Langerhans Cell- and T-Lymphocyte Functions in Patients With Atopic Dermatitis With Disseminated Cutaneous Herpes Simplex Virus Infection

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We studied whether Langerhans cell (LC) - and T-lymphocyte functions of atopic dermatitis (AD) patients are impaired. Our study groups consisted of 6 patients with AD with previous disseminated herpes simplex virus infection (AD + HSV), 8 patients with ordinary AD, and 5 healthy subjects. Suction blisters were performed on abdominal skin and LC isolated on the basis of their attachment to IgG-coated erythrocyte monolayers. Antigen-presenting function of purified LC was studied by measuring the proliferation of HSV-stimulated T cells. Langerhans cells were also used to stimulate T cells in autologous mixed cell reaction (AMCR). In addition, the production of epidermal cell thymocyte-activating factor (ETAF) by crude epidermal cells was measured.

The HSV-induced T-cell proliferation in AD + HSV and AD patients was comparable with that of controls. The AMCR responses of patients with AD + HSV and AD were clearly diminished when compared with healthy controls. Patients with AD also produced significantly less ETAF than controls. Our results suggest that HSV antigen-presenting function of LC from patients with AD + HSV seems to be intact. Defective AMCR may reflect an abnormality in autoregulation and generation of effector cells and this together with decreased ETAF production may have pathogenetic significance in AD. J Invest Dermatol 89:15-18, 1987

**Materials and Methods**

**Patients and Healthy Controls** Our study population consisted of 5 healthy subjects, 8 ordinary patients with AD, and 6 atopic patients with a history of cutaneous herpes virus infections (Table I). Within the last year, 5 of the patients had had at least 1 disseminated HSV-1 infection and 1 patient had suffered from HSV-1 encephalitis and several local cutaneous herpes infections. Disseminated cutaneous infections and encephalitis had been verified by isolation and typing and treated with acyclovir. At the time of the study none of the patients had active herpes infection.

**Enrichment of Epidermal LC** The purification procedure of LC has been previously described in detail [6]. In brief, epidermal sheets were treated with 0.25% trypsin (GIBCO Ltd., Paisley, Scotland) for 30 min at 37°C and then with 0.01% DNase I (Sigma Chemical Co., St. Louis, Missouri) for 5 min at room temperature. The sheets were pipetted vigorously and the cell suspension filtered through a nylon mesh. The LC were isolated in tissue culture dishes by attaching them to sheep red blood cell (SRBC) monolayers coated with anti-SRBC IgG (Cappel Laboratories

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**Abbreviations:**
- AD: atopic dermatitis
- AD + HSV: atopic dermatitis with cutaneous herpes simplex virus infection
- AMCR: autologous mixed cell reaction
- EIA: enzyme immunoassay
- ETAF: epidermal cell thymocyte-activating factor
- IL-1: interleukin 1
- IL-2: interleukin 2
- LC: Langerhans cell(s)
- PMA: 4α-phorbol 12-myristate 13-acetate
- SI: stimulation index
- SRBC: sheep red blood cells
Inc., Cochranville, Pennsylvania). To enhance the binding of LC to erythrocyte monolayers the plates were centrifuged; this was followed by incubation at 4°C. Langerhans cell–enriched cells were obtained after removal of nonadherent cells by rocking the plates and lysing the erythrocytes by hypotonic shock. The average purity of LC-enriched fractions was 80% as assessed by staining of OKT6-positive cells.

Separation of T Lymphocytes Mononuclear cells were obtained by Ficoll-Isoopaque centrifugation of peripheral blood. These cells were suspended in 10% human AB serum-RPMI 1640 and incubated in tissue culture dishes at 37°C for 1 h, after which nonadherent cells were removed. Nonadherent cells were treated with carboxyl iron for 1 h, followed by depletion of phagocytic cells with a magnet. The recovered lymphocytes were resuspended twice with 2-aminothylisothiourea (to inhibit antigen processing) and centrifuged by Ficoll-Isoopaque. Contaminating SRBC among T cells were lysed by hypotonic shock. T-cell fractions contained >95% T lymphocytes (stained by OKT3), <3% surface membrane immunoglobulin-positive B lymphocytes, and <0.2% monocytes as assessed by nonspecific esterase staining.

Herpes Simplex Virus–Stimulated Cultures T cells with or without 5% LC were suspended at a density of 0.25–10⁶ cells/ml in RPMI 1640 containing 10% autologous plasma. Volumes (0.1 ml) of these suspensions were pipetted per well of V-bottomed microplates. Thereafter 0.1 ml of various dilutions of HSV-1 and corresponding control antigens (Behringwerke AG, Marburg, F.R.G.) were added per well. The cultures were terminated after 6 days of incubation. Sixteen hours before harvesting, 0.125 μCi of 5-[¹³¹]iodo-2'-deoxyuridine (Amersham, Buckinghamshire, England) containing 1 μM fluorodeoxyuridine was added to each well [7]. The uptake of the isotope was measured with a gamma counter.

Autologous Mixed Cell Reaction Cultures In these cultures T lymphocytes were stimulated with autologous purified LC. In preliminary experiments it was found that optimum stimulator to responder cell ratio was 1:1 and optimum culture time 7 days for both healthy controls and atopic patients. Subsequently, 25–10⁴ T cells were cultured in the presence of 25–10⁴ LC in V-bottomed microplates for 7 days. In some experiments exogenous interleukin 1 (IL-1, final concentration 10 units/ml, Genzyme Corp., Norwalk, Connecticut), interleukin 2 (IL-2, final concentration 5%, Lymphocult-T-LF, Biostec, Offenbach, F.R.G.), or both were added to AMCR cultures. At the end of the 7-day incubation period, proliferation of T cells was measured as described above.

Production and Measurement of ETAF For the production of ETAF crude epidermal cells (10⁶ cells/ml) were incubated in 5% AB serum-RPMI in tissue culture tubes for 2 days. To enhance the elaboration of ETAF, the culture medium was supplemented with 20 ng/ml of 4β-phorbol 12-myristate 13-acetate (PMA, Sigma Chemical Co). The cell-free control tubes contained culture medium with PMA.

Epidermal cell thymocyte-activating factor activity was determined as described by Luger et al [8], using thymocytes from 6- to 8-week-old BALB/c mice. Thymocytes were suspended at a density of 15-10⁶ cells/ml in RPMI supplemented with 20% fetal calf serum and 5×10⁻⁵ M 2-mercaptoethanol. One hundred microliters of thymocyte suspension, 50 μl of culture supernatants (diluted 1:2–1:8) or purified IL-1, and 50 μl of phytohemagglutinin (4 μg/ml of purified PHA, Wellcome, Dartford, England) were pipetted per well of flat-bottomed microplates. Amounts of 2.5, 5, and 10 units/ml of IL-1 were used for determination of standard slope. The plates were incubated at 37°C for 3 days and proliferation of the cells measured as described above. The cpm value of the undiluted sample was calculated on the basis of the dilution giving 50% maximum label incorporation. The results were read from the standard slope and expressed as units/ml.

Other Methods Total IgE values were measured with a commercial RIST kit (Pharmacia, Uppsala, Sweden). The HSV-1 antibodies were measured with an enzyme immunoassay (EIA) as described previously [9]. The HSV isolates were typed using a commercial immunofluorescence kit (Syva Co., Palo Alto, California).

RESULTS

T-Cell Proliferative Responses and Serum Antibodies to HSV-1 Cell-mediated immunity to HSV-1 was assessed in vitro by measuring the proliferation of T lymphocytes stimulated with a commercial antigen preparation. Accessory cells were essential for T-cell blast transformation, and here we supplemented the cultures with 5% purified LC. Table II illustrates the results of cell culture experiments and serum antibody determinations in individual cases. Three subjects (numbers 5, 6, and 12) were
seronegative to HSV-1 and they also showed no response to the antigen preparation in the blast transformation test (stimulation indices less than 3.0 were considered negative). Two subjects (3 and 4) had evidently had primary infection, and their in vitro responses to HSV-1 were relatively low. All seropositive subjects were also positive in the blast transformation test, but there was no correlation between antibody levels and magnitude of lymphocyte responsiveness. Since only 2 subjects with positive HSV-I IgG antibodies were included in our healthy control group, the results on HSV-1-induced proliferation cannot be statistically analyzed among different study groups. However, it seems that seropositive atopic patients with or without previous disseminated HSV-1 infection respond well to herpetic antigens in vitro.

**Autologous Mixed Cell Reaction and ETAF in Patient and Control Groups** Langerhans cells, due to their DR antigen, are able to stimulate autologous T lymphocytes to proliferate. This reaction was significantly impaired (p < 0.01, Mann-Whitney U test) in AD + HSV and AD groups as compared with the controls (2.7 ± 0.7, 2.5 ± 0.5, and 9.7 ± 1.9, mean stimulation index (SI) ± SEM, respectively). The AMCR was negative (SI < 3.0) in 3/6 AD + HSV patients and 5/8 AD patients but positive in all healthy subjects (Table II). Deficient AMCR did not seem to correlate with the severity of AD (Tables I, II).

We also measured ETAF activity in crude epidermal cell cultures (Table II). The mean ± SEM values in units/ml were 16.7 ± 2.5, 8.0 ± 1.1, and 6.0 ± 1.5 in healthy control, AD, and AD + HSV groups, respectively. Both AD and AD + HSV groups differed significantly from the controls (p < 0.01, Mann-Whitney U test). The ETAF values did not correlate with AMCR or activity of AD. Interestingly, our patient with encephalitis had the lowest ETAF and AMCR values in spite of good antibody levels and proliferative responsiveness to HSV-1.

In AMCR, T cells both produce and respond to IL-2 [10]. Interleukin 1 in turn promotes the synthesis of IL-2. We next studied whether supplementation of AMCR cultures with exogenous IL-1, IL-2, or both restores deficient proliferation. With 3 atopic patients the mean AMCR responses were 2.6 (cultures not supplemented with interleukins), 3.4 (in the presence of exogenous IL-1), 5.5 (in the presence of IL-2), and 8.1 (culture supplemented with both IL-1 and IL-2). Interleukin 1 and IL-2 together thus caused the most distinct enhancement of depressed AMCR.

**DISCUSSION**

Here we present evidence that the in vitro T-lymphocyte responsiveness of HSV-1-seropositive atopicies to herpes antigens is intact. However, compared with the healthy control group, patients with AD have defects in ETAF production and AMCR.

Cell-mediated immunity to HSV was measured by T-lymphocyte proliferation. In this system we used epidermal LC as antigen-presenting cells. We and others have previously reported that with several antigens LC are more effective accessory cells than are monocytes [11-13]. All seropositive subjects, whether healthy or atopic, responded by blast transformation to HSV. However, there seemed to be no correlation between serum antibody levels to HSV and magnitude of lymphocyte response. We have previously obtained similar results with mumps virus [14], and are inclined to believe that these findings illustrate a real situation and are not due to technical errors or variability.

It is generally believed that cell-mediated immunity is impaired in patients with AD and increased susceptibility of these patients to disseminated cutaneous viral infections is one manifestation of defective immunity [1]. In vitro studies on cell-mediated immunity in patients with AD have given contradictory results. In many reports, antigen-induced or mitogen-induced T-lymphocyte proliferation has been normal, whereas other investigators have obtained diminished responses, especially in severe forms of AD [15]. It seems plausible that when lymphocytes are activated with strong antigenic or mitogenic stimulants, suble changes in their responsiveness cannot be detected. We believe that the
AMCR used in the present study is suitable for detecting even slight defects in cellular interactions.

In AMCR T lymphocytes are activated by their own DR-positive cells. Dendritic cells have been found to be the most potent autologous activators [16]. Autologous mixed cell reaction has been observed to be impaired in autoimmune, immunoproliferative, and atopic diseases [10,17-19]. We are not aware of earlier reports in which strongly DR-positive LC were used as stimulator cells in AMCR in atopic or other diseases. In any case, our results resemble those obtained by others with non-T cells as autologous stimulators [17-19]. In our hands, defective AMCR could be restored by exogenous IL-1 and IL-2. Impaired AMCR may thus reflect diminished production or response of both interleukins. According to newer concepts, AMCR represents an immune circuit that leads to the activation of helper, cytotoxic, and suppressor T lymphocytes [20]. Defective AMCR may be linked with diminished suppressor and cytotoxic cell functions observed earlier in AD [21-24]. T lymphocytes regulate IgE synthesis, and overproduction of IgE in atopic diseases is believed to be due, at least in part, to deficient suppressor T-cell activity [25]. Furthermore, it has been speculated whether defective cytotoxic T-cell function accounts for the increased susceptibility of patients with AD to viral infections [21].

Some of our patients had had numerous local HSV infection episodes in addition to one or two disseminated ones in spite of the fact that they developed seemingly good humoral and cell-mediated responses to this agent. Herpes infections tend to recur even in healthy individuals, but the infection susceptibility of patients with AD is clearly greater. The diminished capacity of these patients to produce biologically active mediators such as EATA, and their defective autoregulation and generation of effector cells may contribute to impaired defense.

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