

The immunoglobulin superantigen-binding site of HIV-1 gp120 activates human basophils

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Objective: To investigate the mechanism whereby HIV-1 envelope glycoprotein gp120 from four different isolates obtained in three different countries induces proinflammatory mediator release from normal human basophils.

Methods: Histamine, cysteinyl leukotriene C₄ (LTC₄) and interleukin 4 (IL-4) release into the supernatant was measured in gp120-stimulated peripheral blood basophils from HIV-1 and HIV-2 negative subjects.

Results: The HIV glycoprotein was a potent stimulus for release of these mediators in basophils purified from donors negative for HIV-1 and HIV-2. There was also a correlation ($r = 0.58$; $P < 0.01$) between the maximum IL-4 release from basophils induced by gp120 and by anti-IgE. Basophils from which IgE had been dissociated by brief exposure to lactic acid no longer released histamine in response to gp120 and anti-IgE. Anti-IgE specifically desensitized basophils to a subsequent challenge with anti-IgE and gp120. Human monoclonal IgM carrying the V_H3 domain, but not that carrying the V_H6 domain, inhibited gp120-induced secretion of histamine from basophils in a concentration-dependent manner. Synthetic peptides identical to regions distant from the N- and C-termini of gp120_{MN} inhibited its activating capacity.

Conclusions: gp120 acts as a viral superantigen interacting with the V_H3 domain of IgE to induce the release of preformed and *de novo* synthesized mediators from human cells carrying the Fc fragment FcεRI receptor.

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Introduction

HIV-1 and HIV-2 cause the destruction of CD4+ lymphocytes allowing development of AIDS [1–3]. The entry of HIV-1 into immune cells is mediated by the viral envelope glycoproteins, which are organized into oligomeric, probably trimeric, spikes displayed on the surface of the virion [4]. The surface of the spike is composed of gp120, associated by non-covalent interactions with gp41 [4,5]. Sequence analysis of gp120 from different primate HIV viruses has identified five variable regions (V₁–V₅) and five constant regions

(C₁–C₅) [6]. Variability in gp120 probably modulates the glycoprotein's immunogenicity and antigenicity.

Entry of HIV-1 into immune cells involves gp120 binding to the CD4 glycoprotein that serves as the primary receptor [7]. This binding induces conformational changes in gp120, involving the exposure and/or formation of a binding site for specific chemokine receptors, CCR5 and CXCR4, which serve as obligate coreceptors for virus entry [8,9].

In addition to binding to CD4 and to chemokine

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receptors, gp120 possesses an additional binding site for human immunoglobulin [10–13]. The gp120 of HIV-1 binds to normal human non-immune immunoglobulin irrespective of the immunoglobulin class, subclass, allotype or L chain type [11–13]. This binding mainly involves the human V_H3 domain family [11–13]. Because, like staphylococcal protein A [12,14,15], gp120 binds outside the antigen-binding site of antibodies [10,12], it has been allocated to a new group of B cell superantigens [11,12].

HIV-infected patients present a wide spectrum of tissue injuries, not necessarily directly related to the cytotoxic effects of HIV-1 infection [16]. These include skin rashes, pruritus, urticarial reactions involving skin mast cells and lung involvement [17–21]. Human basophils and mast cells, through the release of vasoactive and proinflammatory mediators [histamine, cysteinyl leukotrienes (LTC₄), etc.] [22–24] and the elaboration of various cytokines [interleukin 4 (IL-4), etc.] [24–29], can play a role in these disorders.

This study was undertaken to evaluate the effects of HIV-1 envelope gp120 on the release of preformed (histamine) and *de novo* synthesized chemical mediators (LTC₄ and IL-4) from basophils purified from healthy individuals seronegative for antibodies to HIV-1 and HIV-2.

Materials and methods

Reagents

The following reagents were used: PIPES (1,4-piperazinediethanesulphonic acid) and synthetic LTC₄, (Sigma Chemical Co., St Louis, Missouri, USA); Hanks' balanced salt solution (HBSS), fetal calf serum (FCS) and IMDM (Gibco, Grand Island, New York, USA); RPMI 1640 with 25 mmol/l Hepes buffer and Eagle's minimum essential medium (Flow Laboratories, Irvine, UK); the formylated tripeptide (FMLP) (Calbiochem-Behring Corp., La Jolla, California, USA); [³H]-LTC₄ (168 Ci/mmol) was from New England Nuclear (Boston, Massachusetts, USA); cyclosporin A was a gift of Novartis (Milan, Italy). Recombinant gp120s preparations and synthetic peptides of gp120_{MN} were obtained through the AIDS National Institute of Allergy and Infectious Diseases, Research and Reference Reagent Program, Division of AIDS, N.I.H. (Bethesda, Maryland, USA). Rabbit anti-IgE was a gift of Drs Teruko and Kimishige Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, California, USA). The monoclonal antibody against the α -chain of Fc ϵ RI (anti-Fc ϵ RI) was a gift of Dr John Hakimi (Roche Research Center, Hoffman-LaRoche, Nutley, New Jersey, USA). PIPES buffer contained 25 mmol/l PIPES pH 7.37, 110 mmol/l NaCl, 5 mmol/l KCl.

PCG buffer contained PIPES buffer plus 5 mmol/l CaCl₂ and 1 g/l dextrose [23].

Purification of human monoclonal IgM

Monoclonal IgM were purified from the sera of patients with Waldenstrom's macroglobulinaemia [24]. Variable regions of these monoclonal IgM were determined as described [30].

Purification of peripheral blood basophils

Basophils were purified from peripheral blood cells of healthy volunteers aged 21 to 39 years (mean 32.4 ± 4.1 years) who were seronegative for antibodies to HIV-1 and HIV-2 and undergoing haemapheresis [29]. Yields ranged from 3×10^6 to 10×10^6 basophils, with purity from 74 to 98%, as assessed by basophil staining with Alcian blue and counting in a Spiers-Levy eosinophil counter [22].

Mediator release assays

Basophils ($\approx 6 \times 10^4$ basophils/tube) resuspended in PCG (histamine and LTC₄ release) or IMDM (IL-4 release) were placed in 12×75 mm polyethylene tubes and warmed to 37°C; 0.1 ml of each prewarmed stimulus for release was added, and incubation was continued at 37°C for 45 min (histamine and LTC₄ release) or 4 h (IL-4 secretion). The cell-free supernatants were stored at -20°C for subsequent assay of histamine [31], LTC₄ [23] and IL-4 content [24].

LTC₄ was analysed by radioimmunoassay using an anti-LTC₄ antibody that is highly selective, with less than 1% cross-reactivity to other eicosanoids [21,22]. The supernatants were assayed for IL-4 using the IL-4 Quantikine high-sensitivity kit (R&D Systems, Minneapolis, Minnesota, USA) [24].

Statistical analysis

The results are given as means $\pm$ SEM. The data subjected to linear regression were calculated by the least squares method ($y = a + bx$) in which a was the y axis intercept and b the slope of the line [32].

Results

Effect of gp120 from different HIV-1 isolates on histamine release from basophils

In a first series of 12 experiments we evaluated the histamine-releasing activity of increasing concentrations (1 to 30 nM) of recombinant gp120 on histamine release (12 to 82%) from human basophils ($\approx 1\%$) from individuals seronegative for HIV-1 and HIV-2. Gp120 had no effect on basophil viability (data not shown).

In a series of six experiments, purified basophils (> 95%) from individuals seronegative for HIV-1 and

HIV-2 were challenged with a range of concentrations (1–30 nmol/l) of gp120 from various HIV-1 isolates from different clades (Table 1). Gp120 from different clades induced histamine release in a concentration-dependent manner from enriched preparations of basophils. The concentration–response curves induced by the five different gp120 preparations were essentially superimposable (Fig. 1), implying that the releasing activity is constant over gp120 forms of different sequences and is a general feature of gp120 that is maintained throughout the evolution of the virus.

Effect of gp120 on cysteinyl leukotriene C₄ release from human basophils

To verify that HIV-1 gp120 is a complete secretagogue, the production of newly formed lipid mediator (LTC₄) from basophils was evaluated in response to gp120 (1–30 nmol/l). Purified (> 92%) basophils were challenged in three experiments with increasing concentrations of gp120; *de novo* synthesis of LTC₄ occurred in addition to the release of histamine (Table 2). There was a significant correlation ($r = 0.92$; $P < 0.001$) between histamine and LTC₄ release in response to gp120.

Effect of gp120 on interleukin 4 release from human basophils

The release of histamine and IL-4 from purified (60–96%) human basophils was induced by gp120 (1–30 nmol/l) in a concentration-dependent manner. A significant correlation was found between histamine release and IL-4 secretion induced by gp120 ($r = 0.83$; $P < 0.001$).

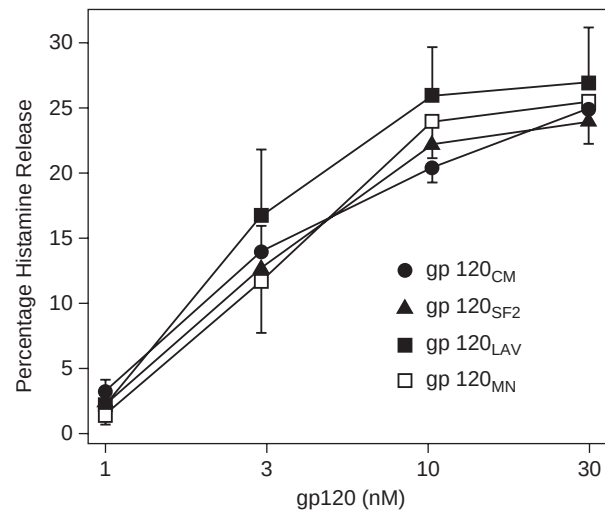


Fig. 1. Effect of increasing concentrations of gp120 from different HIV-1 isolates on histamine secretion from purified human basophils (> 95%) obtained from normal donors ($n = 6$) negative for HIV-1 and HIV-2 antibodies. Values are mean $\pm$ SEM from 12 experiments. Error bars are not shown when graphically too small.

Kinetics of gp120-induced histamine and interleukin 4 release

Figure 2 compares the kinetics of histamine and IL-4 release from three enriched preparations (> 96%) of basophils challenged with an optimal concentration (30 nmol/l) of gp120. Histamine release induced by maximal stimulation was complete within 5 min, whereas IL-4 release was complete in 3–4 h. The average half-time for histamine release was $3.8 \pm$

Table 1. Recombinant envelope glycoprotein gp120 from different isolates of HIV-1 from clades derived from differing geographical areas.

Recombinant envelope glycoprotein	gp120 isolate	Clade	Place of origin	Expression system
gp120 _{MN}	MN	B	USA	Insect cells
gp120 _{SF2}	SF2	B	USA	CHO cells
gp120 _{LAV}	LAV	B	France	Insect cells
gp120 _{CM}	CM	E	Thailand	Insect cells

Table 2. Effect of gp120 on histamine release and *de novo* synthesis of cysteinyl leukotriene C₄ in human basophils.

gp120 (nmol/l)	Experiment 1		Experiment 2		Experiment 3	
	Histamine release (%)	LTC ₄ (ng/10 ⁶ cells)	Histamine release (%)	LTC ₄ (ng/10 ⁶ cells)	Histamine release (%)	LTC ₄ (ng/10 ⁶ cells)
1	4	3	1	0	5	8
3	28	17	21	12	31	26
10	51	38	39	17	50	37
30	56	41	43	20	53	49

LTC₄, cysteinyl leukotriene C₄ *de novo* synthesis.

Cells were incubated with gp120 of varying concentration for 45 min at 37°C; each number is a mean release from duplicate determinations.

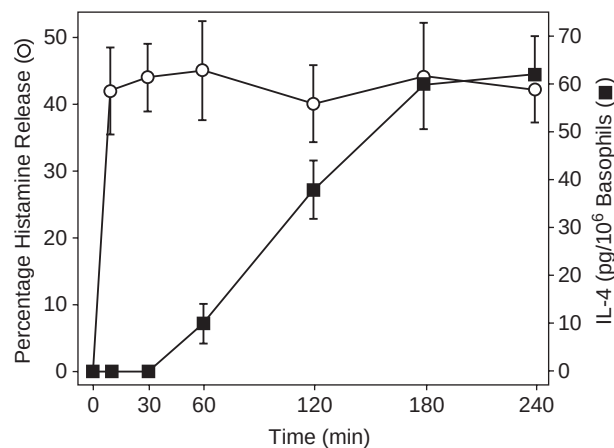


Fig. 2. Kinetics of interleukin 4 (IL-4) and histamine secretion induced by gp120 (30 nmol/l) from purified (> 96%) human basophils from normal donors (n = 3) negative for HIV-1 and HIV-2 antibodies. Each point is the mean $\pm$ SEM of three experiments. Error bars are not shown when graphically too small.

2.4 min, compared with 84 ± 12.4 min for IL-4 ($P < 0.001$).

Cyclosporin A and tacrolimus inhibit gp120-induced mediator release from human basophils

Two immunophilin-binding drugs, cyclosporin A and tacrolimus (FK-506), are potent inhibitors of the IgE-dependent release of proinflammatory mediators from human basophils and mast cells *in vitro* [33–35] and *in vivo* [36]. Preincubation with low concentrations of cyclosporin A (8–800 nmol/l) inhibited the release of histamine and LTC₄ from purified basophils activated by gp120, an effect being seen at as low a concentration as 24 nmol/l (Fig. 3a). The inhibition of histamine release ranged from $\approx 30\%$ at 24 nmol/l to 90–100% at 240 nmol/l, with a median inhibitory concentration of 64.5 ± 10.8 nmol/l, similar to the figure for LTC₄ (64.8 ± 11.5 nmol/l). The effects of tacrolimus on the release of histamine and LTC₄ from basophils activated by gp120 were similar to those for cyclosporin A (Fig. 3b), though tacrolimus was more potent than cyclosporin A. For histamine release, the median inhibitory concentration was 2.7 ± 0.3 nmol/l, similar to the figure for LTC₄ (2.3 ± 0.3 nmol/l).

Correlation between gp120 and anti-IgE effects on histamine release

In eight experiments, basophils (76–96%) were challenged with gp120 (1–30 nmol/l) and anti-IgE (0.03–1 μ g/ml). A significant correlation was found between the maximum histamine release induced by anti-IgE and that induced by gp120 ($r = 0.58$; $P < 0.01$; data not shown), in agreement with the hypothesis that gp120 induces histamine release from basophils by interacting with IgE.

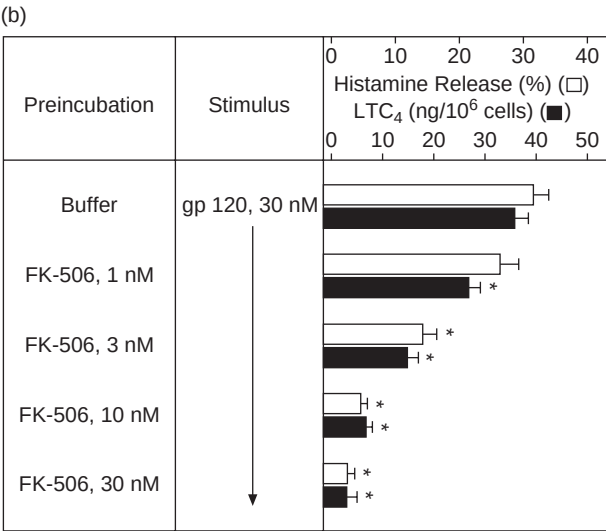
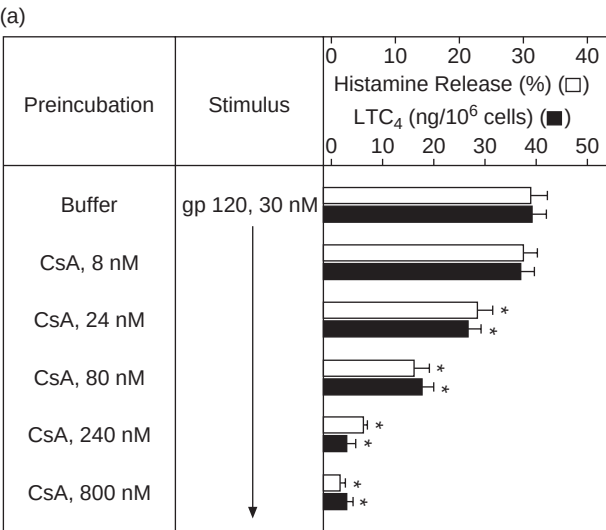


Fig. 3. Effects of various concentrations of immunophilin-inhibiting drugs on histamine and cysteinyl leukotriene C₄ (LTC₄) release from human basophils from normal donors negative for HIV-1 and HIV-2 antibodies. Cells were preincubated for 15 min at 37°C with drug then challenged (45 min at 37°C) with gp120 (30 nmol/l). Each bar shows the mean $\pm$ SEM from four experiments. (a) Cyclosporin A (CsA); (b) tacrolimus (FK-506).

Effect of removal of IgE from human basophils

Brief exposure to low pH removes most of the IgE bound on basophils and markedly reduces the activating properties of IgE-mediated stimuli [22,33]. Figure 4a illustrates three experiments in which brief exposure to lactic acid completely blocks the effects of gp120 and anti-IgE on histamine secretion from basophils. This treatment did not affect the response to a monoclonal antibody cross-linking the α -chain of Fc ϵ RI (anti-Fc ϵ RI α) [24,37].

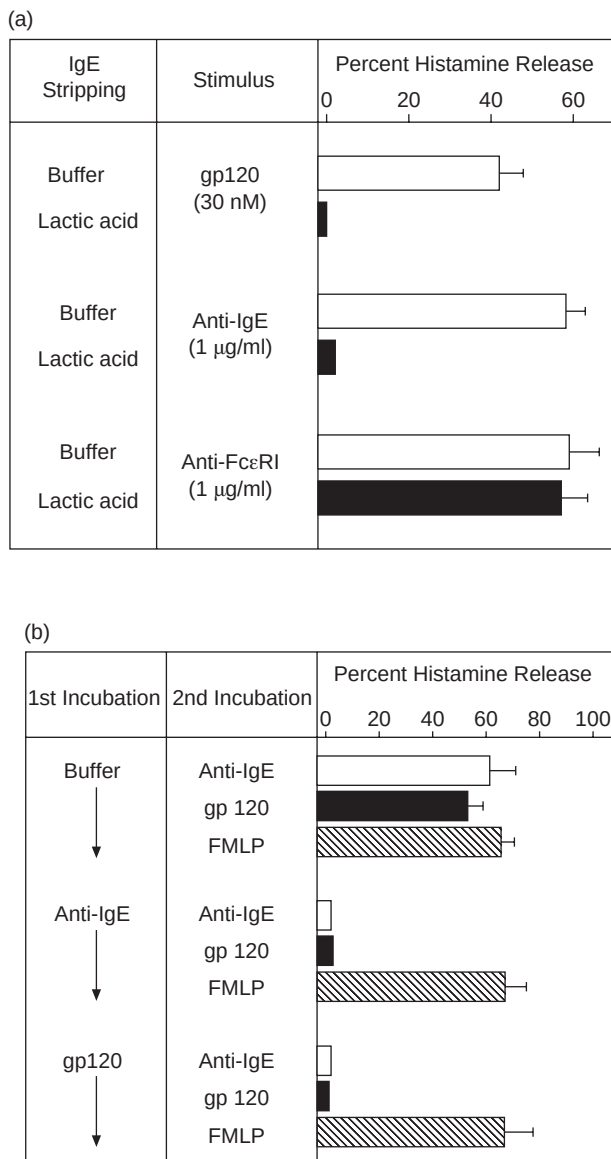


Fig. 4. The effect of cell treatment on histamine release (a) Effect of lactic acid treatment to remove IgE from human basophils on histamine release induced by gp120, anti-IgE and anti-FcεRI. Basophils from three different donors negative for HIV-1 and HIV-2 antibodies were either untreated (buffer) or treated with lactic acid (0.01 mol/l, pH 3.9, 5 min, 22°C) and washed (twice). Basophils were then challenged (45 min at 37°C) with gp120 (30 nmol/l), anti-IgE (1 µg/ml), or anti-FcεRIα (1 µg/ml). (b) Effect of desensitization to stimuli requiring IgE on the response to a second stimulus. Cells were desensitized to IgE using rabbit IgG anti-IgE (anti-IgE, 1 µg/ml) or gp120 (30 nmol/l) by incubation with the stimuli in Ca²⁺-free PIPES–ethylenediamine tetraacetic acid medium for 30 min at 37°C. Cells were washed twice at 4°C, resuspended in PCG (see text), and challenged with an optimal concentration of fresh anti-IgE (1 µg/ml), gp120 (30 nmol/l) or formylated tripeptide (FMLP; 10 µmol/l) for 45 min at 37°C. Each bar shows the mean ± SEM of three experiments. Error bars are not shown when graphically too small.

Cross-desensitization between anti-IgE and gp120

The relationship between IgE and gp120 was examined using rabbit anti-human IgE (anti-IgE) to induce desensitization specific for IgE-mediated stimuli [22,38]. The release caused by secretagogues that do not cross-link IgE on the cell surface are unaffected by this procedure [22,38]. Leukocytes were incubated with anti-IgE (1 µg/ml) or gp120 (30 nmol/l) in PIPES buffer containing 4 mmol/l ethylenediamine tetraacetic acid (EDTA) for 30 min at 37°C. At the end of incubation, cells were washed and resuspended in PCG and rechallenged with the stimuli (anti-IgE, gp120, or FMLP) in the presence of 5 mmol/l Ca²⁺. The subsequent histamine release was measured. Figure 4b shows the results of three experiments in which cells preincubated with buffer alone released histamine normally with both anti-IgE and gp120. Preincubation with anti-IgE abolished the response to subsequent challenge with both anti-IgE and gp120. Similarly, preincubation with gp120 abolished the release on challenge with gp120 and anti-IgE. In contrast, the response to FMLP, which does not act through the IgE receptor [38], was unaffected by preincubation (desensitization) with either anti-IgE or gp120.

Interaction of gp120 with IgM of varying structure

To evaluate the mechanism whereby gp120 activates basophils, the glycoprotein was preincubated with monoclonal IgM of different V_H families [24,30]. In three experiments, one of which is illustrated in Fig. 5a, preincubation of basophils with three preparations of monoclonal IgM that had a V_H3 domain (M3, M11 and LAN) [24,30] inhibited the histamine-releasing activity of gp120 in a concentration-dependent manner. A monoclonal IgM that had a V_H6 domain (M14) had only a marginal effect. These results confirm that binding to the V_H3 domain inhibits gp120 binding to IgE bound to FcεRI on basophils.

Effects of synthetic gp120_{MN} peptides on the activation of basophils by gp120_{MN}

A panel of synthetic peptides encompassing the sequence of the gp120_{MN} core motif (1959, 1960, 1985, 1988 and 1989) and the N- and C-terminal regions (1922 and 2015, respectively) were used to map the IgE-binding sites of gp120 [39] (Table 3). Preincubation of purified (> 94%) basophils with the synthetic peptides of the core motif of gp120_{MN} inhibited gp120_{MN} activity in a concentration-dependent manner, whereas the peptides that span the N- and C-termini did not cause any inhibition (Fig. 5b). These peptides did not affect anti-IgE-induced histamine release from basophils (data not shown).

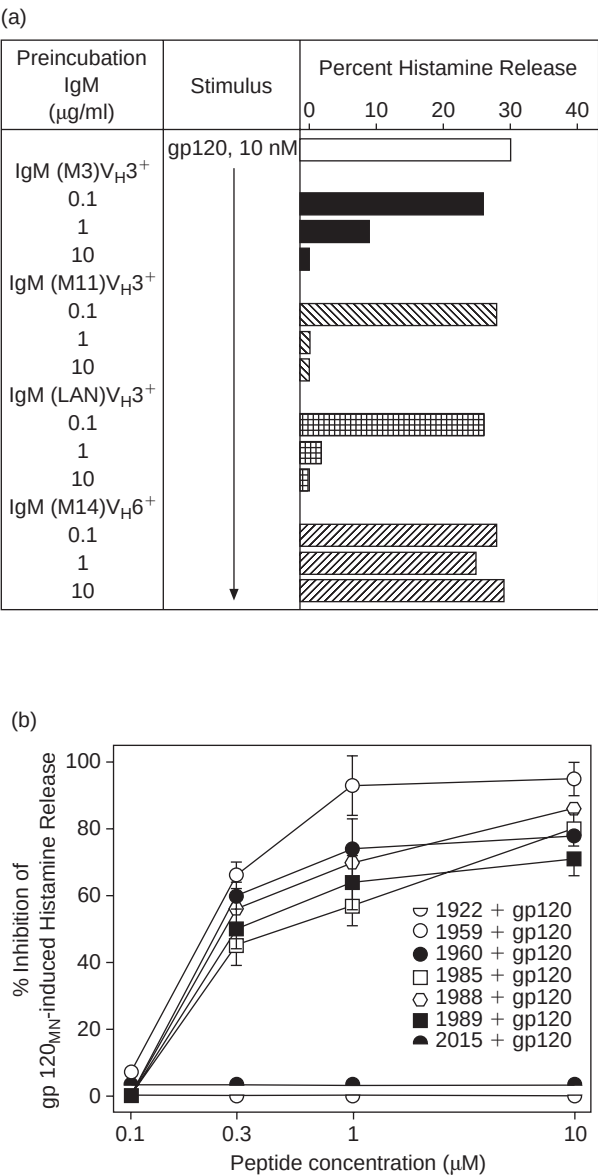


Fig. 5. Activation of basophils by gp120. (a) Effect of preincubation of gp120 with monoclonal IgM on histamine release from human basophils. Gp120 (10 nmol/l) was preincubated for 15 min at 37°C with three human monoclonal IgM preparations that had V_H3 domains, M3 (0.1–10 μg/ml), M11 (0.1–10 μg/ml) and LAN (1–10 μg/ml) or a preparation that had a V_H6 domain, M14 (0.1–10 μg/ml). Basophils were then added and the incubation was continued for another 45 min at 37°C. Each bar shows the mean histamine release from duplicate incubations. (b) Effect of preincubation of human basophils with synthetic peptides from different sites on gp120_{MN} before stimulation with gp120_{MN}. Purified basophils (> 94%) obtained from normal donors (n = 3) negative for HIV-1 and HIV-2 antibodies were preincubated for 15 min at 37°C with the synthetic peptides. Gp120 (10 nmol/l) was then added and the incubation was continued for another 45 min at 37°C. Each bar shows the mean ± SEM of histamine release from three experiments. Error bars are not shown when graphically too small.

Discussion

This study shows that a variety of gp120 from different clades all induce mediator release from human basophils. The releasing activity of gp120 appears to be mediated by interaction with the V_H3 region of IgE on these cells. This is the first indication that the viral envelope glycoprotein gp120 can act as a viral superantigen causing the release of preformed (histamine) and *de novo* synthesized (LTC₄) mediators from human cells carrying FcεRI.

The mechanism of activation of human inflammatory cells possessing FcεRI appears to involve an interaction of gp120 with the V_H3 region of membrane-bound IgE. This is supported by several observations. First, there is a highly significant correlation between the maximal mediator release induced by anti-IgE and that induced by gp120. Second, basophils from which IgE has been dissociated by brief exposure to lactic acid no longer released histamine in response to gp120 and anti-IgE; third, there is complete cross-desensitization between gp120 and anti-IgE. These findings agree with the proposal that gp120 has distinct functional immunoglobulin-binding sites [10–13]. Consequently, gp120 can function as a viral superantigen [10–13] and as a viral superallergen, i.e., reproducing the releasing activity of specific allergens and of anti-IgE on human cells carrying FcεRI [24,33].

Three different monoclonal IgM with a V_H3 domain inhibited the gp120-induced release of histamine from basophils; an IgM with a V_H6 domain had no such effect. This confirms that the releasing activity of gp120 depends on binding to an immunoglobulin structure located not in the C_Hε constant regions but in the V_H3 domain [11–13], a fragment common to all immunoglobulin classes and subclasses [12]. Structural differences between V_H families explain why the monoclonal IgM M14 with the V_H6 domain failed to inhibit the releasing effect on cells carrying FcεRI, while the three monoclonal IgM carrying V_H3 caused concentration-dependent inhibition.

There are seven known human V_H genes encoding human immunoglobulins (V_H1–V_H7) [12]. The V_H3 gene family predominates (> 50%) in the human immunoglobulin repertoire [11–13]; gp120 binds to most of the members of the family [11,13]. Given the overexpression of the V_H3 gene family, the immunoglobulin superantigenic properties of virus-bound or shed gp120 are likely to involve the interaction with the V_H3 domain of IgE bound on human basophils.

Our results provide the first evidence that a viral superantigen, gp120, can induce the secretion of such important mediators as histamine, LTC₄ and IL-4 from human basophils. Gp120 also occurs as a free molecule

Table 3. Peptide sequences of HIV-1_{MN} gp120.

Peptide number	aminoacid residues	gp120 domain	Sequence
1922	31–50	C1	EKLWVTVYYGVVPVWKEATTT
1959	231–250	C2	LKCNDDKKFSGKGCKNVSTV
1960	241–260	C2	KGSSCKNVSSSTVQCTHGIRPVV
1985	301–320	V3	CTRPYNKRRKRIHIGPGRAF
1988	331–350	C3	RQAHNCNISRKWNDRQIV
1989	341–360	C3	KWNDTLRQIVSKLKEQFKNK
2015	501–520	C5-gp41	TKAKRRVVQREKRAAIGALF

in the blood of patients with HIV-1 infection [40]. Interestingly, gp120 is extremely active, which raises the possibility of it having important effects *in vivo*.

The activity of IL-4 has been linked to several immunoregulatory functions including the synthesis of IgE by B lymphocytes [41,42], and the development of T lymphocytes expressing a T helper 2 phenotype [43]. Elevated IgE levels have been found in HIV-1-infected patients [17–20,44–46] and have been associated with progression of the disease [17,44–46]. Consequently, during the early phase of HIV-1 infection when there are high levels of viraemia [1,16], basophils exposed to virus-bound or shed gp120 [40] might serve as an initial source of IL-4, favouring a shift from a T helper-naïve phenotype towards a T helper 2 phenotype [47,48]. Therefore, virus-bound or shed gp120 in the blood of patients with HIV-1 infection might contribute, through the release of IL-4 from cells carrying FcεRI, to the high serum IgE levels found in these conditions [17–20,44–46].

During HIV-1 infection, gp120 is released in the blood [40] and might contribute to some of the manifestations of this condition. Urticaria and pruritus are sometimes seen in HIV-1 infection [16–21], and basophils are activated in these patients [49,50]. Basophils and their vasoactive and proinflammatory mediators presumably play a role in urticarial syndromes [22–24]. This study provides evidence that gp120 can exert a proinflammatory effect on human inflammatory cells, suggesting that the interaction between gp120 and cells carrying FcεRI cells may contribute to some of the manifestations of HIV-1 infection.

In previous *in vitro* [34,35] and *in vivo* [36] studies, we demonstrated that immunophilin-binding drugs (cyclosporin A and tacrolimus) exerted anti-inflammatory effects by inhibiting the IgE-dependent release of proinflammatory mediators from human FcεRI⁺ cells. Here, we reported that these two drugs are potent inhibitors of the gp120-induced release of histamine and LTC₄ from human basophils. It has been suggested that these two drugs inhibit HIV-1 replication and the growth of infected cells [51,52]. There is also some evidence that they might be useful in patients [53].

Several mechanisms have been proposed to explain the antiviral effects of immunophilin-binding drugs [51–53]. Our results propose an additional unrecognized mechanism by which this class of compound could influence the activation of inflammatory cells induced by HIV-1 gp120.

In conclusion, we provide the first evidence that gp120 from different clades can activate human FcεRI⁺ cells to release preformed and *de novo* synthesized mediators, probably through an interaction with the V_H3 region of IgE. This novel interaction between gp120 and FcεRI⁺ cells could contribute to some of the immune dysregulation and clinical manifestations of HIV-1 infection.

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