

Decreased T Cell Reactivity to Trypsinized Group A, Type M22 Streptococci in Psoriasis

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The proliferative responses of peripheral blood mononuclear cells from patients with guttate and chronic plaque psoriasis to streptococcal M protein were investigated using whole and trypsinized group A M22-positive streptococci. Peripheral blood mononuclear cell responses to whole type M22 group A streptococci were significantly increased in guttate, but not chronic plaque, psoriasis patients compared to 17 non-psoriatic controls ($p < 0.05$; $n = 17$). A significant reduction of this response was observed in both guttate ($p < 0.001$; $n = 17$) and chronic plaque ($p < 0.01$; $n = 27$) psoriatic patients, but not in the control group, after repeated trypsinization to remove M protein from the streptococci. Furthermore, the difference between the peripheral blood mononuclear cell response to untrypsinized and trypsinized streptococci was significantly greater in the guttate patients than in the controls ($p < 0.02$). This preliminary study has shown an increased reactivity of T lymphocytes with specificity for trypsin-sensitive protein expressed by type M22 streptococci in the peripheral blood of patients with psoriasis. Key words: peripheral blood; M proteins; proliferation.

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The association between psoriasis and group A, β haemolytic streptococcal infections is well documented. Acute guttate psoriasis is frequently preceded by an acute streptococcal infection about 1–2 weeks before the onset of skin lesions (1, 2). Furthermore, exacerbation of chronic plaque lesions following streptococcal tonsillitis has also been reported (1). In a recent study, 26% of patients with acute guttate psoriasis had positive group A streptococcal throat cultures, compared to 6% of matched controls; raised antibody titres to streptococcal antigens were present in 58% of the group (3). Among 13 isolates of *Streptococcus pyogenes* from the patients in this study, 10 different M serotypes were detected, 7 of which were among the 12 most common strains isolated from the community at the time. More than 80 different M serotypes of group A streptococci have been identified so far with a conserved amino acid sequence at the C-terminus but which vary at the distal N-terminal region (4). These findings suggest that, in contrast to rheumatic fever in which there is an association with less than a dozen M serotypes (5), the ability of group A streptococci to trigger guttate psoriasis may not be serotype-specific (3).

Patients with psoriasis appear to have an altered response to streptococcal antigens *in vivo* (6, 7), whilst *in vitro*, both guttate (8) and chronic plaque (9) psoriatic patients have been shown to have enhanced lymphocyte responses to group A streptococcal antigens. Furthermore, group A streptococcal antigen-specific T

cell lines (TCL) have been consistently isolated from the skin of patients with streptococcal-associated guttate psoriasis (10). The response of T cell clones isolated from one of these guttate TCL to whole group A streptococcal preparations was shown to be HLA-DR restricted and inhibited by anti-DR in a dose-dependent manner (10).

The aim of this preliminary study was, therefore, to investigate whether peripheral blood T lymphocytes from patients with guttate or chronic plaque psoriasis showed an altered proliferative response to trypsin-sensitive streptococcal (M) protein compared to non-psoriatic individuals. Since a purified preparation of M22 protein was not available, whole group A, M22-positive streptococci, which have previously been shown to be stimulatory for T cells isolated from skin lesions of guttate psoriasis (10), were used before and after trypsinization to remove M protein.

MATERIALS AND METHODS

Patient samples

Heparinized blood was taken from 27 patients with chronic plaque psoriasis of varying activity, and 17 patients with acute guttate psoriasis (some of which also had chronic plaque lesions). Guttate psoriasis was defined as the sudden eruption of small lesions on the trunk. The age of the patients ranged from 17–56 years (guttate) and 19–75 years (chronic plaque). The majority of the patients had received no topical treatment for at least 1 week, or systemic treatment for at least 1 month prior to the study, with exception of 7 patients receiving topical steroids (3 guttate, 4 chronic plaque), 2 PUVA (chronic plaque) and 1 chronic plaque patient treated with dithranol. It should be noted that the responses of the 2 patients receiving PUVA showed no difference to those of the untreated patients; their responses were therefore included in the study.

Control subjects consisted of 14 healthy adult members of staff and 3 patients attending the Dermatology clinic who had no known history of psoriasis.

Streptococcal antigen preparations

Strep-M22 was a group A streptococcal throat isolate typed as M22, T12, from a patient with psoriasis which was trypsinized 3 times to remove M protein and designated as Strep-M22T.

These group A streptococcal preparations, gifts of Dr I. Jonsdottir, Landspítalinn, Reykjavik, were added to cell cultures at dilutions of $1:10^2$, $1:10^3$ and $1:10^4$.

Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were isolated from 20 ml of heparinized blood by Ficoll-Hypaque centrifugation (Lymphoprep, Nyegaard & Co, A/S Oslo, Norway). The cells were washed twice in RPMI 1640 containing 2 mg/ml bicarbonate supplemented with 2 mM glutamine, 0.1 mg/ml streptomycin, 0.6 mg/ml benzylpenicillin and resuspended at 10^6 cells/ml in the same medium plus 10% heat-inactivated A + serum.

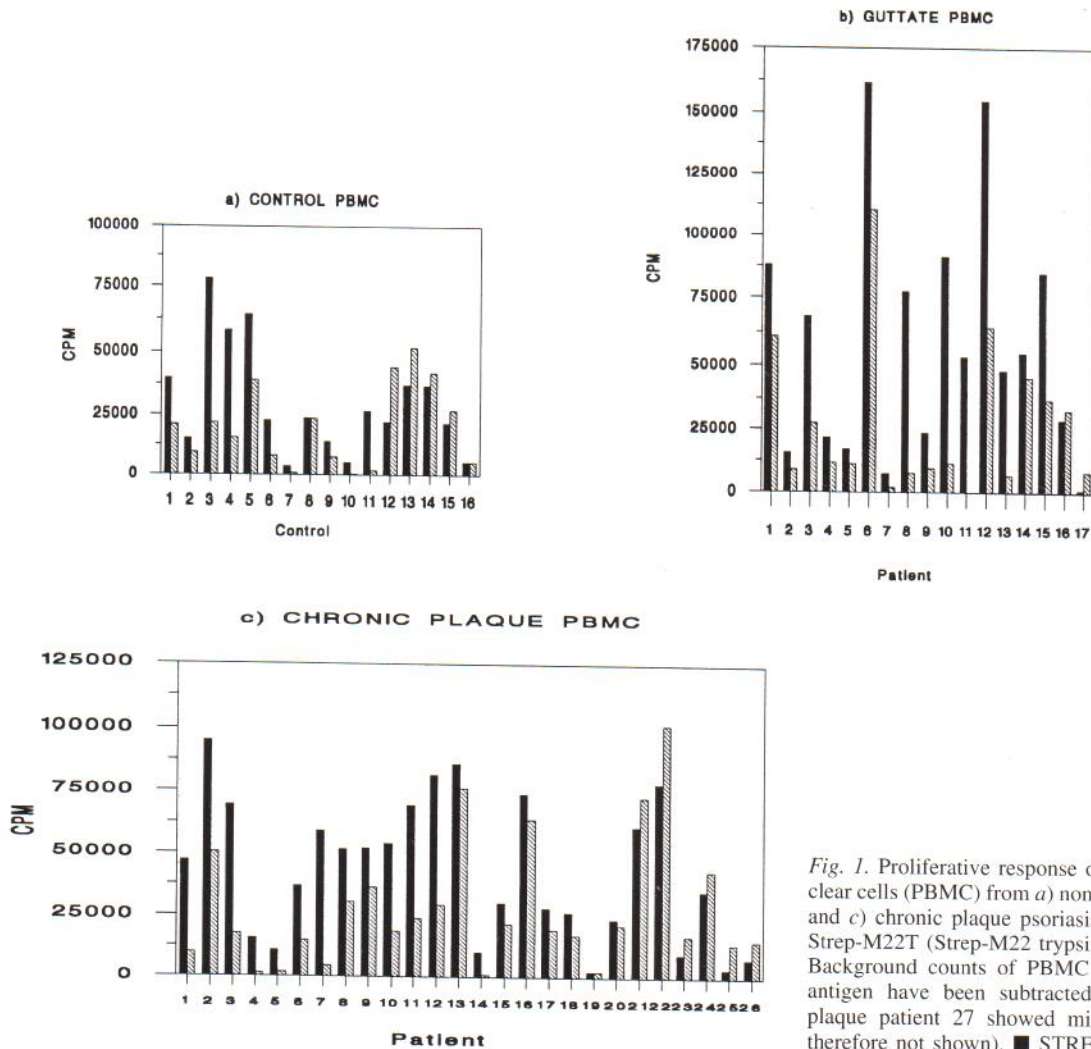


Fig. 1. Proliferative response of peripheral blood mononuclear cells (PBMC) from *a*) non-psoriatic controls, *b*) guttate and *c*) chronic plaque psoriasis patients to Strep-M22 and Strep-M22T (Strep-M22 trypsinized to remove M protein). Background counts of PBMC cultured in the absence of antigen have been subtracted. (Control 17 and chronic plaque patient 27 showed minimal proliferation and are therefore not shown). ■ STREP-M22 ▨ STREP-M22T.

Proliferation assay

Triplicates of 10^5 PBMC were set up with 100 μ l of each antigen (total volume = 200 μ l) in flat-bottomed 96-well plates (Streilin, Hounslow, UK) and cultured for 7 days in a humidified incubator with 5% $\text{CO}_2/95\%$ air at 37°C. Six hours before harvesting, each well was pulsed with 4 $\mu\text{Ci/ml}$ of tritiated thymidine, specific activity 5 Ci/mmol (Amersham Int., Amersham, Bucks). Proliferation was assessed by thymidine incorporation measured in a β plate scintillation counter and expressed as mean cpm \pm SD.

Statistical analysis

The differences between groups of data were analysed by the Wilcoxon unpaired or signed rank sum tests.

RESULTS

Strong PBMC proliferative responses to whole type M22 group A streptococci (Strep-M22) were observed in the majority of both normal and psoriatic individuals tested, but the extent of the response varied widely (Fig. 1). As a group, the PBMC responses of the patients with guttate psoriasis were significantly increased compared to the non-psoriatic controls ($p < 0.05$) (Table I). The chronic plaque PBMC responses were also increased but did not reach statistical significance (Table I).

After repeated trypsinization to remove M protein, the re-

sponse to Strep-M22 was significantly reduced in both guttate ($p < 0.001$; $n = 17$) and chronic plaque ($p < 0.01$; $n = 27$) groups (Fig. 1, Table I). A decrease was observed in the majority of patients in both groups (15/17 guttate; 20/27 chronic plaque). Furthermore, the difference between the response to Strep-M22 and Strep-M22T by PBMC from the guttate psoriatic patients was significantly greater than that observed for the non-psoriatic patients ($p < 0.02$).

However, Strep-M22T induced substantial proliferation of PBMC in most of the psoriatic patients (Table I) and, furthermore, in 2 guttate and 7 chronic plaque psoriasis patients an increase in response to Strep-M22T compared to Strep-M22 was observed (Fig. 1).

In contrast, in the non-psoriatic control group no consistent pattern of response to Strep-M22T compared to Strep-M22 was noted, with only approximately half of the individuals showing decreased proliferation compared to the untrypsinized preparation (Fig. 1).

DISCUSSION

This study has shown increased T cell reactivity to a trypsin-sensitive protein expressed by M22-positive group A strepto-

Table I. Median peripheral blood mononuclear cell (PBMC) responses of control and psoriatic individuals to Strep-M22 and trypsinized Strep-M22 (Strep-M22T)

Range of responses in cpm are shown below the median values. Background counts of PBMC cultured in the absence of antigen have been subtracted.

PBMC	STREP-M22	STREP-M22T
CONTROLS (n = 17)	22,868 86–78,208	15,593 90–52,314
GUTTATE (n = 17)	*53,527 963–161,725	***11,146 0–110,478
CP (n = 27)	36,943 197–95,151	**19,678 799–101,809

* $p < 0.05$ guttate vs controls

** $p < 0.01$ Strep-M22 vs Strep-M22T

*** $p < 0.001$ Strep-M22 vs Strep-M22T

cocci in the blood of patients with guttate and chronic plaque psoriasis compared to that of normal individuals.

Only a limited number of the > 80 different M serotypes have been purified, sequenced and subsequently cloned, for example M5, M6 and M24 (11–13). As it has not been possible to obtain purified M protein preparations, whole and trypsinized streptococci have been used to study psoriatic T lymphocyte responses to M22 antigens; strains expressing the M22 serotype have previously been isolated from patients with psoriasis (3).

PBMC from patients with guttate, and to a lesser extent chronic plaque, psoriasis showed increased proliferation compared to non-psoriatic individuals to whole type M22 group A streptococci. PBMC from chronic plaque patients have previously shown a significantly increased proliferative response to a mixture of 4 group A streptococci (including M types 4 and 12) (9).

After repeated trypsinization, a significant reduction in the response to Strep-M22 was observed in the majority of psoriatic patients of both clinical types. Although similar decreases were observed in approximately half of the normal individuals, this was not significant for the group. The response to the trypsinized streptococci was, however, similar in the patients and controls; this response was probably directed against trypsin-resistant, non-M streptococcal proteins and/or M protein that had not been removed by trypsinization.

These findings suggest that the increased response of patients with psoriasis to the whole type M22 streptococci may be due to an increased T cell reactivity to trypsin-sensitive streptococcal proteins. M-like proteins, such as type-specific M or non-type-specific M-associated proteins (17) are major surface proteins of group A streptococci that are known to be trypsin-sensitive, although other trypsin-sensitive proteins cannot be excluded. In order for us to obtain evidence in support of T cell specificity for M protein, anti-M22 antibody was added, in the presence of whole type M22 streptococci, to PBMC from psoriatic patients (data not shown). However, although inhibition of the proliferative response to the streptococci could be demonstrated, it was not possible to confirm the antibody's specificity. Although PBMC from normal and psoriatic individuals proliferate in response to crude extracts of M22 proteins (unpublished obser-

vations), confirmation of increased numbers of M protein-specific T cells in psoriatic patients will require the availability of purified M protein.

Thus this study has shown an increased T cell reactivity of patients with guttate and chronic plaque psoriasis to a trypsin-sensitive antigen on M22-positive streptococci. This might reflect an increased number of M protein-specific memory T cells in the blood of psoriatic patients which, if recruited to the skin, could play a part in the pathogenesis of this disease.

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