Reduced production of histamine-induced suppressor factor (HSF) by atopic mononuclear cells and decreased prostaglandin E₂ output by HSF-stimulated atopic monocytes

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To characterize the defect responsible for abnormal histamine-induced suppressor cell function observed in atopic subjects, we studied histamine-induced suppressor factor (HSF) production and augmented prostaglandin E₂ production. In addition, we exogenously provided interleukin 1 to determine whether abnormal histamine-induced suppressor cell function could be corrected by this monokine. Mononuclear cells from 16 asymptomatic atopic subjects generated significantly less (p < 0.01) histamine-induced suppressor activity than cells from 10 nonatopic controls, and mean suppressor-factor production was also significantly reduced (p < 0.01). The latter two responses were not corrected by interleukin 1. Atopic monocytes produced significantly less (p < 0.01) prostaglandin E₂ in response to suppressor factor than did control monocytes. The atopic group had phenotypically normal numbers of T-helper cells, T-suppressor cells, and ratios of these cells. These data indicate that despite having phenotypically adequate numbers, T-suppressor cells from atopics produce fewer suppressor signals than cells from nonatopics and their monocytes produce less prostaglandin E₂ even if the suppressor signals are provided. (J ALLERGY CLIN IMMUNOL 72:359-364. 1983.)

Histamine-induced suppressor T cell function is mediated in part through the production of a suppressor lymphokine termed HSF. The latter is produced by stimulating a subpopulation of H₂ receptor-bearing T lymphocytes with histamine.¹ HSF in turn augments the production of prostaglandins by monocytes.² These latter compounds are thought to be intimately involved in the effector stage of this reaction and may directly suppress lymphocyte proliferation or lymphokine production or further activate suppressor T cells.³ In addition, indomethacin, a cyclo-oxygenase pathway inhibitor, can reverse histamine-induced suppression.⁴ ⁵ In addition to its role in the effector stage, the monocyte is also known to be required in the activation of histamine-induced suppressor cells.⁶ ⁷ A monokine, IL-1, has been shown to augment lymphocyte activation in response to histamine.⁸

Atopic subjects have recently been documented to have reduced histamine-induced (but not Con A-induced) suppressor T cell function and to have decreased phenotypic expression of T cell H₂ receptors but not of T cell H₁ receptors, when compared to nonatopic controls.⁹ Since proper functioning of the above suppressor system involves obligatory interactions between lymphocytes and monocytes,¹⁰ it is possible that a defect in lymphocyte and/or monocyte function or a defect in the response to, or production of, monokines, such as IL-1 and prostaglandins, could explain the abnormal histamine-suppressor response seen in atopic subjects. In the present study, we have analyzed the in vitro function of histamine-activated mononuclear cells from atopic patients with

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regard to their production of the suppressor lymphokine (HSF) and a monocyte function, namely HSF-augmented production of PGE₂. In addition, we have exogenously provided IL-1 to in vitro mononuclear cell cultures to determine whether the abnormal co-culture histamine-induced suppressor cell response in atopics could be restored to normal and whether HSF production could also be restored if abnormal.

**METHODS**

**Subjects**

We studied 16 atopic patients in whom a history of seasonal allergic rhinoconjunctivitis and/or asthma was obtained and who were found to have at least two positive tests of immediate-type skin reactivity that correlated with their symptoms. None had received immunotherapy within the previous 5 yr, none had been receiving bronchodilators, antihistamines, or corticosteroids for several weeks at the time of the study, and they were studied 1 to 2 mo after the season and were asymptomatic. There were 10 age- and sex-matched nonatopic control subjects. Informed consent was obtained from all the subjects, and the study was in accordance with the standards of the Committee on Human Experimentation at New England Medical Center Hospital.

**Co-Culture suppressor cell assay**

The methods for histamine and Con A activation of suppressor cells with the use of a co-culture technique have been previously reported. PBMC were isolated by density centrifugation and cultured at a concentration of 6 × 10⁵ cells/ml in medium TC-199 (Gibco, Grand Island, N. Y.), supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), and HEPES buffer (25 mM). This solution (TC-199 HPS) contained 15% normal human AB serum in the absence (control) or presence (suppressor) of 10⁻⁴M histamine (Sigma Chemical Co., St. Louis, Mo.) or Con A (20 µg/ml) and was incubated for 48 hr at 37°C in a 5% carbon dioxide-air atmosphere. The control and suppressor cells were then treated with mitomycin (50 µg/ml) for 1 hr at 37°C, washed three times in TC-199 HPS, and combined with 1 × 10⁶ autologous indicator cells. The final mixture of suppressor and indicator cells was suspended in 1 ml of TC-199 HPS containing 15% AB serum in a final ratio of 1:2 (suppressor:indicator). In some experiments, T cells (more than 98% purity) were purified by passage of PBMC over nylon wool (3 gm) columns as previously described.

At the time of co-culture, the indicator cells were stimulated with Con A (10 µg/ml), and the cell mixture was placed in microtiter plates (2 × 10⁶ cells/well) and incubated for 72 hr. Eighteen hours before culturing was completed, [³H]thymidine (sp. act. 6.7 Ci/mmol; New England Nuclear, Boston, Mass.) was added (1 µCi/well). The incorporation of [³H]thymidine into cellular DNA was determined by harvesting the cultures on glass wool filter paper with a MASH II harvester (M.A. Bioproducts, Walkersville, Md.), and the radioactivity was determined by liquid scintillation counting.

The percent suppression of [³H]thymidine uptake was calculated according to the following formula:

\[
\% \text{ suppression} = 1 - \frac{\text{cpm in presence of histamine or Con A}}{\text{cpm in absence of histamine or Con A}} \times 100
\]

The mean number of cpm ± S.E. was determined from quadruplicate wells. The variability of the replicates was less than 10%.

**HSF production and assay**

The method for the production and assay of HSF factor has been published previously. PBMC were isolated as above, and the cells were washed in TC-199 HPS and resuspended in the same medium containing 15% normal human AB serum to a concentration of 2 × 10⁶ cells/ml. The cells were incubated for 24 hr at 37°C in a 5% CO₂-air atmosphere in the absence (control) or presence (HSF) of 10⁻⁴M histamine. Cell-free supernatants were harvested and dialyzed to remove histamine, sterilized by Millipore filtration (Millipore Corp., Bedford, Mass.), and used as culture medium to resuspend fresh mononuclear cells for the second stage of the assay. For the purposes of the experiments to be described below (PGE₂ generation), control and HSF supernatants were generated in serum-free TC-199 in the presence of 1 µg/ml indomethacin.

Fresh PBMC from normal subjects were resuspended in the presence of control or HSF supernatants. The cell concentration was adjusted to 1 × 10⁶ cells/ml (containing 15% human AB serum) and stimulated with Con A (10 µg/ml) for 72 hr. The cells were placed in microtiter plates, with 2 × 10⁵ cells in 200 µl/well, in quadruplicate. The plates were incubated at 37°C in a 5% CO₂-air atmosphere for 3 days. [³H]thymidine was added as above, and the incorporation of [³H]thymidine into cellular DNA was measured by liquid scintillation counting. The percent suppression was determined by the following formula:

\[
\% \text{ suppression} = 1 - \frac{\text{cpm in HSF}}{\text{cpm in control}} \times 100
\]

**HSF-augmented PGE₂ production by normal and atopic monocytes**

Monocytes (5 × 10⁶ cells/ml) from atopics and controls were isolated by density centrifugation in Percoll (Pharmacia, Uppsala, Sweden) gradients as recently described. Briefly, 120 × 10⁶ mononuclear cells/2 ml were carefully layered onto a discontinuous gradient (40%, 52%, 60%, 70%, 80% Percoll).
Reduced production of histamine-induced suppressor factor

Atopic MNC

Non-atopic T cells

FIG. 1. Histamine-induced suppression by atopic mononuclear or T cells cultured in the absence or presence of IL-1 employing the co-culture technique. The suppressor-cell response by atopic cells was significantly reduced (p < 0.01) compared to cells from control subjects. The addition of IL-1 did not substantially increase the suppressor activity.

70%) of Percoll contained in glass 50 ml conical centrifuge tubes. The cell suspension was centrifuged for 10 min at 500 x g at room temperature. Cells at the 52% Percoll-interphase were removed by Pasteur pipette and washed three times in TC-199. More than 95% of the cells from this layer were positive for nonspecific esterase and ingested latex particles. These cells were then resuspended in control or HSF supernatants derived from one common pool (originally generated in serum-free TC-199 in the presence of indomethacin 1 μg/ml and dialyzed before use) and incubated for 24 hr at 37°C with a 5% CO₂-air atmosphere. The cell-free supernatants from these cultures were harvested and assayed for their content of PGE₂ by using a radioimmunoassay (²¹H Prostaglandin E radioimmunoassay kit; Clinical Assays, Cambridge, Mass.). The radioimmunoassay measures the conversion of PGE to PGB by means of alkaline treatment of the supernatants. Since serum-free conditions were maintained throughout, protein extraction of the supernatant before assay was not found to be necessary. The amount of PGE₂ present in the supernatants was calculated from a standard curve and expressed in picograms per 5 x 10⁶ cells. Net PGE₂ production was calculated by subtracting the levels of PGE₂ found in the control supernatants from that contained in the HSF supernatants.

Addition of IL-1 during the generation stage of the co-culture and HSF production

Highly purified⁷ IL-1 was kindly provided by Dr. Charles Dinarello (Tufts University School of Medicine). Optimum amounts (1:1 dilution with TC-199) of IL-1 were added to either unfractionated mononuclear cells or nylon wool-purified T cells during the generation stage (histamine stimulation part of the co-culture technique). In addition, IL-1 was added to mononuclear cells that were stimulated with histamine for the purpose of generating HSF.

Determination of T cell subsets

T cell subset determinations were performed in the laboratory of Dr. Richard Rudders, Hematology-Oncology Division, New England Medical Center Hospital. Murine monoclonal antibodies designated Leu 1, Leu 2a, Leu 3a, Leu 4, and HLA-Dr (kindly provided by Becton, Dickinson & Co., Sunnyvale, Calif.) were used in this study. Functionally, Leu 1 and Leu 4 define the majority of peripheral T cells, Leu 2a defines the suppressor/cytotoxic T cell subset, and Leu 3a defines the helper/inducer T cell subset. HLA-Dr is found on B cells, monocytes, macrophages, and some activated T cells.

With the use of a direct immunofluorescent staining method, after labeling PBMC (more than 90% depleted of monocytes by adherence to glass Petri dishes) with these monoclonal antibodies, the percentage of each subset was determined by passing the cells through a fluorescent activated cell sorter (FACS Analyzer; Becton Dickinson). Each cell subset was reported as a percent of total PBMC.

Statistical analysis

Student's t test was used to compare mean data from each group, and p values below 0.05 were considered statistically significant.

RESULTS

Co-culture suppressor cell assay

PBMC from atopic patients and controls were preactivated with histamine or Con A, and their ability to suppress lectin-induced lymphocyte proliferation was measured (Fig. 1). Although cells from some atopics had normal amounts of inhibitory activity, the mean (± S.E.M.) percent of suppression by histamine–stimulated atopic mononuclear cells (19.9% ± 4.6) was significantly reduced (p < 0.01) compared to the mean suppression exerted by mononuclear cells from normal subjects (43.2% ± 3.1). The addition of
IL-1 to either PBMC or nylon wool-purified T cells did not restore the histamine-suppressor response of atopics to the normal range.

The mean suppressor activity of mononuclear cells from atopic patients (30.4% ± 3.3) that was generated by Con A was similar to that of normal controls (32.8% ± 5.2) (data not shown).

HSF production

PBMC from atopic patients and controls were stimulated in the absence or presence of 10^{-4}M histamine, and the supernatants were tested for their inhibitory activity on lectin-induced proliferation of indicator cells (PBMC) from normal subjects. As shown in Fig. 2, although there was some overlap between individuals in both groups, the mean (±S.E.M.) suppression induced by HSF supernatants from atopic PBMC (4.9% ± 1.6) was significantly reduced (p < 0.01) compared to that from normal PBMC (27.3% ± 3.1). The exogenous addition of IL-1 to the atopic cultures did not significantly increase HSF activity to control levels.

Monocyte PGE₂ production

Monocytes from atopic patients and control subjects were incubated with control and HSF-containing supernatants (generated in the presence of indomethacin) for 24 hr and then were assayed for their content of PGE₂. The results, expressing the net PGE₂ production (pg/5 × 10^6 monocytes), are summarized in Fig. 3. Monocytes from atopic patients synthesized a net of 47 ± 28.5 pg/5 × 10^6 cells compared to 567 ± 94.4 from monocytes of control subjects. The difference between these two means was statistically significant (p < 0.01). It should be noted that differences in the baseline levels of PGE₂ synthesized by monocytes from atopic patients and control subjects did not account for the reduction observed in the atopic group (data not shown).

Enumeration of T cell subsets

As shown in Fig. 4, the atopic and control groups did not have a statistically significant difference in their T cell subset percentages (expressed as a percent of total PBMC). In addition, the ratio of T-helper and T-suppressor cells was similar in both groups.

DISCUSSION

These results have confirmed our earlier finding that atopic patients have reduced suppressor cell function in response to histamine but not to Con A as measured by a co-culture assay. In order to investigate a potential lymphocyte and/or monocyte defect that could account for the abnormal histamine-induced suppressor cell function observed in atopics, we measured HSF production by atopic lymphocytes and examined whether the addition of IL-1 was able to correct this response. Further, we measured the production of PGE₂ by monocytes from atopics in response to a suppressor signal (HSF).

The abnormal response of atopic suppressor cells to histamine does not appear to represent an absence of
IL-1 production by their monocytes, since the exogenous addition of IL-1 to unfractionated mononuclear cells did not restore their activity to normal levels in the co-culture assays. Also, under conditions in which activation of the suppressor cells becomes IL-1 dependent, that is, when monocytes are removed by passing PBMC over nylon wool columns, T cells from atopics patients still did not respond to histamine plus IL-1. However, our studies do not rule out the possibility that these lymphocytes fail to respond to IL-1 in an appropriate fashion. The latter possibility seems unlikely, since atopics do have normal Con A suppressor responses.

HSF production by atopic mononuclear cells was significantly reduced compared to that for control subjects (Fig. 2). These results suggest that there is inadequate stimulation of atopic lymphocytes by histamine. It has recently been shown that atopics have a decreased phenotypic expression of T cell H<sub>2</sub> receptors, and therefore the latter could account for the decreased production of HSF, since HSF is derived from H<sub>2</sub> receptor-bearing T cells. An intrinsic defect in the cells, more rapid degradation of the suppressor factor, production of a functionally defective HSF molecule, or inhibition of its activity by other as yet unknown factors are other mechanisms that might also account for this defect. Such possibilities are currently under study.

If reduced HSF production alone was responsible for the abnormal histamine suppressor response by atopics, then the exogenous addition of this suppressor signal might be expected to stimulate atopic monocytes to increase their production of prostaglandins. However, we have shown that even when the suppressor signal (HSF) is provided exogenously, atopic monocytes fail to respond normally, producing approximately 10-fold less PGE<sub>2</sub> than monocytes from nonatopic subjects (Fig. 3). The explanation for the decreased PGE<sub>2</sub> production by atopic monocytes is unknown, but the decrease could be caused by reduced amounts of available substrate, a basic difference in the way atopic monocytes metabolize arachidonic acid, or alternatively a heretofore unrecognized hereditary or acquired enzymatic deficiency(ies) located in the arachidonic acid metabolic pathways.

Exogenous prostaglandins have been shown to be potent inhibitors of several lymphocyte functions, including proliferation, antibody production, lymphokine production, and cytolysis. Since macrophages and monocytes appear to be the major source of prostaglandins produced by PBMC during immune responses, it is reasonable to predict that prostaglandins derived from these cells may act as physiologic inhibitors of various lymphocyte functions. The relationship of prostaglandins and their importance in regulating IgE production in humans remain to be clarified.

The majority of patients with active atopic dermatitis have been observed to have a selective deficiency of suppressor/cytotoxic cells in their peripheral blood when compared to nonatopic controls, patients with other skin diseases, or patients with respiratory allergy. This suggests that deficient T-suppressor cell function may play a role in the pathogenesis of this disease. None of our subjects had active atopic dermatitis, and we did not observe differences in T cell subset percentages in our atopic group when compared to the control group. This finding emphasizes the importance of performing functional studies in addition to phenotyping T cell subsets, particularly since, in our case, the histamine-responsive suppressor cell has been shown to be contained in the OKT8<sup>+</sup> subpopulation. Therefore the abnormal histamine-induced suppressor cell response does not appear to be attributable to a quantitative deficiency in the T-suppressor cell population.

In summary, lymphocytes from atopic patients fail to produce normal amounts of a suppressor factor lymphokine (HSF) when stimulated by histamine. This response cannot be corrected by the exogenous addition of IL-1. If HSF is exogenously provided to cultures of atopic monocytes, their production of PGE<sub>2</sub> is reduced as compared to the amount produced from monocytes of normal subjects. These data suggest that there may be an underlying defect in the immunoregulatory system of the mononuclear cells of atopic patients.

REFERENCES


