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Clinical and Laboratory Investigations

Inflammation in acne scarring: a comparison of the responses in lesions from patients prone and not prone to scar

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Summary

Background Many patients with inflammatory acne suffer from significant scarring, which is disfiguring and difficult to treat. A cell-mediated immune response is considered to be involved in the pathogenesis of acne, although the extent of this response has been found to differ among patients.

Objective To assess whether there were differences in the cell-mediated immune responses at different time points in inflamed lesion development and resolution in patients who were prone (S patients) and those with the same degree of inflamed acne who were not prone (NS patients) to develop scarring.

Methods Cellular and vascular markers were investigated using standard immunohistochemical techniques on biopsies of inflamed lesions of known duration, i.e. < 6 h ($n = 14$), 24 h ($n = 14$), 48 h ($n = 10$), 72 h ($n = 10$) and 6–7 days ($n = 11$) from the backs of acne patients.

Results In early lesions from NS patients there was a large influx of CD4+ T cells, macrophages and Langerhans cells with a high number of cells expressing HLA-DR. Also there was significant angiogenesis and vascular adhesion molecule expression. Cell recruitment peaked in 48 h lesions, after which leucocyte numbers decreased and vascular activity returned to normal. Of the T cells, only 50% were memory/effector (CD45RO+) and naive (CD45RA+) cells, while the remainder were unclassified (CD45RO–, CD45RA–). In early lesions from S patients, CD4+ T cell numbers were smaller, although a high proportion were skin homing memory/effector cells. Langerhans cell numbers and cellular HLA-DR expression were low, while numbers of macrophages, blood vessels and vascular adhesion molecules were high. In resolving lesions angiogenesis remained high, with a further influx of macrophages and skin homing memory/effector cells and increased cellular HLA-DR expression.

Conclusions The cellular infiltrate was large and active with a greater nonspecific response (few memory T cells) in early lesions of NS patients, which subsided in resolution. In contrast, a predominantly specific immune response was present in S patients, which was initially smaller and ineffective, but was increased and activated in resolving lesions. Such excessive inflammation in healing tissue is conducive to scarring and suggests that the use of topical anti-inflammatory treatments would be appropriate for these patients.

Key words: acne, macrophages, scarring, skin homing memory/effector T cells

Acne is a common skin disorder, presenting as clinical disease in 28% of adolescents and occurring at a time when sensitivity to appearance is greatest. For some patients, however, scarring, the sequel to inflammatory

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acne, is more devastating and frequently causes social handicap and psychological problems.[1] Very little information is available about the prevalence and clinical development of acne scarring, apart from a study in 1994 by Layton *et al.*[2] Scarring was found to be present in 95% of acne patients, while more significant or clinical scarring ensued in approximately 30% of patients. Many therapeutic approaches have been used to treat acne scarring, including invasive (surgical and laser) and noninvasive methods, but with unquantified differences in efficacy and cost, and often lack of success.[3] Not until the pathogenesis of scarring is understood and predisposing factors have been identified will treatments improve.

Acne scarring is a consequence of the damage that occurs in and around the pilosebaceous follicle during inflammation. However, the precise mechanisms and factors which govern the initiation and exacerbation of inflammation are not fully known. It is known that there are both humoral and cellular immune components which correlate with the severity of the disease and that antigens of *Propionibacterium acnes* play a central role. Normal individuals are immunologically sensitized to *P. acnes*, but patients with acne, particularly those with moderate and severe forms, are sensitized to a greater extent. Patients with severe acne have significantly higher titres of total IgG than do control subjects; these are attributable to increased IgG1, IgG2 and IgG3 subclass antibodies to *P. acnes*.[4,5] Cell-mediated immune involvement in response to *P. acnes* in patients with severe acne was demonstrated in earlier studies.[6–8] They found that cellular immunity was related to the length and level of exposure to *P. acnes* and that it arose as a late event rather than being a factor in the initiation of the disorder. As a high level of sensitivity was not found in all patients, these authors concluded that cellular immunity was an unpredictable event and dependent on the host's immunological response to *P. acnes*. More recent evidence implicates *P. acnes* in the initiation of Th1-mediated immune responses by its role in activating the monocyte/macrophage release of interleukin (IL)-12 via Toll-like receptors (TLR2) in acne.[9] Immunohistochemical data[10,11] are also consistent with inflammation initiated by CD4+ T cells representing a cell-mediated immune response in early developing lesions. Whether this is specifically an antigenic response or whether mitogenic mechanisms are involved, as proposed by Jappe *et al.*,[12] is not clear. Certainly, a specific antigen response is confirmed by the presence of a Th1 mRNA cytokine profile in some very early lesions (unpublished data) and by the fact that although most Vβ families are represented by T cells infiltrating acne lesions, the CDR3 size diversity of expressed Vβ genes is restricted to the mono- or oligoclonal expansions indicative of an antigen-driven response.[13]

Not only can cell-mediated immunity contribute to the clearance of antigens, but it can also contribute to tissue damage; no attention has been paid to the influence that the cellular immune response may have on the resolution of acne lesions. Both Th1 and Th2 mRNA cytokine profiles have been found in established inflamed lesions from different acne patients (unpublished data), which suggests that acne is not a homogeneous disease and that patients may generate different types of immune response. Whether this difference contributes to the predisposition of some patients to scar was investigated in this study. Immunohistochemical methods were used to determine the cell-mediated immune response in developing and resolving inflamed lesions by examining the prevalence and activation states of lymphocyte subsets, macrophages and endothelial cells, the major components of this response, present in two groups of patients with the same degree of inflamed acne but differing in their propensity to scar.

Methods

Biopsies

With local ethics committee approval, 4-mm punch biopsies of inflamed lesions (papules) were obtained. They were taken under local anaesthesia from the interscapular region of the backs of consenting adolescent patients with acne. Patients who were not prone to scar (NS) ($n = 8$; four males and four females) and had no evidence of atrophic or hypertrophic or keloidal scars on the face, back and chest, and patients who were prone to scar (S) ($n = 11$; eight males and three females) and had scarring over these areas, were included. Scarring was quantified using a scar count-based grading system. Three patients had mild macular atrophic scarring and one patient had mild ice pick scarring with a total of between six and 25 scars on the face, back and chest. The remaining seven patients had moderate macular atrophic scarring with between 26 and 100 scars.

A mapping technique[11] was used to obtain biopsies of inflamed lesions of known duration of up to 6 h, 24 h, 48 h, 72 h and 6–7 days. Biopsies of lesions up to 6 h and 24 h were considered to be early lesions, 48 h and 72 h established or ageing lesions, and

6–7 days old resolving lesions. For each time group, up to six biopsies were obtained from NS patients, up to eight biopsies from S patients and, also, 10 control biopsies were taken of normal skin from adolescent subjects who had never suffered from acne. Biopsy samples were frozen and stored in liquid nitrogen. When required they were embedded in optimum cutting temperature compound and longitudinal serial sections of 6 μm were cut through the whole sebaceous follicle or acne lesion. Sections were mounted on silane-treated glass slides, air-dried for 12–24 h, fixed in 100% v/v acetone for 10 min, dried for 10 min and stored at − 70 °C until use.

Primary antibodies for immunohistochemistry

Monoclonal antibodies UCHT1 (anti-CD3; specific for all T cells; dilution 1 : 50), MT310 (anti-CD4; helper/inducer T cells and some monocyte/macrophages; 1 : 8), DK25 (anti-CD8; cytotoxic/suppressor T cells; 1 : 25), 4KB5 (anti-CD45RA; naive/resting T cells; 1 : 25), UCHL1 (anti-CD45RO; memory/effector T cells; 1 : 50), NAI/34 (anti-CD1a; Langerhans cells; 1 : 100), DK22 [anti-HLA-DR; major histocompatibility complex (MHC) class II; 1 : 25] were purchased from Dako Ltd (Amersham, U.K.). Monoclonal antibody Ki-M6 (anti-CD68; monocytes/macrophages; 1 : 100) was obtained from Serotec Ltd. (Oxford, U.K.) and HECA-45 [anticutaneous lymphocyte antigen (CLA); 1 : 25] was from BD PharMingen (San Diego, CA, U.S.A.). Sanbio (Uden, the Netherlands) supplied the monoclonal antibody to EN4 (specific for endothelial cells; used at 1 : 50 dilution). Monoclonal antibodies to adhesion molecules were obtained from R&D Systems (Abingdon, U.K.) and included BBIG-E4 (anti-E-selectin; 1 : 100), BBIG-I1 [anti-intercellular adhesion molecule (ICAM)-1; 1 : 1000] and BBIG-V1 [antivascular cell adhesion molecule (VCAM)-1; 1 : 1000].

Immunohistochemistry

Frozen sections were stained by standard immunoperoxidase techniques using monoclonal antibodies and Vectastain Elite® ABC kits (Vector Laboratories, Peterborough, U.K.). As all sections had been numbered consecutively after cutting, it was possible to stain three sections, i.e. one from the beginning, centre and end of each follicle or lesion with each antibody. Briefly, the technique involved the rehydration of sections in PBS pH 7·4 for 10 min, followed by addition of blocking serum (Vectastain Elite kit) for 15 min and then incubation in primary antibody for 45 min. Sections were washed in phosphate buffered saline (PBS) for 3 min ×3. The appropriate biotinylated secondary antibody was added for 30 min, followed by washing in PBS for 3 min ×3 and then the appropriate ABC–peroxidase complex was added for 30 min. After further washes in PBS, the remaining peroxidase activity was visualized using hydrogen peroxide (120 μL) and diaminobenzidine (DAB: 1 mL in 400 mL PBS) as the substrate. Sections were counterstained with methyl green for 10 min, dehydrated in 100% v/v ethanol for 4 min ×2 and xylene for 4 min ×2 before mounting in Eukitt (Merck, Lutterworth, U.K.).

Quantitative assessment

Skin sections were analysed at ×200 magnification, using a Zeiss Axioplan microscope and photographed using a digital camera (Nikon Coolpix 950). The whole section was examined and the total number of positively immunolabelled cells and blood vessels was counted by two observers independently for each section. As three sections per lesion or follicle were stained with each antibody, a mean count of immunolabelled cells and blood vessels was determined. Computer-aided image analysis was used to measure the section area. The areas of three different sections through each lesion or follicle were determined. A black and white image of each section area was captured via a charge-coupled device camera attached to a microscope (Leitz Laborlux S; Leitz, Wetzlar, Germany) with ×2·5 magnification. Image analysis software (Image Pro Plus; Media Cybermetics, Silver Spring, MA, U.S.A.), which was calibrated to measure in square millimetres, was then used to obtain section areas. The mean of the three values was taken as the section area for that follicle or lesion. Positive immunolabelled cells and blood vessels were finally quantified as counts mm^{-2} per lesion or follicle.

Statistical analysis

The data are represented as the mean ± SEM. As sample numbers in each group were small, the data were analysed with the nonparametric Mann–Whitney *U*-test and *P*-values of $< 0·05$ were considered significant [Microsoft Excel (Microsoft, Reading, UK) and Astute Add-in for Microsoft Excel (DDU Software, Leeds, U.K.) programs].

Results

Patients

As the two groups of acne patients were distinguished by their proneness to scarring, other clinical features were matched. There were no significant differences between the mean ages of the patients in the two groups, with S = 21·4 ± 1·6 years and NS = 22·5 ± 2·8 years ($P = 0{\cdot}8$); or the duration of their acne (S = 6·5 ± 1·8 years; NS = 8·3 ± 3·0 years) ($P = 0{\cdot}8$); or the length of time they had suffered from active inflammatory disease (S = 3·3 ± 2·0 years; NS = 2·4 ± 0·8 years) ($P = 0{\cdot}5$). All patients had superficial inflamed acne with no deep nodular lesions and, according to the Leeds grading system,[14] total grades for the face, back and chest (S = 1·4 ± 0·2; NS = 0·8 ± 0·1), although greater in patients who scarred ($P = 0{\cdot}03$), were inclusive of patients with a moderate degree of acne. No patients had severe acne. On the back, where the biopsies were taken, the same degree of inflammatory acne was present (grade for S = 0·6 ± 0·1; NS = 0·4 ± 0·1) ($P = 0{\cdot}32$).

T cells

CD3+ (pan T cells), CD4+ (helper T cells) and CD8+ (cytotoxic T cells). In both S patients and NS patients extensive mononuclear cell infiltration was evident in early, established and resolving acne lesions with CD3+ and CD4+ T-cell numbers greater than those in normal follicles from control skin without acne. CD8+ T cells constituted only a small proportion of the infiltrate and the numbers in lesions from both patient groups and controls were similar (Fig. 1). The T-cell infiltrates were located predominantly in the dermis, in and around the blood vessels and pilosebaceous follicles, although a small number of CD3+ and a greater number of CD4+ cells were also found in the epidermis. The increased numbers of CD4+ cells is accounted for by the fact that the CD4 epitope is present not only on helper T cells but also on Langerhans cells. Overt rupture of the follicle wall was not observed in the lesions examined, although this does not mean that subcellular dissociation with subsequent permeability changes were not present.

In NS patients, CD3+ and CD4+ cell numbers were high initially and increased further in lesions of up to 48 h of age, after which these cells decreased in number with resolution. In contrast, in lesions from S patients, smaller numbers of CD3+ and CD4+ T cells were present and their numbers remained constant from early lesion development through to resolution. There were clear differences between patient groups in developing lesions, i.e. < 6 h, 24 h, 48 h and 72 h, with a larger CD3+, CD4+ T-cell infiltrate present in lesions from NS patients than in those from S patients (in 48 h lesions, $P = 0{\cdot}028$) but in resolving 6–7 day lesions the numbers of these cells in the infiltrate were similar.

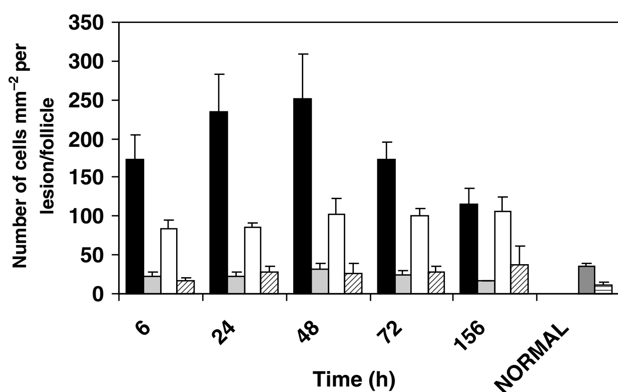

Figure 1. A comparison of the numbers of CD4+ [■, nonscarring (NS); □, scarring (S)] and CD8+ (dotted, NS; hatched, S) T cells at different time points during lesion development and resolution. For CD4+ cells (NS) $n = 6, 6, 5, 5, 3$ and for (S) $n = 7, 7, 4, 4, 7$ for consecutive time points. For normal skin $n = 10$. For CD8+ cells (NS) $n = 4, 5, 3, 3, 2$ and for (S) $n = 6, 8, 4, 4, 6$, for consecutive time points. For normal skin $n = 6$. Values represent the mean number ± SEM of cells mm^{-2} per lesion/follicle.

CD45RA+ (naive T cells), CD45RO+ (memory/effector T cells) and cutaneous leucocyte antigen+ (skin homing T cells). T cells were classified into naive and memory/effector cells. During lesion development and resolution the numbers of both subsets were increased compared with normal skin where CD45RO+ cells were predominant. There were no differences between the numbers of lesional CD45RA+ T cells, although there were differences within the lesional CD45RO+ populations. Similar numbers of CD45RO+ T cells were present in lesions up to the age of 48 h, but after this time in 72-h and 6–7-day-old lesions there were significant decreases in CD45RO+ T-cell numbers in NS patients, whereas in S patients the levels remained high ($P < 0{\cdot}014$) (Fig. 2A).

Another striking difference was that in lesions from S patients a high proportion of the CD3+ T-cell population were CD45RO+ memory/effector cells, whereas in NS patients the numbers of CD45RO+ cells represented less than half of the total CD3+ cells (Table 1).

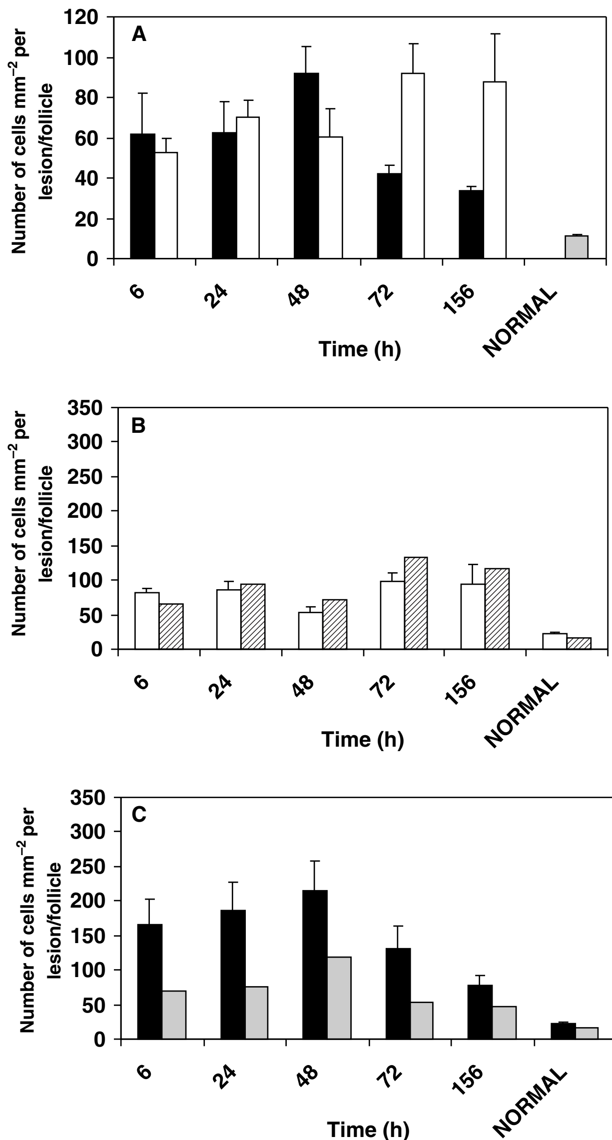

Figure 2. (A) A comparison of the memory/effector cells (CD45RO+) present in developing and resolving lesions from non-scarring (NS) (■) and scarring (S) (□) patients. For NS, $n = 6, 6, 5, 5, 3$ and for S, $n = 6, 6, 3, 4, 7$ for consecutive time points. For normal skin (dotted), $n = 10$. Values represent the mean number ± SEM of cells mm^{-2} per lesion/follicle. (B) The mean number ± SEM of total T cells (CD3+) (□) compared with the combined mean values of naive (CD45RA+) and memory/effector (CD45RO+) T cells (hatched) present in developing and resolving lesions from S patients. For CD3+, $n = 6, 6, 4, 5, 8$, and for CD45RA+ plus CD45RO+ cells, $n = 3, 4, 2, 2, 4$ for consecutive time points. For normal skin $n = 10$. (C) The mean number ± SEM of total T cells (CD3+) (■) compared with the combined mean values of naive (CD45RA+) and memory/effector (CD45RO+) T cells (dotted) present in developing and resolving lesions from NS. For both CD3+ and CD45RA+ plus CD45RO+ cells, $n = 6, 6, 5, 5, 3$ for consecutive time points. For normal skin $n = 10$.

Further differences were revealed when the sum total of CD45RA+ and CD45RO+ T cells was compared with the total number of CD3+ T cells. In lesions from S patients the sum of the two subpopulations of cells was equivalent to or greater than the total number of T cells (Fig. 2B), whereas in NS patients these combined values were much smaller (Fig. 2C), with at least 50% of the CD3+ cells not classified as either naive or memory/effector T cells.

CLA+ T cells are a subset of memory/effector cells, which are primed by skin-associated antigen(s) and bind specifically to the vascular adhesion molecule E- selectin. They were present in high numbers in all lesions from S patients compared with normal skin. These numbers were similar to the numbers of CD45RO+ T cells found in the lesional infiltrate of S patients and represented a high proportion of the total CD3+ T cells (Table 1). In contrast, in lesions from NS patients the numbers of CLA+ cells were lower and similar to those found in normal skin. They represented only 50% of the total CD45RO+ cells and a very small proportion of the total CD3+ cells. In normal skin, the proportion of CLA+ cells in the total T-cell population was far higher than in lesions of NS patients (Table 1).

Macrophages (CD68+)

Macrophages were present in large numbers in acne lesions compared with control skin ($P < 0{\cdot}01$) and were predominantly found in perivascular and perifollicular areas, with single cells also scattered through the papillary dermis. The numbers of macrophages were comparable in < 6 h, 24 h and 48 h lesions in both S and NS patients. After this time the numbers declined, returning to normal levels in 6–7 day lesions of NS patients, but in S patients macrophage numbers increased significantly in 72 h lesions compared with those in the lesions of NS patients ($P = 0{\cdot}028$). Even in resolving 6–7 day lesions macrophage numbers were still greatly elevated and had not returned to normal (Fig. 3).

Langerhans cells (CD1a+)

In acne lesions, cells with a dendritic morphology and a strong CD1a positivity, considered to be Langerhans cells, were found mainly within the epidermis and follicle wall, but some were present in the periductal infiltrate with isolated cells scattered through the papillary dermis. In NS patients a higher cell density

Table 1. Numbers of T-helper cells and memory/effector cells present in developing and resolving lesions from nonscarring and scarring patients.

	Numbers of cells mm^{-2}					
Age of lesion	6 h	24 h	48 h	72 h	6–7 days	Normal control skin
Inflamed lesions from nonscarring patients						
CD3+	165·5 ± 37·6	186·3 ± 44·1	214·4 ± 44·1	131·8 ± 32·9	77·7 ± 14·5	23·5 ± 1·6
CD45RO+	61·5 ± 20·7	62·3 ± 15·7	91·8 ± 13·3	42·2 ± 3·8	34·0 ± 1·5	11·2 ± 0·7
CLA+	21·7 ± 5·0	27·8 ± 5·2	36·8 ± 3·7	19·8 ± 2·8	15·7 ± 0·3	13·1 ± 1·1
Inflamed lesions from scarring patients						
CD3+	81·8 ± 6·8	86·3 ± 12·2	52·5 ± 8·3	98·6 ± 12·1	94·0 ± 29·2	23·5 ± 1·6
CD45RO+	52·8 ± 6·6	70·0 ± 8·4	60·7 ± 13·9	91·8 ± 14·6	88·0 ± 23·4	11·2 ± 0·7
CLA+	56·4 ± 8·0	78·4 ± 12·7	55·3 ± 15·4	91·0 ± 23·6	56·9 ± 10·2	13·1 ± 1·1

Mean values ± SEM.

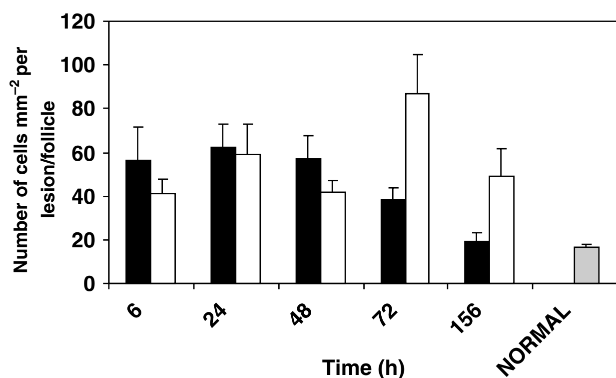

Figure 3. A comparison of the number of macrophages (CD68+) present in developing and resolving lesions from nonscarring (NS) (■) and scarring (S) (□) patients. For NS, $n = 6, 6, 5, 5, 3$ and for S, $n = 6, 6, 5, 4, 8$, for consecutive time points. For normal skin (dotted), $n = 10$. Values represent the mean number ± SEM of cells mm^{-2} per lesion/follicle.

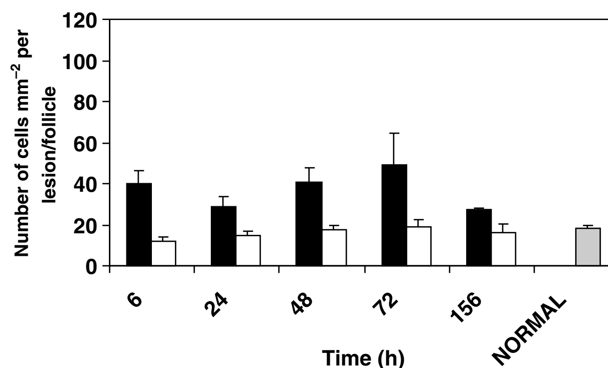

Figure 4. A comparison of the number of Langerhans cells present in the perifollicular and interfollicular dermis in developing and resolving lesions from nonscarring (NS) (■) and scarring (S) (□) patients. For NS, $n = 6, 6, 5, 5, 3$ and for S, $n = 5, 6, 4, 5, 8$ for consecutive time points. For normal skin (dotted), $n = 10$. Values represent the mean number ± SEM of cells mm^{-2} per lesion/follicle.

of CD1a+ cells was observed than in normal skin and this was due to an approximately twofold increase in numbers of CD1a+ cells present in the perifollicular dermis (Fig. 4). In contrast, in lesions from S patients CD1a+ cell numbers in both the epidermis and dermis were similar to normal values.

Human leucocyte antigen HLA-DR+ cells

In addition to vascular expression of HLA-DR, large numbers of cells within the lesional dermis in the perivascular and periductal cell infiltrates expressed HLA-DR. A few HLA-DR+ cells were also scattered through the suprabasal layers of the epidermis and follicle wall in lesions from NS patients, although staining of the basal cells was rarely seen in lesions of either patient group. In < 6 h to 48 h lesions of S patients, expression levels were similar to those in

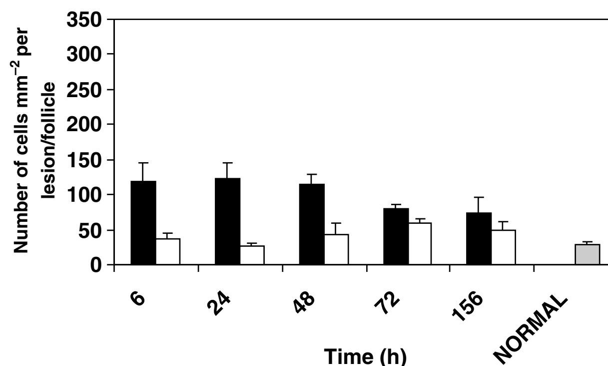

Figure 5. A comparison of HLA-DR+ cellular expression present in developing and resolving lesions from nonscarring (NS) (■) and scarring (S) (□) patients. For NS, $n = 6, 6, 5, 5, 3$ and for S, $n = 6, 6, 5, 5, 8$ for consecutive time points. For normal skin (dotted), $n = 10$. Values represent the mean number ± SEM of cells mm^{-2} per lesion/follicle.

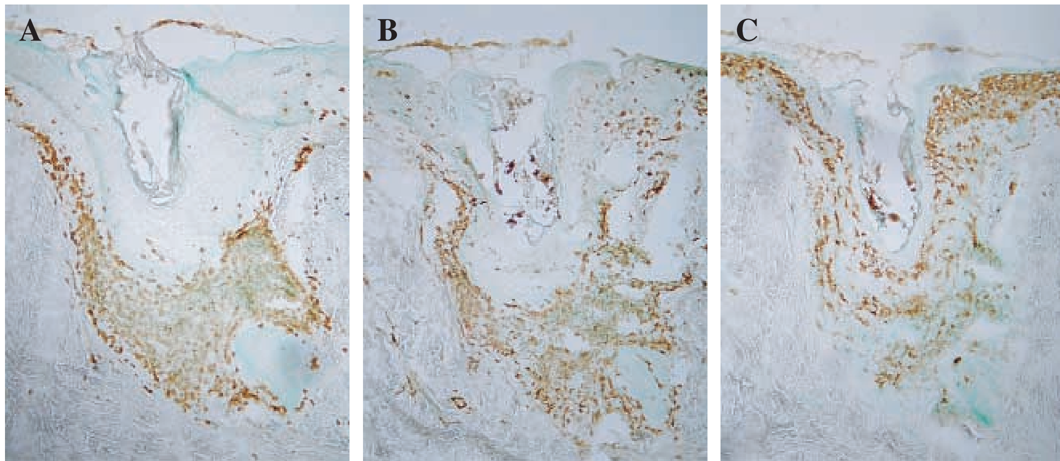

Figure 6. A comparison of the location of the (A) CD3+ T-cell population ($n = 186{\cdot}3 \pm 41{\cdot}4$), (B) cellular HLA-DR+ infiltrate ($n = 129{\cdot}3 \pm 22{\cdot}1$), and (C) CD1a+ (Langerhans) cell population (total $n = 76{\cdot}2 \pm 13{\cdot}2$, dermal $n = 28{\cdot}5 \pm 5{\cdot}4$) within a 24 h lesion from a nonscarring acne patient. Original magnification ×200.

normal skin, but in 72 h lesions HLA-DR expression greatly increased ($P = 0{\cdot}005$). In contrast, in NS patients HLA-DR expression was elevated above normal in all lesions. Thus differences between cells expressing HLA-DR in < 6 h to 48 h lesions were observed with much higher numbers in NS patients than S patients (at 6 h, $P < 0{\cdot}006$), while in 6–7-day-old lesions the expression of this marker was similar in both patient groups (Fig. 5).

Cells were not double-labelled and it was difficult to say with certainty which cells expressed HLA-DR. However, from the morphological appearance and distribution of the positive cells, HLA-DR expression correlated more with dermal T cells and macrophages than CD1a+ Langerhans cells (Fig. 6). Langerhans cells express HLA-DR constitutively, but surface levels are very low on immature cells,[15] which may account for the low level of labelling and indicate that in this study many CD1a+ cells were inactive, i.e. sampling and processing the local environment.

Both activated CD45RO+ cells and activated macrophages express high levels of HLA-DR and, taking this into consideration, the summation of the totals of CD68+ and CD45RO+ cells when compared with the total number of HLA-DR+ cells was found to be similar in NS patients (Fig. 7A). This was not the case in S patients, where HLA-DR+ cells represented only half the total number of the combined cells and were equivalent to either individual CD45RO+ or CD68+ cell numbers (Fig. 7B). This would indicate that either T cells or macrophages or a mixture of small numbers of both cell types were expressing HLA-DR in S patients.

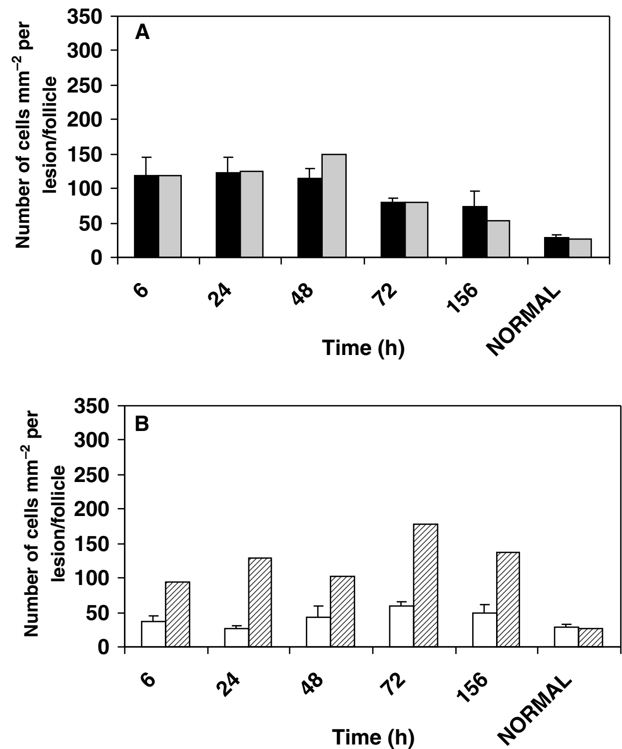

Figure 7. (A) The mean number ± SEM of cells expressing HLA-DR (■) compared with the combined mean number of macrophages (CD68+) and memory/effector cells (CD45RO+) (dotted) present in developing and resolving lesions from nonscarring patients. For HLA-DR+ cells, $n = 6, 6, 5, 5, 3$ and for CD68+ plus CD45RO+ cells, $n = 6, 6, 5, 5, 3$ for consecutive time points. For normal skin, $n = 10$. (B) The mean number ± SEM of cells expressing HLA-DR (□) compared with the combined mean number of macrophages (CD68+) and memory/effector cells (CD45RO+) (hatched) present in developing and resolving lesions from scarring patients. For HLA-DR+ cells, $n = 6, 6, 5, 5, 8$ and for CD68+ and CD45RO+ cells, $n = 6, 6, 3, 4, 7$ for consecutive time points. For normal skin, $n = 10$.

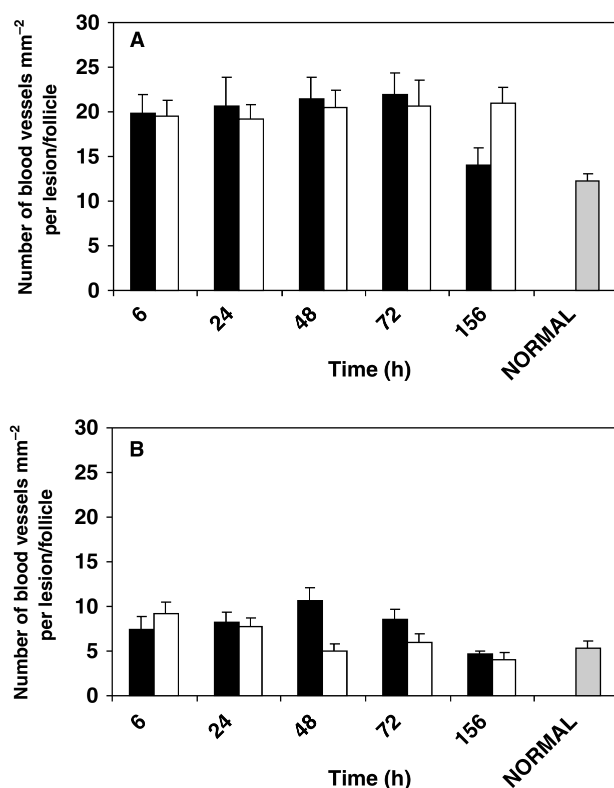

Figure 8. (A) A comparison of the expression of the endothelial cell marker EN4 on the dermal vasculature of developing and resolving lesions from nonscarring (NS) (■) and scarring (S) (□) patients. For NS, $n = 6, 6, 5, 5, 3$ and for S, $n = 6, 6, 4, 5, 8$ for consecutive time points. For normal skin (dotted) $n = 10$. (B) A comparison of the expression of the cell adhesion marker E-selectin, on dermal blood vessels of developing and resolving lesions from NS (■) and S (□). For NS, $n = 6, 6, 5, 5, 3$ and for S, $n = 6, 6, 5, 5, 8$ for consecutive time points. For normal skin (dotted) $n = 10$. Values represent mean number ± SEM of positive staining blood vessels mm^{-2} per lesion/follicle.

Vascular markers

A marked increase in the number of EN4+ labelled blood vessels and single cells was found in all lesions up to the age of 72 h from both S and NS patients, while after this time the number of blood vessels returned to normal levels in NS patients, but remained significantly higher in S patients ($P = 0{\cdot}0005$) (Fig. 8A).

There was upregulation of VCAM-1, HLA-DR and ICAM-1 expression on dermal vascular endothelial cells in developing lesions from < 6 h to 48 h of age, with levels of expression returning to normal in resolution in both patient groups.

In contrast to the other vascular markers, upregulation of E-selectin expression was evident only in < 6 h lesions in S patients, a time when the major influx of CLA+ cells was also seen, while in NS patients increases did not occur until later in lesion development, with strong expression at 24 h, 48 h and 72 h (Fig. 8B) coinciding with the times for the greatest recruitment of CLA+ cells, i.e. 24 h and 48 h.

Discussion

This study has identified clear differences between the cellular infiltrate present in inflamed lesions from two groups of acne patients clinically distinguished by their proneness to scarring (Fig. 9). In lesions of NS patients the time course was typical of a type IV delayed hypersensitivity response. In evolving lesions there was significant angiogenesis and vascular adhesion molecule expression with preferential recruitment of large numbers of CD4+ T cells and an influx of macrophages and Langerhans cells. The high cellular HLA-DR expression, equivalent to that expressed on the total memory/effector and macrophage populations, infers that the majority of these cells were activated and that there was presentation and clearance of the causal antigen(s). Also, high numbers of Langerhans cells within the lesional dermis indicates possible cellular interactions and movement of these cells to the skin-draining lymph node. Cell recruitment peaked at 48 h and was followed by a reduction in numbers of leucocytes, blood vessels and vascular adhesion molecules in older lesions.

Within the CD4+ T-cell population less than half the cells were memory/effectors, with an even smaller number being specifically skin homing, which suggests that these patients, not prone to scar, are not highly sensitized to the antigen(s) responsible for acne. A novel

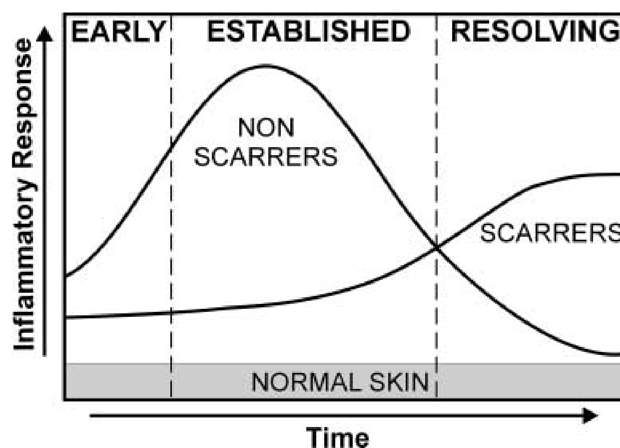

Figure 9. Schematic of the inflammatory responses in acne lesions.

finding was the fact that the other half of the CD4+ T-cell infiltrate could not be classified as either naive or memory/effector cells. Evidence in the literature indicates that subsets of cells with properties between T cells and natural killer (NK) cells exist and since NK T cells have been implicated in the inflammatory skin disease psoriasis,[16,17] one could speculate that these uncharacterized cells may fall into such a category. Thus it is possible that there is effective removal of the causal antigen(s) and the satisfactory resolution of the inflammatory response by mechanisms straddling both the innate and adaptive immune systems.

In lesions from patients who scar, the scenario was different. The level of cellular HLA-DR expression in early lesions was low, indicating an inactivated and ineffective response. Also, the number of CD4+ T cells in the infiltrate was smaller, but steady, with no downregulation in older lesions in the repair phase. Rather there was an upregulation of the cellular response with a further influx of macrophages and skin homing memory/effector cells and an increase in cellular activation promoting tissue/bacterial/antigenic degradation and presentation. In addition, significant levels of angiogenesis also persisted, thus facilitating a prolonged inflammatory response. However, the majority of CD4+ T cells were skin homing memory/effector cells, with the absence of unclassified cells, suggesting that these patients are more susceptible to the causative antigen(s) and that theirs is purely an adaptive immune response. Thus, it may be interpreted that this is a chronic delayed type hypersensitivity reaction provoked by a persistent antigenic stimulus which these patients were initially unable to eliminate.

An individual's T-cell responsiveness to antigen is influenced by their MHC genotype, and certainly genetic predisposition factors have been considered to be involved in acne.[18,19] The findings in this study support this idea, as the different inflammatory cell profiles elicited by the two patient groups are indicative of a difference in sensitivity to the causal antigen(s). Such differences could explain the different qualities of repair observed between these groups, as the inflammatory response has been implicated as an important component in the development of scars. Embryonic wounds, which lack an intense inflammatory response, heal rapidly without scar formation,[20] while reduced scarring has been found in the elderly, who have impaired inflammatory responses.[21] We suggest that the type and magnitude of the inflammatory response in resolving lesions from S patients would lead to the abnormal healing and pathological scarring in these patients. Macrophages sustain T-cell activity and their strong presence represents a dominant force in this response. Lymphocytes and macrophages secrete an extensive array of cytokines and growth factors which are known to modulate dermal fibroblast recruitment, proliferation and phenotype, for example IL-1, transforming growth factor-β, platelet-derived growth factor, IL-4 and IL-6 are considered to be fibrogenic, while IL-2, tumour necrosis factor-α and interferon-γ are antifibrogenic.[22–24] During the course of wound healing there is a tight balance between pro- and anti-fibrinogenic cytokines and growth factors and any imbalances will drive fibroblasts into abnormal extracellular matrix protein synthesis and affect the fibroblast functions such as wound remodelling and contraction which are contributory factors in scarring.

Thus this quantitative comparative study has exposed inherent differences between two groups of patients and emphasizes that acne is a disorder which embraces a number of pathologies. It also signals that the treatment of acne should be more towards agents which influence the regulation of immune mechanisms and that management of either numbers or activation states of inflammatory cells may help to control scarring.

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