

HIV-1 gp120 Induces IL-4 and IL-13 Release from Human FcεRI⁺ Cells Through Interaction with the V_H3 Region of IgE¹

Vincenzo Patella, Giovanni Florio, Angelica Petraroli, and Gianni Marone²

HIV-1 glycoprotein (gp) 120 from different clades is a potent stimulus for IL-4 and IL-13 release from basophils purified from healthy individuals seronegative for Abs to HIV-1 and HIV-2. IL-4 mRNA, constitutively present in basophils, was increased after stimulation by gp120 and was inhibited cyclosporin A and tacrolimus. IL-4 and IL-13 secretion from basophils activated by gp120 was not correlated. There was a correlation between the maximum gp120- and anti-IgE-induced IL-4 release from basophils. The average $t_{1/2}$ gp120-induced IL-4 release was lower than for IL-13 release. Basophils from which IgE had been dissociated by brief exposure to lactic acid no longer released IL-4 in response to gp120 or to anti-IgE. The response to a mAb cross-linking the α -chain of high-affinity receptor for IgE (FcεRI) was unaffected by this treatment. Three human V_H3⁺ monoclonal IgM inhibited gp120-induced secretion of IL-4 from basophils. In contrast, V_H6⁺ monoclonal IgM did not inhibit the release of IL-4 induced by gp120. Synthetic peptides distant from the NH₂ and COOH termini of gp120_{MN} inhibited the activating property of gp120_{MN}. These results indicate that gp120, which acts as a viral superantigen, interacts with the V_H3 region of IgE to induce the release of IL-4 and IL-13 from human FcεRI⁺ cells. *The Journal of Immunology*, 2000, 164: 589–595.

The existence of functionally polarized human CD4⁺ T helper responses based on their profile of cytokine secretion has been established (1). T_H1 cells produce IFN- γ and IL-2, whereas T_H2 cells produce IL-4, IL-5, and IL-13. A type 2 cytokine profile secreted by CD4⁺ T cells stimulates IgE levels (2, 3). Serum IgE levels are increased in adults and in children with HIV-1 infection (4–8). Elevated IgE levels in HIV-1-infected children and adults have been associated with the progression of HIV-1 disease (9–11). Thus, IgE levels could be a marker of poor prognosis in some patients in the early or late stages of HIV-1 infection (9–11).

These observations generated great interest and some controversy. Clerici et al. (12, 13) suggested that, during the early stages of HIV-1 infection, there is a switch from “T_H1-like” toward a “T_H2-like” pattern of cytokine production. This hypothesis was indirectly supported by the observation that IL-4 gene-targeted mice lacking T_H2 responses do not develop murine AIDS (14). However, the association with another gene product is a prerequisite for nondevelopment of murine AIDS (15). Graziosi et al. (16) did not detect an overall shift in the cytokines pattern toward the T_H2 subset in lymph nodes of HIV-1-infected individuals. In another study, Maggi et al. (17) did not find a bias toward T_H2-like cytokine patterns in T cell clones from HIV-1-infected individuals during the progression to AIDS. They demonstrated a preferential depletion of CD4⁺ T_H2-type cells in the advanced phases of

HIV-1 infection and found that HIV-1 replicates preferentially in T_H2 rather than in T_H1 clones (17). Subsequent reports have added to the controversy (18–21). The apparently conflicting results could be due to 1) technical reasons, 2) the production of T_H2-like cytokines by cell types other than lymphocytes, 3) stimulation by specific superantigens, or 4) cytokines other than IL-4. In fact, most studies have focused on IL-4 and IL-10, whereas recent data show that other cytokines such as IL-13 are critical for T_H2 cell polarization (22–24).

Basophils and mast cells are the only cells that synthesize histamine and express high-affinity receptors for IgE (FcεRI)³ (25, 26). Immunologic activation of human basophils generate and secrete a restricted profile of cytokines (IL-4 and IL-13) (27–30) that are critical for T_H2 cell polarization (1–3, 22–24). Also, immunologically activated human mast cells synthesize IL-4 and IL-13 (31–33). Moreover, HIV Ags induce histamine release from basophils (34).

HIV-1 and HIV-2 destroy CD4⁺ lymphocytes, which leads to AIDS (35, 36). The entry of HIV into host cells is mediated by sequential interaction of the viral envelope glycoprotein (gp) gp120, with the CD4 gp (36, 37) and chemokine receptors on the cell surface (38–40). HIV-1 gp120 is a new member of the Ig superantigen family (41–43). Ig V_H3 gene products are the ligand for gp120 (44), and this interaction might explain the superantigen activation of human B lymphocytes in patients with AIDS (45). Protein Fv, an endogenous superantigen stimulated by viral infections in humans (46), interacts with the V_H3 domain of IgE to induce the release of IL-4 and IL-13 from human FcεRI⁺ cells (47, 48). In this study, we demonstrate that HIV-1 gp120 interacts with the V_H3 domain of IgE to induce the release of IL-4 and IL-13 from human FcεRI⁺ cells, thus acting as a viral superantigen.

University of Naples Federico II, Division of Clinical Immunology and Allergy, Naples, Italy

Received for publication August 27, 1999. Accepted for publication October 25, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by grants from the Consiglio Nazionale delle Ricerche (Target Project Biotechnology 99.00401.PF49 and 99.000216.PF31), Ministero della Sanità-Istituto Superiore Sanità (AIDS Project 1998), Ministero dell'Università e della Ricerca Scientifica Tecnologica (Rome, Italy), and by a fellowship from the Ministero della Sanità (to V.P.).

² Address correspondence and reprint requests to Dr. Gianni Marone, Division of Clinical Immunology and Allergy, University of Naples Federico II, Via S. Pansini 5, 80131 Napoli, Italy. E-mail address: marone@unina.it

³ Abbreviations used in this paper: anti-FcεRI α , monoclonal Ab anti- α -chain of high-affinity receptor for IgE; gp, glycoprotein; CsA, cyclosporin A; ICS, internal calibration standard.

Materials and Methods

Reagents

The following were purchased: 60% HClO₄ (Baker, Deventer, The Netherlands); BSA, PIPES, hyaluronidase, collagenase, chymopapain, elastase type I, human serum albumin (Sigma, St. Louis, MO); HBSS, IMDM, and FCS (Life Technologies, Grand Island, NY); deoxyribonuclease I (Calbiochem, La Jolla, CA); RPMI 1640 with 25 mM HEPES buffer, Eagle's MEM (Flow Laboratories, Irvine, Scotland); and Dextran 70, Percoll, and protein A-Sepharose (Pharmacia Biotech, Uppsala, Sweden). Rabbit anti-human-Fcε Ab was a generous gift from Drs. Teruko and Kimishige Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, CA). The mAb against the α-chain of FcεRI was a generous gift from Dr. John Hakimi (Roche Research Center, Hoffman-LaRoche, Nutley, NJ).

Buffers

The PIPES buffer used in these experiments was made up of 25 mM PIPES (pH 7.37), 110 mM NaCl, and 5 mM KCl. The mixture is referred to as P. PCG contained, in addition to P, 5 mM CaCl₂ and 1 g/L dextrose (47); pH was titrated to 7.4 with sodium bicarbonate. PBS contained 8.0 g/L NaCl, 2.89 g/L Na₂HPO₄ · 7H₂O, 0.2 g/L KH₂PO₄, 0.2 g/L KCl, (pH 7.3); TCF contained 8.0 g/L NaCl, 0.2 g/L KCl, 0.05 g/L NaH₂PO₄, 0.28 g/L NaHCO₃, and 1.0 D-glucose (pH 7.3); TGMD contained 0.25 g/L MgCl₂ · 6H₂O, 10 mg/L DNase, and 1 g/L gelatin in addition to TCF (pH 7.3) (46).

Purification of human monoclonal IgM

Monoclonal IgM were purified from the sera of patients with Waldenström's macroglobulinemia by gel permeation, as described (46). Variable regions of these monoclonal IgM were determined using a well-characterized panel of primary sequence-dependent V_H and V_K family-specific reagents that identify framework regions previously described (46).

Purification of peripheral blood basophils

Basophils were purified from peripheral blood cells of normal subjects, aged 19–47 years (mean age, 36.1 ± 5.2 years), undergoing hemapheresis. Buffy coat cell packs from healthy volunteers provided by the Immunohematology Service at the University of Naples Federico II were reconstituted in PBS containing 0.5 g/l human serum albumin and 3.42 g/L sodium citrate and loaded onto a countercurrent elutriator (model J2-21; Beckman Instruments, Fullerton, CA). Several fractions were collected, and fractions containing basophils in large numbers (>20 × 10⁶ basophils) and of improved purity (>15%) were further enriched by discontinuous Percoll gradients (49). Yields by this technique ranged from 3 to 10 × 10⁶ basophils, with a purity from 74 to 98%, as assessed by basophil staining with Alcian blue and counting in a Spiers-Levy eosinophil counter (49).

Isolation and purification of human lung mast cells

Macroscopically normal lung tissue obtained from patients undergoing thoracotomy and lung resection, mostly for lung cancer, was dissected free from pleura, bronchi, and blood vessels, minced into 3- to 8-mm fragments, and dispersed into single-cell suspensions as described (46). Yields with this technique ranged between 3 × 10⁶ and 20 × 10⁶ mast cell, with a purity between 1 and 8%. The cells were resuspended and incubated overnight in RPMI 1640 containing 25 mM HEPES, 2 mM L-glutamine, 1% gentamicin, and 10% FCS as described previously (46). Mast cells isolated from lung parenchyma were fractionated in a Beckman elutriator (Beckman Instruments). Elutriation fractions with the greatest percentage of mast cells were pooled, and further purified by flotation in Percoll density gradients as described (46). The fractions rich in mast cells were then counted by Alcian blue stain.

Histamine and cytokine release assay

Basophils (~6 × 10⁴ basophils/tube) or mast cells (~3 × 10⁴ cells/tube) resuspended in PCG (histamine release) or IMDM (cytokine release) and 0.1 ml of the cell suspension were placed in 12 × 75-mm polyethylene tubes and warmed to 37°C; 0.1 ml of each preformed stimulus for release was added, and incubation was continued at 37°C for 45 min (histamine release), 4 h (IL-4 secretion), or 18 h (IL-13 secretion) (47, 48). At the end of this step, the reaction was stopped by centrifugation (1000 × g, 22°C, 2 min), and the cell-free supernatants were stored at -20°C for subsequent assay of histamine and cytokine content. The cell-free supernatants were assayed for histamine with an automated fluorometric technique (50). Total histamine content was assessed by lysis induced by incubation of cells with 2% perchloric acid before centrifugation. To calculate histamine release as a percentage of total cellular histamine, the spontaneous release of histamine from basophils (0–8% of the total cellular histamine) and mast cells

(4–18% of the total cellular histamine) was subtracted from both the numerator and denominator (49). All values are based on means of duplicate or triplicate determinations. Replicates differed from each other in histamine content by <10%.

IL-4 and IL-13 ELISA

The harvested supernatants were assayed for IL-4 or IL-13 by using the IL-4 or IL-13 Quantikine high sensitivity kit (R&D Systems, Minneapolis, MN). The standard curve for these kits was run in the same medium used for the release experiments (47, 48).

Isolation of cellular mRNA

RNA was isolated by harvesting the basophils from culture wells and centrifuging for 30 s at 10,000 × g. After removal of the supernatants, the cell pellet was extracted with RNAzol B (Tel-Test, Friendswood, TX), which is a modified guanidinium thiocyanate single-step procedure, as described (47).

RT-PCR and quantitative PCR

An aliquot of total cellular mRNA was reversed transcribed to cDNA and PCR expanded using the GeneAmp RNA PCR Core kit (Perkin-Elmer International, Nieuwerkerk, The Netherlands), as described (47). In this protocol, RT was performed on 2 μl of the RNA extract (10–20% of the total RNA extracted). The RT mix (5 mM MgCl₂) was incubated for 20 min in a Perkin-Elmer/Cetus thermocycler (Perkin-Elmer/Cetus, Norwalk, CT) followed by 2 min at 95°C to inactivate the RT. Buffers, dNTPs (final concentration, 0.4 mM each), Amplitaq polymerase (1 U/50 μl reaction), and paired primers (0.5 μM each) were added to RT tubes (bringing MgCl₂ to 2 mM), and the PCR reaction was cycled according to the following protocol: denaturation at 95°C for 15 s, annealing at 60°C for 15 s and at 72°C for 30 s. IL-4 was cycled 56 times before a 15-min incubation at 72°C for a final extension. The primers for IL-4 and IFN-γ was performed and synthesized with commercial source (Life Technologies) based on the known cDNA sequences for the cytokines.

An aliquot (10 μl) of the reaction product was visualized on 2% agarose in buffer containing 0.5 μg/ml of ethidium bromide. As a negative control, an aliquot of each RNA sample was subjected to PCR amplification without the RT step.

In some experiments, an aliquot of total cellular mRNA was reversed-transcribed to cDNA and PCR expanded for quantitative PCR using a Cytopress Detection kit (BioSource International, Camarillo, CA). This technique is a competitive PCR in which a known copy number of an exogenous synthesized DNA, known as the internal calibration standard (ICS). The ICS was constructed to contain PCR primer binding sites identical to the IL-4 cDNA and a unique capture binding site that allows the resulting ICS amplicon to be distinguished from the IL-4 amplicon. The Cytopress kit contains IL-4 primers, one of, which is biotinylated, to be included in the PCR mix. During amplification, biotin-labeled primer is incorporated into both ICS and IL-4 amplicons. After PCR, the amplicons are denatured and hybridized to either ICS or IL-4 sequence-specific capture oligonucleotides. Capture oligonucleotides are prebound to microtiter wells. The captured biotinylated sequences are detected and quantified by the addition of an enzyme-streptavidin conjugate HRP, followed by the addition of the substrate. The signal generated in the reaction is proportional to the amount of amplicon present. Because the ICS is amplified at an efficiency identical to the IL-4 cDNA, it can serve as a standard for IL-4 cDNA quantitation. The number of copies of IL-4 in each PCR reaction is calculated from the ratio of the total OD for the IL-4-specific well to the total OD for the ICS well and the input copy number of the ICS. The following formula is used to calculate the starting copies of IL-4 cDNA in the PCR reaction: (total IL-4/total ICS OD) × 2 × input copy number of ICS = starting copy number of IL-4 cDNA. Factor 2 is used to correct for double-stranded DNA ICS. The copy number is adjusted for any dilution done on cDNA before amplification according to the manufacturer's protocol.

Statistical analysis

The results are means ± 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 (51).

Results

Effects of gp120 on IL-4 synthesis and release from human basophils

For our experiments we purified (>98%) human peripheral blood basophils from healthy individuals who were seronegative for Abs

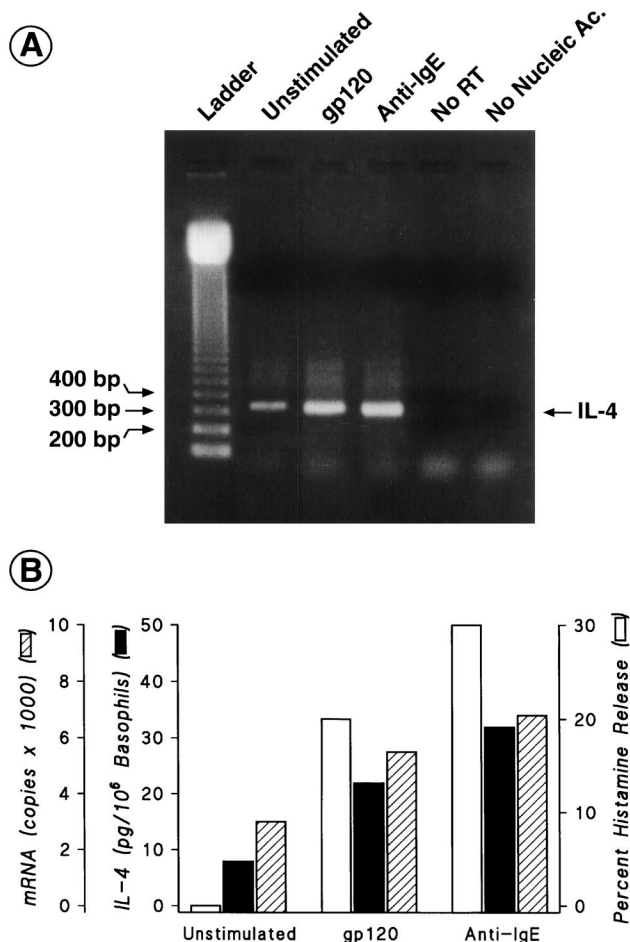


FIGURE 1. A, RT-PCR quantitative analysis of IL-4 mRNA expression in human basophils stimulated with anti-IgE (1 μ g/ml) or gp120 (10 nM). Purified basophils (> 98%) obtained from normal donors negative for HIV-1 and HIV-2 Abs were preincubated (16 h at 37°C) with recombinant human IL-3 (10 ng/ml), washed, and then incubated with recombinant human IL-3 (10 ng/ml) with or without anti-IgE or gp120 for 4 h at 37°C. IL-4 mRNA was amplified by a quantitative RT-PCR. The cDNA subjected to electrophoresis was visualized by ethidium bromide. Without cDNA (no RT) or nucleic acid (no nucleic Ac.), no PCR product was visualized. B, Effects of anti-IgE (1 μ g/ml) and gp120 (10 nM) on intracellular levels of IL-4 mRNA copies, on the extracellular protein levels of IL-4, and on the release of histamine from basophils compared with unstimulated cells.

to HIV-1 and HIV-2. We then cultured basophils with recombinant human IL-3 (10 ng/ml for 16 h), washed the cells, and challenged them with recombinant gp120 from HIV-1 (10 nM) or anti-IgE (1 μ g/ml). Fig. 1 shows a representative result of four experiments in which we examined the effects of gp120 and anti-IgE on the levels of extracellular IL-4 protein, specific IL-4 mRNA, and secretion of histamine. These experiments demonstrated that gp120 and anti-IgE increased specific IL-4 mRNA copies. gp120 and anti-IgE stimulated the release of IL-4 parallel to the secretion of histamine from basophils. In contrast, IFN- γ mRNA was not detected in any of the basophil preparations stimulated with gp120, suggesting that gp120-mediated stimulation of Fc ϵ RI⁺ cells induced only cytokines of the T_H2 profile (data not shown).

Effects of gp120 from divergent isolates of HIV-1 from different viral clades

To verify that HIV-1 envelope glycoprotein gp120 has the capacity to induce basophil activation, we compared four recombinant

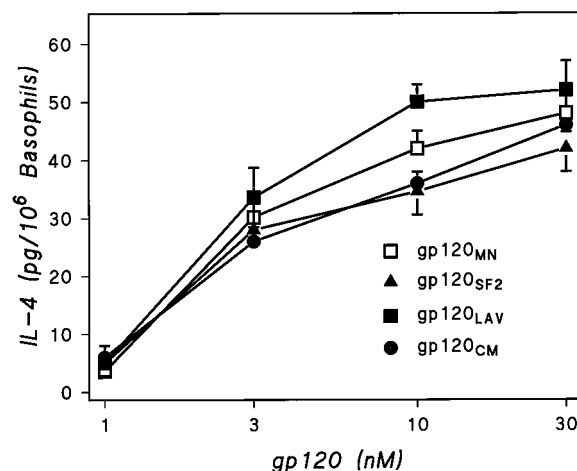


FIGURE 2. Effects of various concentrations of gp120 from four different HIV-1 isolates on IL-4 secretion from human basophils obtained from normal donors negative for HIV-1 and HIV-2 Abs. Each point represents the mean $\pm$ SEM obtained from seven experiments. Error bars are not shown when graphically too small.

gp120 (gp120_{MN}, gp120_{SF2}, gp120_{LAV}, and gp120_{CM}) derived from divergent HIV-1 isolates from different viral clades (B and E) of various geographical origins (United States, France, and Thailand). These highly divergent samples of gp120 induced IL-4 release from basophils. This implies that the capacity to induce cytokine release from basophils is a general feature of gp120, which has been maintained throughout the evolution of the virus (Fig. 2). Two unrelated gps, BSA and human serum albumin (1–100 nM), did not activate any of the basophil preparations tested (data not shown).

Kinetics of gp120-induced IL-4 and IL-13 secretion from human basophils

Fig. 3A compares the kinetics of histamine, IL-4, and IL-13 release from basophils challenged with gp120. Histamine release was complete within 5 min, IL-4 release was complete in 3–4 h, whereas IL-13 release plateaued at 18 h. Lower concentrations of gp120 consistently exerted a more potent effect on the secretion of IL-13 than on IL-4 and histamine release (Fig. 3B). Similar results were obtained with the four gp120 derived from different clades (data not shown). These data demonstrate that nanomolar concentrations of gp120 induce the secretion of IL-13, an important cytokine for the polarization of T_H2 cells (22–24), from basophils.

Cyclosporin A (CsA) and tacrolimus inhibit gp120-induced IL-4 secretion from human basophils

Two immunophilin-binding drugs, CsA and tacrolimus (FK-506), are potent inhibitors of the IgE-dependent release of proinflammatory mediators from human basophils and mast cells (52–54). We compared the effects of preincubation of low concentrations of CsA (24–800 nM) and tacrolimus (1–30 nM) on the release of histamine and the secretion of IL-4 from purified basophils activated by gp120. CsA concentration-dependently inhibited the gp120-induced release of histamine and IL-4 from basophils at concentrations as low as 24 nM (Fig. 4A). The inhibition of IL-4 release ranged from $\approx$ 30% at 24 nM to 90–95% at 240 nM, with an IC₅₀ of 31.6 ± 6.4 nM, similar to that calculated from the inhibition of histamine release (46.0 ± 9.3 nM). The effects of tacrolimus on the release of histamine and the secretion of IL-4 from human basophils were similar to those observed with CsA (Fig. 4B). However, tacrolimus was more potent than CsA with an

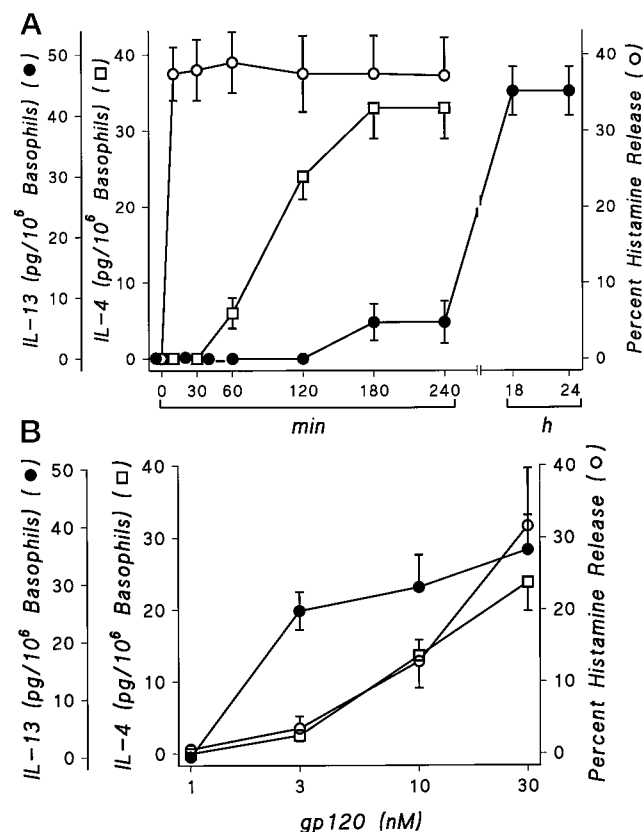


FIGURE 3. A, Kinetics of IL-4, IL-13, and histamine secretion from human basophils induced by gp120 (10 nM). Each point represents the mean $\pm$ SEM obtained from three experiments. Error bars are not shown when graphically too small. B, Effects of various concentrations of gp120 on IL-4, IL-13, and histamine release from human basophils. Each point represents the mean $\pm$ SEM obtained from four experiments. Error bars are not shown when graphically too small.

IC₅₀ for IL-4 release of 2.3 ± 0.2 nM, similar to that calculated from the inhibition of histamine release (2.0 ± 0.4 nM).

Effect of IgE stripping on gp120-induced IL-4 release

Brief exposure to low pH removes most of the IgE bound on FcεRI⁺ cells, thus greatly reducing the activating properties of IgE-mediated stimuli (46). Fig. 5 shows that brief exposure to lactic acid completely blocks the effect exerted by gp120 and by anti-IgE on IL-4 secretion from basophils. In contrast, the response to the mAb cross-linking the α-chain of FcεRI (47) was not affected by this treatment. These data are compatible with the hypothesis that gp120 activates FcεRI⁺ cells through the interaction with IgE bound on basophils.

Interaction between gp120 with different IgM myeloma proteins

To evaluate the mechanism whereby gp120 activates basophils from healthy individuals, we incubated gp120 with monoclonal IgM of different V_H families according to a recently described procedure (47). In these experiments, gp120 (10 nM) was preincubated (15 min, 37°C) with increasing concentrations (0.1–10 μg/ml) of three different preparations of monoclonal IgM V_H3⁺ (IgM M3, IgM M11, and IgM LAN) or monoclonal IgM V_H6⁺ (M14). Basophils isolated from HIV-1- and HIV-2-negative subjects were then added, and the incubation was continued for an additional 4 h at 37°C. At the end of this incubation, IL-4 in the supernatants was assayed. Fig. 6 shows that preincubation of three preparations of monoclonal IgM (M3, M11, and LAN), which pos-

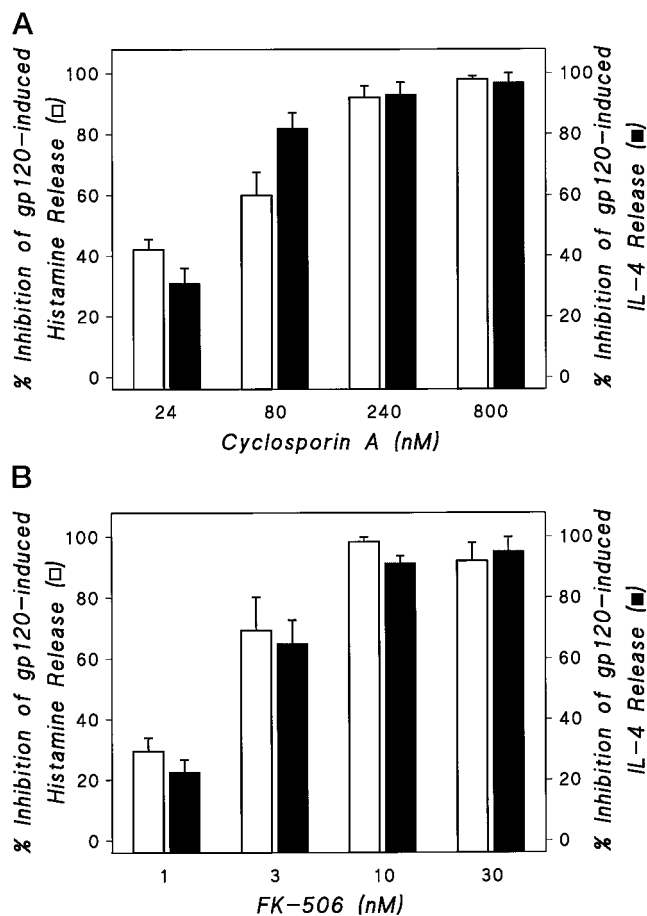


FIGURE 4. A, Effect of various concentrations of CsA on IL-4 and histamine release from human basophils obtained from normal donors negative for HIV-1 and HIV-2 Abs. Cells were preincubated for 15 min at 37°C with the indicated concentrations of CsA and then challenged (4 h at 37°C) with gp120 (3 nM). Each bar represents the mean $\pm$ SEM of percent inhibition of mediator release from three experiments. Error bars are not shown when graphically too small. B, Effect of various concentrations of tacrolimus on IL-4 and histamine release from human basophils obtained from normal donors negative for HIV-1 and HIV-2 Abs. Cells were preincubated for 15 min at 37°C with the indicated concentrations of tacrolimus and then challenged (4 h at 37°C) with gp120 (3 nM). Each bar represents the mean $\pm$ SEM of percent inhibition of mediator release from three experiments.

sess the V_H3 domain, concentration-dependently inhibited the effect of gp120 on IL-4 secretion. In contrast, a monoclonal IgM (M14), which possesses a V_H6 domain, had no effect.

gp120 from different clades also induced mediator release from mast cells isolated and purified from human lung parenchyma, and the releasing activity was inhibited by preincubation with the three V_H3⁺ monoclonal IgM, but not by IgM V_H6⁺ (Fig. 7). Thus, binding to the V_H3 domain inhibits the interaction of gp120 with IgE bound to FcεRI on basophils and lung mast cells.

Effects of synthetic peptides of gp120_{MN} on gp120_{MN}-induced IL-4 release

To map the IgE binding sites on gp120, we tested the inhibitory capacity of a panel of synthetic peptides encompassing the gp120_{MN} sequence (55). The synthetic peptides of the gp120_{MN} core motif (peptides 1959, 1960, 1985, 1988, and 1989), concentration-dependently inhibited the effect of gp120_{MN} on basophils. In contrast, peptides 1922 and 2015, which span the gp120_{MN}

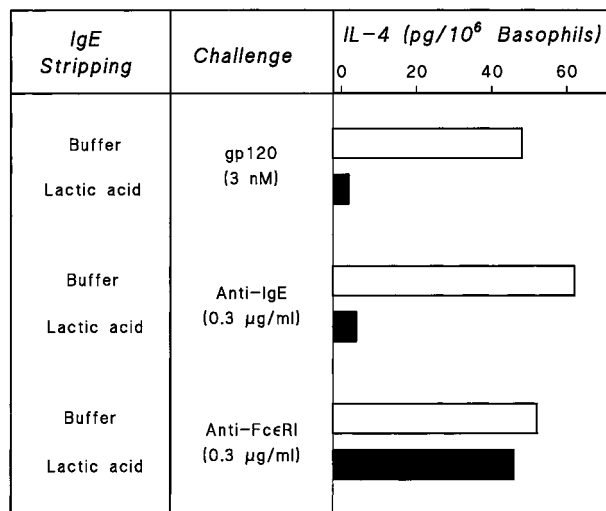


FIGURE 5. Effect of lactic acid on IL-4 release from normal human basophils induced by gp120 and anti-IgE. Purified basophils were either treated with buffer or lactic acid [0.01 M (pH 3.9), 5 min, 22°C] and washed twice. Leukocytes were then challenged (4 h at 37°C) with gp120 (3 nM), anti-IgE (0.3 µg/ml), or anti-FcεRI (0.3 µg/ml). Each bar represents the mean of IL-4 release from duplicate incubations.

amino- and COOH-terminal region, respectively, failed to induce inhibition. Each of these peptides alone neither induced nor inhibited anti-IgE-induced cytokine or histamine release from human FcεRI⁺ cells (data not shown). Our results are consistent with the hypothesis that the superantigen-binding site(s) on gp120 is formed by protein sequences of at least two regions, which span in a discontinuous fashion the constant and variable domains of the molecule (41).

Discussion

This study shows that a variety of gp120 from diverse clades induced the synthesis and release of IL-4 and IL-13 from human

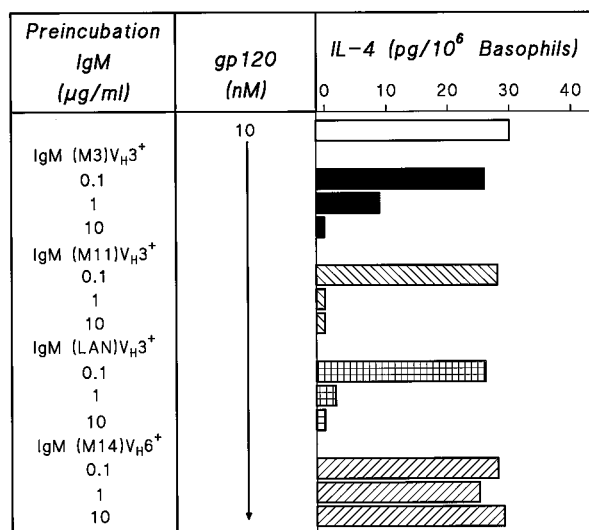


FIGURE 6. Effect of preincubation of gp120 with monoclonal IgM on IL-4 release from normal human basophils. gp120 was preincubated for 15 min at 37°C with increasing concentrations of human monoclonal IgM M3, IgM M11, IgM LAN, or IgM M14. Basophils were then added, and the incubation was continued for an additional 4 h at 37°C. Each bar represents the mean of IL-4 release from duplicate incubations.

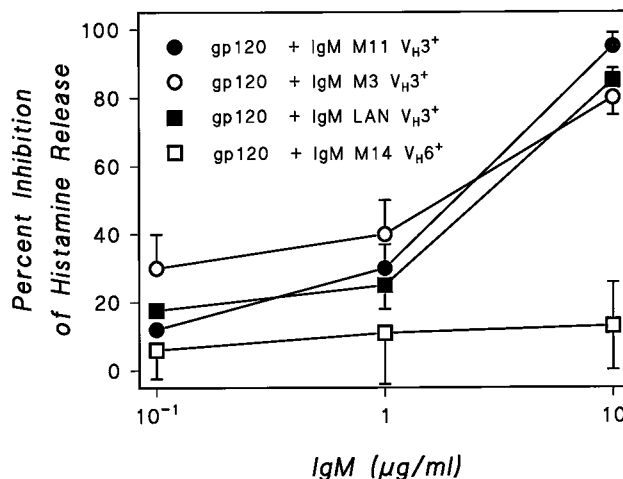


FIGURE 7. Effect of preincubation of gp120 with monoclonal IgM on histamine release from human lung mast cells. gp120 (30 nM) was preincubated for 15 min at 37°C with increasing concentrations of human monoclonal IgM M3, IgM M11, IgM LAN, or IgM M14. Lung mast cells were then added, and the incubation was continued for an additional 30 min at 37°C. Each point represents the mean percent inhibition $\pm$ SEM of histamine release from six experiments. Error bars are not shown when graphically too small.

FcεRI⁺ cells. The activity of gp120 is mediated by interaction with the V_H3 region of IgE present on human basophils and mast cells. This is the first demonstration that gp120 triggers the release of two cytokines critical for T_H2 polarization from human FcεRI⁺ cells.

The relevance of this finding is 3-fold. It suggests that during the early phase of HIV infection, which is associated with high levels of viremia and spreading of the virus (35, 36), basophils exposed to virus-bound or shed gp120 (56) might represent an initial source of IL-4 and IL-13, thereby favoring a shift from a T_H0 toward a T_H2 phenotype. In advanced HIV-1 infection when CD4⁺ T cells are decreased, FcεRI⁺ cells might also represent a significant source of T_H2-like cytokines. Therefore, during both early and advanced HIV-1 infection, basophils and mast cells might be a source of cytokines that contribute to the polarization of CD4⁺ cells toward T_H2 cells. Finally, our findings might be significant also from a quantitative viewpoint. T_H2 cells represent 0.2–2% of CD4⁺ cells (57), whereas basophils represent 1% of peripheral blood leukocytes (25). Viral Ags interact with individual T_H clones; viral superantigen gp120 can produce a rapid and massive activation of basophils via V_H3⁺ IgE. Because the V_H3 family is the largest in the human repertoire (~50%; see Refs. 42–44), it is likely that shed or virus-bound gp120 interacts with a high frequency with V_H3⁺ IgE bound to basophils of normal or early-infected individuals. Finally, the levels of IL-4 produced by human lymphocytes are about 10–20% of those generated by immunologically challenged basophils (58).

IL-4 and IL-13 released from human FcεRI⁺ cells might also indirectly (i.e., through chemokine receptors) play a role in the entry of HIV-1 into CD4⁺ cells. The chemokine receptors CXCR4 and CCR5 are major coreceptors for HIV-1 entry into CD4⁺ T cells (38–40). Up-regulation of CXCR4 by IL-4 (59) facilitates HIV-1 infection of T cells and might even be sufficient to trigger CD4⁺ T cell depletion (60). IL-4 and IL-13 trigger monocytes to produce the T cell chemoattractant protein-1 STCP-1 (or macrophage-derived chemokine), a chemokine selectively active on CCR4 receptor expressed on T_H2 cells (61). These results evoke an amplification loop of polarized T_H2 responses based on induction

of chemokine receptors by gp120-stimulated IL-4 and IL-13 from basophils. The latter observation is important because HIV-1 replicates preferentially in T_H2 rather than in T_H1 cells (17). In this context, basophils may play a role since they can rapidly produce IL-4 and IL-13 in a restricted manner without synthesizing T_H1-type cytokines (e.g., IFN- γ).

We found that the Fc ϵ RI⁺ cell-activating property is well conserved in gp120 from divergent HIV isolates from clades of different geographic locations. Consequently, conservation of these V_H3 binding sites of gp120 between viral clades could be an important mechanism by which the virus can elude the specific immune surveillance of the host.

Our previous in vitro (47, 52, 53) and in vivo (54) studies demonstrated that immunophilin-binding drugs (i.e., CsA and tacrolimus) exert anti-inflammatory effects by inhibiting the IgE-dependent release of proinflammatory mediators from human Fc ϵ RI⁺ cells. In this study, we demonstrate that CsA and tacrolimus concentration-dependently inhibit also the gp120-induced release of cytokines from human basophils. These findings imply that some of the effects exerted by this class of anti-inflammatory/immunosuppressive drugs on HIV-1 replication (62, 63) reflect their actions on IL-4 secretion from Fc ϵ RI⁺ cells. T cells with a T_H2-oriented cytokine profile are more susceptible to HIV-1 infection than are T_H1-oriented cells (17). This is due to up-regulation of CXCR4 receptors induced by IL-4 on T cells (59). The inhibitory effect of CsA and tacrolimus on IL-4 secretion from Fc ϵ RI⁺ cells might be an additional mechanism whereby this class of compounds exerts antiviral activity (62, 63).

In conclusion, we provide the first evidence that gp120 from different viral clades induces the release of IL-4 and IL-13 from human Fc ϵ RI⁺ cells. Because HIV-1 enters the body predominantly through mucosal surfaces and because the early phases of infection are associated with high levels of viremia, mast cells and basophils can be exposed to shed or virus-bound gp120. This suggests that Fc ϵ RI⁺ cells might be a novel source of T_H2 cytokines, thus contributing to the dysregulation of the immune system in HIV-1 infection. The latter observation might reconcile the apparently conflicting results of several investigators (12, 13, 16–21). In fact, it highlights the importance of a specific viral superantigen, gp120, acting on cell types other than lymphocytes in the production of T_H2-like cytokines. This novel observation might be relevant in the design of drugs selectively acting on Fc ϵ RI⁺ cells in the treatment of HIV-1-infected subjects.

Acknowledgments

We thank Dr. J.-P. Bouvet for providing human monoclonal IgM V_H3⁺ and IgM V_H6⁺. We thank the AIDS National Institute of Allergy and Infectious Disease, Research and Reference Reagent Program, Division of AIDS, National Institute of Health for the gift of recombinant gp120s, and Jean Gilder for editing the text. This paper is dedicated to Rita Levi-Montalcini who first suggested an involvement of Fc ϵ RI⁺ cells in HIV-1 infection.

References

- Romagnani, S. 1994. Lymphokine production by human T cells in disease states. *Annu. Rev. Immunol.* 12:227.
- Del Prete, G., E. Maggi, P. Parronchi, I. Chretien, A. Tiri, D. Macchia, M. Ricci, J. Banachereau, J. De Vries, and S. Romagnani. 1988. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their superantigens. *J. Immunol.* 140:4193.
- Abbas, A. K., K. M. Murphy, and A. Sher. 1996. Functional diversity of helper T lymphocytes. *Nature* 383:787.
- Lucy, D. R., R. A. Zajac, G. P. Melcher, C. A. Butzin, and R. N. Boswell. 1990. Serum IgE levels in 622 persons with human immunodeficiency virus infection: IgE elevation with marked depletion of CD4⁺ T-cells. *AIDS Res. Hum. Retroviruses* 6:427.
- Shor-Posner, G., M. J. Miguez-Burbano, Y. Lu, D. Feaster, M. A. Fletcher, H. Sauberlich, and M. K. Baum. 1995. Elevated IgE level in relationship to nutritional status and immune parameters in early human immunodeficiency virus-1 disease. *J. Allergy Clin. Immunol.* 95:886.
- Paganelli, R., E. Scala, I. Mezzaroma, E. Pinter, G. D'Offizi, E. Fanale-Belasio, R. M. Rosso, I. J. Ansotegui, F. Pandolfi, and F. Aiuti. 1995. Immunologic aspects of hyperimmunoglobulinemia E-like syndrome in patients with AIDS. *J. Allergy Clin. Immunol.* 95:995.
- Koutsoukoulis, A., R. P. Nelson, E. Fernandez-Caldas, E. N. Brigino, M. Seleznick, R. A. Good, and R. F. Lockey. 1996. Serum total and specific IgE levels in children infected with human immunodeficiency virus. *J. Allergy Clin. Immunol.* 97:692.
- Secord, E. A., G. I. Kleiner, D. L. Auci, T. Smith-Norowitz, S. Chice, A. Finkielstein, M. Nowakowski, S. Fikrig, and G. Durkin. 1996. IgE against HIV proteins in clinically healthy children with HIV disease. *J. Allergy Clin. Immunol.* 98:979.
- Viganò, A., N. Principi, L. Crupi, J. Onorato, G. V. Zuccotti, and A. Salvaggio. 1995. Elevation of IgE in HIV-infected children and its correlation with the progression of disease. *J. Allergy Clin. Immunol.* 95:627.
- Israël-Biet, D., F. Labrousse, J. M. Tourani, H. Sors, J. M. Andrieu, and P. Even. 1992. Elevation of IgE in HIV-infected subjects: a marker of poor prognosis. *J. Allergy Clin. Immunol.* 89:68.
- Rancinan, C., P. Morlat, G. Chene, S. Guez, A. Baquay, J. Beylot, and R. Salamon. 1998. IgE serum level: a prognostic marker for AIDS in HIV-infected adults. *J. Allergy Clin. Immunol.* 102:329.
- Clerici, M., F. T. Hakim, D. J. Venzon, S. Blatt, C. W. Hendrix, T. A. Wynn, and G. M. Shearer. 1993. Changes in interleukin-2 and interleukin-4 production in asymptomatic, human immunodeficiency virus-seropositive individuals. *J. Clin. Invest.* 91:759.
- Clerici, M., and G. M. Shearer. 1993. A Th1-Th2 switch is a critical step in the etiology of HIV infection. *Immunol. Today* 14:107.
- Kanagawa, O., B. A. Vaupel, S. Gayama, G. Koehler, and M. Kopf. 1993. Resistance of mice deficient in IL-4 to retrovirus-induced immunodeficiency syndrome (MAIDS). *Science* 262:240.
- Morawetz, R. A., T. M. Doherty, N. A. Giese, J. W. Hartley, W. Müller, R. Kühn, K. Rajewsky, R. Coffman, and H. C. Morse III. 1994. Resistance to murine acquired immunodeficiency syndrome (MAIDS). *Science* 265:264.
- Graziosi, C., G. Pantaleo, K. R. Gant, J. P. Fortin, J. F. Demarest, O. J. Cohen, R. P. Sékaly, and A. S. Fauci. 1994. Lack of evidence for the dichotomy of T_H1 and T_H2 predominance in HIV-infected individuals. *Science* 265:248.
- Maggi, E., M. Mazzetti, A. Ravina, F. Annunzio, M. De Carli, M. P. Piccinni, R. Manetti, M. Carbonari, A. M. Pesce, G. Del Prete, and S. Romagnani. 1994. Ability of HIV to promote a T_H1 to T_H0 shift and to replicate preferentially in T_H2 and T_H0 cells. *Science* 265:244.
- Meygaard, L., S. A. Otto, I. P. M. Keet, R. A. W. Van Lier, and F. Miedema. 1994. Changes in cytokine secretion patterns of CD4⁺ T-cell clones in human immunodeficiency virus infection. *Blood* 12:4262.
- Barcellini, W., G. P. Rizzardi, M. O. Borghi, C. Fain, A. Lazzarin, and P. L. Meroni. 1994. TH1 and TH2 cytokine production by peripheral blood mononuclear cells from HIV-infected patients. *AIDS* 8:757.
- Klein, S. A., J. M. Doherty, T. S. Doherty, M. Pape, O. G. Ottman, E. B. Helm, D. Hoelzer, and R. Rossol. 1997. Demonstration of the Th1 to Th2 cytokine shift during the course of HIV-1 infection using cytoplasmic cytokine detection on single cell level by flow cytometry. *AIDS* 11:1111.
- Fakoya, A., P. M. Matear, E. Filley, G. A. W. Rook, J. Stanford, R. J. C. Gilson, N. Beecham, I. V. D. Weller, and A. Vyakarnam. 1997. HIV infection alters the production of both type 1 and 2 cytokines but does not induce a polarized type 1 or 2 state. *AIDS* 11:1445.
- Emson, C. L., S. E. Bell, A. Jones, W. Wisden, and A. N. J. McKenzie. 1998. Interleukin (IL)-4-independent induction of immunoglobulin (Ig)E, and perturbation of T cell development in transgenic mice expressing IL-13. *J. Exp. Med.* 188:399.
- Wills-Karp, M., J. Luyimbazi, X. Xu, B. Schofield, T. Y. Neben, C. L. Karp, and D. D. Donaldson. 1998. Interleukin-13: central mediator of allergic asthma. *Science* 282:2258.
- Grünig, G., M. Warnock, A. E. Wakil, R. Venkayya, F. Brombacher, D. M. Rennick, D. Sheppard, M. Mohrs, D. D. Donaldson, R. M. Locksley, and D. B. Corry. 1998. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 282:2261.
- Galli, S. J., 1993. New concepts about the mast cell. *N. Engl. J. Med.* 328:257.
- Metcalfe, D. D., D. Baram, and Y. A. Mekori. 1997. Mast cells. *Physiol. Rev.* 77:1033.
- Brunner, T., C. H. Heusser, and C. A. Dahinden. 1993. Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. *J. Exp. Med.* 177:605.
- MacGlashan, D., J. M. White, S. K. Huang, S. J. Ono, J. T. Schroeder, and L. M. Lichtenstein. 1994. Secretion of IL-4 from human basophils: the relationship between IL-4 mRNA and protein in resting and stimulated basophils. *J. Immunol.* 152:3006.
- Ochsenberger, B., G. C. Daepf, S. Rihs, and C. A. Dahinden. 1996. Human blood basophils produce interleukin-13 in response to IgE-receptor-dependent and-independent activation. *Blood* 88:3028.
- Li, H., T. C. Sim, and R. Alam. 1996. IL-13 released by and localized in human basophils. *J. Immunol.* 156:4833.
- Bradding, P., I. H. Feather, P. H. Howarth, R. Mueller, J. A. Roberts, K. Britten, J. P. A. Bews, T. C. Hunt, Y. Okayama, C. H. Heusser, G. R. Bullock,

- M. K. Church, and S. T. Holgate. 1992. Interleukin 4 is localized to and released by human mast cells. *J. Exp. Med.* 176:1381.
32. Okayama, Y., C. Petit-Frère, O. Kassel, A. Semper, D. Quint, M. J. Tunon-de-Lara, P. Bradding, S. T. Holgate, and M. K. Church. 1995. IgE-dependent expression of mRNA for IL-4 and IL-5 in human lung mast cells. *J. Immunol.* 155:1796.
 33. Burd, P. R., W. C. Thompson, E. E. Max, and F. C. Mills. 1995. Activated mast cells produce interleukin 13. *J. Exp. Med.* 181:1373.
 34. Pedersen, M., C. M. Nielsen, and H. Permin. 1991. HIV antigen-induced release of histamine from basophils from HIV infected patients. *Allergy* 46:206.
 35. Pantaleo, G., C. Graziosi, and A. S. Fauci. 1993. The immunopathogenesis of human immunodeficiency virus infection. *N. Engl. J. Med.* 328:327.
 36. Fauci, A. S. 1996. Host factors and the pathogenesis of HIV-infected disease. *Nature* 384:529.
 37. Kwong, P. D., R. Wyatt, J. Robinson, R. W. Sweet, J. Sodroski, and W. A. Hendrickson. 1998. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* 393:648.
 38. Feng, Y., C. C. Broder, P. E. Kennedy, and E. A. Berger. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 272:872.
 39. Dragic, T., V. Litwin, G. P. Allaway, S. R. Martin, Y. Huang, K. A. Nagashima, C. Cayan, P. J. Maddon, R. A. Koup, J. P. Moore, and W. A. Paxton. 1996. HIV-1 entry into CD4⁺ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* 381:667.
 40. Ostrowski, M. A., S. J. Justement, A. Catanzaro, C. A. Hallahan, L. A. Ehler, S. B. Mizell, P. N. Kumar, J. A. Mican, T. W. Chun, and A. S. Fauci. 1998. Expression of chemokine receptors CXCR4 and CCR5 in HIV-1-infected and uninfected individuals. *J. Immunol.* 161:3195.
 41. Karray, S., and M. Zouali. 1997. Identification of the B cell superantigen-binding site of HIV-1 gp120. *Proc. Natl. Acad. Sci. USA* 94:1356.
 42. Silverman, G. J. 1997. B-cell superantigens. *Immunol. Today* 18:379.
 43. Karray, S., L. Juompan, R. C. Maroun, D. Isenberg, G. J. Silverman, and M. Zouali. 1998. Structural basis of the gp120 superantigen-binding site on human immunoglobulins. *J. Immunol.* 161:6681.
 44. Berberian, L., L. Goodglick, T. J. Kipps, and J. Braun. 1993. Immunoglobulin V_H3 gene products: natural ligands for HIV gp120. *Science* 261:1588.
 45. Schnittman, S. M., H. C. Lane, S. E. Higgins, T. Folks, and A. S. Fauci. 1986. Direct polyclonal activation of human B lymphocytes by the acquired immune deficiency syndrome virus. *Science* 233:1084.
 46. Patella, V., J.-P. Bouvet, and G. Marone. 1993. Protein Fv produced during viral hepatitis is a novel activator of human basophils and mast cells. *J. Immunol.* 151:5685.
 47. Patella, V., A. Giuliano, J.-P. Bouvet, and G. Marone. 1998. Endogenous superallergen protein Fv induces IL-4 secretion from human FcεRI⁺ cells through interaction with the V_H3 Region of IgE. *J. Immunol.* 161:5647.
 48. Patella, V., A. Giuliano, G. Florio, J.-P. Bouvet, and G. Marone. 1999. Endogenous superallergen protein Fv interacts with the V_H3 region of IgE to induce cytokine secretion from human basophils. *Int. Arch. Allergy Immunol.* 118:197.
 49. Patella, V., V. Casolaro, A. Ciccarelli, G. R. Pettit, M. Columbo, and G. Marone. 1995. The antineoplastic bryostatins affect human basophils and mast cells differently. *Blood* 85:1272.
 50. Siraganian, R. P. 1974. An automated continuous-flow system for the extraction and fluorometric analysis of histamine. *Anal. Biochem.* 57:383.
 51. Snedecor, G. W., and W. G. Cochran. 1980. *Statistical Methods*. Iowa State Univ. Press, Ames.
 52. Cirillo, R., M. Triggiani, L. Siri, A. Ciccarelli, G. R. Pettit, M. Condorelli, and G. Marone. 1990. Cyclosporin A rapidly inhibits mediator release from human basophils presumably by interacting with cyclophilin. *J. Immunol.* 144:3891.
 53. de Paulis, A., R. Cirillo, A. Ciccarelli, M. Condorelli, and G. Marone. 1991. FK-506, a potent novel inhibitor of the release of proinflammatory mediators from human FcεRI⁺ cells. *J. Immunol.* 146:2374.
 54. Casolaro, V., G. Spadaro, V. Patella, and G. Marone. 1993. In vivo characterization of the anti-inflammatory effect of cyclosporin A on human basophils. *J. Immunol.* 151:5563.
 55. Goodlick, L., N. Zevit, M. S. Neshat, and J. Braun. 1995. Mapping the Ig superantigen-binding site of HIV-1 gp120. *J. Immunol.* 155:5151.
 56. Gelderblom, H. R., E. H. S. Hausmann, M. Özel, G. Pauli, and M. A. Koch. 1987. Fine structure of human immunodeficiency virus (HIV) and immunolocalization of structural proteins. *Virology* 156:171.
 57. Andrew, D. P., M. Chang, J. McNinch, S. T. Wathen, M. Rihane, J. Tseng, J. P. Spellberg, and C. G. Elias III. 1998. STCP-1 (MDC) CC chemokine acts specifically activated Th2 lymphocytes and is produced by monocytes on stimulation with Th2 cytokines IL-4 and IL-13. *J. Immunol.* 161:5027.
 58. Schroeder, J. T., B. P. Howard, M. K. Jenkins, A. Kagey-Sobotka, L. M. Lichtenstein, and D. W. MacGlashan, Jr. 1998. IL-4 secretion and histamine release by human basophils are differentially regulated by protein kinase C activation. *J. Leukocyte Biol.* 63:692.
 59. Galli, G., F. Annunziato, C. Mavilia, P. Romagnani, L. Cosmi, R. Manetti, C. Pupilli, E. Maggi, and S. Romagnani. 1998. Enhanced HIV expression during Th2-oriented responses explained by the opposite regulatory effect of IL-4 and IFN-γ on fusin/CXCR4. *Eur. J. Immunol.* 28:3280.
 60. Penn, M. L., J.-C. Grivel, B. Schramm, M. A. Goldsmith, and L. Margolis. 1999. CXCR4 utilization is sufficient to trigger CD4⁺ T cell depletion in HIV-1-infected human lymphoid tissue. *Proc. Natl. Acad. Sci. USA* 96:663.
 61. Bonecchi, R., S. Sozzani, J. T. Stine, W. Luini, G. D'Amico, P. Allavena, D. Chantry, and A. Mantovani. 1998. Divergent effects of interleukin-4 and interferon-γ on macrophage-derived chemokine production: an amplification circuit of polarized T helper 2 responses. *Blood* 92:2668.
 62. Karpas, A., M. Lowdell, S. K. Jacobson, and F. Hill. 1992. Inhibition of human immunodeficiency virus and growth of infected T cells by the immunosuppressive drugs cyclosporin A and FK 506. *Proc. Natl. Acad. Sci. USA* 89:8351.
 63. Thali, M., A. Bukovsky, E. Kondo, B. Rosenwirth, C. T. Walsh, J. Sodroski, and H. G. Göttinger. 1994. Functional association of cyclophilin A with HIV-1 virions. *Nature* 372:363.