T helper 1 to T helper 2 shift in cytokine expression: an autoregulatory process in superantigen-associated psoriasis progression?

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Psoriasis is an inflammatory skin disorder characterized by increased activation of CD4+ T lymphocytes, and systemic and local overexpression of pro-inflammatory cytokines such as interleukin 2 (IL-2), gamma interferon (IFN-γ), IL-6 and tumour necrosis factor alpha, indicating that immunopathogenesis of the disease is T helper 1 (Th1) mediated. Several studies suggest a pivotal role of bacterial superantigens in the initiation and/or exacerbation of this illness. This study was conducted to assess the systemic Th1/Th2 imbalance in Indian psoriasis patients presenting with variable duration of disease by studying systemic superantigen-stimulated peripheral blood mononuclear cell (PBMC) cytokine expression. PBMCs were isolated and stimulated in vitro with superantigens (streptococcal pyrogenic exotoxin A and staphylococcal enterotoxin B), and the cytokines released (IFN-γ for a Th1 response, and IL-4 and IL-10 for a Th2 response) were assayed. In contrast to controls, psoriasis patients in the early course of disease were characterized by significantly increased expression of the pro-inflammatory cytokine IFN-γ, whilst a shift towards IL-10 secretion (Th2 response) was observed in those presenting with increased duration of disease. These observations suggest a possible shift from a Th1 to a Th2 cytokine response with superantigen-associated progression for the duration of psoriasis, perhaps as an adaptive process by the immune system in an attempt to downregulate abnormal inflammatory Th1 immune responses.

INTRODUCTION

Psoriasis is a genetically determined, inflammatory and proliferative disease of the skin (Valdimarsson et al., 1995). Studies suggest that cytokines, released by activated T lymphocytes, initiate and maintain the psoriatic process by stimulating keratinocyte proliferation (Valdimarsson et al., 1986). The precise mechanism by which activated T cells trigger psoriasis is as yet undefined. Increasing evidence suggests an important role for superantigens in the initiation and/or early propagation of this illness. Colonization and infection with Streptococcus pyogenes and Staphylococcus aureus have been shown to trigger psoriasis via release of their superantigenic toxins (Leung et al., 1993). The superantigens bind to major histocompatibility complex class II molecules on antigen-presenting cells and to T-cell-receptor-bearing specific Vβ elements (Marrack & Kappler, 1990). This tri-molecular interaction leads to a massive proliferation of T cells and the elaborate systemic release of pro-inflammatory cytokines, including interleukin 1 (IL-1), IL-6, gamma interferon (IFN-γ), tumour necrosis factor alpha and others (Jupin et al., 1988; Kum et al., 1993; Parsonnet, 1989). Several disorders are known to be associated with an imbalance between the T helper 1 (Th1) and Th2 arms of the cellular immune system (Szegedi et al., 2003). Psoriasis is characterized by a Th1-type cytokine response as IFN-γ and IL-2 are predominantly expressed in the inflamed skin lesions (Uyemura et al., 1993). In contrast, a relatively low level of IL-10, a type 2 cytokine, has been demonstrated, suggesting an ineffective counter-regulatory capacity in psoriasis. However, limited data are available regarding the altered Th1/Th2 balance in Indian psoriasis patients. An understanding of the immunopathological processes in psoriasis would offer the opportunity for more targeted therapeutic interventions and improvements in clinical outcome.
Psoriasis is highly variable in its expression and progression in individuals. However, clinicians tend to initiate empirical treatment for psoriasis irrespective of the prevailing immune status of the patient. Often a patient is unable to afford the follow-up and long-term treatment for this chronic ailment, and the resultant dropout rate is high. The importance of an understanding of the immune status of psoriasis patients needs to be highlighted, as targeting immune responses responsible for the formation of psoriasis plaques will lead to better management and improved clinical outcome for patients. An awareness of the prevailing immunological profile in the individual patient will enable the clinician to tailor the therapy, thus optimizing management. Keeping this in mind, we conducted this pilot study to attempt to define superantigen-provoked cytokine changes (IFN-γ for a Th1 response, and IL-4 and IL-10 for a Th2 response) in psoriasis patients.

METHODS

Patients. A total of 21 patients with psoriasis vulgaris (PV) presenting with initial active disease or a relapse was included in the study. All of the patients had active disease and had not been treated with any antibiotic, steroid or immunosuppressive drugs for at least 4 weeks prior to inclusion in this study. The inclusion and exclusion criteria are outlined in Table 1.

The mean age was 39 years (range 20–65 years) and the patients comprised 15 men and 6 women (Table 2). The duration of disease ranged from 1 week to 25 years (mean duration 54 ± 45.9 months). The number of relapses, which increases with the duration of the disease, varied from 0 to 12.

Throat and nasal swabs were collected from all of the patients upon inclusion in the study. Only one patient had throat carriage of Streptococcus pyogenes, whilst none had a significant anti-streptolysin O titre. Staphylococcus aureus was isolated from the anterior nares of eight patients. Ten age-matched healthy volunteers (six men and four women) were also evaluated as controls. None of the controls had throat carriage of Streptococcus pyogenes or Staphylococcus aureus in their throat or nose.

Reagents. The mitogen phytohaemagglutinin (PHA) and the superantigen staphylococcal enterotoxin B (SEB) were purchased from Sigma Chemicals. The superantigen streptococcal pyrogenic exotoxin A (SPEA) was purchased from Toxin Technology.

Isolation of peripheral blood mononuclear cells (PBMCs) and stimulation with superantigens. PBMCs were isolated from 20 ml heparinized venous blood, drawn from patients and controls upon inclusion in the study, by density-gradient centrifugation with Ficoll–Hypaque (Sigma Diagnostics). In four 15 ml sterile conical centrifuge test tubes, 5 ml Ficoll-Hypaque was added and 5 ml whole blood was layered gently onto the solution along the sides of the walls of the tube to avoid mixing of the two. The tubes were then centrifuged at 400 g for 30 min at room temperature. Lymphocytes and monocytes formed an opaque ring (buffy coat containing PBMCs) at the interface of the gradient and plasma. The upper plasma layer was carefully discarded and the opaque interface was transferred carefully into a fresh conical centrifuge tube with a Pasteur pipette. The separated cells were washed twice in Hank’s balanced salt solution (Sigma Chemicals), and the number of viable cells was determined by trypan blue (a vital stain) exclusion and with a haemocytometer. Viable cells excluded the stain, whilst non-viable cells were stained blue, and cells were counted in each of the four corner squares of a Neubauer counting chamber; cells were then resuspended at a concentration of 1 × 10⁶ cells ml⁻¹ in RPMI 1640 (Sigma Chemicals), supplemented with 10% heat-inactivated fetal calf serum, 2 mmol glutamine 1⁻¹, 100 U penicillin ml⁻¹, 100 µg streptomycin ml⁻¹ and 25 µg amphotericin B ml⁻¹. A volume of 100 µl cell suspension or 1 × 10⁵ cells per well were seeded into a 24-well flat-bottomed plate (Tarson) and cultured in the presence or absence of PHA (1 µg ml⁻¹) or the superantigen SPEA or SEB (both at 10 ng ml⁻¹). Three wells were used for each stimulant. The cells were cultured for 24, 48 and 96 h at 37 °C in a humidified 5% CO₂ atmosphere, and each sample was tested in triplicate.

Cytokine assay. The supernatants of unstimulated and stimulated cultures were harvested after 24, 48 and 96 h of incubation and frozen at −20 °C until assayed for IFN-γ for a Th1 immune response, and IL-4 and IL-10 for a Th2 response, using commercially available ELISA kits (Diaclone Research). The serum was removed rapidly and carefully from the red cells after clotting by centrifugation at approximately 1000 g for 10 min. Aliquots of serum samples were then immediately frozen at −20 °C for cytokine assays. All protocols were performed in triplicate following the manufacturer’s instructions. Results were expressed as pg ml⁻¹.

Statistical analysis. Statistical analysis was performed using SPSS (version 13) to analyse the changes in cytokine levels following challenge with SPEA or SEB compared with controls. IFN-γ levels were expressed as group mean ± sd. A repeated-measures ANOVA design and Dunnett’s test (Dawson-Saunders & Trapp, 1990) were performed to compare the control group with test groups. For unchallenged PBMCs and serum, one-way ANOVA and Dunnett’s test were performed for comparison of the test group with the control group. A Mann–Whitney test was applied to compare IL-10 production in the

Table 1. Inclusion and exclusion criteria in the study

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
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</table>
RESULTS AND DISCUSSION

Measurement of cytokine expression by superantigen-stimulated PBMCs

Patients with early psoriasis
No significant difference was observed in cytokine expression by unstimulated and PHA-stimulated PBMCs of PV patients presenting with up to five episodes of acute psoriasis and normal healthy controls. The PBMCs from these patients exhibited significantly increasing concentrations of IFN-γ in culture supernatants over 24, 48 and 96 h following stimulation with SPEA (group mean peak levels of 121.5 ± 35.13 pg ml⁻¹ at 96 h) compared with normal controls (31.1 ± 3.0 pg ml⁻¹). The lower limit for IFN-γ detection was 5 pg ml⁻¹. Similarly, significantly enhanced levels of IFN-γ were also observed in the culture supernatants of PBMCs of patients stimulated with SEB (group mean peak levels of 62.7 ± 33.4 pg ml⁻¹ at 96 h) compared with controls (30 ± 4.2 pg ml⁻¹), although the rise was twofold higher with SPEA than with SEB (Table 3).

Non-significant expression of IL-4 was observed following stimulation of PBMCs of patients and controls with PHA. IL-4 levels were below the detectable limit of the ELISA (0.5 pg ml⁻¹) in the culture supernatants of both patients and controls after stimulation with the superantigens SPEA and SEB, and no differences were observed (data not shown).

IL-10 expression by PBMCs stimulated with SPEA and SEB showed no significant difference between the patients (median levels at 96 h were 13.0 and 12.5 pg ml⁻¹, respectively) and normal controls (12.5 pg ml⁻¹ for each superantigen) (Table 4). The lower limit of IL-10 detection by ELISA was 5 pg ml⁻¹. These data suggested that patients with early psoriasis are characterized by significantly increased secretion of Th1 cytokines, without any significant difference in expression of Th2 cytokine, compared with the control group.

Patients with long-standing psoriasis
There was no significant difference in expression of cytokines following stimulation with the mitogen PHA among any of the study groups. Patients who presented with six or more relapses failed to express a significant rise in IFN-γ levels following challenge of their PBMCs with either SPEA or SEB compared with healthy controls (Table 3). The elevated IL-10 levels observed were significant (P<0.05) in the PBMC culture supernatants of these patients compared with controls. This rise in IL-10 concentration was detected over 24–96 h of culture with SPEA (median value at 96 h was 16.0 pg ml⁻¹) and to a lesser extent with SEB (15.0 pg ml⁻¹) (Table 4). The results therefore reflected the fact that, with disease progression, Th1 cytokine expression decreases, whilst Th2 cytokine expression is higher compared with controls.

Table 2. Demographic profile of the clinical characteristics of PV patients and controls

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Age (years)</th>
<th>Men</th>
<th>Women</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean (± SD)</td>
<td>No.</td>
<td>Percentage</td>
</tr>
<tr>
<td>PV patients</td>
<td>20–65</td>
<td>39 (9.5)</td>
<td>15</td>
<td>71.4</td>
</tr>
<tr>
<td>Controls</td>
<td>20–50</td>
<td>32 (6.5)</td>
<td>6</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 3. Group IFN-γ levels (mean ± SD in pg ml⁻¹) in PV patients and controls

The ANOVA P value (F-test) was <0.001 for all groups. Values in parentheses indicate P values for Dunnett’s test.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>Unchallenged PBMCs</th>
<th>PBMCs challenge with:</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SPEA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>0–5 relapses (PV)</td>
<td>12</td>
<td>31.6 ± 5.4 (0.316)</td>
<td>38.42 ± 6.03 (&gt;0.05)</td>
<td>55.7 ± 16.6 (&lt;0.05)</td>
</tr>
<tr>
<td>&gt;5 relapses (PV)</td>
<td>9</td>
<td>13.6 ± 0.4 (0.001)</td>
<td>13.44 ± 0.46 (&lt;0.05)</td>
<td>13.44 ± 0.46 (&lt;0.05)</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>29.2 ± 4.3</td>
<td>32.10 ± 4.41</td>
<td>29.00 ± 2.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>SPEA</td>
<td></td>
<td></td>
<td>42.08 ± 8.8 (&gt;0.05)</td>
<td>39.38 ± 5.5 (&gt;0.05)</td>
</tr>
<tr>
<td>SEB</td>
<td></td>
<td></td>
<td>13.00 ± 0.43 (&gt;0.05)</td>
<td>13.63 ± 1.05 (&gt;0.05)</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td>28.8 ± 2.9</td>
<td>30.60 ± 3.17</td>
</tr>
<tr>
<td>SPEA</td>
<td></td>
<td></td>
<td>36.4 ± 7.0</td>
<td></td>
</tr>
<tr>
<td>SEB</td>
<td></td>
<td></td>
<td>13.5 ± 0.5 (0.001)</td>
<td></td>
</tr>
</tbody>
</table>
Significantly higher levels of IFN-\(\gamma\) were observed in patients with fewer than six relapses (36.4 ± 7 pg ml\(^{-1}\)) compared with controls (23.1 ± 4.2 pg ml\(^{-1}\)), whilst IL-10 levels were low in these patients (13.0 pg ml\(^{-1}\)). In contrast, in patients with more than five relapses, IL-10 levels were significantly higher (34.0 pg ml\(^{-1}\)) than controls (12.5 pg ml\(^{-1}\)). IFN-\(\gamma\) levels were significantly decreased in these patients (Tables 3 and 4).

Serum cytokines

It is well established that psoriasis is a T-cell-dependent immune disease (Wrone-Smith & Nickoloff, 1996). The effector T cells involved in this hyperimmune response are predominantly of the Th1 phenotype (Uyemura et al., 1993). Our results also demonstrated a similar Th1-type immune response in the serum and in vitro PBMC cultures of psoriasis patients. Circulating levels of the cytokines IFN-\(\gamma\) and IL-8 were also observed to be elevated in psoriasis patients by Jacob et al. (2003), and correlated with the parameters of disease severity, whilst IL-10 and IL-12 levels were decreased. An important observation in our study was the significant elevation of IFN-\(\gamma\) levels in patients presenting with either the first episode or up to five relapses of the disease. This finding indicates an active Th1 (type 1) immune response occurring during the early course of the disease. However, it was observed that the IFN-\(\gamma\) levels waned in patients presenting with more than five relapses of the disease, associated with a significant rise in IL-10 production. This suggests a shift in the polarization from a Th1- to a Th2-type immune response as the disease progresses in duration (persistent and late-stage disease). High IL-10 positivity has been also reported by Austin et al. (1999) and Szegedi et al. (2003) in psoriatic CD4\(^+\) and CD8\(^+\) cells.

It is known that effector Th cell subsets regulate the activity of one another (Wrone-Smith & Nickoloff, 1996). Thus, the production of IFN-\(\gamma\) by Th1 effector cells inhibits Th2 cell differentiation and proliferation; simultaneously, the Th1 cells themselves lose functional responsiveness to IFN-\(\gamma\) at the receptor level. The production of cytokines by Th1-differentiated cells is inhibited indirectly by the effect on antigen-presenting cells of IL-10, secreted by Th2 cells (Abbas, 1996; Moore et al., 1993; O’Garra, 1998; O’Garra et al., 1997). IL-10 acts on the activated macrophages to terminate T-cell activation by the pro-inflammatory cytokines and return the system to its resting state as the microbial infection is eradicated (Goldsbys, et al., 2002). It also promotes the development of a type 2 cytokine pattern by inhibiting IFN-\(\gamma\) production by T lymphocytes and natural killer cells, and acts as a driving force in the downregulation of the Th1 hyperimmune response, eventually leading to recovery of the host. Therefore, as psoriasis is a typical chronic inflammatory disorder, the shift observed in our study may be an adaptive attempt by the immune system to autoregulate/downregulate abnormal Th1 inflammatory responses.

In our study, this Th2 response was characterized by a rise in IL-10 levels unaccompanied by a rise in IL-4 levels. It has been reported previously that induction of regulatory Th cells following repeated relapses due to superantigen stimulation leads to a hyporesponsive state characterized by clonal deletion and anergy of the remaining superantigen-reactive T-cell subset (Sundstedt, et al., 1997). These anergic/downregulated Th cells characteristically produce high levels of IL-10 and low levels of IL-4, whilst the production of Th1 cytokines, including IL-2, IFN-\(\gamma\) and tumour necrosis factor alpha, are markedly inhibited (Sundstedt et al., 1997). Thus, the regulatory T cell (Tr1) subset seems to play a profound downregulatory role in suppressing the potentially harmful Th1 immune response induced by superantigens (Cameron et al., 2001). T-cell deletion/anergy could also be a possible mechanism underlying repeated relapses, due to either superantigen stimulation or other factors leading to a significant decline in Th1 cytokines; hypo responsiveness of PBMCs to the streptococcal superantigens reported in the past have been suggested to be due to T-cell anergy (Horiuchi et al., 1998). Our results suggest that a type 1 immune response may have a prominent role in initiation/exacerbation of psoriasis skin lesions, whilst type 2 responses may inhibit type 1 cytokine production and thus the inflammatory responses. Thus, the rapidity and magnitude of IL-10 induction may be the critical determinant of the ultimate well-being and/or disease remission in the patient.

SPEA appeared to be able to evoke much greater cytokine release into the culture supernatants of PBMCs compared with SEB in patients with early PV (fewer than six relapses).

**Table 4. Median values for IL-10 levels (pg ml\(^{-1}\)) in PV patients and controls**

Values in parentheses indicate the \(P\) value.

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<tr>
<td>&gt;5 relapses (PV)</td>
<td>9</td>
<td>12.5</td>
<td>13.0</td>
<td>13.5</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>
in our study. Also, the immune response (cytokine release) exhibited by mitogen (PHA)-stimulated PBMCs by patients and normal controls were not significant. Thus, the hyperimmune response mounted to streptococcal superantigen by psoriasis patients included in our study appeared to be specific for this antigen. Patients presenting with a greater duration of disease and an increasing number of relapses appeared to become refractory to stimulation of their PBMCs with SPEA or SEB. This hypothesis would support the initiation of empirical management of patients with early psoriasis with antibiotics to cover Gram-positive bacterial infections (*Streptococcus pyogenes* and *Staphylococcus aureus*) to induce early remission. It also implies that empirical antibiotics to bacteria isolated/identified once locally from the lesions, throat or anterior nares can be initiated at the onset of relapses of psoriasis early in the course of the disease (possibly up to five relapses in our study). However, further clinical studies would be needed for the confirmation of this hypothesis.

**Conclusion**

In conclusion, this study provides evidence for the dominance of a type 1 immune response in early psoriasis and raises the possibility that polarization to a Th2 response with the production of classical anti-inflammatory cytokines, such as IL-10, may occur with the progression and duration of psoriasis. The cytokine imbalance represents an interesting therapeutic target; therefore, evaluation of parameters such as cytokine production *in vitro* and *in vivo* in individual patients may be useful in the diagnosis and monitoring of psoriasis.

**REFERENCES**


Austin, L. M., Ozawa, M., Kikuchi, T., Walters, I. B. & Krueger, J. G. (1999). The majority of epidermal T cells in psoriasis vulgaris lesions can produce type 1 cytokines, interferon-γ, interleukin-2, and tumor necrosis factor-α, defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: a type 1 differentiation bias is also measured in circulating blood T cells in psoriatic patients. *J Invest Dermatol* 113, 752–759.


