Cytokines and soluble intracellular adhesion molecule 1 in the sera of Saudi eczematous children

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ABSTRACT

We report five children with atopic eczema. The level of soluble adhesion molecules is known to correlate with disease severity. On the assumption that atopic eczema is a T-lymphocyte–mediated inflammatory disease, in this study, we aimed to determine the presence of soluble intracellular adhesion molecule (sICAM) 1 and the release of inflammatory cytokines. The patient sera were used to measure sICAM-1 by enzyme-linked immunosorbent assay, and the concentration of interleukin (IL)-4 and IL-5 in whole blood culture supernatant was determined by a flow cytometry microsphere-based assay. sICAM-1 was dramatically higher in these patients than healthy controls. In addition, the results showed an increase in IL-4 and IL-5 concentration compared with control subjects. In conclusion, these results showed that sICAM-1 expression is important in inflammation of epithelial cells and can be used as a useful parameter in monitoring the disease activity. The study suggests that T-helper cell subset plays an important role in initiating eczematous disease and that additional studies are needed to explore such a role. (Allergy Asthma Proc 27:126–129, 2006)

Atopic eczema is a chronic and relapsing inflammatory skin disease that is linked to an increase in serum immunoglobulin E (IgE) levels. IgE was found to be associated with the severity of the disease and a family history of atopy. Etiology of this disease is poorly understood, but usually it disappears in adolescence. Atopic eczema is a common ailment in childhood, affecting ~10% of infants, and is frequently associated with food allergy and the atopic march. The most commonly offending foods are cow’s milk, eggs, corn, wheat, and soy. Besides the elevation of IgE, soluble interleukin (sIL)-4 and sIL-5 and the soluble intracellular adhesion molecule (sICAM) 1 in the sera of patients with atopic eczema can be used to monitor the activity of the disease. In this context, it is well known that ICAM-1, the membrane-bound form of sICAM-1 (sICAM-1/CD54), is highly involved in many immune reactions. In allergic patients, ICAM-1 expression was found to be correlated with the severity of clinical manifestations.

In the pathogenesis of allergic reactions, the balance of T-helper cell types (Th1 versus Th2) seems to be changed. This was confirmed by evidence indicating that atopic disease is controlled by Th2 cells, which mainly produce IL-4. The loss of CD62 L selectin has been described in children with atopic dermatitis. Thus, L-selectin levels may be a marker for leukocyte activation and can correlate with the severity of atopic dermatitis. T lymphocytes can be influenced by various factors in their development into either Th1- or Th2-type cells. Serum levels of IL-4 and IL-5 are thought to be indicators for the deviation of T-cell function. Additionally, sICAM-1 was included in this study, because it has been implicated in the pathogenesis of allergy. ICAM-1 plays an essential role in the early stage of the signal cascade leading to the development of inflammatory responses. The aim of this study was to measure sICAM-1 in atopic eczematous patients and also to investigate cytokine concentrations in their sera.

MATERIALS AND METHODS

Patients Selection

Five patients, aged 2–9 years, who were treated at Riyadh Hospitals, Riyadh, Saudi Arabia for severe atopic eczema, fulfilled the clinical and morphological criteria and were included in this study. Severity of eczema was assessed with topography items (affected skin area), intensity criteria (e.g., erythema and edema), and other subjective parameters (extent of itch and loss of sleep). These patients were treated with topical emollients and steroid preparations at the time of the
investigation. Treatment period lasted for 16 weeks. Biochemical laboratory values and blood pressure were well within the normal range in all patients. The patient sera were compared with sera of three healthy controls. After 3 mL of venous blood was drawn, serum was separated and stored at \(-20^\circ\text{C}\) until use. Written informed consent was obtained in all cases.

**Determination of IgE Antibodies**

Patient sera were analyzed for concentrations of total IgE as determined by using the Pharmacia CAP multidisk system (Pharmacia, Upjohn, Switzerland). The detection limit of the CAP system is 0.35 kU/L.

**Measurement of sICAM-1**

sICAM-1 in serum samples was determined with a Sandwich enzyme-linked immunosorbent assay ELISA kit (R & D System, Minneapolis, MN). The procedures recommended by the manufacturer were followed without modification. The optical absorbance values were read on a micro-ELISA autoreader at 490 nm. All samples were tested in duplicate and assayed on two separate occasions. All samples were coded and read blinded in the assay.

**Measurement of Soluble Cytokine**

The concentration of IL-4 and IL-5 in culture supernatant was measured by commercially available flow cytometer microsphere-based assay (R & D System). A slight modification to the manufacturer’s instructions was made. Monoclonal antibodies specific for IL-4 and IL-5 have been precoated onto a microsphere. Samples were pipetted and any cytokine present (i.e., IL-4 or IL-5) was bound to the captured recombinant human IL-4 and IL-5 Ab. After washing away any unbound substances, fluorescein isothiocyanate (FITC)-conjugated secondary Ab was added. After a wash to remove any unbound Ab-FITC reagent, cells were fixed with 2% paraformaldehyde and checked under an FACScan (San Jose, CA) with an appropriate negative control.

**Light Microscopy and Enumeration of Inflammatory Cells**

Differential blood count was evaluated at presentation by an automated hematology analyzer (Coulter; summarized in Table 1). To quantify the degree of inflammation, skin lesions were obtained from all patients and controls. Quantitative light microscopy technique was used to identify the location and severity of the inflammatory cell infiltration. We examined 10 visual fields at 200× magnification. All slides were blind counted by two pathologists. The mean intraobserver coefficient of variation for repeat counts was <4%. The mononuclear and polymorphonuclear leukocytes were scored semiquantiitatively. These cells were evaluated as follows: NS, no stain or <10%; +, 10–25%; ++, 25–50%; and ++++, >50% (summarized in Table 2).

**Statistical Analysis**

Statistical analysis was performed using Microsoft Excel spreadsheet and the StatView SE + graphics software (Chicago, IL). The probability of a significant difference between groups was determined by Mann-Whitney U test and Wilcoxon signed-rank test. Graphs were plotted using Cricket graph graphics package.
RESULTS

IL-4 and IL-5 and IgE Production

The detailed results are shown in Fig. 1. Spontaneous release of IL-4 and IL-5 was detectable in all atopic patients compared with the healthy controls. The differences between the two groups should reach statistical significance (p < 0.05). Total IgE, which usually is used as a serologic parameter for the diagnosis of atopy, was a trend in these patients (data not shown). In these patients, there was a trend relationship between serum IgE and the magnitude of IL-4 produced from the peripheral blood of atopic patients (data not shown, p = 0.09).

Elevated Levels of sICAM-1 in Patients with Atopic Eczema

Patients with severe atopic eczema showed significantly elevated serum levels of ICAM-1 before treatment as compared with the healthy controls (Fig. 2; p = 0.001). The level of sICAM-1 in the sera of treated patients with atopic eczema was found significantly reduced after 16 weeks of treatment (Fig. 2).

Immunopathological Examinations

Immunopathological examinations were performed on skin lesions obtained from these patients. Indeed, significant cellular changes were detected in all the patients with atopic eczema when compared with healthy controls (see Tables 1 and 2). There was a significant infiltration of mononuclear cells including lymphocytes and mast cells and an increased number of eosinophils dramatically different from control lesions.

DISCUSSION

Both type 1 and type IV hypersensitivity play a role in the induction of the acute and chronic phase of eczematous lesions. In the acute phase, Th2-type cytokines such as IL-4, IL-5, and IL-13 play an important role in mediating local inflammation, whereas Th1-type cytokines, e.g., interferon γ, are required to support the ongoing inflammation.13,14 Our findings indicate that sICAM-1 is useful in monitoring atopic eczema. Clinical improvement in the patients was associated with a significant decrease in sICAM-1 levels. In addition, to the best of my knowledge, this is the first study of sICAM-1 in atopic eczema.

The skin lesions in atopic eczema are infiltrated by eosinophils, indicating the importance of IL-5 and sICAM-1 as essential factors for the eosinophil recruitment and of eosinophil survival.15 Recently, soluble adhesion molecules have been found to be associated with inflammation; however, each soluble molecule may be used as a marker in a certain disease, e.g., sVCAM is linked to systemic lupus erythematosus, whereas sE.selectin, sICAM-1 is not. Nyberg et al.16 showed soluble adhesion molecules elevated in systemic and cutaneous lupus erythematosus. In our study, we showed elevated levels of sICAM-1 in the patients’ serum. Moreover, the treatment of these atopic patients was associated with significant changes in the disease activity. Our findings are in agreement with the recent reports by Hirai et al., which suggest that itching is reflected by sICAM-1 levels.17 Additionally, our study is in parallel with Vignola et al. and others who showed that ICAM-1 expression was significantly higher in atopic than nonatopic subjects.18–20 However, our results contrasted with those of Szegedi et al. who showed an elevation of IL-13 but not IL-4 in atopic patients.21

Atopic eczema displays an overproduction of IL-4 and IL-5, which was not found in healthy controls. In
addition, the release of IL-5 in atopic eczema is associated with eosinophils inflammation. Because of IL-4 rapid breakdown and consumption by other cells, its measurement in the whole blood and peripheral blood mononuclear cells has been proven to be difficult by conventional ELISA. Obviously, our system does not allow us to determine precisely which cell type is involved.22,23 We believe that there is an association between eosinophil inflammation and the magnitude of IL-4 and IL-5 production in these cases. Our results also may reveal a close link between eosinophilic inflammation and IL-4 production. Furthermore, mast cells may be involved in the chronic inflammatory responses by releasing multifunctional cytokines, which can cause the activation of eosinophils.24 We may assume that the atopic eczema confounds the correlation between percent of eosinophils and cytokine concentrations in serum. Bettiol et al. and others showed a significant difference of IL-4 release between atopic and nonatopic individuals, which may be one indicator of the raised serum IgE.25–27

In conclusion, the present cases confirm that sICAM-1 is likely to play an important role in monitoring this disease. Indeed, we showed that a Th2-type immune response is associated with an inflammatory process in atopic eczema. Additional studies should focus on the question of whether sICAM-1 can serve as a marker for prognosis measures.

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REFERENCES