Increased Interleukin 6 Production by T Cells Derived from Patients with Atopic Dermatitis

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Many immunologic aspects of atopic dermatitis have been studied, but basic pathobiologic mechanisms of this disease remain unknown. In this study, we measured the production of interleukin-6 (IL-6) by peripheral blood T cells and monocytes from patients with atopic dermatitis in comparison to normal control subjects and patients with chronic psoriasis. We found that peripheral blood T cells isolated from patients with atopic dermatitis produced significantly higher levels of IL-6 (36.1 ± 5.1 units/ml, n = 22) than T cells derived from either normal subjects (12.6 ± 1.9 units/ml, n = 22) or patients with chronic psoriasis (26.7 ± 4.1 units/ml, n = 7). T-cell activation was also measured in the patients with atopic dermatitis by soluble serum IL-2 receptor levels and were found to be significantly higher (623.7 ± 8.1 units/ml, n = 8) than normal subjects (357.2 ± 26.0 units/ml, n = 8). In contrast to the increased production of IL-6 by T cells in atopic dermatitis, there was no significant difference in the IL-6 production by peripheral blood monocytes derived from patients with atopic dermatitis compared to normal subjects. Thus, peripheral blood T cells derived from patients with AD spontaneously produce increased amounts of IL-6 compared to T cells from normal subjects, which may reflect the increased activation state of T cells in atopic dermatitis. These data support the concept that activated T cells or subsets of T cells may be important effector cells in mediating inflammatory activity in atopic disease. *J Invest Dermatol 100:299–304, 1993*

Atopic dermatitis (AD) is a multifactorial disorder associated with hereditary, environmental, and immunologic abnormalities, including overproduction of serum immunoglobulin E [1], impaired delayed-type hypersensitivity (DTH) responses [2], low proliferative responsiveness of cultured AD lymphocytes to antigen and mitogen stimulation *in vitro* [3,4], and altered natural killer cell activity [5].

Recently, it was reported that peripheral blood mononuclear cells (PBMC) isolated from patients with AD had defects in the secretion of certain cytokines, including decreased monocyte interleukin 1 (IL-1) secretion [6], defective T-cell interferon-gamma (IFN-γ) production [7], and decreased monocyte tumor necrosis factor-α (TNF-α) secretion [8]. Other studies indicated that T cells and T-cell clones from patients with AD secrete increased IL-4 compared to normal subjects [9,10]. The latter report also suggests that fewer IFN-γ–producing T cells in AD might relate both to the inadequate control of B-cell immunoglobulin E production and reduced DTH characteristic of AD. These findings are consistent with Mosmann's hypothesis that subsets of Th cells may be important mediators of certain allergic diseases [11].

Another measurement of T-cell activation is increased T-cell production of cytokines such as IL-6 and increased serum soluble IL-2 receptor (IL-2R) levels. IL-6 is a cytokine with a wide variety of effects on cells mediating inflammatory and immune responses. IL-6 induces acute phase proteins [12], stimulates B-cell differentiation [13,14], promotes the growth of hybridomas [15] and myeloma cells [16], and enhances T-cell proliferation [17,18]. *In vitro* studies have revealed that IL-6 is produced by many cells including fibroblasts [19], endothelial cells [20], epithelial cells/keratinocytes [21], and various lymphoid cells [22–26]. In clinical studies, IL-6 was also thought to be a mediator of the immunocompetent cell interactions in many pathologic processes. Elevated IL-6 levels have been found in serum and culture supernatants of PBMC in many inflammatory conditions such as rheumatoid arthritis [27], multiple myeloma [16], systemic lupus erythematosus [28], and chronic psoriasis [29,30]. Dysregulation of IL-6 production may play an important role in mediating these diseases. However, the precise effects of IL-6 on lymphoid cells and other cytokine-secreting cells within the immunologic network of these diseases is unclear. Little is currently known regarding the contribution of T-cell–derived IL-6 in mediating inflammatory skin diseases. Previous studies have reported that activated T cells may contribute to the pathogenesis of AD [31,32]. Because of its broad inflammatory properties, we have examined the production of IL-6 by peripheral blood T cells isolated from patients with AD in comparison to T cells derived from normal healthy subjects or patients with chronic psoriasis. In addition, as another measurement of T-cell activation we have measured serum levels of soluble IL-2R from patients with AD compared to normal individuals.

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Abbreviations:

ACD: allergic contact dermatitis
AD: atopic dermatitis
DTH: delayed-type hypersensitivity
ELISA: enzyme-linked immunosorbent assay
FCS: fetal calf serum
HTLV-1: human T-cell leukemia virus-1
IFN-γ: interferon-gamma
IL: interleukin
IL-2R: interleukin-2 receptor
MoAb: monoclonal antibody
PBMC: peripheral blood mononuclear cells
rIL-6: recombinant interleukin-6
Th2: type 2 helper T cell
TNF-α: tumor necrosis factor-alpha

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Our results demonstrate that T cells, but not monocytes, isolated from patients with AD produce significantly higher levels of IL-6 compared to normal subjects, perhaps reflecting an increased T-cell–activation state in AD. Consistent with these results were the increased serum levels of IL-2R and increased expression of CD3/CD25 on T cells isolated from PBMC detected in patients with AD. Increased T-cell IL-6 production may be indicative of increased type 2 helper T-cell (Th2) functional activity in AD.

MATERIALS AND METHODS

Subjects Patients with AD were chosen according to well-defined criteria [33]. All were adults with chronic, mild to severe dermatitis, receiving no oral medications. Normal healthy subjects and patients with chronic psoriasis participating in this study had no personal or family history of asthma, allergic rhinitis, or eczema. Extent and severity of dermatitis in the AD patients were graded on five-point scales as previously described [2].

Cytokines and Antibodies Human recombinant IL-1α (specific activity, 1×10^8 units/mg), IL-2 (specific activity, 2.5×10^6 units/mg), granulocyte/macrophage colony-stimulating factor (specific activity, 5×10^7 proliferation units/mg), and IL-6 (specific activity, 1×10^7 units/mg) were purchased from Genzyme Corp. (Boston, MA). Human recombinant IL-4 (specific activity, 1×10^6 units/mg) was a generous gift from Dr. Y.P. Yueng (Amgen, Thousand Oaks, CA). Human recombinant IFN-γ (specific activity, 1.9×10^7 units/mg) was kindly provided by Genentech, Inc. (South San Francisco, CA). The anti-CD3 monoclonal antibody (MoAb) Leu4, the anti-CD11c MoAb, LeuM5, and anti-CD14 MoAb, LeuM3 were purchased from Beckton-Dickinson (Mountain View, CA). Factor XIIIα MoAb was purchased from Calbiochem (La Jolla, CA).

Preparation of the Supernatants PBMC were isolated from heparinized venous blood by Ficoll-Hypaque (Sigma, St. Louis, MO) centrifugation at $400 \times g$ for 30 min. Cells were washed three times with saline, then suspended at 2×10^6 cells/ml in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS, Gibco), 1% L-glutamine (Gibco), 1% penicillin/streptomycin (Gibco) (complete RPMI) and incubated in 24-well culture plates (Corning, New York, NY) at 37°C in humidified atmosphere of 5% CO_2 in air. For T-cell purification, PBMC were incubated at 2×10^6 cells/ml in complete RPMI for 2 h on plastic Petri dishes (Nunc, Roskilde, Denmark) to remove adherent cells. Non-adherent cells were collected and applied to a Human T Cell Recovery Column Kit (Biotex Lab, Edmonton, Canada). T-cell supernatants were obtained by culturing 2×10^6 T cells/ml in complete RPMI at 37°C in humidified atmosphere of 5% CO_2 in air using 24-well culture plates for indicated periods. In some experiments, T-cell supernatants were obtained by culturing 2×10^6 cells/ml of T cells in RPMI supplemented with heat-inactivated human AB serum (Sigma), 1% L-glutamine, and 1% penicillin/streptomycin for 24 h at 37°C in humidified atmosphere of 5% CO_2 in air. Monocyte supernatants were obtained by continuous culture of adherent cells ($5-7 \times 10^5$ cells/ml) after removing the non-adherent cells in new complete RPMI at 37°C in humidified atmosphere of 5% in air for indicated periods. Supernatants were stored at −70°C until they were assayed for IL-6 bioactivity. All culture media used in these studies were screened for contaminating endotoxin by the E-Toxate Limulus amoebocyte bioassay (Sigma Chemical Company, St. Louis, MO). In blocking experiments, supernatants were pre-incubated with 0.5–10 mg/ml of mouse anti-human IL-6 monoclonal antibody (Genzyme) for 1 h at 37°C, 5% CO_2 in air prior to use.

Cell Characterization The PBMC contained 20 to 40% monocytes, 60 to 70% lymphocytes, and 1 to 2% polymorphonuclear cells. T-cell fractions contained 97 to 99% of $CD3^+$ cells and less than 1% non-specific esterase$^+$ monocytes. The monocyte fraction contained more than 98% non-specific esterase$^+$, 95% $CD11c^+$, and more than 96% $CD14^+$ and Factor XIIIα^+1 cells, when determined by staining with MoAbs as described above or α-naphtylacetate esterase (Sigma).

IL-6 Bioassay IL-6 bioactivity was measured by its proliferative action on the murine IL-6–dependent plasmacytoma B9 cell line [18]. The effects of several cytokines other than IL-6 on proliferative action of B9 cells were tested. No proliferation of the B9 cells used in these studies was observed when cultured with human IL-1α, IL-4 (50–100 U/ml), IL-2 (5–100 U/ml), IFN-γ (500–1000 U/ml), or granulocyte/macrophage colony-stimulating factor (500–1000 ng/ml). The specificity of the B9 bioassay for IL-6 in the cell culture supernatants was further confirmed by anti–IL-6 antibody-neutralization studies. Cells were maintained in RPMI supplemented with 5% FCS, 5×10^{-5} M 2-mercaptoethanol (J.T. Baker, Phillipsburg, NJ), 1% of penicillin/streptomycin, and 50 units/ml of human rIL-6. For the assay, B9 cells were harvested by centrifugation and washed three times in Hanks' balanced salt solution. 5×10^3 cells were seeded in 96-well flat-bottomed microtiter plate (Falcon, Lincoln Park, NJ), containing 100 ml of a serial two-fold dilution in media of the supernatants or sera to be tested. Serum samples from a group of AD, psoriatic, and normal subjects were heat-inactivated to 56°C for 30 min just prior to measuring the IL-6 bioactivity. B9 cells were cultured for 48 h in 37°C, the last 6 h in the presence of 1 mCi/well of [^{3}H]-thymidine (New England Nuclear, Boston MA). Incorporation of [^{3}H]-thymidine was determined by liquid scintillation counter and expressed as mean cpm or units/ml of triplicate cultures determined by comparing the samples to a standard bioactivity curve using rIL-6 [34].

Determination of Soluble IL-2R in Serum To assay soluble IL-2R in the serum from AD and normal subjects, an enzyme-linked immunosorbent assay (ELISA) with non-competing murine MoAb against IL-2R was used according to the manufacturer's protocol (T Cell Sciences, Inc., Cambridge, MA). Briefly, 96-well flat-bottomed microtiter plates were coated with a MoAb against IL-2R (2R1.2). After blocking the non-specific binding, plates were washed three times with phosphate-buffered saline, and freshly thawed samples were added in duplicate and incubated for 2 h at 37°C. Samples were then discarded, the plates were washed, and an antibody to the IL-2R conjugated with horseradish peroxidase was added to each well. After another 2 h incubation at 37°C, plates were washed and incubated with O-phenylenediamine-buffered solution containing hydrogen peroxidase. After 30 min at room temperature in the dark, color was developed by the addition of 2N sulfuric acid and the plates were read at 490 nm with microplate autoreader (Bio-Tek Instruments, Inc., Winooski, VT). Units of soluble IL-2R were calculated from a standard curve using soluble human IL-2R.

Statistical Analysis Most data were expressed as mean ± standard error of the mean (SEM). Significance of differences between means were calculated using the Student t test. Differences between values were considered statistically significant at $p < 0.05$. Correlation was performed by linear regression and the strength of the linear relationship was estimated with Pearson product moment coefficient of correlation (r).

RESULTS

IL-6 Production by T Cells Derived from Patients with AD Freshly isolated T cells (2×10^6 cells/ml) from AD, psoriasis, and normal subjects were cultured at 37°C for 24 h in complete RPMI and IL-6 bioactivity in the culture supernatants was measured in the B9 proliferation assay. Unstimulated T-cell culture supernatants from 22 patients with AD contained 36.1 ± 5.1 units/ml of IL-6 compared with 12.6 ± 1.9 units/ml of IL-6 in supernatants from T cells isolated from 22 normal subjects. For comparison we measured IL-6 activity in T-cell supernatants from patients with psoriasis and detected 26.7 ± 4.1 units/ml of IL-6 (Fig 1). Our studies indicated that freshly isolated T cells from patients with AD constitutively released significantly higher levels of IL-6 compared to equal numbers of T cells from normal subjects ($p < 0.005$) and T

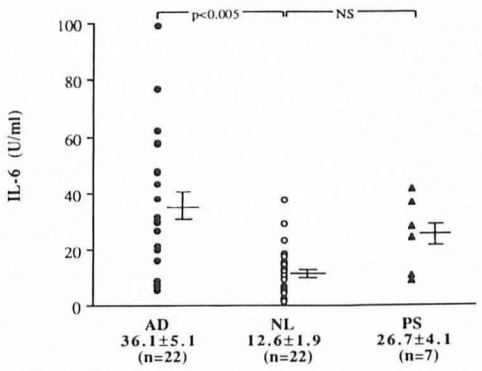

Figure 1. IL-6 production (mean ± SEM) by T cells from normal control subjects (NL, n = 22), patients with atopic dermatitis (AD, n = 22), and chronic psoriasis (PS, n = 7) was determined in the B9 proliferation assay. T-cell supernatants were obtained by culturing purified T cells (2×10^6 cells/ml) in complete RPMI for 24 h at 37°C, 5% CO_2 in air. IL-6 bioactivity of these supernatants (1 : 4 dilution) was measured by the B9 proliferation assay and expressed as units/ml of triplicate cultures determined by comparing the samples to a standard bioactivity curve using rIL-6. NS, not significant.

cells derived from patients with psoriasis ($p < 0.05$). T cells from psoriatic patients also secreted higher levels of IL-6 compared to normal subjects but not to the significant levels of the AD subjects. Peripheral blood T cells from patients with allergic contact dermatitis also secreted similar levels of IL-6 as the T cells derived from normal and psoriatic patients (data not shown). To confirm that the B9 proliferation activity in the cell supernatants was due to IL-6, we pre-incubated supernatants with anti-human IL-6 antibody for 1 h at 37°C. As shown in Fig 2, pre-incubation of the cell supernatants with anti-IL-6 antibody blocked the proliferative action of the supernatants on the B9 cells. In contrast, an anti-IL-4 antibody had no effect on this activity, thus indicating that the supernatant B9 proliferative activity was due to IL-6.

To rule out the possibility that the differential effect of AD supernatants was due to factors in FCS, we carried out controls using serum-free medium or AB serum. We found that AD T cells again produced increased levels of IL-6 compared to normal T cells in medium supplemented with 5% human AB serum instead of 10% FCS. Medium supplemented with either FCS or AB serum did not induce B9 proliferation above baseline cpm (data not shown). It is possible that the differences in IL-6 detected in the AD and normal T-cell supernatants may be due to differences in the secretion of an IL-6 inhibitor; however, normal T-cell supernatants caused no inhibitory effect on recombinant IL-6 activity in the B9 bioassay. Therefore, T cells derived from patients with AD produce signifi-

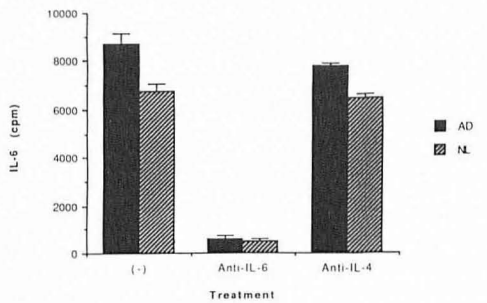

Figure 2. Effects of anti-IL-6 antibody on B9 proliferative response to AD *(solid bars)*, or normal T-cell supernatants (mean ± SEM, n = 4). Supernatants (1 : 4 dilution) were added to the B9 cells alone or pre-incubated with the anti-IL-6 antibody (5 μg/ml) or IL-4 antibody (5 μg/ml) for 1 h and then bioactivity was measured by ^{3}H-thymidine incorporation and quantitated by liquid scintillation spectroscopy.

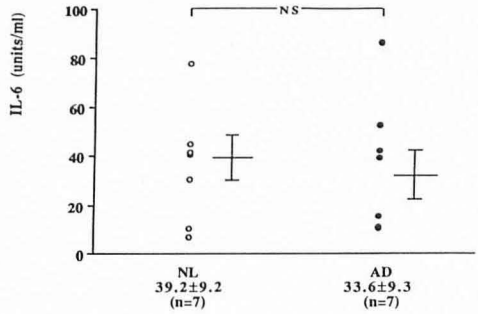

Figure 3. IL-6 production by monocytes from patients with atopic dermatitis (AD, n = 7) and normal (NL, n = 7) subjects. Monocyte supernatants were obtained by culturing purified monocytes ($5-7 \times 10^5$ cells/ml) in complete RPMI at 37°C, 5% CO_2 in air for 24 h. IL-6 bioactivity of these supernatants (1 : 4 dilution) was measured by the B9 proliferation assay and expressed as units/ml of triplicate cultures (mean ± SEM). NS, not significant.

cantly higher IL-6 than those of normal subjects or patients with other non-atopic inflammatory skin diseases.

IL-6 Production by Monocytes Derived from Patients with AD Because monocytes are a major source of IL-6 [23,24], we compared IL-6 production by monocytes derived from AD patients compared to normal subjects. The IL-6 activity in 24-h monocyte-conditioned supernatants from AD and normal subjects were measured in the B9 proliferation assay. As shown in Fig 3, atopic monocytes ($5-7 \times 10^5$ cells/ml, n = 7) released 33.6 ± 9.3 units/ml of IL-6 compared with 39.2 ± 9.2 units/ml of IL-6 from normal donors. Thus, in contrast to T cells, there was not a significant difference in the production of IL-6 bioactivity by monocytes isolated from atopic and normal subjects. However, these results also indicate that monocytes derived from AD and normal subjects produce higher basal levels of IL-6 than the T cells tested in Fig 1. The monocytes (5 to 7×10^5 cells/ml) produced several times more IL-6 on a per cell basis compared to the T cells (2×10^6 cells/ml). This is in agreement with previous studies [23].

Kinetics of IL-6 Production by Freshly Isolated T Cells and Monocytes From AD and Normal Subjects We next examined the time course of IL-6 production by freshly isolated T cells and monocytes from atopic dermatitis and normal subjects. As indicated in Fig 4, there was a significant amount of IL-6 bioactivity in atopic T-cell supernatants as early as 3 h after culturing cells. This activity peaked at 24 h and declined thereafter. In contrast, IL-6 bioactivity in normal T-cell supernatants was also detectable at 3 h but did not peak until 48 h after culturing cells. Again the levels of IL-6 activity in atopic T-cell supernatants was significantly higher than in normal T-cell supernatants at all time points measured (Fig 4*a*).

The kinetics of monocyte IL-6 production were also measured. Purified monocytes ($5-7 \times 10^5$ cells/ml) from both atopic and normal donors also secreted detectable levels of IL-6 after culturing cells for 3 h and this activity peaked after 24 h in tissue culture and then declined (Fig 4*b*). In contrast to the T-cell studies, there were no significant differences of IL-6 production between atopic and normal monocytes at any time point, consistent with the results in Fig 3.

Thus, our results indicate that there are clear differences in both the production and kinetics of freshly isolated T-cell IL-6 production in patients with AD compared to normal subjects. This difference was not detected in the IL-6 production by monocytes from these same donors.

IL-6 Bioactivity in Sera from Atopic Patients and Normal Subjects To determine if the increased IL-6 production by T cells isolated from patients with AD was reflected in increased serum levels of IL-6 in these same patients, we examined IL-6 bioactivity in serum from both atopic and normal subjects in comparison to

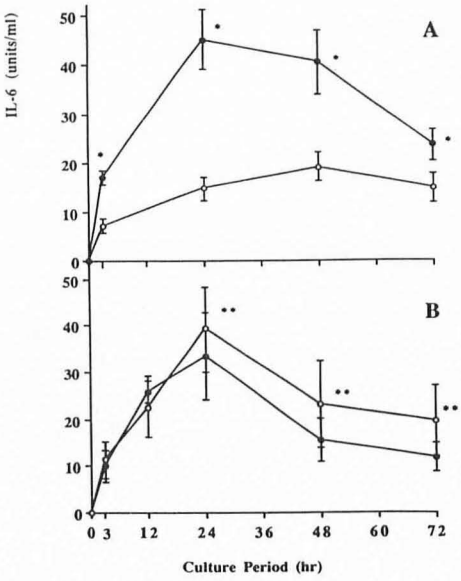

Figure 4. Kinetics of IL-6 production by T cells (*A*) and monocytes (*B*) from patients with AD (●) and normal (○) subjects. Purified T cells (2×10^6 cells/ml) and monocytes ($5-7 \times 10^5$ cells/ml) were cultured in complete RPMI at 37°C, 5% CO_2 in air for the indicated periods. The data represent mean units/ml ± SEM of IL-6 bioactivity in three to seven different donors. *$p < 0.05$; **not significant compared to normal subjects.

patients with chronic psoriasis. Normal human serum contains poorly defined IL-6 inhibitory factors when measured in the B9 bioassay (personal communication, Dr. Thomas Luger, Munster, Germany, 1991); therefore, we measured serum IL-6 bioactivity after first heating sera at 56°C for 30 min. Only low levels of IL-6 bioactivity were detected in atopic and normal sera that was not heat treated (data not shown). After heat inactivation, IL-6 bioactivity was evident in sera from both atopic and normal subjects. However, as indicated in Fig 5, there was not a significant difference in the serum IL-6 activity in AD subjects compared to normal subjects. The serum from patients with psoriasis had a small, slightly significant increase in IL-6 activity compared to normal subjects ($p < 0.05$). Thus, the increased production of IL-6 by atopic T cells *in vitro* was not reflected in increased levels of serum levels of IL-6 in these patients.

Correlation Between IL-6 Production by T Cells and Soluble IL-2R in Serum from Patients with AD and Normal Subjects It has been reported that many patients with AD have an increased level of soluble IL-2R in serum, reflecting the activated

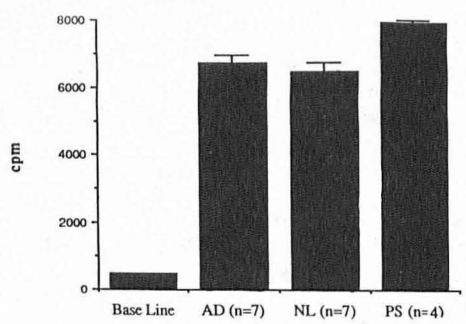

Figure 5. IL-6 bioactivity (mean ± SEM) in serum from patients with AD (n = 7), chronic psoriasis (PS) (n = 4), and normal subjects (n = 7). Sera were heat-inactivated to 56°C for 30 min prior to use and diluted to 1 : 4 with B9 cell medium. The cpm of all unheated sera were less than base line cpm of B9 cells. The value for psoriasis was significantly higher than for normal serum ($p < 0.05$). *Error bar*, SEM.

Table I. Correlation Between IL-6 Production by T Cells and Clinical Severity of Patients with AD

Clinical Severity	IL-6 Bioactivity (units/ml)[a]
Mild	39.1 ± 5.9 (n = 7)
Severe	49.0 ± 9.7 (n = 7)[b]

[a] IL-6 bioactivity was determined by the proliferative action of B9 cells. Data were expressed as mean ± SEM of units/ml of IL-6.
[b] No significant difference of IL-6 bioactivity compared to the patients with mild AD.

state of the T cells in this disease [31,32]. In this study, we measured the serum IL-2R levels of the AD and normal subjects. We found that the mean soluble IL-2R level in serum from these AD patients was significantly higher (623.7 ± 83.1 units/ml, n = 8) than that of the serum IL-2R levels in normal subjects (357.2 ± 26.0 units/ml, n = 8). These results are consistent with an activated T-cell state in AD. The increased serum IL-2R levels in the AD patients was positively correlated with the increased spontaneous IL-6 production by T cells in these patients, as demonstrated in Fig 6 ($p < 0.05$). We also examined the percentage of $CD25^+$ cells as phenotypical analysis of activated T cells in patients with AD using flow cytometry. In the same patients (n = 8), we found a positive correlation between the increased production of T-cell IL-6 production ($r = 0.81$, $p < 0.05$) and the increased $CD25^+$ cells in PBMC. The patients with AD compared to the normal subjects as determined by fluorescence-activated cell sorter analysis (16.6 ± 3.1% for AD [n = 8] and 10.4 ± 2.6% for normals [n = 5], $p < 0.05$).

Correlation Between T-Cell IL-6 Production and Clinical Severity in Patients With AD We questioned whether increased AD T-cell IL-6 production related to disease severity in the subjects tested. IL-6 production levels by AD T cells was compared with the clinical severity of the AD in the study population. Although IL-6 bioactivity in T-cell supernatants from patients with severe AD was higher than in patients with mild AD, the differences were not statistically significant among the 14 patients who could be clearly designated as having mild or severe AD (Table I).

DISCUSSION

This study has provided new and unexpected information relating T-cell IL-6 production and AD. We have demonstrated that freshly isolated unstimulated T cells from peripheral blood of patients with AD consistently release higher levels of IL-6 compared to T cells

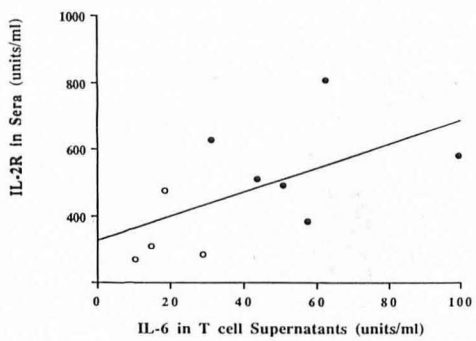

Figure 6. Correlation, by linear regression, between IL-6 production by T cells and soluble IL-2R levels in serum from patients with AD (n = 6, *solid circles*) and normal subjects (n = 4, *open circles*). Serum IL-2R was determined by ELISA using murine MoAb against IL-2R. The data were expressed as units/ml of IL-2R determined by the comparison of samples to a standard curve using human soluble IL-2R.

from normal healthy subjects or patients with other inflammatory skin disorders. It is of particular significance that highly purified (<2% monocytes) atopic T cells produce high levels of IL-6 without the addition of specific exogenous antigens or mitogens. In previous studies, it has been reported that T cells produced IL-6 only after phytohemagglutinin and phorbol stimulation and required the presence of monocytes, whereas normal resting T cells secreted little IL-6 [23,35]. Hori *et al* also reported that normal peripheral blood T cells produce IL-6 in a macrophage-dependent manner, and IL-6 mRNA expression was observed in only mitogen-stimulated T cells but not in resting T cells [23]. In other studies, IL-6 mRNA was demonstrated in human T-cell leukemia virus-1–infected T cells but not in normal T cells [22,26]. In contrast to a previous publication, we consistently detected some IL-6 production by normal T cells, which was significantly increased in AD T cells. This was not due to FCS components because we observed similar proliferative activities when human AB serum was used in our tissue culture medium. Likewise, endotoxin contamination was ruled out as a source of IL-6 induction by routinely screening tissue culture media by the E-toxate assay. The IL-6 produced by unstimulated normal T cells may be due to non-specific activation by culture conditions. Thus, our results demonstrate an example of increased IL-6 production by freshly isolated T cells from patients with AD, which may reflect an activated state of the T cells in this disease.

Because of its multi-functional properties, IL-6 is believed to be an important mediator of host immune and inflammatory responses. Originally, IL-6 was found to be produced by fibroblasts in certain inflammatory conditions [19] and subsequent studies showed that IL-6 was produced by monocytes [24], endothelial cells, epithelial cells [20], and keratinocytes [21]. Monocytes are thought to be a major source of IL-6 in peripheral blood cells. Our findings support this, showing that monocytes produced two to three times more IL-6 on a per cell basis than T cells in AD and normal subjects. However, we found no evidence for increased IL-6 production by monocytes in AD patients compared to normal subjects. Since AD and normal monocytes produced equal amounts of IL-6, it is unlikely that the differences in T-cell IL-6 production can be explained simply by monocyte-derived IL-6 carried into the T-cell cultures by membrane absorption of IL-6. Moreover, we measured the IL-6 bioactivity in T-cell supernatants by culturing T cells (2×10^6 cells/ml) purified by loading PBMC to the T-cell column directly without adherence. AD T cells isolated in this manner also produced significantly higher IL-6 bioactivity than normal T cells and showed levels of IL-6 bioactivity similar to those from T cells obtained after the 2-h adherence removal of monocytes (data not shown). Therefore, although monocytes may exert permissive effects on T-cell IL-6 production, they do not appear to be responsible for abnormal amounts of IL-6 in our AD T-cell studies because they represented less than 2% of T-cell preparations and equal amounts of monocyte IL-6 would be carried in both normal and AD T-cell preparations.

Others have suggested that activated T cells contribute to the pathogenesis of AD [31,32,36]. In the present study in addition to the increased T-cell IL-6 production, T-cell activation is reflected by the increased soluble IL-2R levels in the serum of patients with AD. Thus, our results are consistent with previous studies proposing that activated T cells contribute to AD [31,36]. The elevated T-cell IL-6 production in patients with AD may be a unique sign of T-cell activation in atopy or a predominance of Th2 subsets or both. This cytokine is capable of mediating a number of immunologic and inflammatory responses. The effects of IL-6 on B cells include the stimulation of cell growth, differentiation, and regulation of immunoglobulin isotype class [13,14]. IL-6 is also a mitogen for hepatic acute-phase protein synthesis [12], an endogenous pyrogen, and a potent T-cell activating factor that induces T-cell proliferation [17,18].

We found that peripheral blood T cells from patients with allergic contact dermatitis (ACD) produced levels of IL-6 comparable to normal subjects and less than AD patients. This may reflect the local nature of ACD compared to the systemic manifestations of AD. The consequence of this difference could be much higher numbers of IL-6-producing activated T cells in AD blood compared to ACD. Increased production of IL-6 by atopic T cells or subsets may contribute to the local and systemic immunologic irregularities observed in AD. There are many studies reporting elevated IL-6 production by peripheral blood lymphoid cells in various systemic inflammatory diseases, such as rheumatoid arthritis [27] and systemic lupus erythematosus [28]. Adelman *et al* reported that the serum from patients with common variable immunodeficiency demonstrated elevated levels of IL-6 [34]. Likewise, increased IL-6 activity was reported in suction blister fluid and in keratinocytes from patients with chronic psoriasis [30]. Our results indicated that IL-6 production by peripheral blood T cells isolated from patients with chronic psoriasis was increased above levels of normal subjects but less than AD T cells.

Serum IL-6 levels from AD subjects were similar to those of normal subjects, whereas serum from patients with chronic psoriasis had a small increase in IL-6 bioactivity. This is consistent with findings by other investigators [30], though we are aware of one study in which serum IL-6 was elevated in a subset of AD patients correlating with extensive body surface area of involvement (Dr. J. Krutmann, personal communication, 1992). In our study, normal serum IL-6 levels were detected in AD patients despite cultured AD T cells producing increased amounts of IL-6. It is difficult to accurately measure serum IL-6 levels. Secreted IL-6 is rapidly inactivated in the serum by circulating inhibitors, proteases, and serum binding proteins. Recent reports indicate that IL-6 is rapidly metabolized within a few minutes by serum proteases and is selectively deposited in the skin [37,38]. This tropism of IL-6 for the skin further supports a possible role of T-cell IL-6 in AD. Serum IL-6 may also bind to other proteins thus making it undetectable by available ELISA kits (personal communication, Dr. L. May, Rockefeller University, NYC, NY). Elevated IL-6 production by AD T cells may reflect the increased activation state of these T cells in AD and may contribute to the cutaneous inflammation in this disease by the IL-6 activation of lymphoid and non-lymphoid cells in the skin. The identification of specific T-cell subsets in the skin secreting IL-6 in AD may give added insight in the pathogenesis of this disease.

It is possible that the increased levels of IL-6 produced by AD T cells is due to lower levels of IL-6 inhibitor secreted by T cells in these patients. Although this possibility cannot be absolutely ruled out by our studies, it is unlikely because the T-cell supernatants from normal subjects failed to have any inhibitory activity in the B9 bioassay when the cells were stimulated with recombinant IL-6.

Although there was increased T-cell IL-6 production in severe AD compared to mild AD, we failed to demonstrate a statistical correlation in the small group of patients in our study group. A larger population of AD patients will be needed to adequately address this issue.

There have been several reports concerning the secretion of cytokines by T cells and monocytes in patients with AD. Rasanen *et al* reported that the production of IL-1 by monocytes was impaired in AD and that atopic monocytes failed to induce T-cell activation [6]. Reinhold *et al* reported that a significant proportion of patients with AD had an impaired capacity to produce IFN-γ *in vitro,* and this was correlated with increased serum immunoglobulin E and immunoglobulin G4 levels and associated with an increased proportion of IL-4 producing T cells [8]. Increased T-cell–derived IL-4 may be responsible for increased immunoglobulin E production by B cells characteristic of AD. It has been reported that there are two types of T helper cell subsets in a murine model and more recently in humans, which differ in the pattern of lymphokine release [11,39]. Th1 cells release predominantly IL-2 and IFN-γ and Th2 cells release IL-4, IL-5, and IL-6, but not IFN-γ. The increased IL-6 production by atopic T cells may be the result of the activation of the Th2 subset. This may contribute to the dysregulation of immunoglobulin E production by B cells in AD.

In this study, we have demonstrated that T cells from patients with atopic dermatitis have the capacity to produce significant levels of IL-6 spontaneously, and at higher levels than T cells from

normal subjects. These findings suggest that elevated T cell IL-6 production may contribute to the inflammation in AD. However, the precise role of these IL-6 secreting T cells in the pathogenesis of AD remains to be determined.

REFERENCES

1. Juhlin L, Johansson SGO, Bennich H, Hogman C, Thyresson N: Immunoglobulin E in dermatoses. Levels in atopic dermatitis and urticaria. Arch Dermatol 100:12–16, 1969
2. Elliott ST, Hanifin JM: Delayed cutaneous hypersensitivity and lymphocyte transformation: Dissociation in atopic dermatitis. Arch Dermatol 115:36–39, 1979
3. Hanifin JM, Lobitz WC: Newer concepts of atopic dermatitis. Arch Dermatol 113:663–670, 1977
4. Bousquet J, Andary M, Clot J, Guilhou JJ, Meynadier J: *In vitro* immunological studies in atopic and contact dermatitis. Ann Allergy 41:170–175, 1978
5. Jensen JR, Sand TT, Jorgensen AS, Thestrup-Pedersen K: Modulation of natural killer cell activity in patients with atopic dermatitis. J Invest Dermatol 82:30–34, 1984
6. Rasanen L, Lehto M, Reunala T, Jansen C, Leinikki P: Decreased monocyte production of interleukin-1 and impaired lymphocyte proliferation in atopic dermatitis. Arch Dermatol Res 279:215–218, 1987
7. Reinhold U, Wehrmann W, Kukel S, Kreysel H-W: Evidence that defective interferon-gamma production in atopic dermatitis patients is due to intrinsic abnormalities. Clin Exp Immunol 79:374–379, 1990
8. Reinhold U, Pawelec G, Wehrmann W, Kukel S, Oehr P, Kreysel H-W: Cytokine release from cultured peripheral blood mononuclear cells of patients with severe atopic dermatitis. Acta Derm Venereol (Stockh) 69:497–502, 1989
9. Renz H, Jujo K, Bradley KL, Domenico J, Gelfand EW, Leung DYM: Enhanced IL-4 production and IL-4 receptor expression in atopic dermatitis and their modulation by interferon-gamma. J Invest Dermatol 99:403–409, 1992
10. Wierenga EA, Snoek M, DeGroot C, Chretien L, Bos JD, Jansen HM, Kapsenberg MI: Evidence for compartmentalization of functional subsets of $CD4^+$ T lymphocytes in atopic patients. J Immunol 144:4651–4656, 1990
11. Mosmann TR, Coffman RL: TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Anna Rev Immunol 7:145–173, 1989
12. Gaudie J, Richards C, Harnish D, Lansdorp P, Baumann H: Interferon-β2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc Natl Acad Sci USA 84:7251–7255, 1987
13. Hirano T, Yasukawa K, Harada H, Taga T, Watanabe Y, Matsuda T, Kashiwamura S, Nakajima K, Koyama K, Iwamatsu A, Tsunasawa S, Sakiyama F, Matsui H, Takahara Y, Taniguchi T, Kishimoto T: Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature 324: 73–76, 1986
14. Muraguchi A, Hirano T, Tang B, Matsuda T, Horii Y, Nakajima K, Kishimoto T: The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells. J Exp Med 167:332–344, 1988
15. Aarden LA, DeGroot ER, Schaap OL, Lansdorp PM: Production of hybridoma growth factor by human monocytes. Eur J Immunol 17:1411–1416, 1987
16. Kawano M, Hirano T, Matsuda T, Taga T, Hori Y, Iwato K, Asaoku H, Tang B, Tanabe O, Tanaka H, Kuramoto A, Kishimoto T: Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature 332:83–85, 1988
17. Lotz M, Jirik F, Kabouridis P, Tsoukas C, Hirano T, Kishimoto T, Carson DA: B cell-stimulating factor-2/interleukin 6 is a co-stimulant for human thymocytes and T lymphocytes. J Exp Med 167:1253–1258, 1988
18. Garman RD, Jacobs KA, Clark SC, Raulet DH: B cell-stimulatory factor 2 (β2 interferon) functions as a second signal for interleukin 2 production by mature murine T cells. Proc Natl Acad Sci USA 84:7629–7633, 1987
19. May LT, Helfgott DC, Sehgal PB: Anti-β-interferon antibodies inhibit the increased expression of HLA-B7 mRNA in tumor necrosis factor-treated human fibroblasts: structural studies of the β2 interferon involved. Proc Natl Acad Sci USA 83:8957–8961, 1986
20. Jirik FR, Podor TJ, Hirano T, Kishimoto T, Loskutoff DJ, Carson DA, Lotz M: Bacterial lipopolysaccharide and inflammatory mediators augment IL-6 secretion by human endothelial cells. J Immunol 142:144–147, 1989
21. Kirnbauer R, Kock A, Schwarz T, Urbanski A, Krutmann J, Borth W, Damm D, Shipley G, Ansel JC, Luger TA: IFN-β2, B cell differentiation factor 2, or hybridoma growth factor (IL-6) is expressed and released by human epidermal cells and epidermoid carcinoma cell lines. J Immunol 142:1922–1928, 1989
22. Hirano T, Taga T, Nakano N, Yasukawa K, Kashiwamura S, Shimizu K, Nakajima K, Pyun KH, Kishimoto T: Purification to homogeneity and characterization of human B cell differentiation factor (BCDF or BSFp-2). Proc Natl Acad Sci USA 82:5490–5494, 1985
23. Hori Y, Muraguchi A, Suematsu S, Matsuda T, Yoshizaki K, Hirano T, Kishimoto T: Regulation of BSF-2/IL-6 production by human mononuclear cells. J Immunol 141:1529–1535, 1988
24. Kato K, Yokoi T, Takano N, Kanegane H, Yachie A, Miyawaki T, Taniguchi N: Detection by in situ hybridization and phenotypic characterization of cells expressing IL-6 mRNA in human-stimulated blood. J Immunol 144:1317–1322, 1990
25. Zhang XG, Klein B, Bataille R: Interleukin-6 is a potent myeloma-cell growth factor in patients with aggressive multiple myeloma. Blood 74:11–13, 1989
26. Villiger PM, Cronin MT, Amenomori T, Wachsman W, Lotz M: IL-6 production by human T lymphocytes. J Immunol 146:550–559, 1991
27. Hirano T, Matsuda T, Turner M, Miyasaka N, Buchan G, Tang B, Sato K, Shimizu M, Maini R, Feldmann M, Kishimoto T: Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. Eur J Immunol 18:1797–1801, 1988
28. Tanaka Y, Saito K, Shirakawa F, Ota T, Suzuki H, Eto S, Yamashita U: Production of B cell-stimulating factors by B cells in patients with systemic lupus erythematosus. J Immunol 141:3043–3049, 1988
29. Prens EP, Benne K, Van Damme J, Bakkus M, Brakel K, Benner R, Van Joost T: Interleukin-1 and interleukin-6 in psoriasis. J Invest Dermatol 95:121S–124S, 1990
30. Neuner P, Urbanski A, Trautinger F, Moller A, Kirnbauer R, Kapp A, Schopf E, Schwarz T, Luger TA: Increased IL-6 production by monocytes and keratinocytes in patients with psoriasis. J Invest Dermatol 97:27–33, 1991
31. Kapp A, Piskorski A, Schopf E: Elevated levels of interleukin 2 receptor in sera of patients with atopic dermatitis and psoriasis. Br J Dermatol 119:707–710, 1988
32. Rubin LA, Kurman CC, Fritz ME, Biddison WE, Boutin B, Yarchoan R, Nelson DL: Soluble interleukin 2 receptors are released from activated human lymphoid cells *in vitro*. J Immunol 135:3172–3177, 1985
33. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol Suppl 92:44–47, 1980
34. Adelman DC, Matsuda T, Hirano T, Kishimoto T, Saxon A: Elevated serum interleukin-6 associated with a failure in B cell differentiation in common variable immunodeficiency. J Allergy Clin Immunol 86:512–521, 1990
35. Krutmann J, Kirnbauer R, Kock A, Schwarz T, Schopf E, May LT, Sehgal PB, Luger TA: Cross-linking Fc receptors on monocytes triggers IL-6 production. J Immunol 145:1337–1342, 1990
36. Colver GB, Symons JA, Duff GW: Soluble interleukin 2 receptor in atopic eczema. Br Med J 298:1426–1428, 1989
37. Castell J, Klapproth J, Gross V, Walter E, Andus T, Snyers L, Content J, Heinrich PC: Fate of interleukin-6 in the rat. Eur J Biochem 189:113–118, 1990
38. Ulich TR, Guo K, Remick D, Del Castillo J, Yin S: Endotoxin-induced cytokine gene expression *in vivo*. J Immunol 146:2316–2323, 1991
39. Parronchi P, Macchia D, Piccinni M-P, Biswas P, Simonelli C, Maggi E, Ricci M, Ansari AA, Romagnani S: Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. Proc Natl Acad Sci USA 88:4538–4542, 1991