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Altered Production of Immuno-modulating Cytokines in Patients with Atopic Dermatitis

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Atopic dermatitis (AD) represents an inflammatory skin disorder which is characterized by many signs of immunodeficiency. Particularly, decreased lymphoproliferative responses upon stimulation with mitogens as well as bacterial antigens were reported repeatedly. Since there is increasing evidence for a network of immuno-modulating cytokines playing a crucial role in the regulation of immunity and inflammation, in the present study we investigated whether an altered production of these mediators is one of the pathomechanisms responsible for the altered immune response in AD. For this purpose the 24-h supernatants of LPS- and PHA-stimulated or unstimulated mononuclear cells (MNC) from patients with AD of a moderate to severe disease activity and from nonatopic healthy controls were tested for Interleukin-1 (IL-1) and Interleukin-2 (IL-2) activity. Whereas supernatants of unstimulated MNC of AD patients and controls did not contain significantly different levels of these cytokines, LPS-stimulated MNC of AD patients released significantly less IL-1 in the supernatants. Similarly, the production of IL-2 by PHA-stimulated MNC of AD patients was significantly decreased in comparison to the controls. Moreover, there was a strong correlation between IL-1 and IL-2 levels. These findings indicate that diminished lymphoproliferative responses in AD may partly be caused by a decreased capacity of MNC to release immuno-modulating cytokines, even upon appropriate stimulation.

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Atopic dermatitis (AD) represents an inflammatory skin disorder which is characterized by significant changes in humoral and cell mediated immunity, particularly by alterations in T-cell-related functions (1–7). Clinically, these abnormalities are manifested as an enhanced susceptibility to severe infectious skin diseases, particularly viral infections with herpes simplex and vaccinia. Furthermore, an increased incidence of warts and molluscum contagiosum has been reported (1, 6). Since a network of cytokines including interferons, colony-stimulating factors and interleukins appear to regulate many effects of immunity and inflammation, it has been speculated that these patients have a defect in the capacity to produce interferons in response to viral antigens. However, the production of interferon-alpha and -gamma in whole blood cultures of patients with AD was found to be unchanged (6). In contrast, reduced lymphocyte responses to T cell mitogens or recall antigens in vitro, most evident during severe exacerbations of the disease, have been reported (1, 5–7). Therefore, the present study was performed to investigate whether one of the pathomechanisms responsible for these changes may be due to an altered production of cytokines.

MATERIALS AND METHODS

Patients and controls

The atopic dermatitis (AD) group consisted of 10 patients (1 male, 9 females) with moderate to severe disease activity: $\geq 25\%$ of body surface area was involved, all patients had excoriated skin lesions with intensive pruritus. The diagnosis was established according to the criteria of Hanifin and Rajka (8). The control group consisted of 7 healthy blood donors (6 males, 1 female). Atopy was excluded by history and laboratory findings. Neither control nor patients had received local or systemic steroid therapy, or therapy with ultraviolet light for at least 3 weeks prior to blood collection.

Isolation of mononuclear cells (MNC)

Unfractionated MNC were isolated from heparinized (50 units/ml) peripheral blood of patients and controls as described previously (9). MNC were adjusted at a concentration of 5×10^6/ml in serum-free HEPES-buffered Eagle's minimal essential medium (MEM) containing 50 µg/ml bovine serum albumin (BSA) and incubated in presence of 0.5 µg/ml lipopolysaccharide (LPS), 5 µg/ml PHA or without stimulus at 37°C in 95% humidified air. After 24 h supernatants were collected, centrifuged, sterile filtered and stored at −70°C until testing.

Bioassays

Supernatants were tested for Interleukin-1 (IL-1) activity using the thymocyte costimulator assay and the IL-1 mediated

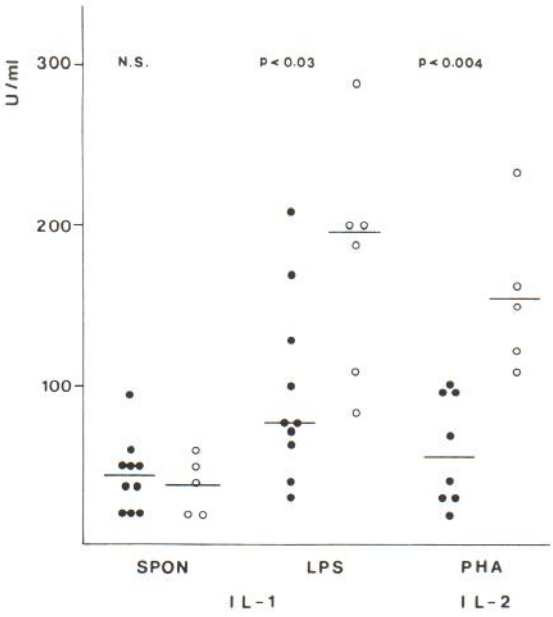

Fig. 1. Spontaneous (SPON) and LPS-stimulated (LPS) release of Interleukin-1 (IL-1) and PHA-stimulated release of Interleukin-2 (IL-2) by MNC of patients with atopic dermatitis (●) and non-atopic controls (○). Bars indicate medians.

proliferation of a murine T cell line (D10) as described previously (10, 11). IL-2 activity was evaluated measuring the proliferation of an IL-2 dependent cytotoxic mouse T-cell line (CTLL-16) as described (12). Results are expressed as U/ml which were calculated by comparing serial dilutions of samples with serial dilutions of a known IL-1 or IL-2 preparation containing 100 U/ml (10).

Reagents and stimuli

Phytohemagglutinin (PHA) was obtained from Wellcome (Burgwedel, FRG). S-form LPS from Salmonella abortus equi was a kind gift of Dr C. Galanos, MPI for Immunobiology, Freiburg, FRG. Purified BSA was from Sigma, Munich, FRG. Culture media were purchased from Biochrom-Seromed, Berlin, FRG.

Statistical analysis

Statistical evaluations were performed by using the Mann-Whitney U-test. For evaluation of correlations Spearman's rank correlation coefficient was calculated.

RESULTS

MNC of AD patients and controls released detectable amounts of IL-1 spontaneously (Fig. 1). However, no significant differences could be detected between patients and controls. In contrast, the levels of IL-1 activity in the supernatants of LPS-stimulated MNC were significantly decreased in AD patients (Fig. 1). Since thymocyte proliferation may be altered by other cytokines present in MNC supernatants IL-1 activity, additionally, was evaluated using the IL-1 sensitive murine T cell line D10 which does not proliferate in response to interleukin-6 and tumor necrosis factor. Using this bioassay similar levels of IL-1 activity were detected in the MNC supernatants tested. Moreover, a monoclonal antibody directed against IL-1 (13) blocked the thymocyte- or the D10 proliferation-inducing capacity of the MNC supernatants suggesting that IL-1, mainly, is responsible for the bioactivity measured (data not shown).

Similarly the release of IL-2 in the supernatants by PHA-stimulated MNC was significantly lower in patients with AD when compared with the control group (Fig. 1). There was no detectable production of IL-2 spontaneously as well as upon stimulation with LPS in patients and controls. Levels of IL-1 and IL-2 correlated significantly ($r=0.791$, $p \leqslant 0.004$).

DISCUSSION

Activation of T cells is dependent on the release of immunomodulating cytokines: Following binding of the T cell to the antigen-presenting cell the T cell stimulates the antigen-presenting cell to produce IL-1 by a mechanism which is not completely clarified at present. IL-1 in association with antigen stimulation induces IL-2 receptors on the T cells and stimulates T cells to release IL-2 which drives antigen activated cells into proliferation (14). Therefore, decreased lymphoproliferative responses could be the result of a diminished capacity of MNC to release IL-1 and IL-2 upon appropriate stimulation. Our data show that MNC from AD patients release significantly less IL-1 and IL-2 following stimulation whereas the basal IL-1 production was unchanged. The results are in agreement with a previous report of Räsänen et al. (15) who could show that purified monocytes of AD patients produced clearly less IL-1 in response to stimulation than monocytes from healthy controls. Therefore, the decreased production of IL-1 is apparently not due to suppressive factors derived from T-lymphocytes. These data support the hypothesis that in addition to T cell-related changes monocyte functions are impaired in AD. Depressed lymphoproliferative responses upon stimulation with T cell mitogens may be caused by diminished production of IL-2 upon stimulation. Our results clearly demonstrate that MNC of AD patients release significantly less IL-2 in

the supernatants following stimulation with the T cell mitogen PHA in comparison to healthy controls.

Levels of IL-1 and IL-2 were significantly correlated suggesting a general defect of MNC from AD patients to produce cytokines. However, production of interferons in whole blood cultures was recently shown to be unchanged in AD (6). Furthermore, serum IL-2 receptor levels were found to be significantly increased in AD (16). Increased serum levels of IL-2 receptor have been detected in different diseases accompanied with T cell activation and changes in the immune system. Therefore, it appears very unlikely that depressed lymphoproliferative responses in AD are the result of a basic defect of patients' MNC to produce immuno-modulating cytokines upon stimulation. We, therefore, suggest that the decreased production of cytokines by blood MNC is due to down-regulation induced by cytokines released from activated cells in the inflamed dermis. Alternatively, the hyporesponsiveness of MNC in vitro could be a sign of "exhaustion" following excessive stimulation in vivo. These suggestions are supported by the finding that altered lymphoproliferative responses normalize with clinical remissions (17).

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