

Seborrhoeic dermatitis and *Pityrosporum* (*Malassezia*) folliculitis: characterization of inflammatory cells and mediators in the skin by immunohistochemistry

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Summary

Background The fact that *Pityrosporum ovale* plays a part in seborrhoeic dermatitis is well established but the mechanism of this relationship has not been established.

Objectives To compare the number and type of inflammatory cells and mediators in skin biopsies from normal and lesional skin from the trunk and scalp in patients with seborrhoeic dermatitis, *Pityrosporum* (*Malassezia*) folliculitis and in normal skin from healthy controls.

Methods The skin biopsies were stained using the labelled Streptavidin–biotin method. The following markers were studied: CD4, CD8, CD68, HLA-DR, NK1, CD16, C1q, C3c, IgG, CD54 (ICAM-1), interleukin (IL) -1 α , IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, tumour necrosis factor- α and interferon- γ .

Results HLA-DR+ cells were seen in the highest number, and were higher in lesional skin compared with normal skin from both patients and healthy volunteers. ICAM-1 expression was also increased in lesional skin. C1q and the interleukins showed an increased cellular and intercellular staining in patients compared with healthy controls and the intercellular staining was often more intense in lesions compared with non-lesional skin. Staining was often more intense when *Malassezia* (*Pityrosporum ovale*) yeast cells were present.

Conclusions An increase in NK1+ and CD16+ cells in combination with complement activation indicates that an irritant non-immunogenic stimulation of the immune system is important. The result with the interleukins showed both an increase in the production of inflammatory interleukins as well as in the regulatory interleukins for both T_H1 and T_H2 cells. Similarities to the immune response described for *Candida albicans* infections indicate the role of *Malassezia* in the skin response in seborrhoeic dermatitis and *Pityrosporum* folliculitis.

Key words: controls, *Malassezia* (*Pityrosporum*), patients, Streptavidin–biotin

There are now many studies indicating that *Malassezia* (*Pityrosporum ovale*) plays an important part in seborrhoeic dermatitis.^{1,2} Many of these are treatment studies that describe effectiveness of antimycotics, paralleled by a reduction in the number of *Malassezia*, whereas recolonization leads to a recurrence of seborrhoeic dermatitis. The increased incidence of seborrhoeic dermatitis in patients with immunosuppressive disorders suggests that the relationship between *Malassezia*

and the immune system is of importance, but the exact mechanism involved is still unknown. It is not within the scope of this paper to discuss the new nomenclature for the *Malassezia* yeasts. However, the *Malassezia* yeasts can be divided into six lipophilic species and one non-lipophilic member; the one which is the most important in seborrhoeic dermatitis and *Pityrosporum* (*Malassezia*) folliculitis is still not known. We will use the genus name *Malassezia* in this article. Studies designed to investigate the relationship between *Malassezia* and the immune system in seborrhoeic dermatitis fall into four areas, namely: (i) the humoral response to *Malassezia*;

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(ii) lymphocyte transformation response to *Malassezia*; (iii) lymphocyte proliferation response to *Malassezia* extracts; and (iv) T-helper (CD4)/T suppressor (CD8) cell ratios. The literature in these areas shows a considerable level of inconsistency.

The humoral immune response to *Malassezia* in seborrhoeic dermatitis can be shown to be elevated,³ lowered² or unchanged.⁴ In seborrhoeic dermatitis the lymphocyte transformation response can be shown to be reduced,^{5,6} enhanced⁷ or unchanged^{8,9} and the same has been reported with the lymphocyte proliferation response to *Malassezia* extracts. Results from our own lab are variable with a low (< 0.7) response to phytohaemagglutinin (PHA) and concanavalin A in 13 of 30 patients,¹⁰ but more recently in a separate study we found no difference in response.⁸ Similarly Ashbee *et al.*⁷ found a normal PHA stimulation response in both seborrhoeic dermatitis patients and controls.

The ratio of CD4/CD8 has been examined by ourselves and others. We found a normal or low CD4/CD8 ratio in 26 of 30 patients with seborrhoeic dermatitis.² Ashbee *et al.*⁷ also found a normal ratio whereas Kieffer *et al.*⁴ describe a lowered CD4/CD8 ratio in 13 of 19 patients with seborrhoeic dermatitis.

Such variability in cellular responses has not been satisfactorily explained thus far. The information about cytokine production in response to *Malassezia* is also variable and incomplete. In a study by Neuber *et al.*⁵ Interleukin (IL) -2 and interferon (IFN) - γ production by lymphocytes from patients with seborrhoeic dermatitis was markedly depressed and IL-10 synthesis was increased after stimulation with *Malassezia* extract. In another study by Kesevan *et al.*¹¹ the *Malassezia* yeast suppressed the production of the proinflammatory cytokines IL-1 α , IL-6 and tumour necrosis factor (TNF) - α . One immunohistochemical study in seborrhoeic dermatitis showed deposits of complement C3c and IgG in the stratum corneum below clusters of *Malassezia*.¹² In another study infiltrates of especially CD4-positive cells were found in lesional skin.¹⁰

In *Pityrosporum* (*Malassezia*) folliculitis, serum IgG antibodies against *Malassezia* have been found in higher titres than in healthy controls.¹³ An increased number of T-helper cells and Langerhans cells has been found in skin lesions from *Pityrosporum* folliculitis.¹²

Given the large amount of inconsistency in available data for both generalized responses to *Malassezia* and the changes in the immune response in patients with seborrhoeic dermatitis, we sought to investigate immune responses at their site of action in the skin. We

hypothesized that the reaction to *Malassezia*, leading to a well characterized clinical skin lesion, is primarily due to the local reaction of the skin to the presence of *Malassezia*. Thus, the aim of this study was to investigate the type of inflammatory cells and the presence of inflammatory mediators in skin biopsies from normal and lesional skin in patients with seborrhoeic dermatitis, *Pityrosporum* folliculitis and in normal skin from healthy controls.

Materials and methods

Patients

Fifteen patients, three females and 12 males (mean age 44 years; range 23–64) with seborrhoeic dermatitis; eight patients, six females and two males (mean age 36 years; range 26–48) with *Pityrosporum* folliculitis and four healthy volunteers, one female and three males (mean age 40 years; range 31–48) were included. The patients with seborrhoeic dermatitis had typical lesions on either the scalp and/or on the trunk (mid-chest or mid-back). The majority also had lesions on the face. Patients with *Pityrosporum* folliculitis had typical lesions on the upper chest or back. The patients were not investigated for human immunodeficiency virus but none of them were receiving immunosuppressive or immunomodulating drugs or had any symptoms or signs of internal diseases.

Procedure

Punch biopsies were taken from: (i) lesional and normal skin from the chest and/or mid-upper back area and the scalp in patients with seborrhoeic dermatitis; (ii) lesional and normal skin from the chest and/or mid-upper back area in patients with *Pityrosporum* folliculitis; and (iii) normal skin from the chest and scalp in healthy controls. In patients with seborrhoeic dermatitis lesions were not always found on both the trunk and the scalp, but in 10 patients biopsies were taken from both the trunk and the scalp. Biopsies were quick frozen in liquid nitrogen and then transferred to a -70°C freezer. As positive controls, biopsies from a tonsil and an allergic skin patch test reaction (72 h) were used.

Antibodies

Antibodies against the following cellular markers were used: CD4, CD8, CD68, HLA-DR, NK1 and CD 16.

Antibodies against the adhesion molecule ICAM-1 (CD54), against complement factors C1q and C3c, as well as against IgG were also included. Antibodies against interleukins were markers for the proinflammatory interleukins IL-1 α , IL-1 β , IL-6 and TNF- α and for the regulatory interleukins for T_H1 cells: IL-2, IL-12 and IFN- γ and for T_H2 cells IL-4 and IL-10. IL-12 also stimulates cells with natural killer cell function. CD4, CD8, CD68, HLA-DR, NK1, CD16 and IgG were obtained from DAKO (Copenhagen, Denmark). CD54 (ICAM-1) was obtained from Calbiochem (San Diego, CA, U.S.A.). C1q, C3c, IL-1 α , IL-1 β , IL-2, IL-4, IL-10, IL-12 and IFN- γ were obtained from Sigma Bio Sciences (St Louis, MO, U.S.A.). IL-6 and TNF- α were obtained from Innogenetics (Zwijndrecht, Belgium). The labelled Streptavidin–biotin (LSAB) kit containing hydrogen peroxide, biotinylated link antibody and peroxidase-labelled streptavidin as well as 3,3'-diaminobenzidine (DAB) was obtained from DAKO (Copenhagen, Denmark).

Immunohistochemistry

The biopsies were cut in 6- μ m sections, placed on a glass slide at room temperature for 30 min and frozen at -70 °C. When ready for staining, the glass slide was kept at room temperature for 30 min and was then fixed with phosphate-buffered formaldehyde solution, 4%, pH 7.4 before staining using the LSAB technique. Three sections were cut and stained from each biopsy. Endogenous peroxide was blocked with 0.6% hydrogen peroxide in methanol for 15 min at room temperature. The primary antibody was applied and the section was incubated for 60 min. Then, first the biotinylated link antibody (15 min) and secondly peroxidase-labelled streptavidin (30 min) was applied. DAB was subsequently applied for 5 min. The sections were counterstained with haematoxylin and finally mounted.

The dilution used for the primary antibodies was: 1 : 10 for CD4 and CD8; 1 : 100 for CD68; 1 : 50 for HLA-DR, NK1, CD54 and CD16; 1 : 20,480 for C1q; 1 : 40,960 for C3c; 1 : 38,400 for IgG; 1 : 500 for IL-1 α and IL-2; 1 : 2000 for IL-1 β ; 1 : 25 for IL-4; 1 : 40 for IL-6; 1 : 125 for IL-10; 1 : 250 for IL-12; 1 : 40 for TNF- α ; and 1 : 10 for IFN- γ . Controls included slides with only the secondary layer antibody and isotype matched primary antibodies.

The number of stained cells in six high power fields ($\times$ 400) from the best of three sections was counted and, when present, the intercellular staining was

assessed using a 0–3 scale (0 = no staining and 3 = maximum staining). Two different observers read the slides and the mean values were used.

Statistics

The difference in number of positive cells and intercellular staining between lesional and non-lesional skin in patients and between patients and healthy controls were compared using the Student–Newman–Keuls test for variables.

Results

Seborrhoeic dermatitis

Biopsies from normal and lesional skin from the trunk and scalp were available in 10 patients from each location. In several biopsies from lesions there were dense cell infiltrates. The cells were lymphocytes, macrophages, monocytes, Langerhans cells and a few granulocytes. The cell infiltrates were found mainly in the upper dermis and in particular perivascular and around the upper parts of hair follicles. However, inflammatory cells were also sometimes seen in the epidermis and in a band at the dermal–epidermal border.

No significant differences in number of immunoreactive cells were seen when skin from the trunk was compared with scalp skin in either the lesional, non-lesional or normal healthy samples. Therefore, the data from the trunk and scalp were taken together in patients with seborrhoeic dermatitis and healthy volunteers. The highest number of cells stained with the cellular markers were seen in lesional skin (Tables 1–4). However, the number of cells stained with the cellular markers was also higher in both lesional and normal skin from patients compared with biopsies from healthy controls. Because of the low number of healthy controls ($n = 4$) a statistical trend for the cellular markers was only seen with the markers for CD4, HLA-DR, NK1 and CD16. The dominating cells were HLA-DR+ cells (Fig. 1). CD4+, NK1+ and CD16+ cells were also present in high numbers. CD68+ cells, a marker for macrophages, monocytes and Langerhans cells, were found in the same numbers in skin from healthy controls and from both lesional and non-lesional skin from patients. The presence of high numbers of NK1+ and CD16+ cells in biopsies from patients, especially in lesional skin, indicate that a non-immunogenic, irritant-like stimulation of the immune

Table 1 Skin biopsies from normal skin of patients with seborrhoeic dermatitis ($n = 10$), *Pityrosporum folliculitis* ($n = 8$) and healthy volunteers ($n = 4$)

Stain	Number of cells (mean $\pm$ SD)			Significance		
	Seborrhoeic dermatitis	<i>Pityrosporum folliculitis</i>	Volunteers	Seborrhoeic vs. volunteers	<i>Pityrosporum</i> vs. volunteers	Seborrhoeic vs. <i>Pityrosporum</i>
CD4	29 $\pm$ 21.0	27 $\pm$ 16.0	13 $\pm$ 7.7	ns	ns	ns
CD8	11 $\pm$ 12.0	6 $\pm$ 3.6	8 $\pm$ 5.7	ns	ns	ns
CD68	14 $\pm$ 11.4	15 $\pm$ 11.4	21 $\pm$ 6.0	ns	ns	ns
HLA-DR	40 $\pm$ 15.2	38 $\pm$ 14.2	18 $\pm$ 11.5	0.0032	0.0032	ns
NK1	22 $\pm$ 11.3	17 $\pm$ 6.4	9 $\pm$ 5.1	0.0076	0.0076	ns
CD16	25 $\pm$ 15.4	15 $\pm$ 9.4	7 $\pm$ 5.2	0.0066	0.0066	ns
C1q	13 $\pm$ 7.0	10 $\pm$ 3.6	3 $\pm$ 2.2	0.0007	0.0007	ns
C3c	29 $\pm$ 20.1	18 $\pm$ 13.2	22 $\pm$ 20.1	ns	ns	ns
IgG	16 $\pm$ 9.9	10 $\pm$ 10.3	5 $\pm$ 7.0	0.031	ns	ns
CD54	31 $\pm$ 13.0	23 $\pm$ 15.0	24 $\pm$ 11.1	ns	ns	ns
IL-1 α	15 $\pm$ 14.9	15 $\pm$ 11.8	4 $\pm$ 4.0	0.0481	0.0481	ns
IL-1 β	22 $\pm$ 14.2	25 $\pm$ 28.4	4 $\pm$ 3.6	0.0436	0.0436	ns
IL-2	21 $\pm$ 15.8	14 $\pm$ 9.7	3 $\pm$ 1.7	0.0085	0.0085	ns
IL-4	30 $\pm$ 12.8	27 $\pm$ 20.6	8 $\pm$ 4.8	0.0025	0.0025	ns
IL-6	22 $\pm$ 14.0	13 $\pm$ 13.9	6 $\pm$ 5.7	0.0147	ns	ns
IL-10	25 $\pm$ 11.7	22 $\pm$ 21.2	5 $\pm$ 3.8	0.0049	0.0049	ns
IL-12	15 $\pm$ 10.1	10 $\pm$ 5.6	2 $\pm$ 1.7	0.0011	0.0011	ns
TNF- α	18 $\pm$ 9.7	21 $\pm$ 20.1	8 $\pm$ 2.7	ns	ns	ns
IFN- γ	18 $\pm$ 12.8	19 $\pm$ 20.1	3 $\pm$ 2.8	0.0312	0.0312	ns

SD, standard deviation.

system may be occurring (Fig. 2). CD54+ (ICAM-1) cells were found in higher numbers in lesional skin in patients with seborrhoeic dermatitis compared with healthy controls.

The complement markers C1q and C3c were expressed on cells and intercellular deposits were found in both

lesional and non-lesional skin from patients. The number of cells stained with C1q, but not C3c, was higher in patients compared with controls. No difference in the number of complement positive cells was found between lesional and non-lesional skin in patients. However, the intercellular staining was more

Table 2 Skin biopsies from lesional skin of patients with seborrhoeic dermatitis ($n = 10$), *Pityrosporum folliculitis* ($n = 8$) and normal skin in healthy volunteers ($n = 4$)

Stain	Number of cells (mean $\pm$ SD)			Significance		
	Seborrhoeic dermatitis	<i>Pityrosporum folliculitis</i>	Volunteers	Seborrhoeic vs. volunteers	<i>Pityrosporum</i> vs. volunteers	Seborrhoeic vs. <i>Pityrosporum</i>
CD4	43 $\pm$ 24.9	44 $\pm$ 26.3	13 $\pm$ 7.7	0.0096	0.0096	ns
CD8	19 $\pm$ 12.7	21 $\pm$ 15.9	8 $\pm$ 5.7	ns	ns	ns
CD68	24 $\pm$ 16.8	36 $\pm$ 19.4	21 $\pm$ 6.0	ns	ns	ns
HLA-DR	59 $\pm$ 14.8	57 $\pm$ 13.9	18 $\pm$ 11.5	0.0001	0.0001	ns
NK1	38 $\pm$ 19.7	27 $\pm$ 10.1	9 $\pm$ 5.1	0.0007	0.0007	ns
CD16	45 $\pm$ 23.8	74 $\pm$ 18.0	7 $\pm$ 5.2	0.0001	0.0001	0.001
C1q	13 $\pm$ 5.4	12 $\pm$ 4.4	3 $\pm$ 2.2	0.0001	0.0001	ns
C3c	27 $\pm$ 20.5	23 $\pm$ 16.7	22 $\pm$ 20.1	ns	ns	ns
IgG	15 $\pm$ 12.5	12 $\pm$ 6.7	5 $\pm$ 7.0	ns	ns	ns
CD54	46 $\pm$ 19.2	43 $\pm$ 26.8	24 $\pm$ 11.1	0.0386	0.0386	ns
IL-1 α	18 $\pm$ 15.7	32 $\pm$ 23.1	4 $\pm$ 4.0	0.0057	0.0057	ns
IL-1 β	23 $\pm$ 23.9	32 $\pm$ 21.9	4 $\pm$ 3.6	0.0377	0.0377	ns
IL-2	17 $\pm$ 15.3	26 $\pm$ 20.1	3 $\pm$ 1.7	0.0147	0.0147	ns
IL-4	28 $\pm$ 15.8	29 $\pm$ 13.1	8 $\pm$ 4.8	0.003	0.003	ns
IL-6	17 $\pm$ 10.8	16 $\pm$ 10.5	6 $\pm$ 5.7	0.0432	0.0432	ns
IL-10	24 $\pm$ 21.4	22 $\pm$ 12.6	5 $\pm$ 3.8	0.0398	0.0398	ns
IL-12	12 $\pm$ 9.9	13 $\pm$ 8.5	2 $\pm$ 1.7	0.0126	0.0126	ns
TNF- α	22 $\pm$ 16.0	32 $\pm$ 18.1	8 $\pm$ 2.7	0.012	0.012	ns
IFN- γ	23 $\pm$ 14.7	18 $\pm$ 9.7	3 $\pm$ 2.8	0.0019	0.0019	ns

Table 3 Skin biopsies from lesional skin and normal skin of patients with seborrhoeic dermatitis ($n = 10$).

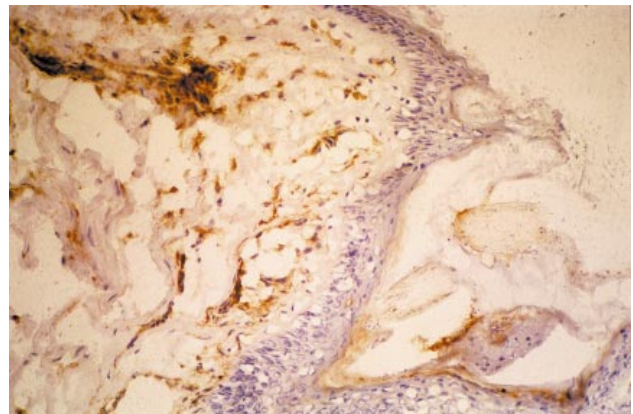
Stain	Number of cells (mean $\pm$ SD)		P-value
	Normal	Lesional	
CD4	29 $\pm$ 21.0	43 $\pm$ 24.9	0.0057
CD8	11 $\pm$ 12.0	19 $\pm$ 12.7	0.05
CD68	14 $\pm$ 11.4	24 $\pm$ 16.8	0.0095
HLA-DR	40 $\pm$ 15.2	59 $\pm$ 14.8	0.0007
NK1	22 $\pm$ 11.3	38 $\pm$ 19.7	0.0063
CD16	25 $\pm$ 15.4	45 $\pm$ 23.8	0.0314
C1q	13 $\pm$ 7.0	13 $\pm$ 5.4	ns
C3c	29 $\pm$ 20.1	27 $\pm$ 20.5	ns
IgG	16 $\pm$ 9.9	15 $\pm$ 12.5	ns
CD54	31 $\pm$ 13.0	46 $\pm$ 19.2	ns
IL-1 α	15 $\pm$ 14.9	18 $\pm$ 15.7	ns
IL-1 β	22 $\pm$ 14.2	23 $\pm$ 23.9	ns
IL-2	21 $\pm$ 15.8	17 $\pm$ 15.3	ns
IL-4	30 $\pm$ 12.8	28 $\pm$ 15.8	ns
IL-6	22 $\pm$ 14.0	17 $\pm$ 10.8	ns
IL-10	25 $\pm$ 11.7	24 $\pm$ 21.4	ns
IL-12	15 $\pm$ 10.1	12 $\pm$ 9.9	ns
TNF- α	18 $\pm$ 9.7	22 $\pm$ 16.0	ns
IFN- γ	18 $\pm$ 12.8	23 $\pm$ 14.7	ns

intense with the marker for C1q in lesional skin from patients with seborrhoeic dermatitis compared with non-lesional skin (Table 5). In several sections yeasts, coated with complement, were observed. The highest number of yeasts were seen in the follicle often surrounded by perifollicular infiltrating cells.

An increased number of interleukin-positive cells was found in both lesional and non-lesional skin from

Table 4 Skin biopsies from lesional skin and normal skin of patients with *Pityrosporum folliculitis* ($n = 8$).

Stain	Number of cells (mean $\pm$ SD)		P-value
	Normal	Lesional	
CD4	27 $\pm$ 16.0	44 $\pm$ 26.3	0.0484
CD8	6 $\pm$ 3.6	21 $\pm$ 15.9	0.0226
CD68	15 $\pm$ 11.4	36 $\pm$ 19.4	0.0092
HLA-DR	38 $\pm$ 14.2	57 $\pm$ 13.9	0.0017
NK1	17 $\pm$ 6.4	27 $\pm$ 10.1	0.0059
CD16	15 $\pm$ 9.4	74 $\pm$ 18.0	0.0001
C1q	10 $\pm$ 3.6	12 $\pm$ 4.4	ns
C3c	18 $\pm$ 13.2	23 $\pm$ 16.7	ns
IgG	10 $\pm$ 10.3	12 $\pm$ 6.7	ns
CD54	23 $\pm$ 15.0	43 $\pm$ 26.8	0.0436
IL-1 α	15 $\pm$ 11.8	32 $\pm$ 23.1	ns
IL-1 β	25 $\pm$ 28.4	32 $\pm$ 21.9	ns
IL-2	14 $\pm$ 9.7	26 $\pm$ 20.1	ns
IL-4	27 $\pm$ 20.6	29 $\pm$ 13.1	ns
IL-6	13 $\pm$ 13.9	16 $\pm$ 10.5	ns
IL-10	22 $\pm$ 21.2	22 $\pm$ 12.6	ns
IL-12	10 $\pm$ 5.6	13 $\pm$ 8.5	ns
TNF- α	21 $\pm$ 20.1	32 $\pm$ 18.1	ns
IFN- γ	19 $\pm$ 20.1	18 $\pm$ 9.7	ns

**Figure 1.** In lesional skin in seborrhoeic dermatitis HLA-DR+ cells are found both perivascular, perifollicular and in a subepidermal band. A few cells are also seen in the epidermis ($\times 200$, scalp skin).

patients with seborrhoeic dermatitis compared with healthy controls (Tables 1 and 2 and Figs 3–5). No difference was observed in the number of positive cells between lesional and non-lesional skin in patients (Tables 3 and 4). The intercellular staining was much stronger in both lesional and non-lesional skin from patients with seborrhoeic dermatitis compared with normal skin from healthy volunteers. In patients with seborrhoeic dermatitis the intercellular staining was more intense in lesional skin compared with non-lesional skin with markers for the interleukins IL-1 α , IL-1 β , TNF- α , IFN- γ , IL-12 and IL-4 (Table 5).

Pityrosporum folliculitis

The results were very similar to the results described for seborrhoeic dermatitis (Tables 1–4). The only significant

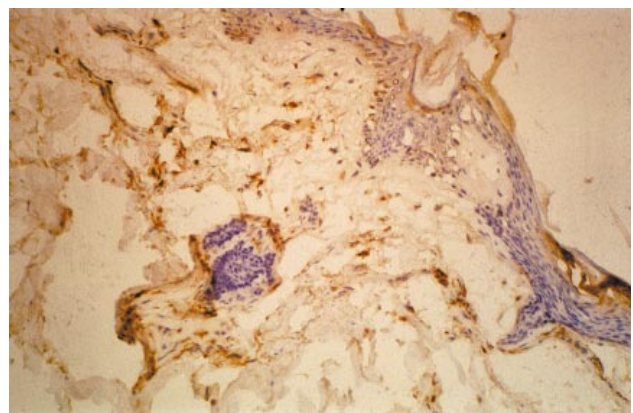
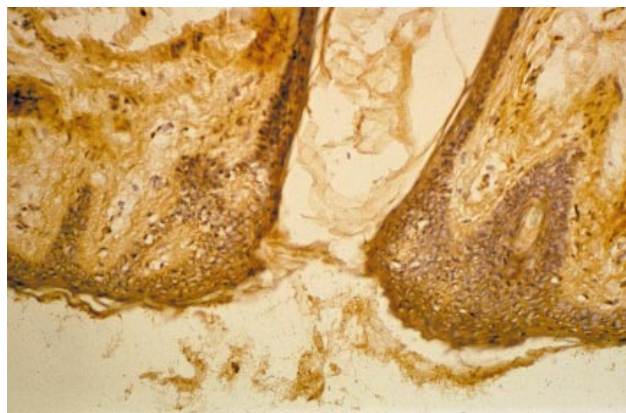
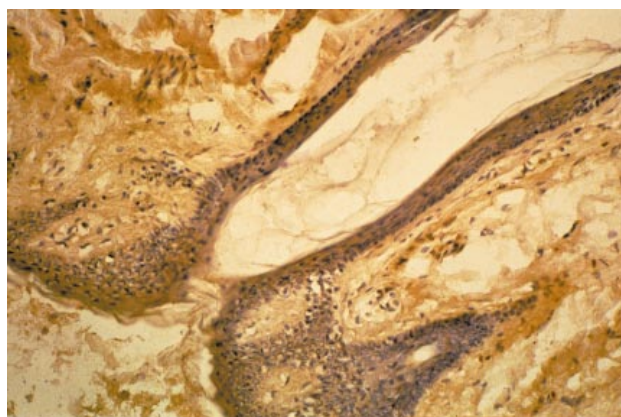
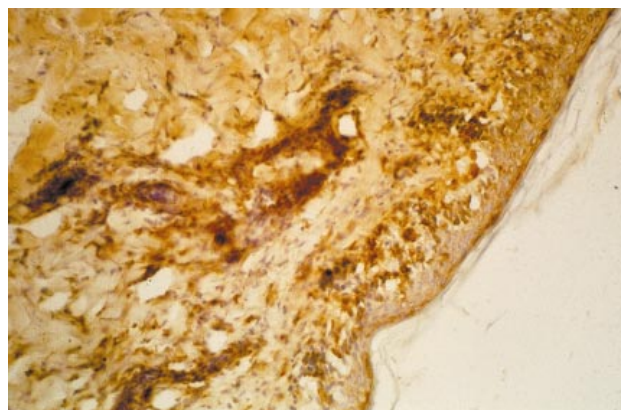
**Figure 2.** CD16, a marker for natural killer cells and monocytes/macrophages, was found in high number in lesional skin in seborrhoeic dermatitis ($\times 200$, scalp skin).

Table 5 Intercellular staining in skin biopsies from lesional and nonlesional skin in patients with seborrhoeic dermatitis.

Stain	Number of cells (mean $\pm$ SD)		P-value
	Normal	Lesional	
C1q	2.4 $\pm$ 0.5	1.9 $\pm$ 0.6	0.0331
C3c	1.3 $\pm$ 1.2	1.4 $\pm$ 1.1	ns
IL-1 α	1.1 $\pm$ 0.3	1.9 $\pm$ 0.8	0.0232
IL-1 β	1 $\pm$ 0	1.9 $\pm$ 0.8	0.0209
IL-2	1 $\pm$ 0	1.3 $\pm$ 0.5	ns
IL-4	1.2 $\pm$ 0.4	1.9 $\pm$ 0.8	0.0497
IL-6	1.2 $\pm$ 0.7	1.7 $\pm$ 0.9	ns
IL-10	1.4 $\pm$ 0.5	1.7 $\pm$ 0.7	ns
IL-12	0.4 $\pm$ 0.5	1.0 $\pm$ 0.7	0.0133
TNF- α	0.8 $\pm$ 0.6	2.0 $\pm$ 0.9	0.0212
IFN- γ	0.7 $\pm$ 0.9	1.3 $\pm$ 0.9	0.025

difference between *Pityrosporum* folliculitis and seborrhoeic dermatitis was a higher number of CD16+ cells found in lesional skin of *Pityrosporum* folliculitis compared with seborrhoeic dermatitis (Fig. 6). CD54+ cells were found in higher number in lesional skin compared with non-lesional skin in patients with *Pityrosporum* folliculitis; in lesional skin in patients with *Pityrosporum* folliculitis the number was higher than in healthy controls. The intercellular staining with the markers for C3c and IL-1 α was more intense in lesional skin from patients with *Pityrosporum* folliculitis compared with non-lesional skin (for C3c, mean = 1.57 vs. mean = 0.86 for non-lesional skin, $P = 0.05$. For IL1 α , mean = 2.14 vs. mean = 1.14 for non-lesional skin, $P = 0.02$). However, the distribution of cell infiltrates was primarily perifollicular and a high number of yeasts was often seen in the follicle, even in the deeper parts. The cellular markers and the

**Figure 3.** In seborrhoeic dermatitis a strong cellular and intercellular staining was found with the marker for interleukin-1 α especially perivascular, perifollicular and in the basal part of the epidermis. The intercellular staining was strongest in lesional skin ($\times 200$, scalp skin).**Figure 4.** This staining with the marker for interleukin-2 in seborrhoeic dermatitis was almost identical with the staining for interleukin-1 α ($\times 200$, scalp skin).**Figure 5.** The staining in seborrhoeic dermatitis with the marker for interleukin-4 almost identical to the staining seen in Figures 3 and 4 ($\times 200$, scalp skin).**Figure 6.** The highest number of CD16+ cells was found perifollicular in *Pityrosporum* folliculitis ($\times 200$).

interleukins, complement and IgG were more intensely immunoreacting in the perifollicular region, compared with seborrhoeic dermatitis.

Discussion

Earlier reports have tried to characterize the inflammatory cells seen in lesional skin from seborrhoeic dermatitis^{2,4,12,14} and *Pityrosporum* folliculitis.¹³ However, this study characterizes the profile in skin biopsies of both various cellular markers and the distribution of IgG, various complement factors and both proinflammatory and regulatory interleukins in seborrhoeic dermatitis and *Pityrosporum* folliculitis compared with healthy controls. With the exception of CD68 all cellular markers examined showed an increased cellular staining in both seborrhoeic dermatitis and *Pityrosporum* folliculitis compared with healthy controls. A high number of HLA-DR+, NK1+ and CD16+ cells was also found. HLA-DR is one of the major expressed products of the human class II region. It is expressed by Langerhans cells, macrophages and activated keratinocytes.

The increased number of NK1+ and CD16+ cells as well as an increase in complement staining indicate that an irritant or non-immunogenic stimulation of the immune system is important in both seborrhoeic dermatitis and *Pityrosporum* folliculitis. The reaction seen with the interleukins is complex, showing both an increase in the production of the inflammatory interleukins IL-1 α , IL-1 β , IL-6 and TNF- α as well as interleukins responsible both for a T_H1 and a T_H2 reaction. Importantly, no major differences were seen in the number of interleukin-associated cells between lesional and non-lesional skin in both seborrhoeic dermatitis and *Pityrosporum* folliculitis. However, the intercellular staining was more intense in lesional skin. The staining was also more intense in patients compared with healthy controls.

An increase in the production of interleukins responsible both for T_H1 and T_H2 responses were seen. IL-12 will not only stimulate a T_H1 reaction but it also stimulates NK cell proliferation, which may explain the increase in NK1+ and CD16+ cells. CD54+ (ICAM-1) cells were increased in lesional skin in both patients with seborrhoeic dermatitis and *Pityrosporum* folliculitis. ICAM-1 expression is driven by IL-1, which was also elevated in a similar way. Positive cells were found both in the dermis (e.g. T cells, monocytes/macrophages, dendritic cells) and in the epidermis (keratinocytes). No statistically significant difference was seen in the

number of interleukin-positive cells in lesional skin compared with non-lesional skin in patients with seborrhoeic dermatitis. However, the intercellular staining was more intense in lesional skin compared with non-lesional skin. The biopsies from non-lesional skin were taken close to a lesion, in order to reduce the difference between various anatomical locations, and microscopically, signs of inflammation might therefore be present.

Earlier studies on both humoral and cellular immune responses to various *Malassezia* antigen preparations show conflicting results, as previously described.^{3-9,14,15} The preparation of the extract is probably of major importance and it has recently been shown that *Malassezia* is capable of suppressing the release of proinflammatory cytokines by peripheral blood mononuclear cells and that removal of lipids from the cell wall negates this ability.¹¹ The local immune response in the skin, therefore, may be different from the results obtained from *in vitro* studies on peripheral blood mononuclear cells and may better explain the nature of the inflammatory reaction seen in both seborrhoeic dermatitis and *Pityrosporum* folliculitis.

In several studies *Malassezia* has been mentioned as an important allergen in atopic dermatitis (reviewed¹⁶). However, the immune response seen in the skin in both patients with seborrhoeic dermatitis and *Pityrosporum* folliculitis is different from that seen in atopic dermatitis.^{10,12,13,16} In atopic dermatitis an increase in the regulatory interleukins for T_H2 cells are found¹⁷ and ICAM-1 expression is increased not only in lesional but also in non-lesional skin in atopic dermatitis.¹⁸

The immune response in the skin of patients with seborrhoeic dermatitis or *Pityrosporum* folliculitis is complex but showed some similarities with the results obtained with *Candida* infections.^{19,20} *Malassezia* is a member of the normal skin flora and all individuals have both a humoral and a cellular immune response to this yeast.¹⁶ This is probably one of the important explanations of why the immune response in the skin is more complex in diseases where this organism is involved.

In summary, we have attempted here to understand the immune response in seborrhoeic dermatitis and *Pityrosporum* folliculitis by examining the *in situ* response in the skin. This was intended to circumvent the issues of inconsistency arising when the humoral and cellular blood responses have been examined by ourselves and others in the field. A key finding is the similarity between non-lesional and lesional skin in seborrhoeic dermatitis. This may be due to the

non-lesional sites having preclinical lesions, or also could be explained by a greater predisposition or generalized sensitivity to *Malassezia* in seborrhoeic dermatitis patients. This is exemplified by the situation in *Pityrosporum* folliculitis where the differences between lesional and non-lesional sites is much greater and more obviously linked to the presence of *Malassezia* in follicles. A second key finding is the increased number of CD16+ and NK1+ cells in conjunction with complement activation. This sort of response is characteristic of an irritant response. The causes of this response are likely to be products released by *Malassezia*. However, the means of ingress into the skin and the nature of the irritant remains to be elucidated.

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