

Cytokine-induced histamine release from basophils of AIDS patients

Interaction between cytokines and specific IgE antibodies

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Basophil leukocytes obtained from AIDS patients, allergic patients and healthy controls were stimulated *in vitro* with interleukin 4, lymphotoxin, tumour necrosis factor alpha and interferon gamma to examine the histamine releasing effect. The cytokines caused histamine release from the basophils of approximately half the AIDS patients and from 8-17 % of the allergic patients. No response was obtained in the control group. Removal of cell surface immunoglobulins abolished the response to cytokines, indicating an Ig-dependent mechanism. Passive sensitization with cell-derived Ig, with Ig deprived of IgE, or with IgG, indicated that cell-bound IgE was responsible for the cytokine-induced histamine release in AIDS patients. This response may be mediated by cytokine-selective IgE antibodies.

Key words. AIDS; basophils; histamine liberation; IgE; interferon gamma; interleukin 4; lymphotoxin; tumour necrosis factor.

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Human immunodeficiency virus (HIV) causes pronounced cellular immunodeficiency along with other abnormal immunoinflammatory reactions (5). In contrast to cells from healthy individuals, basophils from some AIDS patients release histamine after stimulation with interleukin 4 (IL-4), lymphotoxin (LT), tumour necrosis factor alpha (TNF α) and interferon gamma (IFN γ) (10). An equal percentage of AIDS patients and healthy controls respond to stimulation with IL-1 α , IL-1 β , IL-3, colony stimulating factor (CSF) and granulocyte-macrophage-CSF (10). Cells from most AIDS patients release histamine after stimulation with opportunistic microorganisms and HIV, and the mechanism was shown to be IgE-mediated (11-13). The purpose of this study was to investigate the mechanisms by which IL-4, LT, TNF α and IFN γ induce histamine release from basophils of AIDS patients.

MATERIAL AND METHODS

Patients

The study comprised 26 patients, all men, fulfilling one of the CDC AIDS criteria IV A, B, C-I or E (3). The median age was 41 years (range 25-64 years). The time from the diagnosis of AIDS until the investigation was median 4 months (range 0-40 months). One of the AIDS patients suffers from allergic bronchial asthma, which he also did before achieving the HIV infection. Four AIDS patients present adverse reactions to drugs. Furthermore, 12 patients (3 women and 9 men with median age 31 years (range 21-43 years)) with allergic diseases but not suffering from AIDS were included in this study. These patients did

not receive systemic glucocorticoids, and basophils from these patients release histamine (13–58%) when challenged with specific allergens. Healthy, non-allergic volunteers (2 women and 9 men median age 37 years (range 24–62 years)), without evidence of HIV infection served as controls. All subjects included in this study were cellular responders, i.e. their leukocytes responded to anti-IgE antibody stimulation with histamine release.

Methods

The *E. coli*-derived human cytokines were tested at the final concentrations specified below and at 5 consecutive 10-fold dilutions. Recombinant (rIL-4) (max. conc. 5×10^3 pg/ml) was from Genzyme Corp. (Boston, MA, USA). Recombinant tumour necrosis factor alpha (rTNF α), recombinant lymphotoxin (rLT) and recombinant interferon gamma (rIFN γ) were kindly donated by Dr. G. R. Adolf (Vienna, Austria) and tested at max. conc. 10^4 , 10^5 and 3×10^5 pg/ml, respectively. Rabbit anti-human IgE antibodies (ϵ -chain 478,000 IU/ml containing approx. 1 mg protein/ml) was obtained from Behringwerke (Marburg, Germany). Calcium-ionophore A23187 was from Calbiochem (San Diego, CA, USA). All stimulants were kept in small aliquots at -20°C .

Preparation of leukocyte suspensions

Mononuclear cells with approx. 2% basophils were isolated after Ficoll-Hypaque density gradient centrifugation of EDTA blood, as previously described (15). The cells were washed twice (160 g and 110 g for 10 min) in buffer containing Tris (hydroxymethyl) amino methane 25 mM, at pH 7.4, 0.12 M NaCl, 5 mM KCl, 0.6 mM CaCl $_2$, 1.1 mM MgCl $_2$, 0.3 mg/ml human serum albumin and 1 mg/ml glucose (Tris-AMC) and finally resuspended in this buffer.

Basophil histamine release

Histamine release from basophils was examined in 90 μl leukocyte suspensions. The samples

were incubated in polystyrene tubes (Nunc, Roskilde, Denmark) for 40 min at 37°C with 10 μl cytokine, anti-IgE antibody or calcium-ionophore A23187. Anti-IgE antibody was used at final concentrations between 10^{-6} and 10^{-2} mg/ml. Calcium-ionophore A23187 was used at final concentrations of 0.03 and 1.3 μM . The addition of stimulator was omitted in some cell samples to determine spontaneous histamine release. The reaction was terminated by the addition of 3 ml ice-cold Tris-AMC. The cell samples were thereafter centrifugated at 2000 g for 10 min. Besides liberated histamine, the supernatants contained proteins and other substances, which might interfere with the histamine analysis. The supernatants were therefore discarded and residual histamine in the cell sediment was determined by the fluorometric method (15). The release was expressed as a percentage of the total histamine content in the samples. The spontaneous histamine release amounted to up to 10%. Hence, only histamine release $\geq 10\%$ was considered significant.

Cell surface immunoglobulin deprivation and cell surface immunoglobulin fractions from AIDS patients

These procedures have been described previously (11, 14). Briefly, leukocytes were isolated from EDTA-blood. Surface immunoglobulin-deprived cells were obtained by removing the cell surface immunoglobulins (sIg) from the intact cells by the acidification procedure (11). The sIg obtained by the acidification procedure of blood obtained from AIDS patients was dissolved in Tris-AMC. IgG or IgE was selectively removed by incubating the sIg with either Sepharose-bound anti-IgG antibodies or anti-IgE antibodies (14). This procedure removed 75% of IgG $_1$, 85% of IgG $_2$, 85% of IgG $_3$ and 95% of the IgE as estimated by ELISA or RIA.

Passive sensitization by sIg from AIDS patients

Leukocytes obtained from one of the healthy controls were used in all experiments. The cells were deprived of sIg by the acidification procedure.

dures. The cell pellets containing the Ig-depleted (stripped) cells were washed and resuspended in Tris-AMC (pH 7.4). These cells were then incubated at 37°C for 1 h with sIg or sIg fractions from AIDS patients containing intact Ig or Ig deprived of IgG or IgE (see above). Finally, the leukocytes were washed and resuspended in Tris-AMC. Basophil histamine release induced by cytokines was examined as described above.

Statistics

The four-fold table test was used with a significant level of 0.05.

RESULTS

Histamine release induced by rIL-4, rLT, rTNF α and rIFN γ

Basophils were challenged with rIL-4, rLT, rTNF α and rIFN γ and the histamine release was examined in cell suspensions obtained from AIDS patients, from allergic patients with frequently occurring allergic diseases, and from healthy controls. Five consecutive 10-fold dilutions of the cytokines were used. Table 1 shows that 44–50% of the cells of AIDS patients responded with significant histamine release ($\geq 10\%$) after challenge with each of the four cytokines. However, only three AIDS patients responded to all four cytokines, and only one showed no response to any of the cytokines.

Table 1.

Histamine release response to cytokines in cell suspensions from AIDS patients, allergic patients and healthy controls

Stimulator	AIDS	Allergy	Controls
rIL-4	13 (26) ⁺ □	1 (12)	0 (11)
rTNF α	11 (25) □	2 (12)	0 (10)
rLT	13 (27) ⁺ □	1 (12)	0 (11)
rIFN γ	12 (26) ⁺ □	1 (12)	0 (10)

Number of persons in whom the cells responded with significant histamine release. Total number of subjects tested is given in parenthesis. Recombinant interleukin 4: rIL-4; recombinant tumour necrosis factor alpha: rTNF α ; recombinant lymphotoxin: rLT; recombinant interferon-gamma: rIFN γ .

⁺ $P < 0.05$ between AIDS patients and allergic patients.

□ $P < 0.05$ between AIDS patients and healthy controls.

Histamine release was observed in the range $0.5\text{--}5 \times 10^3$ pg/ml IL-4, $10\text{--}10^3$ pg/ml rLT, $1\text{--}10^3$ rTNF α pg/ml and $0.3\text{--}3 \times 10^3$ pg/ml rIFN γ . The maximal release of histamine varied between 11–22%, 11–49%, 11–19% and 11–19%, respectively. 8–17% of the allergic patients responded to the cytokines, and the response was similar in magnitude to that of the AIDS patients. Only one allergic patient responded to all four cytokines, whereas 10 patients showed no response to any of the cytokines. There was no difference in the histamine release response to anti-IgE antibody between allergic patients whether or not they responded to cytokines. None of the cell suspensions from the healthy controls showed significant histamine release to cytokine stimulation, while all responded to anti-IgE antibody stimulation. The number of AIDS patients responding to rIL-4, rLT and rIFN γ differed significantly ($P < 0.05$) from that of the allergic patients as well as from that of the controls. The basophils from AIDS patients did not differ from basophils from controls in regard to spontaneous histamine release.

Activation of intact cells and cells deprived of sIg

The activation mechanism of the cells from AIDS patients was examined by parallel cytokine stimulation of intact cells and cells deprived of sIg. Fig. 1 shows that the intact cells responded to the cytokines with histamine release, whereas cells deprived of sIg did not. These experiments were performed with each cytokine using cells from at least three AIDS patients. Removal of sIgE by the acidification procedure was ascertained by their inability to respond to anti-IgE antibodies (data not shown). The basic cellular function in intact and in Ig-depleted cells was comparable, judged by a similar histamine release after stimulation with calcium ionophore A23187 (data not shown).

Passive sensitization of cells from a control person with sIg from AIDS patients

Sig from three AIDS patients with significant histamine release due to stimulation with 1:

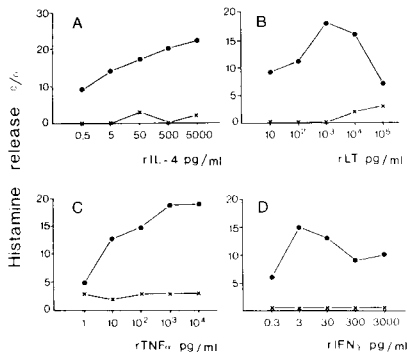


Fig. 1. Histamine release from blood leukocytes from an AIDS patient induced by cytokine stimulation. A: rIL-4; B: rLT; C: rTNFα; D: rIFNγ. Intact cells (●-●), cells deprived of Ig (x-x). Mean of duplicate determination is given. $n = 3$ for each cytokine.

rIL-4 and rTNFα, 2: rIL-4 and rLT and 3: rIL-4, was used for passive sensitization of cells from a healthy control. Fig. 2A shows the first experiment using sIg from the patients who responded to rIL-4 and rTNFα. The sensitized control cells responded to the cytokines with

histamine release, whereas no response was obtained from the intact or the sIg-deprived cells. The figure also shows that only rIL-4 and rTNFα, but not rLT or rIFNγ, caused histamine release, indicating a selective response to the cytokines. In the second experiment, where

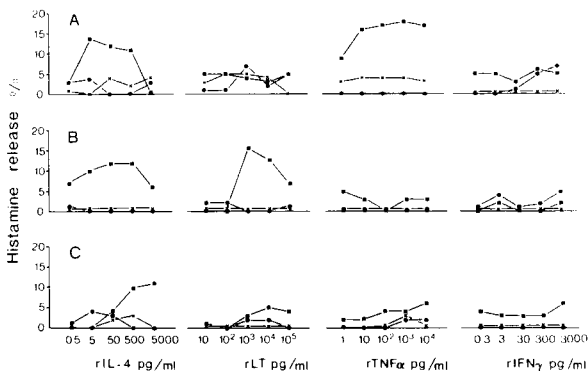


Fig. 2. Histamine release from blood leukocytes from a control person induced by cytokine stimulation. Intact cells (●-●), cells deprived of own Ig (x-x) and with addition of cell surface immunoglobulins (■-■) from AIDS patients whose cells responded to rIL-4 and rTNFα (A), rIL-4 and rLT (B) and rIL-4 (C). Mean of duplicate determination is given.

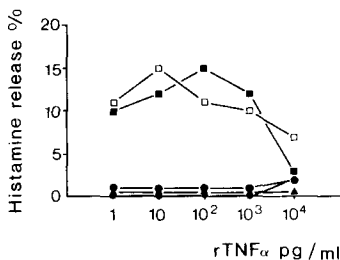


Fig. 3. Histamine release from blood leukocytes from a control person induced by rTNF α . Intact cells (●-●), cells deprived of own Ig (x-x) and with addition of cell surface immunoglobulins from an AIDS patient, i.e. intact Ig (■-■), Ig deprived of IgG (□-□) or IgE (▲-▲). Mean of duplicate determination is given.

the patient responded to rIL-4 and rLT, only these cytokines induced histamine release in the passively sensitized cells (Fig. 2B), and this was also the case in the third experiment, where release was obtained only with rIL-4 (Fig. 2C).

Determination of the immunoglobulin class involved in basophil histamine release by cytokines

The sIg responsible for the histamine release induced by rTNF α and rIFN γ was examined by passive sensitization of cells from a control person. sIg was obtained from two AIDS patients whose cells responded to rTNF α , and from one AIDS patient whose cells responded to rIFN γ . In addition to intact sIg, sIg deprived of IgG or IgE were used for passive sensitization. Fig. 3 shows the results of one of these experiments; analogous results were obtained in the other two experiments. Intact and Ig-depleted cells from the control person showed no release of histamine after challenge with rTNF α . However, histamine release was obtained after passive sensitization of the Ig-depleted cells with intact sIg from the AIDS patient. When IgG was removed from the sIg, histamine release was still obtained, but there was no release of histamine when the IgE molecules were removed.

DISCUSSION

In a previous study we found that basophil leukocytes from AIDS patients responded to the cytokines rIL-4, rLT, rTNF α and rIFN γ with histamine release, whereas no release was obtained from cells of healthy controls (10). In this study the number of AIDS patients was increased and cells from approximately 50% of these patients were found to respond to cytokines with histamine release. These responses did not correlate with any specific medication, including systemic glucocorticoids, of three of the patients. None of the patients receive cytokine therapy. Differences in histamine release between cells from healthy individuals and allergic patients in response to histamine releasing-factor have been claimed by some investigators (7, 8). A group of 12 allergic patients was therefore included in this study, but only one, or in the case of rTNF α two patients responded to the cytokines with histamine release. These responses were not statistically different from the responses of cells from healthy controls. Our results are in accordance with previous investigations (1, 4, 17).

In AIDS patients, sIg appears to be involved in the histamine response to cytokines, because the response was abrogated when using Ig-depleted cells. Further evidence of this mechanism was obtained by the finding that transfer of sIg from AIDS patients to cells from a normal individual rendered these cells responsive to the cytokines. Complete accordance was obtained between the response of the cells from AIDS patients and the capacity of their sIg to sensitize control cells to the individual cytokine. If, for example, the patient's cells responded to rIL-4 but not to other cytokines, passive sensitization of control cells with sIg from the patient's cells rendered the control cells responsive only to rIL-4.

The capability of different sIg fractions from AIDS patients to sensitize cells from a healthy control was next investigated. Transfer of intact sIg, or sIg deprived of IgG, to cells of the control rendered the cells responsive to cytokines. In contrast, none of the cells responded to the cytokines after the transfer of sIg deprived of IgE.

The results of the study indicate that cells from AIDS patients, in contrast to cells from allergic patients and healthy individuals, frequently release histamine after exposure to rIL-4, rIL-1, rTNF α or rIFN γ . The passive sensitization with intact sIg and sIg deprived of IgG or IgE indicates that these reactions are mediated by cell surface IgE antibodies reacting with these human cytokines. Previous studies have demonstrated antibodies against various cytokines of the IgG and IgM class (2, 6, 9, 16). These autoantibodies may be part of a polyclonal B-cell response against cytokines.

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