Changes in numbers of epidermal cell adhesion molecules caused by oral cyclosporin in psoriasis

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Abstract

Aim—To determine the effects of a three month course of low dose cyclosporin on the expression of epidermal cell adhesion molecules.

Methods—Eighteen patients with psoriasis were treated for 12 weeks with either 2.5 or 5 mg/kg/day of oral cyclosporin. Biopsy specimens taken from skin before, during, and after cyclosporin treatment were stained immunohistochemically for CD 54 (ICAM-1), CD 29 (β -1 integrins), and CD18 (β -2 integrins).

Results—There was a highly significant ($p < 0.01$) clinical response after 12 weeks of cyclosporin as assessed by the Psoriasis Area and Severity Index (PASI) score. The staining of CD 29 on keratinocytes of affected and unaffected psoriatic skin was not affected by cyclosporin. Epidermal CD54 was variably expressed in active psoriatic plaques and changed unpredictably after cyclosporin ($p = \text{NS}$). Staining for CD18 on large epidermal dendritic cells was reduced after cyclosporin ($p < 0.02$). The expression of CD18 by large epidermal dendritic cells during treatment correlated strongly with the PASI score at that time and one month after stopping cyclosporin ($p < 0.02$).

Conclusions—Persistence of epidermal staining for CD 54 in psoriasis is compatible with a good clinical response to cyclosporin. Residual staining for CD 18 on large epidermal dendritic cells may be a useful marker for early clinical relapse.

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Low dose cyclosporin, defined as 5 mg/kg/day or less, is an effective treatment for severe chronic plaque psoriasis.¹ Dermatologists are deterred from using it because of its potential nephrotoxicity. Short term treatment may help reduce this risk, but unfortunately most patients tend to relapse after treatment is withdrawn.

The reasons why psoriasis should relapse after responding so well are not clear. The low doses (less than 5 mg/kg/day) required to clear psoriasis in many patients suggest that cyclosporin may be acting primarily by interfering with the release of certain cytokines from activated T lymphocytes.² Cytokines such as γ interferon and tumour necrosis factor induce expression of cell adhesion

molecules such as CD 54 (intercellular adhesion molecule-1; ICAM-1) on keratinocytes.³ CD54 and the integrin family of adhesion molecules are expressed by various cells involved in antigen presentation and T cell activation. Each integrin receptor consists of an α and β subunit. There are at least seven different subunits and four β subunits. The integrins incorporating the β -1 subunit (CD 29), previously named the VLA group, were initially described on lymphocytes but have since been recognised as occurring on most cell types and are thought to be important in maintaining epidermal cohesion.⁴ β -2 integrins (CD11a-c) are present on leucocytes and help control the intercellular interaction during antigen presentation. CD 54 is a ligand for one of the β -2 integrins CD18/CD11a (LFA-1).⁵

Successful treatment of alopecia areata with cyclosporin has been correlated with reduced expression of CD54 on follicular cells.⁶ Skin biopsy specimens taken after short courses of cyclosporin for psoriasis show reduced epidermal staining for CD54.^{7,8} A course of cyclosporin lasting four weeks caused reduced staining of keratinocytes for CD54 and CD11a and b. Staining for CD29 resembled that of normal skin.⁹

Methods

Eighteen patients with chronic plaque psoriasis were treated with a 12 week course of cyclosporin capsules at a dose of either 2.5 mg/kg/day ($n = 7$) or 5 mg/kg/day ($n = 12$). The corresponding mean (SD) trough concentrations of whole blood cyclosporin were 71 (20) and 132 (13) ng/ml. The mean duration of psoriasis was 20 years (range 4-35 years). No systemic treatment had been used for at least 12 weeks before starting cyclosporin treatment. Exclusion criteria for this study were as follows: (1) plasma creatinine concentration above 120 μmol in at least one of two specimens; (2) hypertension controlled by drugs or a supine blood pressure above 130/95 mm Hg measured on two occasions; (3) abnormal liver function defined as hepatic enzymes or bilirubin values over twice the upper limit of normal; (4) a history of malignancy. Six of the eight patients in group 1 had already completed a three month course of cyclosporin of 2.5 mg/kg/day three months before starting the course under study. Salicylic acid ointment at a concentration of up to 10% was permitted during treatment with cyclosporin.

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Patients were seen at the start of treatment and then at two, four, eight and 12 weeks after starting cyclosporin. At each visit the Psoriasis Area and Severity Index (PASI) score was measured by one of two observers (JBO'D and BDE).¹⁰

BIOPSY AND IMMUNOCHEMISTRY

Gluteal punch biopsy specimens (4 mm) were obtained from the centre of an active psoriatic plaque and an area of clinically unaffected skin within 5 cm of the plaque. The specimens were covered in OCT cryoprotectant (Tissue Tek) and snap frozen in liquid nitrogen. Cryostat sections (5 μ m)

Figure 1 Photomicrographs of serial sections of psoriatic plaque before cyclosporin treatment: (A) stained for CD54 (ICAM-1) showing sparse patchy positivity on keratinocytes alone; (B) stained for CD18 (β-2 integrin) showing large epidermal dendritic cells (haematoxylin nuclear counterstain).

were fixed in acetone for 5 minutes and endogenous peroxidase activity was blocked according to the method of Andrew *et al.*¹¹ Immunoperoxidase staining of test sections was controlled by replacing each layer in turn with 0.6% bovine serum albumin in (TRIS-buffered saline (TBS). Frozen sections of normal human tonsil were used as a positive control. The following monoclonal antibodies were used with their optimal working dilutions and origin: CD54 (RR-1/1: 1 in 500; T Springer); CD29 (A-1A5: 1 in 1000; ME Helmer); and CD18 (TSY1b: 1 in 1000; T Springer¹²). The secondary antibodies were biotinylated anti-mouse (Sigma) and the labelling was visualised using an avidin-biotin kit (ABC Kit, Dako) with diaminobenzidine (Sigma) as the substrate. Negative controls consisted of replacing the primary antibody with an irrelevant antibody of the same subclass or non-immune serum at the same dilution and replacement of each layer in turn with TBS.

QUANTITATION

Cells expressing each antigen were counted within an area of the section delineated by marks made with indelible ink on the overlying coverslip. Cells were counted if circumferential staining for CD29 was shown. Cells with dendritic morphology positive for CD18 were counted (fig 1B). We did not attempt to distinguish Langerhans' cells from non-Langerhans' cells or to characterise dermal dendritic cells. Staining of these cells is currently under investigation. Semiautomated quantitation of the length of basement membrane underlying the area of section in which the cells had been counted was made using a VIDS II image analyser. The basement membrane measured included that surrounding islands of papillary dermis which are inevitably included in sections of psoriatic skin. Cell counts were then expressed as cells per unit length of underlying basement membrane.

Significances of difference in cell counts and PASI scores were assessed using Student's two tailed *t* test and Wilcoxon's rank sum test using the Minitab release 6 (Minitab Inc.) statistics program. The PASI scores and cell counts were correlated using Spearman's rank correlation coefficient (r_s). Means and standard errors were used.

Results

CLINICAL RESPONSE

There was a highly significant reduction in PASI score after a course of cyclosporin for 12 weeks from 13.1 ((SEM) 1.8) to 2.7 (0.7) ($p < 0.01$) (Wilcoxon's rank sum test). Two of the seven patients in group A had a poor response and the mean reduction in PASI score was only 40%. Ten of the 11 patients in group B had greater than 80% improvement in their PASI scores. The mean PASI score rose significantly in the four weeks after therapy from 2.7 (0.7) to 5.5 (0.9) ($p < 0.01$) (Wilcoxon's rank sum test). One month after

Figure 2 ICAM-1 expression by keratinocytes in affected and unaffected skin (mean number of cells per mm of basement membrane; 16 patients). Although an overall reduction in the mean number of ICAM-1 positive cells was seen after treatment, the level of ICAM-1 did not always parallel clinical response so that there was no significant difference in staining before and after cyclosporin (affected skin $p = 0.09$; unaffected skin $p < 0.05$).

(A)

(B)

Figure 3 Photomicrographs of skin successfully treated with cyclosporin: (A) stained for CD54 to show persistent expression of CD54 despite considerable clinical and histological improvement. The morphologically normal capillaries in the upper dermis are positive for CD54. (B) Stained for CD18 ($\beta 2$ integrin) (haematoxylin nuclear counterstain).

stopping treatment all but two patients showed some recrudescence of their disease. At three months 17 out of 19 patients had attained PASI scores comparable with baseline with a sustained clinical improvement in only two (whole group PASI score: 11.7 (1.8)). As biopsy specimens had been taken from buttock skin PASI scores for the lower limbs might reflect histological changes with more accuracy. Lower limb PASI scores declined in parallel with the whole body score during cyclosporin treatment (week 0: 5.9 (0.9); week 12: 1.4 (0.4)).

Biopsy specimens taken at the end of treatment showed a return to normal, with reductions in acanthosis, parakeratosis, and inflammation.

ADHESION MOLECULE EXPRESSION

CD54 was expressed by clusters of keratinocytes and lymphocytes infiltrating the epidermis but was rarely seen expressed by large dendritic cells (fig 1A). The keratinocytes of 13 patients stained positive for CD54 and all patients with strong expression showed a decrease in staining after cyclosporin treatment. CD54 expression on keratinocytes was higher in affected than unaffected skin and usually decreased during treatment ($p = 0.09$) in affected skin (fig 2). It was, however, variable and some of the pretreatment biopsy specimens showed very little expression even though they came from active plaques in patients with high PASI scores. Some post-treatment biopsy specimens showed CD54 expression despite histological and clinical improvement (fig 3A). The two patients whose clinical response was poor showed no decrease in CD54 or CD18 expression. Two patients showed a pronounced increase in CD54 staining after cyclosporin despite a greater than 85% reduction in PASI score.¹³ Endothelial cells stained positive for CD54 in all epidermal sections before, during, and after cyclosporin treatment.

CD18 was expressed by lymphocytes and large epidermal dendritic cells, which, morphologically, resembled antigen presenting cells (fig 1B). It was not expressed by keratinocytes. Numbers of CD18 positive large epidermal dendritic cells had increased in most biopsy specimens before treatment. These cells were more abundant in affected than unaffected skin and usually decreased in number after treatment with cyclosporin ($p < 0.008$ affected skin) (figs 3B and 4). In two patients an excellent clinical response was followed by a rapid and severe relapse of their psoriasis within one month of stopping cyclosporin. These patients with an excellent generalised clinical response to treatment continued to have CD18 positive large epidermal dendritic cells in their skin which clinically looked almost normal.

There were equal numbers of keratinocytes positive for CD29 in both affected and unaffected skin and these were not significantly affected by cyclosporin.

To see whether the histological expression of these cell adhesion molecules changed in parallel with clinical response, cell counts and

Figure 4 Significant reduction in the number of large epidermal dendritic cells which stain for β -2 integrin in affected skin. (Mean number of cells per mm of underlying basement membrane; 18 patients; affected skin $p < 0.008$, unaffected skin $p = 0.6$).

Figure 5 Positive correlation between PASI score and staining score for β -2 integrin on large epidermal staining cells after 12 weeks of cyclosporin ($r = 0.812$; $p < 0.02$). Note two patients with a very good PASI score and significant number of β -2 positive cells remaining in the epidermis.

PASI scores were compared before, during, and after treatment. The expression of CD18 after 12 weeks was significantly correlated with the PASI score both at that time and one month later, after treatment had been withdrawn ($p < 0.02$) (fig 5). No significant correlation between staining for CD54 and the PASI score could be shown.

Discussion

It has been predicted that drugs such as cyclosporin, which inhibits production of γ interferon and subsequent induction of CD54, should interrupt the chronic and self-perpetuating interaction between T lymphocytes and keratinocytes in psoriasis to allow them both to return to the resting state.¹⁴ It is reasonable to assume that the decreased numbers of epidermal inflammatory cells in resolving psoriasis should be preceded by loss of CD54 expression by keratinocytes.¹⁵ Although our clinical experience confirms the efficacy of cyclosporin in improving psoriasis in most patients, the decrease in expression of CD54 did not always parallel the clinical improvement.

Previous studies of intralesional cyclosporin have shown a decrease in epidermal

and endothelial CD54 staining which parallels the clinical improvement in psoriasis with cyclosporin.^{7,8} A reduction in CD 54 in papillary microvessels in biopsies performed two weeks after starting oral cyclosporin (5 mg/kg/day) was restricted to those patients who had responded to treatment.⁸ Biopsy specimens from three patients with psoriasis responsive to psoralen and ultraviolet A treatment (PUVA) showed an even greater reduction in infiltrating T cells yet no change in papillary endothelial staining for CD54.⁸ Paradoxically, PUVA treatment can increase the staining of keratinocytes for CD54.¹⁶

Positive staining of keratinocytes for CD54 has been described in psoriasis and other inflammatory dermatoses.¹⁷ Intense staining for CD54 was found in at least 50% of our patients. Nevertheless, it is curious that some patients showed very little CD54 staining in pre-treatment biopsy specimens despite extensive psoriasis. It was particularly striking to observe that staining of epidermal CD54 could increase after treatment despite a good clinical and histological response in which the skin had returned almost to normal.¹² If CD54 expression remains on keratinocytes after cyclosporin this may indicate persistent activation which would be expected to lead to prompt clinical relapse.

In contrast with a previous study we found there was no staining for CD18 on keratinocytes.⁹ The expression of CD18 on epidermal dendritic cells is increased in psoriasis.¹⁸ We confirm that large epidermal dendritic cells stained for CD18 can be easily counted. This contrasts with HLA-DR which stains both keratinocytes and dendritic cells.¹⁹ Expression of CD1 was not used as a marker to replace β -2 integrin because the reported percentage of epidermal dendritic cells which are CD1 positive varies between 70–98%.^{20,21} The proportion of dendritic cells which are CD1 positive is decreased in psoriatic epidermis when compared with normal epidermis.¹⁸ To exclude CD1 negative cells is relevant because these cells are crucial in the mixed lymphocyte response and presumably also in antigen presentation in vivo.²² Other workers have shown that 14 days of cyclosporin reduces preferentially the number of epidermal CD1 negative cells compared with CD1 positive cells as well as the overall activity of antigen presenting cells.^{21,23} CD18 is expressed by lymphocytes but their smaller size and lack of dendrites means they can be easily excluded from the cell counts. As recently shown in a smaller study using cyclosporin for only two weeks, we confirm that CD18 positive dendritic cells usually are decreased in psoriatic epidermis after cyclosporin treatment.^{9,16} Persistence of CD18 positive cells seemed to be a predictor of rapid relapse in two patients who had an excellent response to treatment.

Different members of the CD29 (β -1) family of integrin adhesion molecules are receptors for different components of the extracellular matrix.²⁴ CD29 expression in normal skin is confined to the basal and first

suprabasal layers.²⁵ In psoriasis CD29 integrin expression is increased which is thought to be due mainly to increased CD49_c/CD29 integrins.^{24,25} The present study shows an equal amount of β -1 expression in both affected and unaffected skin from patients with psoriasis which suggests that abnormal keratinocytes occur in clinically normal skin. Cyclosporin treatment did not affect CD29 expression: this remained the same in biopsy specimens from affected and unaffected skin from patients with psoriasis. We are currently studying the effect of cyclosporin on the distribution of CD49_{a-f} in psoriasis.

The expression of CD54 by keratinocytes can persist despite an excellent response to treatment. This suggests that this adhesion molecule is not involved in initiating the inflammatory response in psoriasis. It would be useful to know why psoriasis relapses so quickly once cyclosporin is stopped and if markers could be found to predict this relapse. Such a marker could be persistence of staining for CD18 on dendritic cells in skin biopsy specimens. Unfortunately, absence of CD18 integrin staining does not necessarily indicate remission. Nevertheless, our results suggest that significant residual staining for CD18 after successful treatment with cyclosporin may herald the rapid relapse of psoriasis.

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