

# Determination of immunoglobulins responsible for histamine release induced by microbial antigens in AIDS patients

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AIDS patients in contrast to healthy individuals show basophil histamine release by stimulation *in vitro* of their blood leukocytes with *Candida albicans* (CA) and Herpes simplex virus Type I (HSV-I). Passive sensitization of cells from healthy individuals with the patient serum or surface immunoglobulins (Ig) derived from leukocytes of AIDS patients caused histamine release by the following stimulation of the cells with CA or HSV-I. When IgE was removed from the surface Ig no response was obtained, whereas a removal of IgG did not diminish the response. In contrast, no passive sensitization was obtained by serum derived from healthy individuals. The results show that IgE is responsible for the histamine release caused by CA and HSV-I in AIDS patients.

**Key words:** AIDS; *Candida albicans*; Herpes simplex virus; histamine release; IgE; RAST.

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## CLINICAL ASPECTS

*Candida albicans* and Herpes simplex virus Type I infections can induce Type I allergy in AIDS patients. Histamine and other mediators released from basophils and mast cells by the Type I allergic reaction could be an important factor in the course of the infections. Moreover, histamine is known to influence various immunological reactions. It might therefore be tempting to consider a treatment of these patients with anti-allergic or immunomodulating drugs.

Patients with acquired immunodeficiency syndrome (AIDS) suffer from a variety of opportunistic infections and malignancies (11). The infections include fungus, parasites, vira and some bacteria. Previous *in vitro* investigations show that basophil leukocytes from AIDS patients often release histamine due to stimulation with microbial antigens, such as *Candida albicans* (CA), Herpes simplex virus Type I (HSV-I) and *Pneumocystis carinii* (8, 9). IgE and probably IgG<sub>4</sub> have the ability to activate the basophil leukocytes and mast cells by

combining with specific antigen, resulting in a release of mediators such as histamine (4, 6, 10). Immunoglobulins of the IgG class directed against CA and HSV are seen in healthy individuals and in AIDS patients (3, 5, 12). However, CA and HSV-I did not result in basophil histamine release in healthy individuals (8). The aim of this study was to classify the immunoglobulins (Ig) responsible for the basophil histamine release caused by challenge with CA and HSV-I in AIDS patients.

## MATERIAL AND METHODS

### *Patients*

The investigation included 10 AIDS patients according to the criteria of Centers for Disease Control. The mean age was 36 years (range 28–54). The patients were examined from 0–18 months after the diagnosis of AIDS. Nine patients presented severe *Candida albicans* infections in the throat and/or in the esophagus, three patients had recurrent mucocutaneous Herpes simplex infections, eight patients suffered from *Pneumocystis carinii* pneumonia and two patients had Kaposi's sarcoma. All patients presented IgG antibodies against HIV (human immuno deficiency virus) and HSV-I. Ten healthy men with a mean age of 33 years (range 27–58) formed a control group.

### *Microbial antigen preparations and stimulators*

*Candida albicans* (CA) was cultured on Sabouraud dextrose agar for 48 h at 37°C and the colonies were scraped off, washed twice and resuspended in saline (0.154 M NaCl) to a final concentration of 1 g wet weight/ml (1).

*Herpes simplex virus Type I* (HSV-I). Human embryonic lung fibroblast cells were grown in minimal essential medium with 10% foetal calf serum. The fibroblasts were infected with 6 plaque-forming unit per cell of HSV-I strain F, which was obtained from Professor B. Roizman, University of Chicago. The infection was made under standard conditions and the cells were maintained in minimal essential medium with 1% foetal calf serum (7). Twenty hours after the infection the monolayer cells were washed in phosphate-buffered saline (PBS), scraped off into 10 ml PBS and pelleted by centrifugation. The cells were solubilized by sonication in a 0.020 M glycine including 0.0076 M Tris-buffer (pH 8.6) and 1% Triton X-100. Triton was used to obtain a more efficient extraction of infected cell proteins and to eliminate infectious virus particles by destroy-

ing their envelope. The antigen preparation was clarified by ultracentrifugation at 100,000 *g* at 4°C. The concentration of total protein was 6–7 mg/ml.

Calcium ionophore A23187 was from Calbiochem AG, Switzerland, Sepharose 4B from Pharmacia, Sweden, goat anti-IgG and goat anti-IgE (1 mg/ml) from Tago, USA. All antigens and stimulators were kept in small aliquots at –20°C.

### *Preparation of leukocyte suspension*

Mononuclear cells with approx. 2% basophils were isolated from EDTA-blood obtained from the patients and control persons by the Ficoll-Hypaque gradient method, as previously described (8, 14). The cells were washed twice (160 *g* and 110 *g* for 10 min) in Tris-AMC (albumin, magnesium, calcium) buffer containing Tris (hydroxymethyl) amino methane 25 mM, at pH 7.4, NaCl 0.12 M, KCl 5 mM, CaCl<sub>2</sub> 0.6 mM, MgCl<sub>2</sub> 1.1 mM, human serum albumin 0.3 mg/ml, and glucose 1 mg/ml, and finally resuspended in this buffer.

### *Basophil histamine release*

Basophil histamine release was examined in 90 µl leukocyte suspension containing 700–1000 basophils. The samples were incubated in plastic tubes at 37°C for 40 min with 10 µl of microbial antigen preparations. *Candida albicans* was used in final concentrations of 10<sup>–9</sup> to 10<sup>–3</sup> mg protein/ml and HSV-I in final dilutions of 10<sup>–3</sup> to 10<sup>–7</sup>. For determination of the spontaneous histamine release and the total histamine content the addition of agent was omitted in some 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 supernatants contained proteins and other substances, which might interfere with the histamine analysis. The supernatants were therefore discarded and residual histamine was determined in the cell

sediment (14). The release was expressed as a percentage of the total histamine content of the sample. The spontaneous histamine release amounted to up to 10%, hence only histamine release exceeding 10% was considered significant.

#### *Preparation of cell surface Ig fractions from AIDS patients*

Leucocytes were obtained from 90 ml EDTA-blood from the AIDS patients, as described above. The surface Ig was isolated according to the procedure previously described (8) and the Ig was dissolved in Tris-AMC. IgG or IgE were removed from the Ig by binding to anti-IgG or anti-IgE Sepharose gels, respectively. For the preparation of gels 200 µl goat anti-IgG or 200 µl goat anti-IgE were coupled to 60 mg CNBv (cyano bromide) activated Sepharose 4B, and the gels were resuspended in 2 ml PBS ( $\text{Na}_2\text{HPO}_4$  0.1 M and NaCl 0.15 M, pH 7.4). Half a ml of the Ig solution was incubated in polypropylene tubes with 200 µl anti-IgG Sepharose or anti-IgE Sepharose for 24 h at 4°C. After centrifugation the supernatants represented Ig deprived of IgG or IgE. Seventy-five per cent of IgG<sub>1</sub>, 85% of IgG<sub>3</sub>, 85% of IgG<sub>4</sub>, and 95% of the IgE were removed by the Sepharose gels, estimated by ELISA and RIA, respectively. The removal of IgG<sub>2</sub> was not determined. 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 Ig from AIDS patients*

Leucocytes obtained from one control person were used in all experiments. The cells were deprived of Ig by the acidification procedure, as previously described (8). The cell pellet containing the Ig-deprived cells was washed and resuspended in Tris-AMC (pH 7.4). After centrifugation samples of cell pellets were resuspended and incubated at 37°C for 1 h with 0.5 ml cell surface immunoglobulin fraction

from three 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 was examined as described above. Further, samples were challenged with calcium ionophore A23187 in final concentrations of 0.32 and 1.28 µM.

#### *Passive sensitization by serum from AIDS patients*

Passive sensitization was performed according to the method of Ebbesen et al. (2). Cells obtained from one control person were used in all the experiments. In brief, washed blood cells were deprived of Ig by adjusting the pH to 3.6 for 5 min at 4°C, whereafter dissociated Ig was removed by washing and resuspending the cells in Pipes-AMC (pH 7.4). Thereafter, passive sensitization was obtained by incubating the Ig-deprived cells with equal volumes of patient serum for 2 h at 37°C. The cells were washed three times and resuspended in Pipes-AMC (pH 7.4).

Basophil histamine release was examined in 50 µl samples of the blood cell suspension by incubation (37°C for 1 h) with 20 µl of CA or HSV-I. The released histamine was determined by the new glass microfibre method (2, 15) (International patent application No. WO 83/00229). In brief, during the incubation released histamine is bound with high affinity to the glass microfibre-prepared microtiter plates. The microtiter plates are washed free of cells and interfering substances and the fibre-bound histamine were released from the fibres and determined by the fluorometric o-phthalaldehyde method (2, 15). Significant histamine release was  $\geq 0.15$  ng.

#### *RAST*

Specific IgE against CA was determined by RAST (Pharmacia, Sweden).

#### RESULTS

The immunoglobulins responsible for the *in vitro* basophil histamine release induced by CA

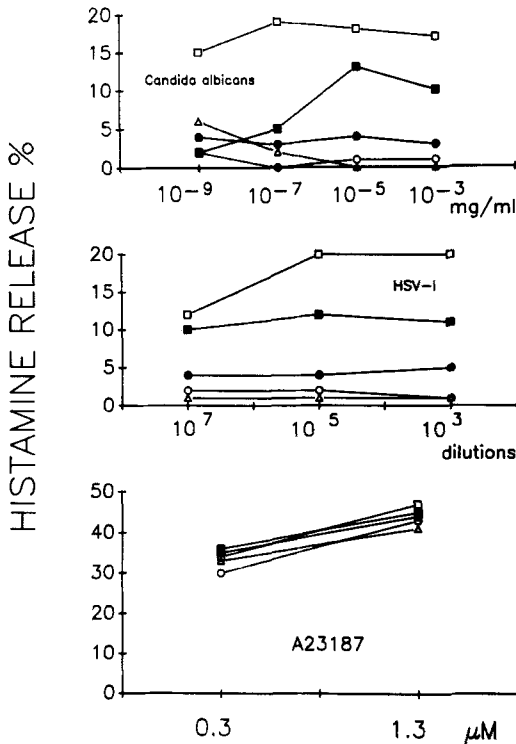


Fig. 1. Histamine release induced by *Candida albicans* (CA), Herpes simplex virus type I (HSV-I), and calcium ionophore A23187 in leukocyte suspensions from a control person. Intact cells (●—●), cells deprived of own Ig (○—○) and with addition of cell surface immunoglobulins from an AIDS patient, i.e. intact Ig (■—■), Ig deprived of IgG (□—□) or IgE (△—△). Duplicate determinations are given.

and HSV-I in AIDS patients were examined by passive sensitization. Three AIDS patients, who responded to CA and HSV-I with histamine release, were included in these experiments. Cell surface Ig was obtained from the leukocytes and intact Ig, Ig deprived of IgG and Ig deprived of IgE were used for passive sensitization. In all three experiments cells from one control person were used for comparing the effect of the immunoglobulins.

Fig. 1 shows representative results from one of the three patients and all results are given in Table 1. Leucocytes from the control person did not release histamine by challenge with CA or HSV-I. This was also the case when Ig was removed from the cells prior to stimulation with these microbial antigens. However, when the Ig-deprived cells were passively sensitized with cell surface Ig from the AIDS patients, CA and HSV-I caused basophil histamine release. The same applied to passive sensitization with Ig deprived of IgG, whereas no release of histamine was obtained with Ig deprived of IgE.

The basic cellular function was elucidated by the non-immunological histamine release response to calcium ionophore A23187, which was used in concentrations of 0.3 and 1.3 μM. 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

Table 1

Histamine release (%) induced by *Candida albicans* (CA) and Herpes simplex virus Type I (HSV-I) in leukocyte suspensions from a control person

| Control cells                        | CA               |                  |                  |                  | HSV-I            |                  |                  |
|--------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                                      | 10 <sup>-9</sup> | 10 <sup>-7</sup> | 10 <sup>-5</sup> | 10 <sup>-3</sup> | 10 <sup>-7</sup> | 10 <sup>-5</sup> | 10 <sup>-3</sup> |
| Intact                               | 2, 0, 4          | 0, 0, 3          | 2, 5, 4          | 2, 2, 3          | 4, 6, 4          | 2, 4, 4          | 6, 0, 5          |
| Deprived of Ig                       | 3, 0, 2          | 3, 0, 0          | 2, 1, 1          | 0, 0, 1          | 3, 3, 2          | 1, 2, 2          | 2, 1, 1          |
| *With addition of patient Ig, intact | 7, 10, 2         | 9, 10, 5         | 15, 4, 13        | 12, 12, 10       | 9, 6, 10         | 12, 14, 10       | 9, 0, 11         |
| Deprived of IgE                      | 0, 0, 6          | 0, 7, 2          | 0, 6, 0          | 0, 4, 0          | 0, 0, 0          | 0, 3, 0          | 0, 5, 0          |
| Or deprived of IgG                   | 6, 5, 15         | 13, 8, 19        | 14, 14, 18       | 13, 16, 17       | 11, 0, 12        | 13, 12, 20       | 8, 14, 20        |

\*Passive sensitization with immunoglobulins derived from cells of three AIDS patients. Histamine release >10% is significant

with patient Ig or patient Ig deprived of IgG or IgE (Fig. 1), indicating that the cellular function was not changed by these procedures.

Passive sensitization by patient serum was performed to examine functional Ig in serum capable of inducing basophil histamine release in response to CA and HSV-I. When blood cells from the control person were passively sensitized with sera from the patients and then challenged with CA or HSV-I, the microbial antigens were found to induce histamine release. A positive histamine release response to CA was obtained by sera from eight of the 10 patients and by HSV-I stimulation half of the sera gave a positive response (Table 2). In contrast, no response was found after passive sensitization with sera from the 10 controls (Table 2). A dose-response curve after passive sensitization with serum from one of the responding patients is shown in Fig. 2. The figure also

shows that no response was obtained by omitting the passive sensitization with patient serum, i.e. using the intact cells, the cells deprived of Ig and after incubation with autologous serum from the control.

The passive sensitization was compared with the response obtained in leukocyte suspensions from the AIDS patients and the controls. Table 2 shows that the eight patients whose sera caused passive sensitization also released histamine when their leukocytes were challenged with CA. A less pronounced relationship was obtained by HSV-I, since of eight leukocyte responders only five showed passive sensitization. However, in the 10 control persons no response was obtained by either passive sensitization or by challenge of their leukocytes with CA or HSV-I. RAST showed CA-specific IgE in serum in four of the eight responding AIDS patients, but in none of the controls (Table 2).

Table 2  
Comparison between passive sensitization, basophil histamine release (BHR) and RAST

| AIDS patients No. | Histamine release (ng/sample) from blood cells passively sensitized with patient or control serum |       | BHR (%) in leukocyte suspension from patients or controls |       | RAST class |
|-------------------|---------------------------------------------------------------------------------------------------|-------|-----------------------------------------------------------|-------|------------|
|                   | CA                                                                                                | HSV-I | CA                                                        | HSV-I | CA         |
| 1                 | 0.25                                                                                              | 0.25  | 24                                                        | 21    | 2          |
| 2                 | 0.15                                                                                              | 0.15  | 18                                                        | 14    | 3          |
| 3                 | 0.30                                                                                              | 0.15  | 34                                                        | 20    | 2          |
| 4                 | 0.15                                                                                              | 0     | 20                                                        | 12    | 2          |
| 5                 | 0.15                                                                                              | 0.15  | 25                                                        | 20    | 0          |
| 6                 | 0.30                                                                                              | 0.45  | 28                                                        | 18    | 0          |
| 7                 | 0.30                                                                                              | 0     | 12                                                        | 18    | 0          |
| 8                 | 0.25                                                                                              | 0     | 26                                                        | 24    | 0          |
| 9                 | 0                                                                                                 | 0     | 6                                                         | 2     | 0          |
| 10                | 0                                                                                                 | 0     | 0                                                         | 0     | 0          |
| Control No.       |                                                                                                   |       |                                                           |       |            |
| 1                 | 0                                                                                                 | 0     | 5                                                         | 7     | 0          |
| 2                 | 0                                                                                                 | 0     | 6                                                         | 4     | 0          |
| 3                 | 0                                                                                                 | 0     | 6                                                         | 4     | 0          |
| 4                 | 0                                                                                                 | 0     | 6                                                         | 7     | 0          |
| 5                 | 0                                                                                                 | 0     | 5                                                         | 2     | 0          |
| 6                 | 0                                                                                                 | 0     | 3                                                         | 3     | 0          |
| 7                 | 0.05                                                                                              | 0     | 5                                                         | 8     | 0          |
| 8                 | 0.05                                                                                              | 0.05  | 2                                                         | 1     | 0          |
| 9                 | 0.10                                                                                              | 0     | 0                                                         | 0     | 0          |
| 10                | 0                                                                                                 | 0     | 0                                                         | 0     | 0          |

Maximal histamine release is given. Histamine release  $\geq 0.15$  ng per sample or  $> 10\%$  of the total histamine content of the sample is significant

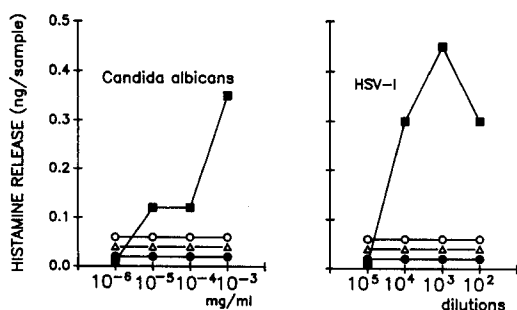


Fig. 2. Histamine release induced by CA and HSV-I in blood cell suspensions from a control person. Intact cells (●—●), cells deprived of own Ig (○—○) and after passive sensitization with serum from an AIDS patient (■) or with own serum (△). Duplicate determinations are given.

## DISCUSSION

HIV infection induces severe decreased cellular immunodeficiency (13). Other immune functions are also affected, such as polyclonal activation of B-lymphocytes, resulting in increased levels of IgA and IgG indicating an alteration of the immunoregulatory functions. In contrast to healthy individuals, AIDS patients show basophil histamine release by stimulation *in vitro* with *Candida albicans*, Herpes simplex virus Type I and *Pneumocystis carinii* (8, 9). The response is possibly caused by Type I allergic reactions directed against these microbial antigens, since by removal from and fixation to the cell surface of immunoglobulins the response was abolished and regained, respectively (8, 9). To classify the responsible Ig, cell-bound Ig was removed from blood leukocytes of AIDS patients and transferred to leukocytes of healthy individuals (controls). Cells stripped of Ig were used to ensure a maximum of free receptors. By this passive sensitization it was possible to study the Ig class responsible for the histamine release response. When the intact, cell-derived Ig was used for passive sensitization the cells responded to CA and HSV-I with histamine release. However, a removal of IgE from the Ig by anti-IgE Sepharose gel completely abolished the response, whereas the response was still obtained using the Ig deprived of IgG, indicat-

ing that IgE but not IgG was responsible for the CA- and HSV-I-induced histamine release.

To verify that the basic cellular function of the cells was not changed during these procedures the histamine response to calcium ionophore A23187 was examined. No change was obtained between intact cells, stripped cells and after passive sensitization with intact Ig and Ig deprived of IgE or IgG.

The presence of circulating Ig in serum capable of inducing basophil histamine release in response to CA and HSV-I was shown by passive sensitization of blood cells from a control person with patient serum. No histamine release was obtained by omitting the passive sensitization or by using sera from controls. The results of positive and negative response were in accordance with those obtained by challenge of leukocytes from patients and controls with CA, whereas a less pronounced relationship was obtained by HSV-I. RAST showed CA-specific IgE in sera of half of the responding patients, but in none of the controls.

The experiments with passive sensitization using cell-bound Ig or serum from AIDS patients and the selective removal of IgE or IgG from the cell-bound Ig demonstrate that IgE was responsible for the basophil histamine release caused by CA and HSV-I in AIDS patients, whereas IgG seemed to be of no importance.

The results of this study suggest that AIDS patients produce specific IgE antibodies against microbial antigens, at least *Candida albicans* and Herpes simplex virus. Increased antigen presentation for the immune system in connection with its dysfunction might be the explanation.

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