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CD23/FcεR11 expression in contact sensitivity reactions: a comparison between aeroallergen patch test reactions in atopic dermatitis and the nickel patch test reaction in non-atopic individuals

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SUMMARY

The immunopathology of patch test reactions to aeroallergens in patients with atopic dermatitis (AD) has been compared to that of contact sensitivity reactions to nickel in non-atopic individuals. Both reactions were found to exhibit equivalent erythema and induration on gross examination at 48 h. Four millimetre punch biopsies were obtained at 48 h frozen, and cryostat sections stained with a panel of MoAbs. The distribution of macrophages, dermal dendritic cells, Langerhans cells, T lymphocytes and the expression of CD23 antigen was recorded. Increased numbers of dermal dendritic cells, macrophages, T lymphocytes and Langerhans cells were found in the dermal infiltrates of both the nickel patch test reactions and the aeroallergen patch test reactions compared with their respective controls. There were no significant differences between atopic patch test reaction and nickel patch test reaction samples in the tissue distribution of these cell types. There was a significant increase in CD23 expression on Langerhans cells and dermal dendritic cells in the atopic patch test reactions, whereas an increase was only observed on dendritic cells in nickel patch test reactions. No significant difference in CD23 expression was observed in the control skin samples taken from patients with AD, nickel-sensitive patients and normal controls. This study supports the hypothesis that the aeroallergen patch test reaction in atopic dermatitis is a delayed hypersensitivity reaction, yet is distinct from the contact sensitivity reaction to nickel in terms of raised expression of CD23.

Keywords atopic dermatitis low affinity IgE receptors aeroallergens cell-mediated immunity contact allergic sensitivity

INTRODUCTION

Allergic contact dermatitis is regarded as a classical delayed hypersensitivity reaction. The cellular and molecular mechanisms governing this reaction are now well understood [1,2].

The histology of lesional skin in patients with atopic dermatitis (AD) demonstrates a dermal mononuclear cell infiltrate consisting of activated T lymphocytes and Langerhans cells [3]. This is thought to represent a cell-mediated immune reaction. Many investigators have shown a reduced capacity to manifest DTH reactions to inorganic allergens in patients with AD [4–7], in particular when the disease is active [8]. Despite this, patch testing with aeroallergens produces a delayed type eczematous response at 48 h in many patients with AD [9–12]. Histological examination of such reactions shows the presence of increased numbers of Langerhans cells and activated T lymphocytes within the dermal infiltrate [13,14]. Aeroallergen patch test reactions have previously been compared with lesional skin from patients with AD and demonstrated an identical immunopathological picture [12]. These parameters reflect a DTH reaction. Subsequent studies of the distribution of $CD23^+$ cells (low affinity receptor for IgE) revealed that a shift in CD23 expression occurred from macrophages in the control samples to Langerhans cells and dermal dendritic cells, both in the developing atopic patch test reaction and in lesional skin. Bruynzeel-Koomen *et al.* [15] have compared an allergen-induced patch test reaction with thiuram-induced reaction in the same patient, and observed that the main difference between the two reactions was the presence of infiltrating eosinophils in the allergen-induced reaction. It is not known, however, whether the changing distribution of CD23 on dendritic cells is a feature of delayed hypersensitivity reactions in general or a feature unique to atopic dermatitis.

It has recently been demonstrated *in vitro* that the presence of IgE on Langerhans cells is important for antigen presentation

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in the atopic patient [16], but there is no information concerning the role of IgE receptors on antigen-presenting cells in non-atopic individuals. In order to answer these questions, this study makes a direct comparison between the atopic patch test reaction in patients with AD and the delayed hypersensitivity reaction promoted by nickel in patients with allergic contact dermatitis.

PATIENTS AND METHODS

Patients

Fifty-six patients (37 male, 19 female) with atopic dermatitis (age range 22–38 years) fullfilling the diagnostic criteria of Hanifin & Rajka [17] were studied. Patients had moderate to severe eczema requiring daily topical corticosteroids. Seven of the patients had bronchial asthma and 20 suffered from seasonal allergic rhinitis. All patients were skin prick tested on the volar aspect of the non-dominant forearm to the following common inhaled allergens: *Cladosporium herbarum*, *Alternaria tenuis*, *Candida albicans*, *Dermatophagoides pteronyssinus*, *D. farinae*, cat and dog dander, birch extract, plane tree extract, rye grass and timothy grass pollens (Biodiagnostics, Ltd) using 1% histamine as a positive control and diluent of 0·4% phenol and 50% glycerin in 0·9% saline as a negative control. The allergen concentrations are expressed in biological units (BU). All the allergens have 50 000 BU/ml equivalent to approximately 5000 protein nitrogen units (PNU)/ml. All atopic individuals developed a wheal and flare reaction (wheal diameter > 3 mm) to one or more allergens. Eight individuals with allergic contact dermatitis to nickel with no personal or family history of atopy and negative skin prick test reactivity were chosen for comparison.

Patch testing and skin biopsies

All patients had not applied topical corticosteroids to the areas to be patch tested for 2 weeks before testing and none had received ultra-violet B irradiation, PUVA or treatment with immunosuppressive agents in the previous month.

Epicutaneous patch testing in the atopic group was performed with 8 mm Finn Chambers (Epitest Ltd, Helsinki, Finland) containing aeroallergens in the same concentrations as used for skin prick testing. The chambers, containing patch test filter paper impregnated with 50 μl of allergen, were applied to clinically normal skin on both sides of the upper back, one side having been prepared by Sellotape stripping 15 times. One chamber on either side contained diluent as control.

Patients with allergic contact dermatitis were patch tested on clinically normal skin on the upper back (without prior Sellotape stripping) with 5% nickel sulphate, one chamber containing white petrolatum as control The patches were removed at 48 h. The positive reactions in both patch test groups were graded according to established criteria [18]. Four millimetre punch biopsies were taken from positive patch test reactions and the patch test control sites in eight patients from each group.

Biopsies were also taken from non-Sellotape stripped skin on the upper back from five age- and sex-matched normal controls with no personal or family history of atopy, and who were skin prick test-negative to the above aeroallergens. All biopsies were placed in OCT embedding compound (Brights, Instruments, UK) snap frozen in liquid nitrogen and stored at $-70°C$. Sections (6 μm) were cut using a cryostat, air dried for 1 h, fixed in chloroform:acetone (1:1) for 10 min and stored at $-20°C$.

Immunostaining

The following panel of MoAbs were used: CD1, a marker for thymocytes and Langerhans cells (Dakopatts, Ltd); CD23, FCεR11/low affinity IgE receptors (Dakopatts); RFD1, a marker for dendritic antigen-presenting cells [19] (Royal Free Hospital School of Medicine, London, UK (RFHSM)); RFD7, a mature tissue macrophage marker (RFHSM); RFT-mix, a pan T cell marker (RFHSM) [19]. For the staining of individual cell types the indirect alkaline phosphatase anti-alkaline phosphatase method was used. The primary antibody diluted in Tris-buffered saline (TBS) was added to the sections and incubated for 30 min. After washing in TBS for 2 min a second layer of rabbit anti-mouse immunoglobulin (Dakopatts) was applied and incubated for 30 min, washed for 2 min and followed by a third layer of an alkaline phosphatase mouse anti-alkaline phosphatase complex (APAAP; Dakopatts) for a further 30 min. Fast red (Sigma) was used as developer. Sections were counterstained in haematoxylin and mounted in PBS/glycerol (9:1). For controls the first layer was replaced by TBS. Double staining was performed for CD1/CD23, RFD7/CD23 using APAAP and peroxidase methods sequentially. Here peroxidase staining identified CD23 expression and APAAP that of macrophages ($RFD7^+$) and Langerhans cells ($CD1^+$). Immunofluorescence was performed using a second layer conjugated with FITC and tetramethylrhodamine isothiocyanate (TRITC) to identify $RFD1^+/CD23^+$ and $RFD1^+/CD1^+$ cells.

Quantification of the distribution of specific cell types was performed using an image analyser. At least six areas of tissue, viewed at $\times 400$ magnification, were defined and measured and counts made of the positively stained cells. The results were expressed as the number of cells per unit area ($\times 10^5$ μm^2). The number of $RFD1^+$ cells was estimated in at least six areas of dermis per section, viewed under a fluorescence microscope, and the results expressed as the number of cells per high power field ($\times 400$).

Statistical analysis

Where appropriate, significance was determined using the Wilcoxon sign rank test for paired data. $P < 0{\cdot}05$ was considered significant.

RESULTS

Clinical/histology appearance

The clinical appearance of the positive aeroallergen and nickel patch test reactions were identical and consisted of erythema, papules and induration in all cases, with vesicle formation in some. No urticarial lesions were observed in either group at 48 h. The histology of these positive reactions was also similar in both groups, with varying degrees of epidermal spongiosis and a dermal mononuclear cell infiltrate in the perivascular and intervascular areas. An increased number of eosinophils were observed in dermal infiltrate of the aeroallergen patch test reactions compared with the aeroallergen patch test control. The *stratum corneum* was intact on all the biopsies taken from Sellotape-stripped sites.

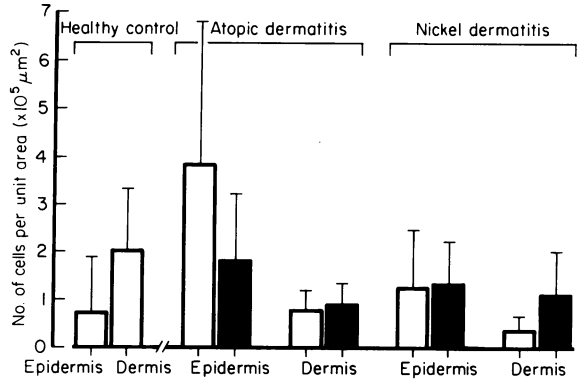

Fig. 1. Distribution of Langerhans cells (CD1$^+$) within the epidermis and dermis of normal healthy control skin, atopic patch test reaction, atopic patch test control, nickel patch test reaction and nickel patch test control. Mean values ± s.d. are shown. □, Patch test control; ■, patch test reaction.

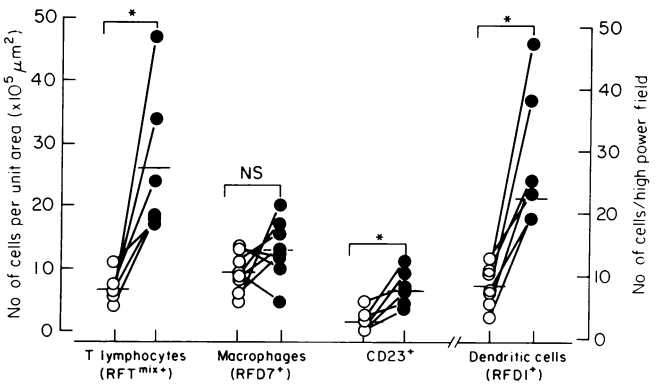

Fig. 3. Number of T lymphocytes (RFT^{mix+}), macrophages (RFD7$^+$) and CD23$^+$ cells per unit area, and the number of dendritic cells (RFD1$^+$) per high power field in the nickel patch test control (○) and nickel patch test reaction biopsies (●) from individual patients. *$P < 0.05$; NS, not significant. Horizontal bars = median values.

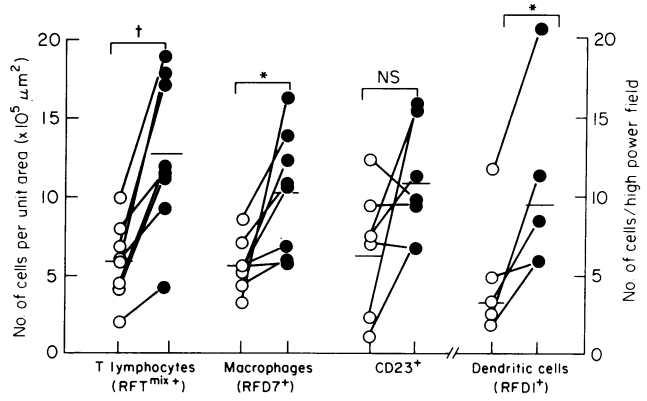

Fig. 2. Number of T lymphocytes (RFT^{mix+}), macrophages (RFD7$^+$) and CD23$^+$ cells per unit area, and the number of dendritic cells (RFD1$^+$) per high power field in the atopic patch test control (○) and atopic patch test reaction (●) biopsies from individual patients. *$P < 0.05$; †$P < 0.001$; NS, not significant. Horizontal bars = median values.

Table 1. CD23 expression on dendritic cells in control biopsies

	Macrophages RFD7 (%)	Langerhans cells CD1 (%)	Dendritic cells RFD1 (%)
Normal control	30 ± 5	8 ± 3	2 ± 2
Atopic patch test control	36 ± 10	11 + 1	4 + 1
Nickel patch test control	25 ± 6	3 ± 2	2 ± 3

Mean (± s.d.) values for the percentage of macrophages (RFD7$^+$), Langerhans cells (CD1$^+$) and dendritic cells (RFD1$^+$) in the dermis of the control samples coexpressing the CD23 receptor.

Immunohistology

Figure 1 shows the distribution of Langerhans cells (LC) in the epidermis and dermis of the biopsy specimens. There was no difference in the number of epidermal and dermal LC in the nickel patch test reactions and their controls. In atopic patch test reactions there was no significant difference in number of dermal LC compared with atopic controls, while there was a trend towards reduced numbers of epidermal LC in the atopic patch test reactions compared with atopic control. There was a significant increase in the number of epidermal LC in the Sellotape-stripped atopic patch test compared with normal control skin (Fig. 1).

There were significant increases in the number of T lymphocytes ($P < 0.001$), macrophages (RFD7$^+$) ($P < 0.05$), dendritic cells (RFD1$^+$) ($P < 0.05$) in all atopic patch test reaction biopsies compared with the atopic patch test controls (Fig. 2). An increase in the number of CD23$^+$ cells was found in 5/7 atopic patch test reactions compared with atopic control biopsies, although this was not significant. When the nickel patch test reations were analysed, a similar trend was observed in that there was an increase in the number of T lymphocytes ($P < 0.05$), macrophages (RFD1$^+$) and dendritic cells (RFD1$^+$) ($P < 0.05$) in the nickel patch test reactions compared with nickel patch test controls. A significant increase in CD23$^+$ cells was observed in nickel patch test reactions compared with nickel patch test controls (Fig. 3).

The CD4:CD8 ratio within the dermal infiltrate was increased in both positive patch test reactions compared with their respective controls: 4:1 in the nickel patients and 3:1 in the atopic dermatitis patients (data not shown).

Double staining was used to investigate the expression and distribution of the low affinity IgE receptor (CD23) on LC (CD1$^+$), dendritic cells (RFD1$^+$) and mature tissue macrophages (RFD7$^+$). There was no statistical difference in the percentage of macrophages (RFD7$^+$), LC (CD1$^+$) and dendritic cells (RFD1$^+$) expressing CD23 in the dermis of normal healthy control, nickel patch test controls and atopic patch test control samples respectively (Table 1). In atopic patch test reactions, however, there was a smaller percentage of macrophages (RFD7$^+$) (10%) which were CD23$^+$ compared with normal control sections (36%), while there was a marked increase in the percentage of LC (80%) and dendritic cells (66%) expressing this antigen compared with normal controls (8% and 4% respectively) (Fig. 4) ($P < 0.05$). In contrast, there was a slight reduction in the proportion of macrophages (RFD7$^+$) (15%)

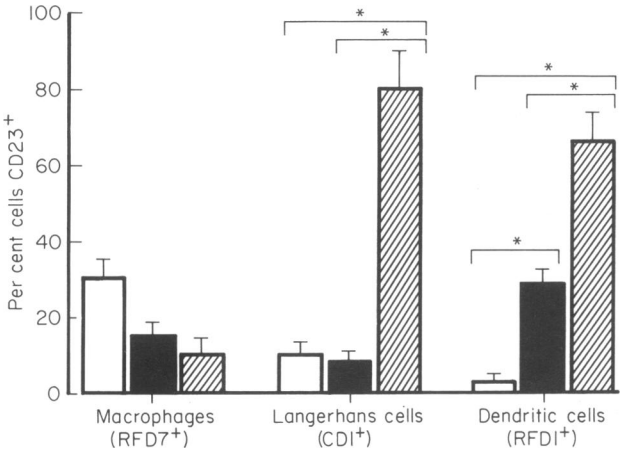

Fig. 4. The proportion of macrophages (RFD7$^+$), Langerhans cells (CD1$^+$) and dendritic cells (RFD1$^+$) expressing the CD23 antigen in normal healthy control skin, nickel patch test reactions and atopic patch test reactions. Mean $\pm$ s.d. is shown. $^*P < 0{\cdot}05$. □, Healthy controls; ■, nickel patch test reaction; ▨, atopic patch test reaction.

and LC (8%) and a significant increase ($P < 0{\cdot}05$) in the proportion of dendritic cells (RFD1$^+$) (25%) expressing CD23$^+$ in nickel patch test reactions compared with normal controls (Fig. 4). Within the dermis, 60% of the CD1$^+$ cells in the atopic patch test reaction samples were RFD1$^+$ compared with 73% in the nickel patch test reactions and 24% in normal control samples.

DISCUSSION

These results show that the delayed aeroallergen patch test reaction in patients with atopic dermatitis and that due to nickel in non-atopic individuals have similar clinical and immunocytochemical features, but differ in the distribution of the low affinity receptor for IgE on antigen-presenting cells.

In a delayed hypersensitivity reaction the lymphocytic infiltrate is composed of a mixture of CD4$^+$ and CD8$^+$ T lymphocytes that occur in a ratio of 2:1 [20]. This ratio is essentially the same as that in the circulation [21], thus implying that in terms of CD4/CD8 subset ratio, no preferential emigration or selection process occurs during the early stages of delayed hypersenstivity reactions. In our studies, a slightly increased CD4:CD8 ratio was seen in both delayed reactions, the ratio being greater in the nickel patch test than in the atopic patch test (4:1 and 3:1, respectively). It has previously been shown that the aeroallergen patch test reaction reflects the lesional skin in atopic dermatitis [12], and the present study provides strong clinical and immunohistochemical evidence to support the view that lesional skin in atopic dermatitis represents a DTH reaction.

It was observed that in the epidermis of atopic patch test reactions the number of LC were decreased while the number of LC were slightly increased in the epidermis of nickel patch test reactions compared with their controls. The number of dermal LC were increased in both atopic and nickel patch test reactions compared with their controls. Interestingly, there was a greater number of epidermal ($P < 0{\cdot}05$) and dermal CD1$^+$ cells in the atopic patch test reactions compared with nickel patch test reactions and normal control skin. This is unlikely to be due to Sellotape stripping, since in a previous unpublished study we compared Sellotape-stripped (15 times) and non-stripped uninvolved skin from patients with AD and found no difference in the number of epidermal and dermal LC. The increase in the number of LC observed in this study suggests that in the normal-appearing skin of the atopic control there is a higher level of activity of LC than in normal control skin. In keeping with this notion is the finding of a greater number of macrophages and activated T lymphocytes in the dermal infiltrate of atopic patch test control compared with normal control skin. Moreover, these findings support recent observations which demonstrated a reduction in the density of epidermal LC and a simultaneous increase in dermal LC in negative patch test reactions in normal individuals [22].

A low level of CD23 expression was detected on dendritic cells in the normal controls. This finding is consistent with the findings of Sakamoto *et al.* [23], who also demonstrated that the expression of this antigen is not unique to patients with atopic dermatitis. Interestingly, increased CD23 staining was detected in the nickel patch test biopsies compared with normal controls.

The significance of these low affinity IgE receptors on dendritic cells, particularly in the context of a delayed type reaction, remains unclear. In the atopic patch test biopsies there was a marked increase in the percentage of both LC and dendritic cells which were CD23$^+$, while there was a reduction in CD23 expression on macrophages compared with control samples, suggesting that this shift in the expression of CD23 may play an integral part in antigen presentation in the atopic patient. This suggestion is consistent with the *in vitro* studies, which demonstrated the importance of IgE on LC in antigen presentation of organic allergens in atopic dermatitis [16]. The only study which really demonstrates CD23 on epidermal LC is by Torresani *et al.* [24], who demonstrated that approximately a third of these cells obtained from normal skin expressed CD23. Recently, epidermal LC have been shown to express high IgE-affinity IgE receptors in normal skin [25,26] and skin from AD patients [27], suggesting that it is this receptor rather than CD23 which may be of importance in antigen presentation.

In the nickel patch test reactions, in contrast, the percentage of CD1$^+$ LC and RFD7$^+$ macrophages expressing the CD23 antigen did not differ from the control biopsies, although there was an increase in CD23$^+$ RFD1$^+$ dendritic cells. This finding could be taken to indicate that although IgE receptors are expressed on antigen-presenting cells in the nickel patch test reaction, they may not be playing a role in the promotion of the cell-mediated response, as no shift in CD23 expression from macrophages to dendritic cells was observed. This latter increase in CD23 expression on dendritic cells may be due to a non-specific up-regulation of CD23 by T cell-derived cytokines.

In keeping with other reports, we have demonstrated weak but positive CD23 staining on LC of both the dermis and the epidermis of uninvolved skin of our atopic patients [16,28]. We have demonstrated that the immunopathology of the aeroallergen patch test reaction in atopic dermatitis reflects the delayed hypersensitivity reaction observed in classical contact allergic reaction. The significant difference in the distribution of the low affinity receptor for IgE on antigen-presenting cells in the individual with AD suggests that antigen may be presented to T cells in association with IgE.

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