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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

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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: 5160–5165.

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 α6β1 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 IFN-γ. 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-
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 up-regulated 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 old, 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, in particular systemic 1 y before the study entry and after 2, 12, and 24 mo. The patients’ need for anti-psoriasis 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 (PSI) (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, 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 then selected from 16 peptide pools, 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% CO2 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 150 μl cold 4% (w/v) 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 FACS Calibur 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>
<thead>
<tr>
<th>Table I. Baseline demographic information and disease characteristics of the patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX Group</td>
</tr>
<tr>
<td>(n = 15)</td>
</tr>
<tr>
<td>Males/females</td>
</tr>
<tr>
<td>Age, y (±SD)</td>
</tr>
<tr>
<td>Body mass index ± SD</td>
</tr>
<tr>
<td>Duration of psoriasis, y (±SD)</td>
</tr>
<tr>
<td>Age at onset, y (range)</td>
</tr>
<tr>
<td>Family history (n)</td>
</tr>
<tr>
<td>Psoriatic arthritis (n)</td>
</tr>
<tr>
<td>Psoriatic nails (n)</td>
</tr>
<tr>
<td>HLA-Cw*0602-positive</td>
</tr>
<tr>
<td>PASI score ± SD</td>
</tr>
<tr>
<td>Prior treatments (n)</td>
</tr>
</tbody>
</table>

Values are means unless specified otherwise.
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 sub-populations and then expressed as an average for each patient after square root normalization.

Data were tested for normality using the Kolmogorov–Smirnov 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 and when 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 improvement was observed in the controls (D).

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

<table>
<thead>
<tr>
<th>Treatment 6-24 mo</th>
<th>TX Group (n = 15)</th>
<th>Control Group (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical, n (%)</td>
<td>4 (27)</td>
<td>12 (86)</td>
</tr>
<tr>
<td>Phototherapy, n (%)</td>
<td>3 (20)</td>
<td>3 (21)</td>
</tr>
<tr>
<td>Systemic, n (%)</td>
<td>1 (7)</td>
<td>1 (7)</td>
</tr>
</tbody>
</table>

*Corticosteroid creams and vitamin D analog creams.*
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 tonsillar 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).

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
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 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).

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 ($r = -0.023, p = 0.896$). 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 psoriasis lesions do not contain discrete populations of Th1 and Th17 T cells. This is probably partly because epitope spreading makes it difficult to identify primary and dominant autoepitopes in humans 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 immunomodulatory 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

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