

Immunologia. — *Envelope gp120 of HIV-1 binds to and activates human basophils.* Nota di VINCENZO PATELLA, GIOVANNI FLORIO, ANGELICA PETRAROLI, MASSIMO TRIGGIANI e GIANNI MARONE, presentata (*) dal Socio G. Vecchio.

ABSTRACT. — Envelope glycoprotein gp120 isolated from different clades of HIV-1 is a potent stimulus for IL-4 and IL-13 release from basophils purified from healthy donors seronegative for antibodies to HIV-1 and HIV-2. The expression of IL-4 mRNA, constitutively present in basophils, was increased after stimulation with gp120 and was inhibited by cyclosporin A and tacrolimus. A significant correlation was found between the maximum release of IL-4 induced by gp120 and by an antibody anti-human IgE. IgE removal from basophils by brief exposure to lactic acid abolished IL-4 release in response to both gp120 and anti-IgE. Preincubation of gp120 with three different human monoclonal IgM V_H3^+ but not with monoclonal IgM V_H6^+ inhibited gp120-induced secretion of IL-4 from basophils. Preincubation of basophils with synthetic peptides distant from the NH_2 and $COOH$ -termini of gp120_{MN} resulted in the inhibition of gp120_{MN} induced IL-4 release. These results indicate that gp120 acts as a viral immunoglobulin superantigen by interacting with the V_H3 region of IgE and induces the expression and the release of IL-4 and IL-13 from human basophils.

KEY WORDS: AIDS/HIV; Basophils/Mast Cells; Cytokines; Infectious Immunity; Virus.

RIASSUNTO. — *La glicoproteina gp120 del virus HIV-1 induce la sintesi di citochine dai granulociti basofili umani.* La glicoproteina gp120 ottenuta da differenti cladi del virus HIV-1 è un potente attivatore della secrezione di interleuchina 4 (IL-4) ed interleuchina 13 (IL-13) dai basofili purificati dal sangue periferico di donatori sieronegativi per anticorpi anti-HIV-1 e anti-HIV-2. L'incubazione con gp120 aumenta l'espressione di RNA messaggero per IL-4, costitutivamente presente nei basofili umani. L'attivazione indotta da gp120 viene inibita dalla preincubazione dei basofili con ciclosporina A e con tacrolimus. La cinetica della secrezione di IL-4 indotta dalla gp120 è sovrapponibile a quella indotta da un anticorpo monoclonale anti-IgE umane. Diversi tipi di gp120 inducono la secrezione di istamina dai basofili e dai mastociti umani isolati dal parenchima polmonare. La rimozione delle IgE di membrana, attraverso una breve incubazione dei basofili o dei mastociti con acido lattico, abolisce la risposta di queste cellule alla gp120 ed all'anticorpo anti-IgE. La preincubazione della gp120 con IgM monoclonali umane V_H3^+ , ma non con IgM monoclonali V_H6^+ , inibisce la secrezione di IL-4. La preincubazione dei basofili con peptidi sintetici che riproducono sequenze distanti dall'estremo carbossi- ed amino-terminale della struttura primaria della gp120 inibiscono la secrezione di IL-4 indotta dalla gp120. Questi risultati dimostrano che la gp120 agisce come un superantigene virale interagendo con la regione V_H3^+ delle IgE di membrana delle cellule $Fc_\epsilon RI^+$. La sintesi di IL-4 e di IL-13 dai basofili e dai mastociti umani potrebbe svolgere un ruolo importante nella replicazione di HIV-1 e nella progressione dell'AIDS.

INTRODUCTION

Envelope glycoprotein of human immunodeficiency virus type 1 (HIV-1) is synthesized as a precursor, gp160, which is subsequently cleaved by proteinases to gp120 and gp41 (Kowalski *et al.*, 1987). Diverse clades of HIV-1 identified from seropositive patients from divergent geographic area synthesize different types of gp120. Gp120

(*) Nella seduta del 9 marzo 2001.

plays a central role in HIV-1 infection by binding to CD4 glycoprotein on immune cells (Fauci, 1996; Kwong *et al.*, 1998). Upon binding to CD4, gp120 undergoes conformational changes that allow its interaction with several chemokine receptors (CCR5, CXCR4, CCR3, etc.) expressed on human T cells and macrophages (Dragic *et al.*, 1996; Feng *et al.*, 1996; Ostrowski *et al.*, 1998).

It is well established that human CD4⁺ T cells produce functionally polarized responses based on the profile of cytokines released (Abbas *et al.*, 1996). T_H1 cells produce IFN- γ and IL-2, whereas T_H2 cells produce predominantly IL-4, IL-5, and IL-13 (Del Prete *et al.*, 1988). Cytokine produced by T_H2 cells have been associated with an increased IgE production (Lucey *et al.*, 1990; Shor-Posner *et al.*, 1995). Serum IgE levels are often increased in children and adults with HIV-1 infection (Lucey *et al.*, 1990; Paganelli *et al.*, 1995; Shor-Posner *et al.*, 1995; Koutsonikolis *et al.*, 1996; Secord *et al.*, 1996) and an increased IgE level has been associated with the progression of HIV-1 disease (Lucey *et al.*, 1990; Shor-Posner *et al.*, 1995). Thus, IgE level is a marker of poor prognosis in the early or late stages of HIV-1 infection (Israël-Biet *et al.*, 1992; Viganò *et al.*, 1995; Rancinan *et al.*, 1998).

Clerici and colleagues suggested that, in the early stage of HIV-1 infection, there is a switch from «T_H1-like» toward a «T_H2-like» pattern of cytokine production (Clerici and Shearer, 1993; Clerici *et al.*, 1993). However Graziosi, Fauci, and collaborators were unable to detect an overall shift to T_H2 cytokine pattern in lymph nodes of HIV-1-infected individuals (Graziosi *et al.*, 1994). In addition, Maggi and collaborators did not consistently find a T_H2-like cytokine profile produced by T-cell clones obtained from HIV-1-infected individuals during progression to AIDS (Maggi *et al.*, 1994). Although it has been reported that HIV-1 replicates preferentially in T_H2 rather than in T_H1 clones (Maggi *et al.*, 1994; Rancinan *et al.*, 1998), this hypothesis is, at least in part, still controversial (Kanagawa *et al.*, 1993; Morawetz *et al.*, 1994). These conflicting results may be due a number of reasons, including the production of T_H2 cytokines by cells other than lymphocytes, T cell activation by specific HIV-1 superantigens, or the production of other cytokines critical for T_H2 cell polarization such as IL-13.

Basophil and mast cell are the only cells in humans constitutively expressing the high affinity receptor for IgE (Fc ϵ RI) (Marone, 1995*a,b*; Marone *et al.*, 2000). Several studies have demonstrated that Fc ϵ RI⁺ cells are involved in defence against host invasion parasites and bacterial and viral infections (Florio *et al.*, 2000; Patella *et al.*, 2000). Increasing evidence suggests that basophils and mast cells may have a role in HIV-1 infection. Furthermore, immunologically activated basophils (Brunner *et al.*, 1993; Ochensberger *et al.*, 1996) and mast cells (Bradding *et al.*, 1992; Burd *et al.*, 1995) produce IL-4 and IL-13, two key cytokines for the induction of a T_H2-like response (Abbas *et al.*, 1996). Interestingly basophils and mast cells may be activated by superantigens interacting with the V_H3 domain of surface IgE. For example, protein Fv, an endogenous superantigen produced in patient with viral infection, induced IL-4 and IL-13 secretion from human basophils (Patella *et al.*, 1998). It has been shown that glycoprotein of HIV-1 gp120

is a superantigen that binds to V_H3 region of immunoglobulin inducing a polyclonal activation of human B cells (Karray and Zouali, 1997; Karray *et al.*, 1998). In addition, HIV-1 antigens induce histamine release from human basophils (Pedersen *et al.*, 1991). Together, these observations suggest a possible interaction between gp120 and human Fc_εRI⁺ cells (basophils and mast cells). We have studied this interaction and tested the hypothesis that gp120 may induce the expression and release of IL-4 and IL-13 from Fc_εRI⁺ cells purified from healthy individuals seronegative for antibodies to HIV-1 and HIV-2.

MATERIALS AND METHODS

Reagents.

The following were purchased: PIPES buffer (Sigma Chemical Co., St. Louis, MO); Hanks' balanced salt solution (HBSS), FCS and IMDM (GIBCO, Grand Island, NY); RPMI 1640 with 25 mM Hepes buffer, Eagle's minimum essential medium (Flow Laboratories, Irvine, Scotland) (Calbiochem-Behring Corp., La Jolla, CA); Cyclosporin A was a gift of Novartis (Milan). Recombinant gp120s 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, MD). Rabbit anti-IgE was a gift of Drs. Teruko and Kimishige Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, CA). The monoclonal antibody against the α-chain of Fc_εRI (anti-Fc_εRI) was a gift of 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, 5 mM KCl. The mixture is referred to as P. PCG contains, in addition to P, 5 mM CaCl₂ and 1 g/liter dextrose (Patella *et al.*, 1995).

Purification of human basophils.

Basophils were purified from «buffy coat» cell packs from healthy donors seronegative for HIV-1 and HIV-2 antibodies provided by the Immunohematology Service of the University of Naples «Federico II». Leukocytes resuspended in PBS containing 0.5 g/l human serum albumin (HSA) and 3.42 g/l Na citrate, were loaded onto a counter-current elutriator (model J2-21, Beckman Instruments, Inc., Fullerton, CA). Fractions containing the largest number of basophils ($> 10 \times 10^6$) of the highest purity ($> 15\%$) were further enriched by flotation over discontinuous Percoll gradients (Patella *et al.*, 1995). Recovery of basophils with this technique ranged from 3 to 10×10^6 cells and basophils purity was between 74% to 98%, as assessed by staining with Alcian blue (Patella *et al.*, 1995). Basophils were cultured (37 °C, 16 h) in the presence of human recombinant IL-3 (10 ng/ml) before the addition of stimuli.

Purification of lung mast cells.

Macroscopically normal lung tissue from patients undergoing thoracotomy and lung resection, was dissected free from pleura, bronchi, and blood vessels, minced into 3-8 mm fragments and dispersed into single cell suspension as described (Patella *et al.*, 1993). Recovery of mast cells ranged between 8×10^6 and 20×10^6 , 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% gentamycin, and 10% FCS as previously described (Patella *et al.*, 1993). Mast cells were enriched by countercurrent elutriation and fractions with the highest percentage of mast cells were pooled and further purified by flotation over Percoll density gradients as described (Patella *et al.*, 1993). At the end of these procedures, recovery of mast cells was $2-6 \times 10^6$ with a 75-95% purity.

Human monoclonal IgM.

Monoclonal IgM were purified from the sera of patients with Waldenstrom's macroglobulinemia by gel permeation, as described (Patella *et al.*, 1993). 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 (Patella *et al.*, 1998).

Histamine and cytokine release assay.

Basophils ($\approx 6 \times 10^4$ cells/tube) or mast cells ($\approx 3 \times 10^4$ cells/tube) were resuspended in Pipes buffer containing 1 mM CaCl_2 (histamine release) or IMDM (cytokine release). Gp120 isolated from different clades was added and incubation was continued at 37 °C for 45 min (histamine release), 4 hours (IL-4 secretion), or 18 hours (IL-13 secretion) (Patella *et al.*, 1998, 1999). The reaction was stopped by centrifugation ($1000 \times g$, 22 °C, 2 min) and the cell-free supernatants were stored at -20 °C for subsequent assay of histamine and cytokine content. Histamine assay was performed by automated fluorometric technique (Siraganian, 1974). IL-4 and IL-13 assays were performed using high sensitivity ELISA kits (R&D Systems, Minneapolis, MN) (Patella *et al.*, 1998).

Reverse-transcription-PCR and quantitative PCR.

At the end of each experiment, basophils were centrifuged for 30 s at 10,000 *g*. RNA was extracted the cell pellet with the RNazol B technique (Tel-test Inc., Friendswood, TX) (Patella *et al.*, 1998). 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, NL). In some experiments, an aliquot of total cellular mRNA was reversed-transcribed to cDNA and PCR expanded for quantitative PCR using a CytospressTM Detection Kit (BioSource International Inc., Camarillo, CA, USA). This technique is a competitive PCR in which a known copy number of an exogenously

synthesized DNA, indicated as the «internal calibration standard» (ICS), is amplified. 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 Cytotpress™ 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 pre-bound to microtiter wells. The captured biotinylated sequences are detected and quantified by the addition of an enzyme-streptavidin conjugate horseradish peroxidase (HRP), followed by the addition of the substrate. The signal generated in the reaction is proportional to the amount of amplicon present. Since 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:

$$\frac{\text{Total IL-4 OD}}{\text{Total ICS OD}} \times 2 \times \text{Input copy number of ICS} = \text{Starting copy number of IL-4 cDNA}$$

Factor 2 is used to correct for double-stranded DNA ICS. The copy number is adjusted for any dilution done on cDNA prior to amplification according to the manufacturer's protocol.

Statistical analysis.

The results are expressed as the mean \pm SEM.

RESULTS AND DISCUSSION

Gp120 Induces the Synthesis and Release of IL-4 and IL-13 from Human Basophils.

Purified human basophils from healthy donors seronegative for antibodies anti- HIV-1 and -HIV-2, were cultured with rhIL-3 and challenged with recombinant gp120 from HIV-1 (30 nM) or anti-IgE (1 μ g/ml). Figure 1 shows a representative experiment in which we examined the effects of gp120 and anti-IgE on the release of IL-4 protein and secretion of histamine. Both gp120 and anti-IgE induced the rapid release of IL-4 as well as the secretion of histamine from basophils. In addition, gp120 and anti-IgE increased the expression of IL-4 mRNA (fig. 2). In contrast, IFN- γ mRNA was not detected in any of the basophil preparations stimulated with gp120, suggesting that gp120 may selectively induce T_H2-type cytokines in basophils (data not shown).

To confirm this hypothesis, experiments were performed to determine whether gp120 also activated normal human basophils to produce IL-13, another T_H2-inducing cytokine (Emson *et al.*, 1998; Grünig *et al.*, 1998; Wills-Karp *et al.*, 1998). The results of these experiments indicate that, in addition to histamine and IL-4, gp120 also induces the

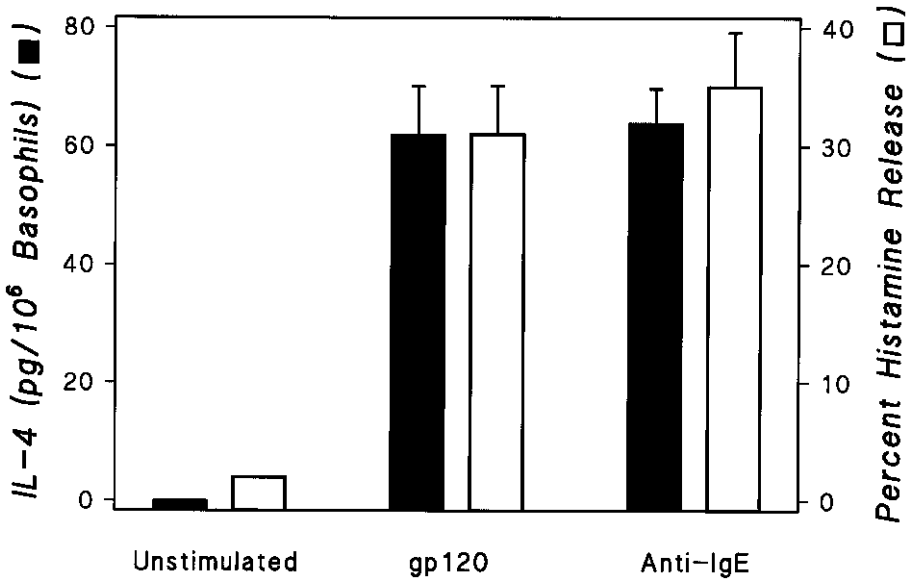


Fig. 1. – Effects of anti-IgE and of gp120 on the extracellular protein levels of IL-4 and on the release of histamine from basophils, compared with unstimulated cells. Purified basophils (> 98%) obtained from normal donors negative for HIV-1 and HIV-2 antibodies were preincubated (16 h at 37 °C) with recombinant human IL-3 (10 ng/ml), washed, and then incubated with recombinant human IL-3 (10 ng/ml) with or without anti-IgE (1 µg/ml) or gp120 (30 nM) for 4 h at 37 °C.

release of IL-13. However, while histamine release was complete within 5 min, the release of IL-4 and of IL-13 was maximal 4 and 18 hours, respectively, after the addition of gp120 (fig. 3). These results suggest that, during the early phase of HIV infection, associated with high levels of viremia and spreading of the virus (Pantaleo *et al.*, 1993; Fauci, 1996), basophils exposed to virus-bound or shed gp120 (Gelderblom *et al.*, 1987) might represent an initial source of IL-4 and IL-13. These cytokines may favour a shift from a T_H0 toward a T_H2 phenotype. In addition, human basophils may represent an additional source of IL-4 and IL-13 in advanced HIV-1 infection, when CD4⁺ T-cells are decreased.

Effect of gp120 from Divergent Isolates of HIV-1 from Different Viral Clades.

In another series of experiments we examined the effect of four recombinant gp120 derived from HIV-1 isolates from different viral clades (gp120_{MN}, gp120_{SF2}, gp120_{LAV}, and gp120_{CM}). The clade and geographical origin of these proteins are shown in table I. All these highly divergent samples of gp120 induced IL-4 release from basophils (fig. 4). Two unrelated glycoproteins, bovine serum albumin and human serum albumin (1 – 100 nM) do not activate any of the basophil preparation tested (data not shown). These results implies that the capacity to induce cytokine release from basophils is a general feature of HIV-1 gp120 that has been maintained throughout the evolution of the virus.

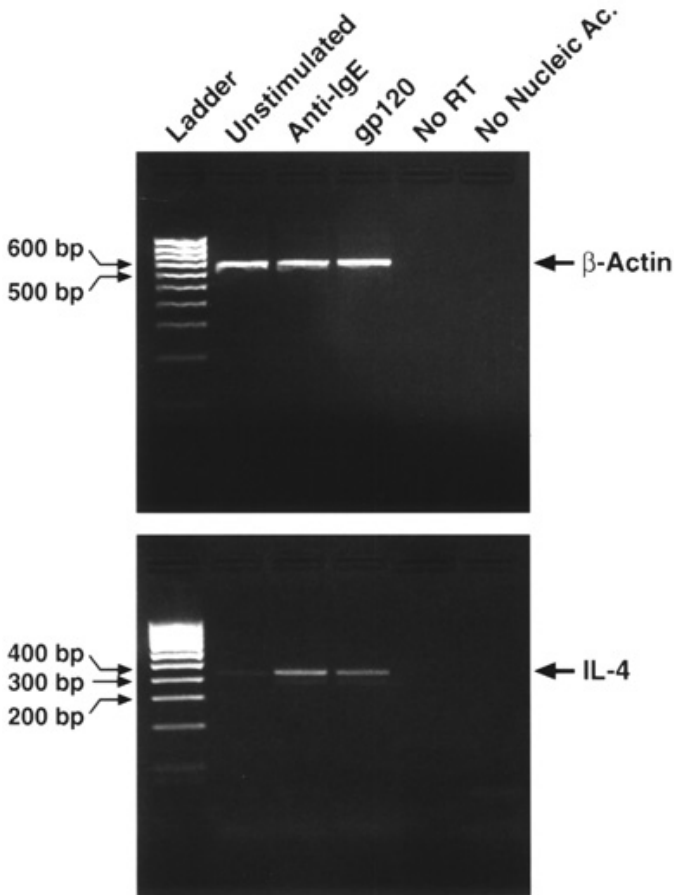


Fig. 2. – Reverse-transcription-PCR quantitative analysis of IL-4 mRNA expression in human basophils stimulated with anti-IgE (0.3 μ g/ml) or gp120 (30 nM). IL-4 mRNA was amplified by a quantitative RT-PCR. The cDNA subjected to electrophoresis was visualized by ethidium bromide. Without cDNA (No RT) or nucleic acid (No Nucleic Ac.), no PCR product was visualized.

TABLE I. – Recombinant envelope glycoprotein gp120 from divergent isolates of HIV-1 from clades derived from different places.

Recombinant envelope protein	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

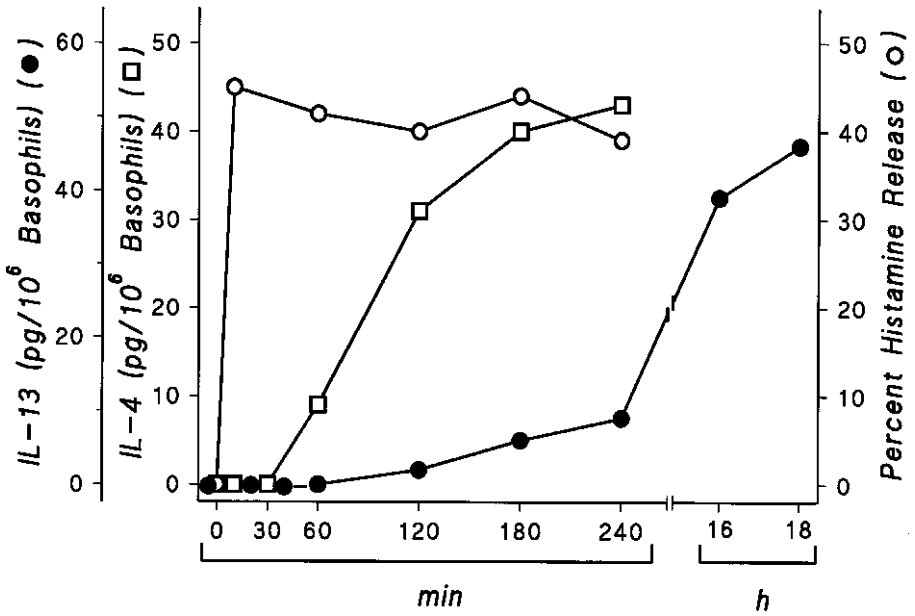


Fig. 3. – Kinetics of IL-13, IL-4 and histamine secretion from human basophils induced by gp120 (30 nM). Each point represents the mean of IL-13, IL-4 and histamine release from duplicate incubations.

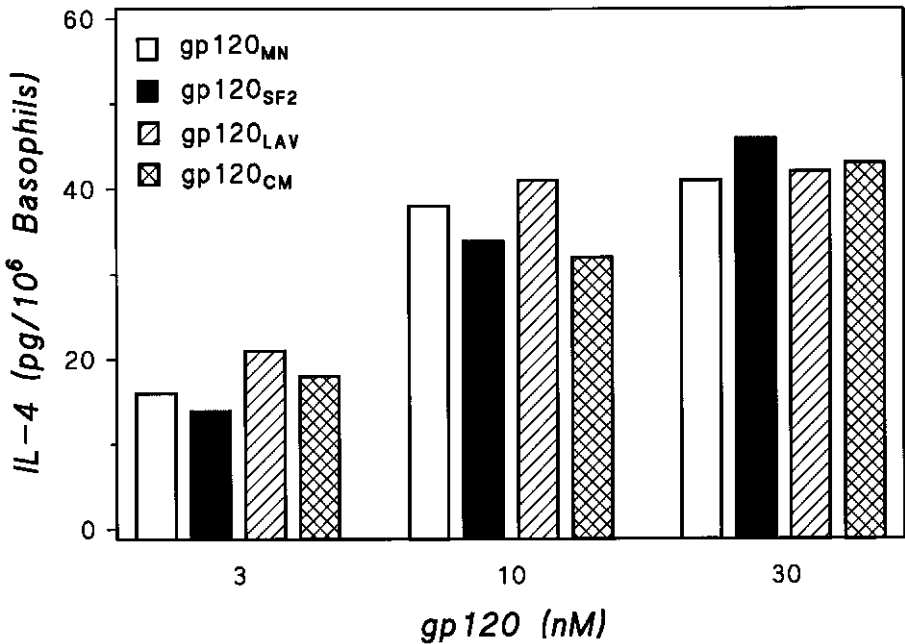


Fig. 4. – Effects of various concentrations of gp120 from four different HIV-1 isolates on IL-4 secretion from human basophils obtained from normal donors negative for HIV-1 and HIV-2 antibodies. Each bar represents the mean of IL-4 release from duplicate incubations.

Cyclosporin A and Tacrolimus Inhibit gp120-induced IL-4 Secretion from Human Basophils.

Studies from our group demonstrated that immunophilin-binding drugs (*i.e.*, CsA and tacrolimus) inhibited the IgE-dependent release of proinflammatory mediators from human Fc_εRI⁺ cells (basophils and mast cells) both *in vitro* (Cirillo *et al.*, 1990; de Paulis *et al.*, 1991; Patella *et al.*, 1998) and *in vivo* (Casolaro *et al.*, 1993). To explore whether gp120-induced activation of basophils was sensitive to immunophilin-binding drugs, we preincubated the cells with CsA (24-800 nM) or tacrolimus (24-800 nM) before the addition of gp120. CsA concentration-dependently inhibited the gp120-induced release of histamine and IL-4 from basophils at concentrations as low as 24 nM. The inhibition of IL-4 release ranged from \approx 30% at 24 nM to 95% at 240 nM. Tacrolimus was even more potent than CsA in inhibiting gp-120-induced histamine and IL-4 release from basophils (data not shown).

This class of anti-inflammatory/immunosuppressive drugs has been shown to influence HIV-1 replication (Karpas *et al.*, 1992; Thali *et al.*, 1994). The observation that T_H2 cells are more susceptible to HIV-1 infection than T_H1 cells (Maggi *et al.*, 1994) has been recently explained by the upregulation of CXCR4 receptors induced by IL-4 on T_H2 cells (Galli *et al.*, 1998). Therefore, our data provide a novel mechanism for the antiviral activity of immunophilin-binding drugs (Karpas *et al.*, 1992; Thali *et al.*, 1994), suggesting that it may be related, at least in part, to the inhibition of IL-4 secretion from basophils.

IgE Stripping Inhibits gp120-induced IL-4 and IL-13 Release from Basophils.

A short incubation of basophils with lactic acid removes most of the IgE bound on Fc_εRI⁺ cells and inhibits basophil and mast cell activation by IgE-mediated stimuli (Patella *et al.*, 1993). IgE stripping by lactic acid completely blocked IL-4 and IL-13 secretion induced by gp120 and by anti-IgE (data not shown). In contrast, this treatment did not affect the response to the cross-linking the of α -chain of the high affinity IgE receptor (Fc_εRI) by a monoclonal antibody anti-Fc_εRI (Patella *et al.*, 1998). These data strongly support the hypothesis that gp120 activates Fc_εRI⁺ cells through the interaction with surface IgE.

Specificity of the Interaction between gp120 and IgE.

Another set of experiment was designed to confirm the interaction of gp120 with IgE bound on human basophils and to explore the specificities of the V_H region recognized by gp120. V_H3⁺ is the most frequent immunoglobulin V_H gene product in the human repertoire and it is expressed in approximately 50% of human serum immunoglobulins (Berberian *et al.*, 1993; Silverman, 1997; Karray *et al.*, 1998). In these experiments, gp120 (30 nM) was preincubated (15 min, 37 °C) with increasing concentrations (0.1 to 10 μ g/ml) of 3 different human monoclonal IgM V_H3⁺ (IgM M3, IgM M11, and IgM LAN) or with a monoclonal IgM V_H6⁺ (M 14). Basophils were then added and the incubation was continued for 4 h at 37 °C. At the end of the incubation, IL-4 was measured in the supernatants. Figure 5 shows that preincubation

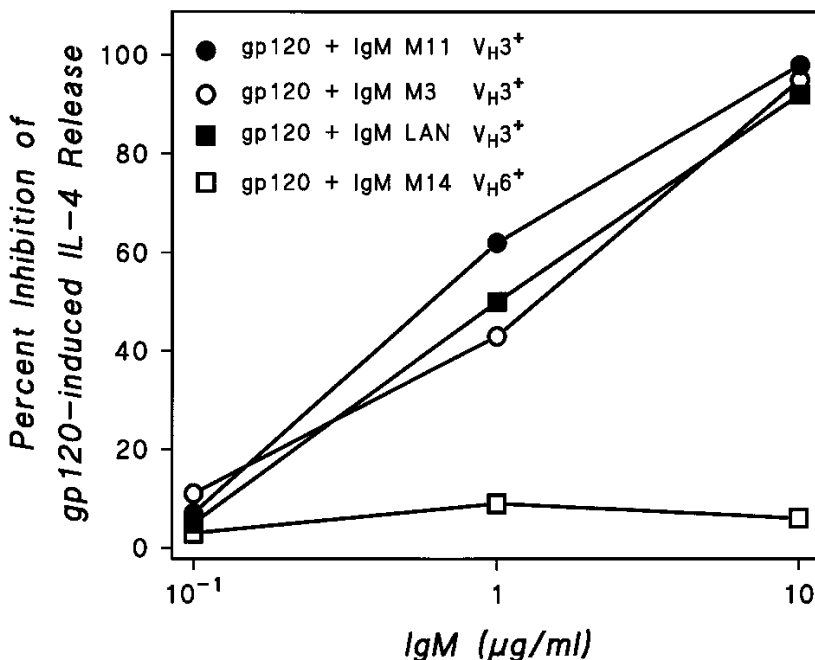


Fig. 5. – Effect of preincubation of gp120 with monoclonal IgM on IL-4 release from human basophils. Gp120 (30 nM) was preincubated for 15 min at 37 °C with increasing concentrations of human monoclonal IgM M11, IgM M3, IgM LAN (V_H3⁺) or IgM M14 (V_H6⁺). Basophils were then added and the incubation was continued for an additional 4 h at 37 °C. Each point represents the mean percent inhibition of IL-4 release from duplicate incubations.

of gp120 with all monoclonal IgM (M3, M11, and LAN) possessing the V_H3 domain concentration-dependently inhibited gp120-induced IL-4 secretion. In contrast, preincubation of gp120 with a monoclonal IgM (M14), possessing a V_H6 domain, had no effect on IL-4 release. These data support the hypothesis that gp120 binds to human IgE by recognizing the region expressing the V_H3, but not the V_H6 gene product.

Similar data were obtained using human mast cells isolated from the lung parenchyma. Gp120 isolated from different HIV-1 clades induced histamine release from human lung mast cells. Also in mast cells, preincubation with the three V_H3⁺ monoclonal IgM, but not with IgM V_H6⁺, inhibited gp120-induced mediator release (data not shown). Together, these data indicate that gp120 activates human mast cells as well as human basophils. The effect of gp120 is mediated by an interaction restricted to the V_H3 region of IgE bound on the surface of Fc_εRI⁺ 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 (Goodlick *et al.*, 1995). The biochemical characteristics of peptides used in these experiments are indicated in table II.

TABLE II. – Peptide sequences of HIV-1_{MN} gp120.

Peptide No.	Amino acids	Gp120 Domain	Sequence
1922	31-50	C1	EKLWVTVYYGVPVWKEATTt
1959	231-250	C2	LKCNDKKFSGKGSCKNVSTV
1960	241-260	C2	KGSCKNVSTVQCTHGIRPVV
1985	301-320	V3	CTRPNYNKRKRRIHIGPGRAF
1988	331-350	C3	RQAHCNISRAKWNDTLRQIV
1989	341-360	C3	KWNDTLRQIVSKLKEQFKNK
2015	501-520	C5-gp41	TKAKRRVVQREKRAAIGALF

Preincubation of basophils with the synthetic peptides of the gp120_{MN} core motif (1959, 1960, 1985, 1988 and 1989) concentration-dependently inhibited IL-4 release induced by gp120_{MN}. In contrast, peptides 1922 and 2015, which span the gp120_{MN} amino and COOH terminal region respectively, had no effect. The inhibitory peptides alone did not induce histamine release and they did not influence anti-IgE-induced IL-4 or histamine release from basophil and mast cells (data not shown). These 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 (Karray *et al.*, 1997).

Taken together our results demonstrate that gp120 isolated from diverse HIV-1 clades induces the synthesis and release of IL-4 and IL-13 from human Fc_εRI⁺ cells. The activity of gp120 is mediated by an interaction with the V_H3 region of the 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 cells other than T lymphocytes. IL-4 and IL-13 released from human Fc_εRI⁺ cells might also indirectly play a role in the entry of HIV-1 into CD4⁺ cells. In fact, IL-4 induces the upregulation of the chemokine receptor CXCR4 (Galli *et al.*, 1998) that, together with CCR5, is a major co-receptor facilitating HIV-1 entry into CD4⁺ cells (Dragic *et al.*, 1996; Feng *et al.*, 1996; Ostrowski *et al.*, 1998). Engagement of CXCR4 is also sufficient to trigger CD4⁺ T cell depletion (Penn *et al.*, 1999). In addition, IL-4 and IL-13 activate monocytes to produce macrophage-derived chemokine (MDC), a chemokine selectively active on CCR4 receptor expressed on T_H2 cells (Bonecchi *et al.*, 1998). Thus, IL-4 and IL-13 are important not only for T_H2 switching of T cell responses but also for HIV-1 infection and replication in T_H2 cells (Maggi *et al.*, 1994; Rancinan *et al.*, 1998). In this context, the production of IL-4 and IL-13 by cells other than T lymphocytes may have a crucial role in both the early and late phase of HIV infection. Two of these cells may well be the basophil and the mast cell (Fc_εRI⁺ cells) that selectively produce IL-4 and IL-13 when activated by gp120. The relevance of this observation is further strengthened by considering that basophils represent 0.5-1% of peripheral blood leukocytes (Marone, 1995*a,b*) whereas T_H2 cells constitute only 0.2-2% of CD4⁺ cells (Andrew *et al.*, 1998).

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 $_{\epsilon}$ 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 (Fauci, 1996), mast cells and basophils can be exposed to shed or virus-bound gp120. This suggests that Fc $_{\epsilon}$ RI $^{+}$ cells might be a novel source of T $_{H}2$ 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 (Clerici *et al.*, 1993; Barcellini *et al.*, 1994; Graziosi *et al.*, 1994; Maggi *et al.*, 1994; Meyaard *et al.*, 1994; Fakoya *et al.*, 1997; Klein *et al.*, 1997). In fact, it highlights the importance of a specific viral superantigen, gp120, acting on cell types other than lymphocytes in the production of T $_{H}2$ -like cytokines. This novel observation might be also relevant in the design of drugs selectively acting on Fc $_{\epsilon}$ RI $^{+}$ cells in the treatment of HIV-1 infected subjects.

ACKNOWLEDGEMENTS

This paper is dedicated to Rita Levi-Montalcini who first suggested an involvement of Fc $_{\epsilon}$ RI $^{+}$ cells in HIV-1 infection.

This work was supported by grants from the Ministero della Sanità, Istituto Superiore di Sanità (AIDS Projects No. 40.B.64 and 40.A.67), Consiglio Nazionale delle Ricerche (Target Project Biotechnology No. 99.00401.PF49 and 99.000216.PF31) and by a fellowship from the Ministero della Sanità to V.P.

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Pervenuta il 30 agosto 2000,

in forma definitiva il 27 settembre 2000.

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