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Interleukin-1 Release From Peripheral Blood Monocytes and Soluble Interleukin-2 and CD8 Receptors in Serum from Patients with Atopic Dermatitis

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In 31 adult patients with atopic dermatitis, the capacity to secrete interleukin-1 (IL-1) from peripheral blood mononuclear cells and from purified monocytes was investigated following stimulation with lipopolysaccharide. We also measured soluble interleukin-2 receptor levels (sIL-2R) and CD-8 receptor in serum from some of the patients in order to estimate the degree of lymphocyte stimulation in vivo. We observed that purified monocytes from patients with atopic dermatitis released more IL-1 than unseparated blood mononuclear cells did and also had significantly greater IL-1 activity than non-atopic donors. Addition of histamine in concentrations of 10^{-7} to 10^{-4} M did not suppress, but rather augmented the IL-1 activity release. An increased monocyte-IL-1 release could lead to increased T lymphocyte activity. We observed that 60% of the patients had increased sIL-2R concentrations in serum. There was no correlation between serum IgE and sIL-2R. Our observations indicate that monocytes in atopic dermatitis patients release increased quantities of IL-1, supporting an augmented T lymphocyte activation in the patients.

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Atopic dermatitis (AD) is a chronic skin disease characterized by a fluctuating clinical course and having a high incidence of type I allergies and skin infections. Immunohistological studies of skin from AD patients have shown that most cells in the skin are T lymphocytes of the CD4+ subset which express HLA-antigens as a sign of activation (1).

Recently there have appeared two reports of significantly reduced IL-1 release from mononuclear blood cells following in vitro stimulation with LPS, indicating a suppression of or lack of stimulation in the stage of T cell activation (2, 3).

We have also studied IL-1 release from LPS-stimulated non-purified blood mononuclear cells, but found IL-1 release to be within the normal range (5). However, many patients with AD have increased numbers of soluble interleukin-2 receptors in serum, indicating concurrent lymphocyte activation (5–7). We therefore decided to further explore the IL-1 activity from LPS-stimulated purified monocytes, and to measure soluble interleukin-2 and CD-8 receptor levels in patients with AD both in vivo and in vitro.

PATIENTS AND METHODS

Altogether 31 patients participated in the study. All had active AD and were being treated either as in-patients, due to severe exacerbation of their disease, or as out-patients. Treatment was topical and consisted of tar baths and topical steroids (in-patients) or topical steroids alone (out-patients). Control subjects were healthy staff from our Department.

Interleukin-1 assay

From 11 patients (3 men, 8 women, mean age of 28 years) blood was drawn and mononuclear cells were isolated on Lymphoprep®, washed three times in Hanks' Balanced Salt Solution with 1% serum and suspended in RPMI 1640 with 1% human serum. Some cells were incubated in 50-ml tissue culture flasks, where monocytes were allowed to adhere to the bottom during a 1-h incubation at 37°C. Non-adhering cells were then discarded and the adhering monocytes were secured by placing the tissue culture flask at 4°C for 30 min. This gives a purity of monocytes around 80–85% as judged microscopically and by the esterase staining method.

Both cell populations, mononuclear cells and monocytes, were now incubated for 24 h with or without LPS 10^{-5} M in LPS-free RPMI 1640 with 1% serum at a cell concentration of 1×10^{6}/ml. Supernatants were collected and assayed for IL-1 activity using the C_3H mouse thymocyte assay as described elsewhere (8). We also studied the effect of histamine on the in vitro release of IL-1 from monocytes by adding from 10^{-4} to 10^{-7} M histamine to the cultures of

Table I. *Interleukin-1 assay of culture supernatants following 24-hour LPS stimulation 10^{-5} M of human purified monocytes*

Parameter	Atopic eczema		Control persons	
	−LPS	+LPS	−LPS	+LPS
Mean	358	1.597	85	221
S.D.	446	2.054	38	117
N	13	13	7	7
Range	<30–1.550	225–5.935	<30–137	<30–325

either purified monocytes or mononuclear cells before monocyte purification.

Interleukin-2 receptor levels

Serum was collected from 20 patients (10 men, 10 women, mean age 24.5 years) and 9 control subjects and stored at −20°C until assayed. The concentration of soluble interleukin-2 receptors (sIL-2R) was determined by an enzyme-linked immunosorbent assay (ELISA) *ad modum* Rubin et al. (9–11) as supplied in kit form (Cellfree® Interleukin-2 Receptor Test kit, T Cell Sciences Inc., Cambridge, Mass.). The test employs two non-competing murine mAb, 7G7/B6 (II) and anti-Tac (III) (or equivalent) towards the α-chain (p55) of the human IL-2R. According to the manufacturer's instructions, a 96-well microtitre plate was coated with the first antibody (anti-Tac), washed and blocked. Then 50 µl serum was applied with 100 µl diluent and after 2 h of incubation at 37°C the wells were washed and horseradish peroxidase conjugated second antibody (7G7/B6) added. After another 2 h at 37°C the wells were washed and *O*-phenylenediamine (OPD) was added. The reaction was quenched after 30 min at room temperature with 2 N sulphuric acid and the absorbance at 490 nm measured. SIL-2R concentrations are expressed in Units/ml (U/ml). 1.000 units is defined as the amount of sIL-2R present in 1.0 ml of a reference preparation of culture supernatant from phytohemagglutinin (PHA) stimulated peripheral blood cell lines. The detection limit is approx. 50 U/ml.

Statistics

We used linear regression analysis and the Wilcoxon test for two samples. A $p < 0.05$ was considered to be significant.

RESULTS

Interleukin-1

Unstimulated purified monocytes from patients with AD released on average 358 units/ml of IL-1, compared with 85 units/ml of IL-1 from control subjects (Table I). This is a 4.2-fold higher IL-1 release, which increased to 7.3-fold higher mean IL-1 activity following LPS stimulation (Table I, Fig. 1). Thus, purified monocytes from patients with AD release significantly more of IL-1 activity, both unstimulated and when stimulated with LPS.

Fig. 2 shows the results of seven experiments, where IL-1 was measured from LPS-stimulated unseparated cells or monocytes, and monocytes with histamine added. It is confirmed that purification of monocytes leads to an increased release of IL-1 and that histamine apparently can augment IL-1 release in 5 of 7 patients. The augmenting histamine concentrations were 10^{-6} to 10^{-5} M, whereas larger amounts of histamine lead to a reduction of IL-1 release. Similar changes were not observed in non-atopics or patients with psoriasis.

Soluble IL-2 and CD-8-receptors

Fig. 3 shows the results of studies on receptor levels in serum. The normal range of sIL-2 receptors is 175–490 U/ml and it is seen that 12 patients (60%) had increased sIL-2 in serum. There was no correla-

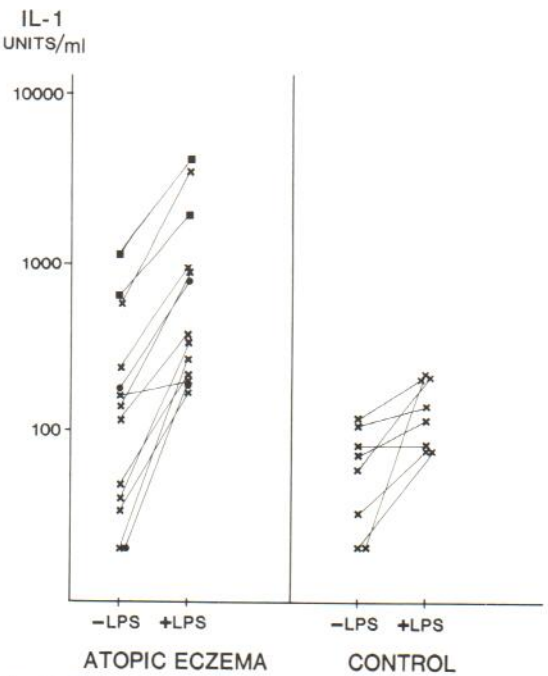

Fig. 1. Figures denote interleukin-1 (IL-1) release from purified monocytes of atopic dermatitis patients ($n = 11$) or control subjects ($n = 8$). Two patients with atopic eczema were investigated twice (●--● and ■--■). Lipopolysaccharide (LPS) stimulation 10^{-5} M induced a statistically significant increase in IL-1 release ($p < 0.01$) in both populations. IL-1 release was significantly increased from purified monocytes from patients with atopic dermatitis ($p < 0.05$).

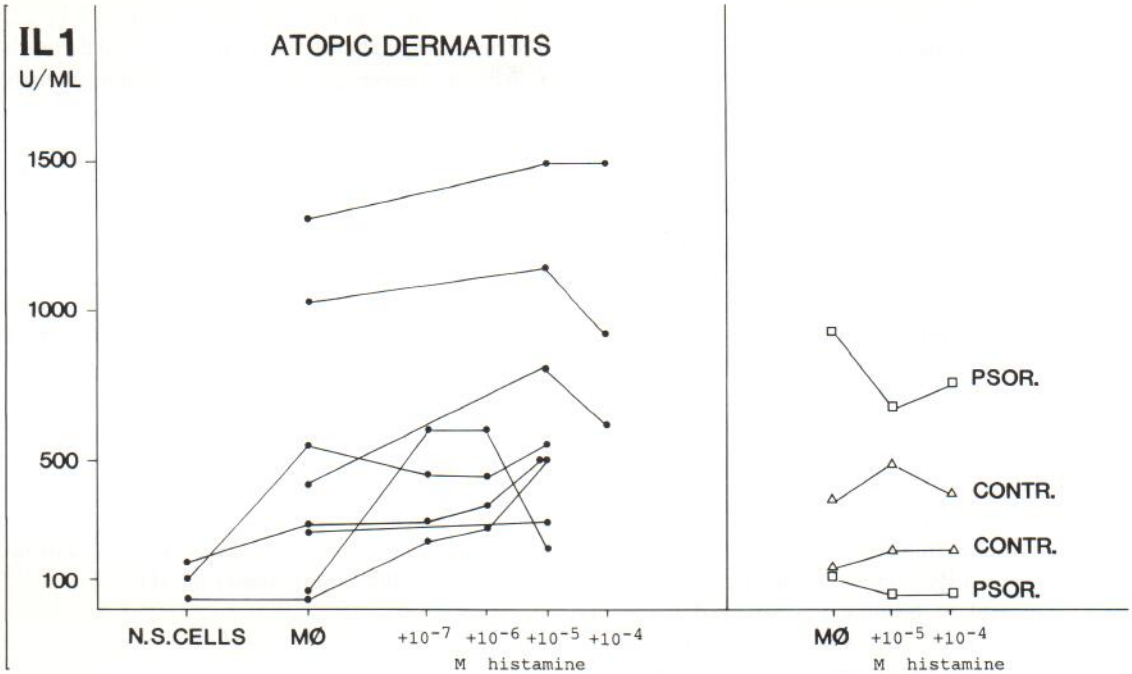

Fig. 2. Mononuclear cells (N.S. cells) or purified monocytes (MØ) were stimulated with LPS, 10^{-5} M, with or without addition of histamine.

tion between the sIL-2 level and IgE. Four patients had more than one investigation. It was found that clinical improvement of the eczema leads to a decline in the elevated sIL-2 values during therapy (systemic prednisone and/or azathioprine). Thus, the sIL-2 receptor level may reflect disease activity (Fig. 4). CD-8 receptor levels were not increased in any of the patients studied (results not shown).

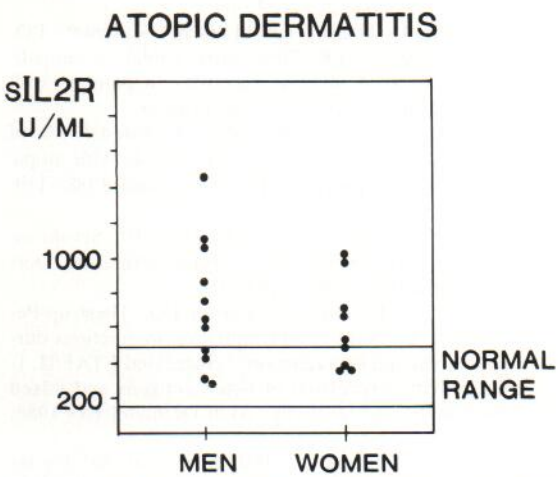

Fig. 3. Serum levels of interleukin-2 receptor (sIL-2R) in 20 patients with atopic dermatitis. Normal range indicated.

DISCUSSION

IL-1 can be expressed by a wide variety of cells, but the largest producers are LPS-stimulated monocytes. IL-1 acts as a secondary growth or stimulatory factor for a subset of T helper cells, the T_h2 lymphocytes, which release interleukines 4, 5 and 6 as autocrine growth factors following antigen or lectin stimulation (2).

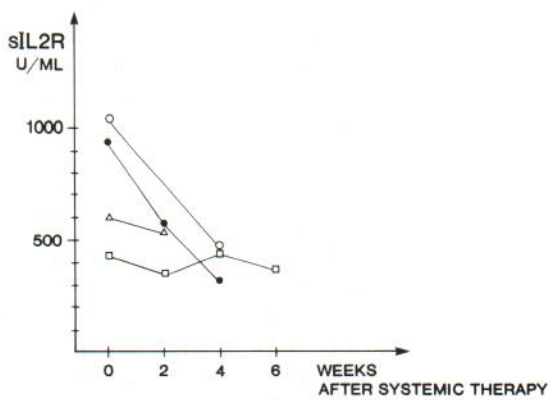

Fig. 4. The increased sIL-2R levels tend to normalize following treatment and clinically improvement of the disease (4 patients).

We and others have previously shown that unseparated, mononuclear cells release reduced (3, 4) or normal (5) IL-1 activity upon LPS stimulation. This was also found in the present study (Fig. 2, non-separated cells). Purification of monocytes from AD patients does, however, reveal an increased release of IL-1 activity (Fig. 1). It is known that adherence purification of monocytes augments their release of interleukines. However, this should then also be reflected among the non-atopics. It is therefore likely that monocytes from patients with very active AD are activated in vivo. This is apparently not detectable – or down-regulated – in non-separated cell suspensions.

The recurrent skin infections in AD may stimulate monocytes in the skin and thus lead to a stimulation of T helper cells. An additional factor for increased IL-1 may be histamine, which augmented the IL-1 release from LPS-stimulated monocytes (Fig. 2). The skin level of histamine is believed to be increased in patients with AD (12). A further histamine source may be basophils in peripheral blood, which demonstrates increased histamine release following addition of IL-1, IL-3 and granulocyte-macrophage colony-stimulating factor – at least in vitro (13, 14). Histamine has, however, been found to suppress the IL-1 release from purified monocytes from healthy blood donors by approx. 50%, with an optimum of 10^{-5} M (15). This effect may only be seen when the assay is performed in medium containing D_2O (16). However, we can not confirm these results using our culture system (Fig. 2).

The net effect for unseparated, peripheral blood mononuclear cells from AD patients seems to be a normal release of IL-1 (5) or even decreased level (3, 4). T lymphocytes may have a negative feed-back on IL-1 release from monocytes, because increased IL-1 release stimulates the T cell subset, which uses IL-4, -5 and -6 as autocrine growth factors upon antigen stimulation (2), and IL-4 has been found to suppress IL-1 release, tumour necrosis factor alfa, and prostaglandin E_2 from monocytes (17). It may be anticipated, however, that such a regulation is not taking place in the skin, where infection may start the increased T cell stimulation.

We can confirm that approx. 60% of patients with AD have increased levels of sIL-2R in serum (5–7). Resting lymphocytes do not express the 55 kD L chain, whereas activation leads to its expression and release from both T and B lymphocytes (9–11). Increased sIL-2R levels are seen in a wide variety of diseases with increased immune reactivity (18–22) and are not specific for atopic dermatitis. The levels reflect the degree of immune stimulation. Weekly observations showed fairly stable individual levels in 9 AD patients with clinically active disease (5). It is therefore anticipated that therapy will reduce the elevated sIL-2R level, as was also observed in some patients (Fig. 4).

The augmented IL-1 release may be one factor in the immune stimulation in AD. T cells are also most probably stimulated through other signals including a potential specific immune stimulation from allergens or mitogens.

Our observations confirm that augmented immune activity is taking place in patients with AD. The sIL-2 receptor parameter may be used to evaluate the activity of AD. Further studies are necessary in order to assess the significance of skin infections and other interleukines in AD.

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