Production of Interleukin-2 by Mononuclear Cells \textit{In vitro} in Patients with Atopic Dermatitis and Psoriasis

Comparison with Serum Interleukin-2 Receptor Levels*

ALEXANDER KAPP,1 PETER NEUNER,2 JEAN KRUTMANN,1 THOMAS A. LUGER2 and ERWIN SCHÖPF1

1Dept. of Dermatology, Univ. of Freiburg, F.R.G. and 2nd Dept. of Dermatology, Univ. of Vienna, and LBI-DVS, Lab. for Cellbiology, Austria and Dept. of Dermatology, Univ. of Münster, F.R.G.

Atopic dermatitis is associated with profound immunological alterations, in particular decreased lymphoproliferative responses upon stimulation with T-cell mitogens. T-cell blastogenesis involves the production of the soluble cytokine interleukin-2 (IL-2), which in turn upregulates the expression of its own receptor. To investigate the potential role of this cytokine for the pathomechanisms present in atopic dermatitis, 24-h supernatants of PHA-stimulated peripheral blood mononuclear cells from patients with atopic dermatitis (n = 30) of a moderate to severe disease activity were tested for IL-2 activity. In addition, serum concentrations of soluble interleukin-2 receptor (IL-2R) were measured. Non-atopic healthy controls (n = 19) and patients with psoriasis (n = 20), an inflammatory skin disorder with distinct pathogenesis, served as controls. In comparison with psoriasis patients and normal controls, PHA-stimulated mononuclear cells of atopic dermatitis patients released significantly less IL-2 into supernatants. Moreover, there was an inverse correlation between IL-2 concentrations and body surface involvement or serum IgE levels. In contrast, serum IL-2R levels were significantly elevated in both atopic dermatitis and psoriasis, as compared with healthy controls. Furthermore, IL-2R levels in atopic dermatitis patients showed a significant correlation with IgE levels and body surface involvement. The data indicate that T cell activation may occur in both skin diseases. Atopic dermatitis, however, is further characterized by the decreased capacity of mononuclear cells to release IL-2 upon stimulation \textit{in vitro}.

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A. Kapp, Department of Dermatology, University of Freiburg, Hauptstrasse 7, D-7800 Freiburg, F.R.G.

Although atopic dermatitis (AD) represents an inflammatory skin disorder which has been well-known since the beginning of the century, its pathogenesis is not yet clearly understood (1). In addition to a multifactorial determined genetic disposition, AD is characterized by a number of changes in humoral and cell-mediated immunity (1-6). In the last decade a variety of cellular dysfunctions of immune cells have been reported, particularly in the T cell compartment. There is increasing evidence that these alterations may be associated with imbalances within the cytokine network (7). Since T-cell activation is regulated by immuno-modulating cytokines, it appears very likely that an altered production of these mediators may be the cause of changes of the immune response. Accordingly, the production of interleukin (IL)-1 (8, 9) by mononuclear cells of AD patients \textit{in vitro} was found to be significantly decreased upon appropriate stimulation. Moreover, the release of the inflammatory mediator tumour necrosis factor (TNFα) was significantly diminished (10). In contrast, an unchanged generation of lymphotoxin (TNFβ) as well as of interferon-α and γ could be observed (10-12). In addition, the release of IL-6 upon stimulation was found to be normal (13). Reduced lymphocyte responses to T cell mitogens or recall antigens \textit{in vitro} (1, 5-6), most evident during severe exacerbations of the disease, have been reported.

In the present study the production of IL-2 by mononuclear cells and the concentration of serum IL-2 receptors was measured in AD patients to correlate the T cell activation \textit{in vitro} with signs of T cell activation \textit{in vivo}. The results obtained with AD patients were compared with a group of normal non-atopic controls as well as a group of patients with psoriasis, representing an inflammatory skin disorder of distinct pathology (14, 15).
Isolation of mononuclear cells (MNC) and generation of supernatants (SUP)

Unfractionated MNC were isolated from heparinized (50 units/ml) peripheral blood of patients and controls as described previously (18). MNC were adjusted at a concentration of 5×10^6/ml in serum-free HEPES-buffered Eagle's minimal essential medium containing 50 μg/ml bovine serum albumin and incubated in presence of 5 μg/ml PHA or without stimulus at 37°C in 95% humidified air. The absolute number of monocytes in MNC preparations of patients and controls was determined routinely by nonspecific esterase staining (Technikon, Bad Vilbel, F.R.G.) and no significant differences between patients and controls could be detected. After 24 h, supernatants were collected, centrifuged, sterile filtered and stored at −70°C until testing.

Bioassays

IL-2 activity in supernatants was determined as proliferation of the IL-2 dependent cytotoxic mouse T-cell line (CTLL-16) as described earlier (19, 20). Results are expressed as U/ml which were calculated by comparing serial dilutions of samples with serial dilutions of a known IL-2 preparation containing 100 U/ml (21).

Measurement of interleukin 2 receptor in vivo

Serum IL2R was measured by an ELISA technique using Cellfree® interleukin 2 receptor kit (T cell sciences, Cambridge, Mass., USA) in serum samples from patients and controls (22, 23). All determinations were carried out using ELISA kits with the same lot number.

Reagents and stimuli

Phytohemagglutinin (PHA) was obtained from Wellcome (Burgwedel, F.R.G.). Purified bovine serum albumin was from Sigma, Münich, F.R.G. Culture media were purchased from Biochrom-Seromed, Berlin, F.R.G.

Statistical analysis

Statistical evaluations were performed by using Mann-Whitney's U-Test. For evaluation of correlations Spearman’s rank correlation coefficient was calculated and tested for statistical significance.

RESULTS

IL-2 production in vitro

In patients with AD, significantly decreased amounts of IL-2 bioactivity in SUP of PHA-stimulated (p ≤ 0.01) MNC were observed in comparison with non-atopic controls (Fig. 1). There was no significant difference in IL-2 secretion between psoriatic patients and the control group (Fig. 1). In addition, there was no significant spontaneous release of IL-2 by MNC of patients and controls, respectively (data not shown). A significant inverse correlation was found between IL-2 levels and body
Production of Interleukin-2 by Mononuclear Cells

U/ml

\[ p < 0.005 \quad p < 0.01 \]

100

500

1000

2000

AD

PS

C

IL-2 Receptor

Fig. 2. Serum levels of Interleukin-2 receptor in patients with atopic dermatitis (AD), psoriasis (PS) and in non-atopic healthy controls (C). Bars indicate medians.

surface involvement \( (R = -0.67, p \leq 0.01) \) in AD patients. Furthermore, a significant inverse correlation could be detected between IL-2 and IgE levels \( (R = -0.57, p \leq 0.0002) \).

Serum IL-2 receptor

Levels of IL-2R were markedly elevated in atopic dermatitis \( (p \leq 0.005) \) and psoriasis patients \( (p \leq 0.01) \) (Fig. 2). A significant positive correlation was found between IL-2R levels and body surface involvement \( (R = 0.62, p \leq 0.01) \). Furthermore, a significant positive correlation could be detected between IL-2R and IgE levels \( (R = 0.45, p \leq 0.05) \). In addition, IgE levels were significantly correlated with body surface involvement \( (R = 0.55, p \leq 0.03) \).

DISCUSSION

The study provides clear evidence that patients with AD of moderate to severe disease activity are characterized by an impaired capacity of their T cells to release IL-2 in vitro. This deficiency may be relatively specific, since no significant changes could be detected in patients with severe plaque-type psoriasis. Moreover, decreased IL-2 production in AD was significantly correlated with the eczematous involve-

ment of patient's skin. IgE levels were significantly correlated with the severity of skin disease \( (1) \), but inversely correlated with IL-2 production in vitro.

T lymphocyte activation represents a very complex process which is known to involve a variety of cytokines, including IL-1 and IL-2 \( (24) \). Specifically, IL-1 is released by monocytes and thought to represent one of the accessory cell-derived signals required for efficient T cell activation. In contrast, IL-2 is produced by activated T cells and known to upregulate the expression of its own receptor. Functional alterations of T cells, such as decreased lymphoproliferative responses \( (5, 6, 11) \), therefore, could be the result of a diminished capacity of mononuclear cells to release IL-1 and IL-2 upon appropriate stimulation. As shown previously, monocytes from AD patients released significantly less IL-1 following stimulation, whereas the basal IL-1 production was unchanged \( (8, 9) \). The data presented in this study indicate that depressed lymphoproliferative responses upon stimulation with T cell mitogens observed in AD \( (5, 6, 11) \) may be caused by diminished production of IL-2 upon stimulation. This may be due to either a defective regulation of accessory cell function or a T cell defect.

Based on the diminished production of the monocyte-derived cytokines IL-1 \( (8, 9) \) and TNFα \( (10) \), in contrast to the 'normal' production of the T-cell products interferon-γ \( (11) \) and TNFβ \( (10) \), a decreased capacity of monocytes to release immunomodulating cytokines could be assumed for AD patients in contrast to psoriatic patients. However, since there are no signs of an altered production of other monocyte-derived cytokines, such as interferon-α \( (11) \) or IL-6 \( (13) \), a relatively specific dysregulation of monocyte functions may be suggested.

Moreover, as previously shown \( (25) \), sera of AD patients as well as psoriatic patients contained significantly increased levels of the IL-2 receptor (IL-2R), as compared with healthy controls. In the present study it could be demonstrated that IL-2R levels in AD patients showed a significant correlation with IgE levels and body surface involvement. Increased concentrations of IL-2 receptors in serum have been interpreted as a sign for T cell activation \( (25) \). Thus, the finding of increased IL-2 receptor levels is in apparent conflict with decreased lymphoproliferative responses in vitro \( (5, 6, 11) \). Therefore, a supposed T cell defect which could be shown by functional assays in the peripheral blood is in direct contrast to signs of hyperactivation.
Usually, investigations on immunologic parameters of AD were done with peripheral blood leukocytes. One possible explanation for the contradictory findings would be that the inflammatory process in AD is focused on the skin. Therefore, it is tempting to speculate that the decreased production of cytokines by blood mononuclear cells is due to a down-regulation of the immune response in the peripheral blood induced by cytokines released from hyperactivated immune cells in the inflamed skin. Alternatively, the hyporesponsiveness of mononuclear cells in vitro could be a sign of 'exhaustion' following excessive stimulation in vivo. These suggestions are supported by the finding that alterations of distinct T cell functions normalized with clinical remissions (5, 26–28). In view of recent findings these alterations in the peripheral blood of AD patients may be attributed at least partially to profound dysregulations within their skin immune system (7).

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REFERENCES