimulation by streptococci in the tonsils gives rise to a set of pathogenic skin-homing (CLA^+) T cells (27). The link between streptococcal throat infections and psoriasis is supported by several lines of research, including increased T-cell responses to streptococcal-derived peptides (28–30), shared T cell receptor rearrangements in psoriasis tonsil and skin-homing and skin-resident T cells (31), increased streptococcal-reactive immunoglobulin G (IgG) titres in the blood of patients with plaque psoriasis (32) and increased throat carriage rate of streptococci among patients with psoriasis (13, 14). Once generated in the tonsils, skin-homing T cells can migrate to the dermis and epidermis, where they are thought to cross-react with skin-derived epitopes, such as keratins (28, 29, 33, 34), maspin, ezrin, PRDX2, hsp27 (30) or melanocyte-derived peptides (35), driving the cutaneous inflammation characteristic of psoriasis. Several studies have indicated that psoriasis can improve after tonsillectomy (26, 36), but indications for such treatment remain to be established. However, most of the patients who have been treated in this way had a history of psoriasis exacerbation in association with sore throats and/or streptococcal throat infections.

Table IV. *Effects of tonsillectomy reported by 56 patients with psoriasis who were tonsillectomized after onset of their psoriasis*

Improvement after tonsillectomy, % (*n*)	48 (27/56)
Plaque psoriasis	49 (18/37)
Guttate psoriasis	75 (3/4)
Guttate and plaque psoriasis	55 (6/11)
Not sure or no improvement, % (*n*)	52 (29/56)

We report here that 42% of patients with plaque psoriasis experienced worsening of their disease in association with sore throat. Furthermore, 72% of the participants with plaque psoriasis and confirmed streptococcal throat infections reported exacerbation of their skin lesions. This is a higher frequency than previously reported by Wardrop et al. (11), where 33% of patients with plaque psoriasis associated worsening of psoriasis with sore throat compared with 3% of matched eczema controls. Note, our study was designed as a retrospective questionnaire and could therefore be limited by recall bias. Sore throat-associated psoriasis aggravation was more common among women ($p = 0.001$). It is not clear why this gender difference exists, but it has been observed that women are more frequently affected with recurrent tonsillitis than men (37). Female sex hormones or altered skin corticosteroid levels might be involved, but we are not aware of any reports on this issue. To that end, it might be interesting to assess prospectively whether postmenopausal women are less sensitive to psoriasis exacerbation after a sore throat and/or a streptococcal throat infection. Patients reporting sore throat-associated aggravation of psoriasis also noted worsening in relation to various other aggravation factors, such as general malaise, stress, alcohol or cold weather. Notably, such associations were not reported by those participants who did not associate sore throat with psoriasis exacerbation. Chronic plaque psoriasis has previously been subdivided into stable and dynamic types (38). Patients with the dynamic type have a more fluctuating course, appear to be more influenced by the various exacerbating factors listed above, and are more often carriers of HLA-Cw6 than patients with a relatively stable disease. This form of plaque psoriasis has even been considered somewhat similar to guttate psoriasis (39). Beside the association between sore throat-associated psoriasis aggravation and other psoriasis exacerbating factors, our data also show that patients with early-onset psoriasis are more prone to sore throat-induced psoriasis aggravation. This suggests that these patients have the dynamic phenotype of plaque

psoriasis, and thus might be appropriate candidates for tonsillectomy. However, our study cohort was limited to Icelandic patients with psoriasis, and Iceland's geographical isolation might have influenced the development of patients more affected by environmental trigger factors, such as streptococcal throat infections.

The tonsils are a major target for streptococcal infections in humans, which are the most common cause of bacterial pharyngitis (40). The high level of streptococcal throat carriage and infections in patients with plaque psoriasis is noteworthy, with the carrier rate for groups A, C and G streptococci as high as 44% (14). Long-term treatment with antibiotics has not been effective for psoriasis (41). Streptococci can exist in both the extracellular and intracellular spaces, forming intracellular reservoirs inside endothelial cells and macrophages within the tonsils (42). At best, antibiotic therapy only manages to reduce the bacterial load in the tonsils, leaving quiescent intracellular streptococci in the tonsillar epithelia and macrophages (42). These streptococci can reactivate, re-colonize and cause symptoms again, whereas tonsillectomy might remove this pool of streptococci.

This, and a number of other studies, support the association between psoriasis and streptococci (11–13, 20). Despite the lack of large controlled clinical trials, tonsillectomy is commonly advocated for patients with recurrent guttate psoriasis. Furthermore, according to a European expert group consensus, tonsillectomy may now be indicated for juvenile psoriasis patients with a positive streptococcal culture and more than 3 recurrent infections (43). Our findings might help to identify patients with plaque psoriasis who could benefit from tonsillectomy, but they need to be confirmed in prospective and more structured studies.

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The authors declare no conflicts of interest.

REFERENCES

1. Tsoi LC, Spain SL, Knight J, Ellinghaus E, Stuart PE, Capon F, et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. Nat Genet 2012; 44: 1341–1348.
2. Tsoi LC, Spain SL, Ellinghaus E, Stuart PE, Capon F, Knight J, et al. Enhanced meta-analysis and replication studies identify five new psoriasis susceptibility loci. Nat Commun 2015; 6: 7001.
3. Nair RP, Stuart PE, Nistor I, Hiremagalore R, Chia NV, Jenisch S, et al. Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. Am J Hum Genet 2006; 78: 827–851.
4. Eyre RW, Krueger GG. Response to injury of skin involved and uninvolved with psoriasis, and its relation to disease activity: Koebner and 'reverse' Koebner reactions. Br J Dermatol 1982; 106: 153–159.
5. Naldi L, Parazzini F, Brevi A, Peserico A, Veller Fornasa C, Grosso G, et al. Family history, smoking habits, alcohol consumption and risk of psoriasis. Br J Dermatol 1992; 127: 212–217.
6. Mallbris L, Larsson P, Bergqvist S, Vingård E, Granath F, Ståhle M. Psoriasis phenotype at disease onset: clinical characterization of 400 adult cases. J Invest Dermatol 2005; 124: 499–504.
7. Fry L, Baker B. Triggering psoriasis: the role of infections and medications. Clin Dermatol 2007; 25: 606–615.
8. Winfield JM. Psoriasis as a sequel to acute inflammations of the tonsils: a clinical note. J Cutan Dis 1916; 34: 441–443.
9. Whyte H, Baughman R. Acute guttate psoriasis and streptococcal infection. Arch Dermatol 1964; 89: 350–356.
10. Telfer N, Chalmers R, Whale K, Colman G. The role of streptococcal infection in the initiation of guttate psoriasis. Arch Dermatol 1992; 128: 39–42.
11. Wardrop P, Weller R, Marais J, Kavanagh G. Tonsillitis and chronic psoriasis. Clin Otolaryngol Allied Sci 1998; 23: 67–68.
12. Bartenjev I, Rogl Butina M, Potocnik M. Subclinical microbial infection in patients with chronic plaque psoriasis. Acta Derm Venereol 2000; Suppl 211: 17–18.
13. Gudjonsson J, Thorarinsson A, Sigurgeirsson B, Kristinsson K, Valdimarsson H. Streptococcal throat infections and exacerbation of chronic plaque psoriasis: a prospective study. Br J Dermatol 2003; 149: 530–534.
14. Sigurdardottir SL, Thorleifsdottir RH, Valdimarsson H, Johnston A. The association of sore throat and psoriasis might be explained by histologically distinctive tonsils and increased expression of skin-homing molecules by tonsil T cells. Clin Exp Immunol 2013; 174: 139–151.
15. de Waal AC, van de Kerkhof PC. Pustulosis palmoplantaris is a disease distinct from psoriasis. J Dermatolog Treat 2011; 22: 102–105.
16. Mrowietz U, van de Kerkhof PC. Management of palmoplantar pustulosis: do we need to change? Br J Dermatol 2011; 164: 942–946.
17. Andrews GC, Machacek GF. Pustular bacterids of the hands and feet. Arch Derm Syphilol 1935; 32: 837–835.
18. Yokoyama M, Hashigucci K, Yamasaki Y. Effect of tonsillectomy in patients with pustulosis palmaris et plantaris. Acta Otolaryngol 2004; 124: 1109–1110.
19. Takahara M. Clinical outcome of tonsillectomy for palmoplantar pustulosis and etiological relationship between palmoplantar pustulosis and tonsils. Adv Otorhinolaryngol 2011; 72: 86–88.
20. Thorleifsdottir RH, Sigurdardottir SL, Sigurgeirsson B, Olafsson JH, Sigurdsson MI, Petersen H, et al. Improvement of psoriasis after tonsillectomy is associated with a decrease in the frequency of circulating T cells that recognize streptococcal determinants and homologous skin determinants. J Immunol 2012; 188: 5160–5165.
21. Eysteinsdóttir JH, Ólafsson JH, Agnarsson BA, Lúðvíksson BR, Sigurgeirsson B. Psoriasis treatment: faster and long-standing results after bathing in geothermal seawater. A randomized trial of three UVB phototherapy regimens. Photodermatol Photoimmunol Photomed 2014; 30: 25–34.
22. Henseler T, Christophers E. Psoriasis of early and late onset:

characterization of two types of psoriasis vulgaris. J Am Acad Dermatol 1985; 13: 450–456.
23. Swindell WR, Xing X, Stuart PE, Chen CS, Aphale A, Nair RP, et al. Heterogeneity of inflammatory and cytokine networks in chronic plaque psoriasis. PLoS One 2012; 7: e34594.
24. Elder JT, Bruce AT, Gudjonsson JE, Johnston A, Stuart PE, Tejasvi T, et al. Molecular dissection of psoriasis: integrating genetics and biology. J Invest Dermatol 2010; 130: 1213–1226.
25. Valdimarsson H, Thorleifsdottir R, Sigurdardottir S, Gudjonsson J, Johnston A. Psoriasis – as an autoimmune disease caused by molecular mimicry. Trends Immunol 2009; 30: 494–501.
26. Sigurdardottir SL, Thorleifsdottir RH, Valdimarsson H, Johnston A. The role of the palatine tonsils in the pathogenesis and treatment of psoriasis. Br J Dermatol 2013; 168: 237–242.
27. Sigmundsdottir H, Gudjonsson JE, Valdimarsson H. Interleukin-12 alone can not enhance the expression of the cutaneous lymphocyte associated antigen (CLA) by superantigen-stimulated T lymphocytes. Clin Exp Immunol 2003; 132: 430–435.
28. Sigmundsdottir H, Sigurgeirsson B, Troye-Blomberg M, Good M, Valdimarsson H, Jonsdottir I. Circulating T cells of patients with active psoriasis respond to streptococcal M-peptides sharing sequences with human epidermal keratins. Scand J Immunol 1997; 45: 688–697.
29. Johnston A, Gudjonsson J, Sigmundsdottir H, Love T, Valdimarsson H. Peripheral blood T cell responses to keratin peptides that share sequences with streptococcal M proteins are largely restricted to skin-homing CD8(+) T cells. Clin Exp Immunol 2004; 138: 83–93.
30. Besgen P, Trommler P, Vollmer S, Prinz JC. Ezrin, maspin, peroxiredoxin 2, and heat shock protein 27: potential targets of a streptococcal-induced autoimmune response in psoriasis. J Immunol 2010; 184: 5392–5402.
31. Diluvio L, Vollmer S, Besgen P, Ellwart J, Chimenti S, Prinz J. Identical TCR beta-chain rearrangements in streptococcal angina and skin lesions of patients with psoriasis vulgaris. J Immunol 2006; 176: 7104–7111.
32. El-Rachkidy RG, Hales JM, Freestone PP, Young HS, Griffiths CE, Camp RD. Increased blood levels of IgG reactive with secreted Streptococcus pyogenes proteins in chronic plaque psoriasis. J Invest Dermatol 2007; 127: 1337–1342.
33. Shen Z, Wang G, Fan J-Y, Li W, Liu Y-F. HLA DR B1*04, *07-restricted epitopes on Keratin 17 for autoreactive T cells in psoriasis. J Dermatol Sci 2005; 38: 25–39.
34. Ferran M, Galvan AB, Rincon C, Romeu ER, Sacrista M, Barboza E, et al. Streptococcus induces circulating CLA(+) memory T-cell-dependent epidermal cell activation in psoriasis. J Invest Dermatol 2013; 133: 999–1007.
35. Arakawa A, Siewert K, Stohr J, Besgen P, Kim SM, Ruhl G, et al. Melanocyte antigen triggers autoimmunity in human psoriasis. J Exp Med 2015; 212: 2203–2212.
36. Rachakonda TD, Dhillon JS, Florek AG, Armstrong AW. Effect of tonsillectomy on psoriasis: a systematic review. J Am Acad Dermatol 2015; 72: 261–275.
37. Kvestad E, Kvaerner KJ, Røysamb E, Tambs K, Harris JR, Magnus P. Heritability of recurrent tonsillitis. Arch Otolaryngol Head Neck Surg 2005; 131: 383–387.
38. Gudjonsson J, Johnston A, Sigmundsdottir H, Valdimarsson H. Immunopathogenic mechanisms in psoriasis. Clin Exp Immunol 2004; 135: 1–8.
39. Gudjonsson J, Karason A, Runarsdottir E, Antonsdottir A, Hauksson V, Jónsson H, et al. Distinct clinical differences between HLA-Cw*0602 positive and negative psoriasis patients – an analysis of 1019 HLA-C- and HLA-B-typed patients. J Invest Dermatol 2006; 126: 740–745.
40. Cunningham MW. Pathogenesis of group A streptococcal infections. Clin Microbiol Rev 2000; 13: 470–511.
41. Owen C, Chalmers R, O'Sullivan T, Griffiths C. A systematic review of antistreptococcal interventions for guttate and chronic plaque psoriasis. Br J Dermatol 2001; 145: 886–890.
42. Osterlund A, Popa R, Nikkila T, Scheynius A, Engstrand L. Intracellular reservoir of Streptococcus pyogenes in vivo: a possible explanation for recurrent pharyngotonsillitis. Laryngoscope 1997; 107: 640–647.
43. Stahle M, Atakan N, Boehncke WH, Chimenti S, Dauden E, Giannetti A, et al. Juvenile psoriasis and its clinical management: a European expert group consensus. J Dtsch Dermatol Ges 2010; 8: 812–818.

Supplementary material to article by R. H. Thorleifsdottir et al. "Throat Infections are Associated with Exacerbation in a Substantial Proportion of Patients with Chronic Plaque Psoriasis"

Appendix S1. *Questionnaire on frequency of psoriasis exacerbation during or after a sore throat.*

The frequency of exacerbation of psoriasis during or after a sore throat

Please answer as accurately as possible by writing or ticking the appropriate boxes for each question

1. **How old are you:**_____
2. **What is your gender:**
   - ○ Male
   - ○ Female
3. **How old were you when you first noticed you had psoriasis:**_____
4. **Did the first signs of your psoriasis appear during or within 3 weeks of having a sore throat (painful inflammation or infection or in the throat) or a streptococcal throat infection (strep throat)?**
   - ○ Yes
   - ○ No
   - ○ Unsure
5. **Has a dermatologist confirmed that you have psoriasis?**
   - ○ Yes
   - ○ No
   - ○ Unsure
6. **Which of the following statements describe your psoriasis best? (You may tick more than one box)**
   - ○ I have plaque psoriasis with raised, circular-to-oval red patches covered with silvery scales, for example on my scalp and/or knees, elbows and lower back
   - ○ I have guttate psoriasis with small (1-10mm), dot-like, fine scaled rash, mostly on my trunk and/or upper arms and legs
   - ○ I have palmoplantar pustulosis (PPP) that appears as white blisters (pustules) surrounded by red skin in my palms and/or soles
   - ○ I have psoriasis in my nails (all or some of these changes);
     - Pitting (small nail depressions, less than 1 mm in diameter)
     - Discolouration (circular areas resembling an oil drop)
     - Subungual hyperkeratosis (thickening of the nails)
     - Onycholysis (separation of the nail from the nail bed)
   - ○ I have psoriasis arthritis diagnosed by a dermatologist or rheumatologist
   - ○ None of the above applies to my psoriasis
7. **Have you experienced worsening of your psoriasis during or within 3 weeks after having a sore throat?**
   - ○ Yes
   - ○ No
   - ○ Unsure
8. **How many times during the past 12 months have you had a sore throat?**

○ Never	○ 4	○ 8
○ 1	○ 5	○ 9
○ 2	○ 6	○ >10
○ 3	○ 7	

9. **Have you been diagnosed with a streptococcal throat infection (strep throat) by a physician, a throat swab or a rapid strep test?**
   - ○ Yes
   - ○ No
   - ○ Unsure
10. **If yes, how many times**_____
11. **Did you notice worsening of your psoriasis during or within 3 weeks after having a streptococcal throat infection (strep throat)?**
   - ○ Yes
   - ○ No
   - ○ Unsure
12. **Have you experienced worsening of your psoriasis in association to the following? (You may tick more than one box)**
   - ○ General sickness
   - ○ Stress
   - ○ Cold weather
   - ○ Alcohol
   - ○ Food, what food_____
   - ○ Pregnancy
   - ○ Medication, which medication?_____
   - ○ Other, what?_____
13. **Have you had your tonsils removed (had tonsillectomy)?**
   - ○ Yes
   - ○ No
   - ○ Unsure
14. **If yes, at what age were your tonsils removed**_____
15. **Did your psoriasis improve after the tonsillectomy?**
   - ○ Yes
   - ○ No
   - ○ Unsure