

HIV antigen-induced release of histamine from basophils from HIV infected patients

Mechanism and relation to disease progression and immunodeficiency

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Basophil leukocytes from 39 HIV-infected patients with various degrees of immunodeficiency and disease progression were stimulated with an HIV antigen preparation. Cells from 19 of 22 patients with AIDS and all of six patients with milder degrees of HIV-related disease showed significant histamine release. In contrast, cells from 11 asymptomatic HIV-infected patients and 11 healthy control persons released no histamine. The histamine release induced by HIV antigen was found to be inversely correlated to the number of CD4 positive T lymphocytes. These results indicate that the histamine release was related to both the clinical stage of disease and the degree of immunodeficiency. Passive sensitization experiments showed that IgE, but not IgG, was responsible for the induction of histamine release, indicating the reaction to be type 1 allergic. The histamine release caused by HIV might be involved in the development of disease because of the immunomodulating properties of this mediator.

Key words: AIDS; basophils; histamine liberation; IgE; HIV.

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Basophil leukocytes from patients with the acquired immunodeficiency syndrome (AIDS) often show release of histamine *in vitro* when stimulated with microorganisms causing opportunistic infections (10, 11). In general, antigen-induced activation of basophil leukocytes and mast cells, resulting in release of mediators such as histamine, is known to be mediated by IgE (5) and probably also by IgG₄ (2, 14). The histamine release induced by *Candida albicans* and *Herpes simplex* virus type I from basophils from AIDS patients is mediated by surface IgE molecules (12). The causative agent for AIDS, the human immunodeficiency virus (HIV), has been found to be an effective inducer of histamine release from basophil leukocytes from AIDS patients, but not from cells from healthy individuals (13). The aim of this study was to

examine the HIV-induced histamine release from basophil leukocytes obtained from HIV-infected patients with various degrees of immunological dysfunction. In addition, the mechanism of HIV-induced activation of the basophils was examined.

MATERIAL AND METHODS

Patients

The investigation comprised 22 patients median age 39 years (range 25-58) with AIDS according to the criteria of the Centers for Disease Control (CDC) (1, 15). Seventeen patients fulfilled the AIDS criteria by presenting opportunistic infection and were classified in Group IV

C-1, three patients having Kaposi's sarcoma were classified in Group IV D and two patients fulfilled the criteria for both Group IV C-1 and IV D. Further, six patients with median age 45 years (range 27-72) classified in CDC Group IV C-2 and 11 patients with asymptomatic HIV infection (CDC Group II) or persistent generalized lymphadenopathy (CDC Group III) median age 38 years (range 22-57), were included in this study. Thirty-seven of the 39 HIV-infected patients were homo- or bisexual. One AIDS patient was heterosexual and was infected in Africa and one AIDS patient received HIV infection by blood transfusion. All the patients had IgG antibodies against HIV demonstrated by ELISA and Western blot analysis. Three healthy women and eight men with median age 34 years (range 28-58), from the laboratory staff and not belonging to known risk-groups were used as a control group.

HIV antigen preparation

HIV (CDC, strain 451) was propagated in a continuously infected HUT-78 derived clone (CDC, clone 6D5) (4). Virus was concentrated from culture supernatant by PEG-precipitation and purified by double density gradient ultracentrifugation in Nycodenz (Nyegaard, Oslo). The antigenic peak from the second gradient was pelleted through 20% sucrose and resuspended in buffer (100 mM NaCl, 10 mM Tris, 1 mM EDTA pH 8.0 and 1% Triton X-100). The antigen was finally incubated for 1 h at 56°C, sonicated and sterile filtered. The protein concentration was 0.5 mg/ml in the Biorad protein assay. This antigen is in the following referred to as HIV antigen. Crude cellular HIV antigen was prepared from the continuously HIV-infected 6D5 cells. The cells were washed in PBS, resuspended in PBS with 1% Triton X-100, heat-inactivated at 56°C for 1 h and sonicated. The antigen preparation was centrifuged at 100,000 g for 1 h and the supernatant was used as antigen. A similar crude antigen preparation was prepared from uninfected 6D5 cells and served as control antigen. All preparations were stored at -80°C.

Rabbit antihuman IgE (ϵ -chain 400,000 IU/ml containing approx. 1 mg protein/ml) was obtained from Behringwerke (Marburg, Germany). Calcium-ionophore A23187 was from Calbiochem (San Diego, USA). All stimulators were kept in small aliquots at -20°C.

Preparation of leukocyte suspension

Mononuclear cells with approx. 2% basophils were isolated after Ficoll-Hypaque density gradient centrifugation of EDTA blood, as previously described (17). 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 suspension. The samples were incubated in plastic tubes for 40 min at 37°C with 10 μl HIV antigen or other stimulators. HIV antigen was used in final dilutions of 10^4 to 10^{18} of the original preparations. Anti-IgE was used in final concentrations of 10^{-6} - 10^{-3} mg/ml. Calcium-ionophore was used in final concentrations of 0.08 to 1.3 μM . Addition of stimulator was omitted in some of the cell samples. The reaction was terminated by addition of 3 ml ice-cold Tris-AMC. The cell samples were thereafter centrifuged at 2000 g for 10 min. Besides liberated histamine, the supernatant contained proteins and other substances which might interfere with the histamine analysis. The supernatants were therefore discarded and residual histamine in the cell sediments of stimulated and unstimulated cell samples were determined by the fluorometric method (17). The histamine release was expressed as a percentage of the total histamine content of unstimulated samples. The spontaneous histamine release amounted to up to 10%, hence only

histamine release $\geq 10\%$ was considered significant.

Preparation of cell surface immunoglobulin fractions from AIDS patients.

This procedure has previously been described in detail (12). Briefly, leukocytes were isolated from EDTA-blood obtained from three AIDS patients. Immunoglobulins (Ig) were dissociated and removed from the cells by the acidification procedure (10) and the Ig was dissolved in Tris-AMC. IgG or IgE was removed from the Ig by incubating the Ig with particle-bound anti-IgG or anti-IgE (12). After centrifugation these supernatants represent Ig deprived of IgG or IgE. 75 % of IgG₁, 85 % of IgG₃, 85 % of IgG₄ and 95 % of the IgE were removed as estimated by ELISA and RIA, respectively. The removal of Ig was selective, since no IgE was removed by the anti-IgG Sepharose gel and vice versa.

Passive sensitization by cell surface immunoglobulins from AIDS patients

Leukocytes obtained from control persons were used in all experiments. The cells were deprived of Ig by the acidification procedure, as previously described (10). The cell pellet containing the Ig-deprived (stripped) cells was washed and resuspended in Tris-AMC (pH 7.4). The stripped cells were incubated at 37°C for 1 h with cell surface immunoglobulin fractions from AIDS patients containing intact Ig or Ig deprived of IgG or IgE (see above). Thereafter the leukocytes were washed and resuspended in Tris-AMC (pH 7.4). Basophil histamine release induced by HIV antigen was examined as described above. Further, samples were challenged with calcium-ionophore A23187 in final concentrations of 0.08 to 1.3 μ M.

Statistics

The rank sum test for unpaired samples and Spearman's rank correlation coefficient was used. $P < 0.05$ was considered significant.

RESULTS

HIV antigen-induced histamine release from basophil leukocytes

Mononuclear cells with basophil leukocytes obtained from HIV-infected patients and healthy control persons were incubated with HIV antigen and the histamine release was determined. To ensure a relevant response all persons were in advance verified as cellular responders by means of significant histamine release due to stimulation with anti-IgE since antigen-induced histamine release can only be expected in this category. The HIV antigen was used in final dilutions of 10^4 to 10^{18} of the original preparation. Fig.1 shows that among 22 patients with AIDS, basophils from 19 pa-

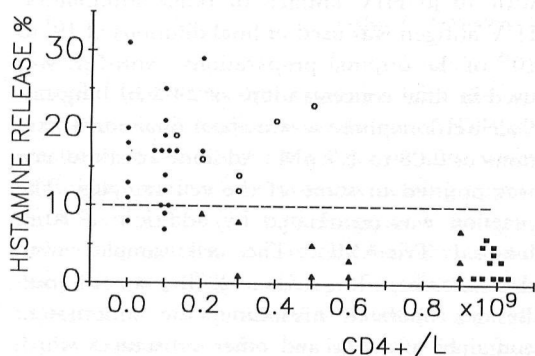


Fig. 1. HIV antigen-induced histamine release in leukocyte suspensions from HIV-infected patients in relation to their number of CD4⁺ cells. HIV-infected patients representing AIDS CDC Group IV C-1 and/or IV D (●), HIV-infected patients from CDC Group IV C-2 (○), HIV-infected patients from CDC Group II or III (▲), and healthy control persons (■). Maximal histamine release is given independently of the antigen dilution. Mean of duplicate determination is given. CD4⁺ cells were not determined for the healthy controls.

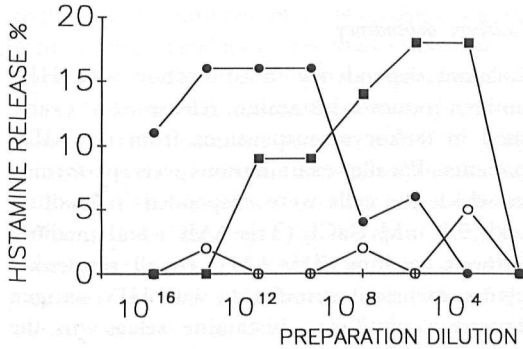


Fig. 2. Basophil histamine release, in leukocyte suspension from an AIDS patient, triggered by HIV antigen (●-●), crude HIV antigen (■-■) and the latter preparation not infected with HIV (control) (○-○). Mean of duplicate determination is given.

tients (86 %) show significant histamine release (median 17 %, range 7–31 %) when challenged with HIV. Cells from all six patients with HIV-related symptoms (CDC Group IV C-2) but not fulfilling the AIDS criteria also released a significant amount of histamine (median 16 %, range 12–23 %). There was no significant difference between the histamine release in these two groups. None of the leukocyte suspensions from 11 HIV-infected patients, representing the CDC Groups II or III, showed significant histamine release due to stimulation with HIV antigen and this was also the case with the 11 healthy controls. The spontaneous histamine release from basophils obtained from AIDS patients and from basophils from healthy controls was indistinguishable. The degree of immunodeficiency estimated by the number of CD4⁺ cells was found to be positively correlated to HIV-induced histamine release since decreased cell number coincided with an increase in histamine release (Fig. 1) (Spearman's rank correlation coefficient $r_s = -0.47$, $P < 0.01$).

To elucidate the specificity of the response to HIV antigen, six AIDS patients showing histamine release to HIV antigen were further examined. The cells were stimulated in parallel with HIV antigen, crude HIV antigen and a similar crude antigen prepared from uninfected cells (control). No significant histamine re-

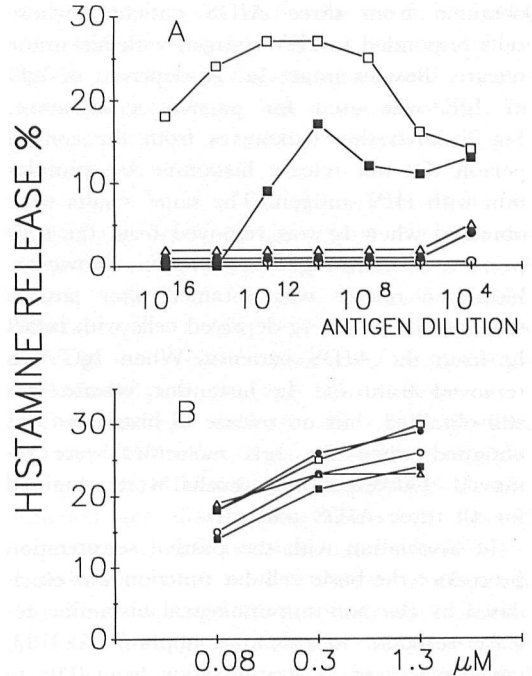


Fig. 3. Histamine release induced by HIV antigen (A) and calcium ionophore A23187 (B) in leukocyte suspensions from a control. Intact cells (●-●), cells deprived of own Ig (○-○) and with addition of cell surface immunoglobulins from an AIDS patient, i.e. intact Ig (■-■), Ig deprived of IgG (□-□) or IgE (△-△). Mean of duplicate determination is given.

lease, i.e. $< 10\%$ was obtained with the control antigen, whereas the purified and the crude HIV antigen preparations both caused a significant release of histamine. Fig. 2 shows that the maximal release of histamine was similar for the HIV antigen and the crude HIV antigen, but as expected a shift to the left of the dose-response curve was obtained by the purified and more concentrated HIV antigen preparation.

Determination of immunoglobulins involved in basophil histamine release by HIV antigen

The immunoglobulins responsible for the histamine release induced by HIV antigen were examined by passive sensitization of cells from the control group. Cell surface Ig was

obtained from three AIDS patients, whose cells responded to HIV antigen with histamine release. Besides intact Ig, Ig deprived of IgG or IgE was used for passive sensitization. Fig. 3 shows that leukocytes from the control person did not release histamine by stimulation with HIV antigen. The same results were obtained when Ig was removed from the cells prior to HIV antigen stimulation. However, histamine release was obtained after passive sensitization of the Ig-deprived cells with intact Ig from the AIDS patients. When IgG was removed from the Ig histamine release was still obtained, but no release of histamine was obtained when the IgE molecules were removed. Corresponding results were obtained for all three AIDS patients.

In association with the passive sensitization procedure the basic cellular function was elucidated by the non-immunological histamine release response to calcium-ionophore A23187, which was used in concentration from 0.08 to 1.3 μ M. A similar release of histamine was obtained in intact leukocytes and in cells deprived of Ig as well as in Ig-deprived cells passively sensitized with patient Ig or patient Ig deprived of IgG or IgE (Fig. 3), indicating that the cellular function was not changed by these procedures.

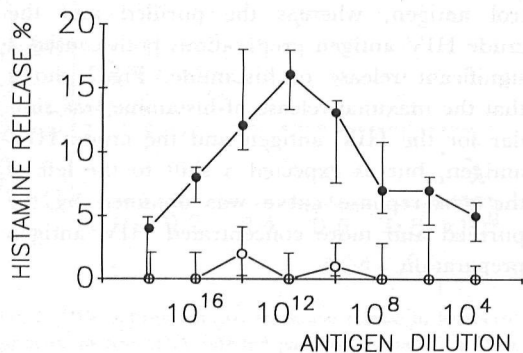


Fig. 4. Histamine release induced by HIV antigen in leukocyte suspensions obtained from 6 AIDS patients. Histamine release in the presence of 0.6 mM Ca^{++} (●-●) and in the absence of calcium (○-○). The median histamine release and fractiles 17-83% are given. Histamine release in the presence and absence of calcium was statistically different ($P < 0.05$).

Calcium dependency

Calcium dependency in connection with HIV antigen-induced histamine release was examined in leukocyte suspensions from six AIDS patients. Parallel examinations were performed in which the cells were suspended in medium with 0.6 mM CaCl_2 (Tris-AMC) and medium without calcium (Tris-AM). In all six leukocyte suspensions stimulation with HIV antigen caused a significant histamine release in the presence of 0.6 mM CaCl_2 , whereas no release was obtained in cell suspensions with no calcium (Fig. 4), indicating that the histamine release by HIV antigen depends on calcium.

DISCUSSION

In contrast to healthy individuals patients with AIDS show basophil histamine release by stimulation with antigen preparations from the microorganisms causing opportunistic infections, such as *Candida albicans*, *Herpes simplex* virus type I and *Pneumocystis carinii* (10, 11). In a previous investigation we found a similar release of histamine when using the causative agent for AIDS, the human immunodeficiency virus (HIV) (13).

In the present study we examined whether the HIV antigen-induced histamine release in cell suspensions from AIDS patients was related to the degree of immunodeficiency and/or to the clinical manifestations of the HIV disease. It was clearly demonstrated that HIV-induced histamine release from basophil leukocytes was related to the clinical stage of HIV-related diseases since histamine release was only seen in cell suspensions from AIDS patients and patients with milder forms of HIV-related disease, whereas clinically "asymptomatic" HIV-infected patients and healthy control persons did not release histamine in response to HIV antigen stimulation. The percentage of histamine release obtained by HIV stimulation was found to be low compared with histamine release obtained from patients with allergic diseases, possibly explained by lower amount of specific IgE antibodies in HIV-infected patients. When the histamine release was compared with the degree of the patients' immunodeficiency esti-

mated by the number of CD4⁺ cells a significant inverse correlation was found between the number of CD4⁺ cells and the histamine release. In contradistinction, there was no correlation between histamine release and HIV (p24) antigen levels in serum (data not shown).

The histamine release was not caused by or influenced by any specific therapy, since the medication given to the AIDS patients was multifarious and indeed different from that of the patients classified in Group IV C-2, whose cells showed a similar response to HIV antigen. Among the 22 AIDS patients 11 received Zidovudine and nine of these showed significant histamine release to HIV stimulation. In the group of patients without Zidovudine treatment similar results were obtained.

The HIV antigen-induced histamine release was found to be specific, i.e. caused by HIV and not by other substances from the culture since histamine release was obtained only by challenge with purified HIV antigen and crude HIV antigen preparation in contrast to the latter preparation without HIV infection (control).

We have previously shown that histamine release depends on the cellular immunoglobulins (Ig) since removal of Ig from the patient cells before stimulation with HIV antigen resulted in abolition of the response, and transfer of the Ig to cells from normal individuals rendered these cells able to respond to HIV antigen (13). Furthermore, the removal and fixation of Ig were followed by disappearance and reappearance of the response to anti-IgE. It was therefore suggested that the histamine release was caused by a type I allergic reaction (13).

In this study we obtained direct evidence for classifying the responsible Ig as IgE. The capability of Ig fractions to sensitize cells from healthy individuals (controls) was investigated using cells stripped of Ig to ensure a maximum of free receptors for the passive sensitization. Transfer to the cells of intact Ig or Ig deprived of IgG rendered the cells able to respond to HIV antigen, but no cell response was obtained after the transfer of Ig deprived of IgE molecules. That the abolition of the response was due to the lack of responsible IgE, and not to a disturbance of the basic cellular function due to

the procedure, was verified by the finding that the cellular response to calcium ionophore A23187, which bypasses receptors and triggers histamine release by influx of calcium (6, 7), was comparative in intact, stripped, and passively sensitized cells.

A type I reaction was also supported by the finding that the histamine release response was dependent on calcium, analogous to the response caused by specific antigens (7, 8). Whether there might be other substances than HIV in the purified antigen preparation that could modify the response is not known.

Our findings indicate that IgE directed against HIV is a frequent finding among patients with HIV-induced immunological dysfunction and disease progression. An altered regulation of IgE production among HIV-infected patients has been described by others (9, 16). They suggested this dysregulation to be due to their findings of increased proportions of T-cell clones able to produce interleukin-4 and decreased proportion of T-cell clones able to produce interferon-gamma in cells from AIDS patients.

The results of this study show that HIV antigen frequently induces histamine release from cells from patients with HIV-related diseases and immunological dysfunction. The activation of basophil leukocytes has been shown to be mediated by cell-surface IgE molecules. It seems reasonable to argue that HIV-induced histamine release could be a pathogenetic co-factor for the immunological dysfunction, since histamine is known to possess immunomodulating properties (3).

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