

# ***In Vivo* Treatment with Fusion Inhibitor Enfuvirtide Leads to Increased IL-12 Production by Autologous *in Vitro* Activated Monocytes from HIV-infected Individuals**

Andrea Vecchi<sup>1</sup>, Hamid Hasson<sup>1</sup>, Andrea Galli<sup>1</sup>,  
Antonella Castagna<sup>1</sup>, Adriano Lazzarin<sup>1,2</sup>, Priscilla Biswas<sup>1,\*</sup>

<sup>1</sup>San Raffaele Scientific Institute and Hospital, Milan, Italy

<sup>2</sup>Università Vita-Salute San Raffaele, Milan, Italy

## **Abstract**

Enfuvirtide inhibits IL-12 synthesis *in vitro*. We investigated IL-12 production in monocytes from thirteen chronic, late-stage patients who underwent ENF treatment *in vivo* for at least 12 weeks. Peripheral blood mononuclear cells from frozen samples and flow cytometry was used. Interestingly, a statistically significant increase of the percentage of IL-12-producing activated monocytes was documented after treatment with the ENF-containing antiretroviral regimen.

In about 50% of the subjects the increase of IL-12 co-existed with increase of CD4 T lymphocyte count and, unexpectedly, serum IgE and with decrease of HIV-1 viremia. Lack of a true association could be due to the retrospective nature and small sample size of our study. Nevertheless, *in vitro* evaluation of IL-12 production in response to antiretroviral drugs should be taken into consideration as it could reflect the status of at least one arm of innate immunity and complement the information derived solely by the CD4 T lymphocyte count.

**Keywords:** IL-12; Enfuvirtide; Gp41; IgE; CD4 counts; HIV-1 viremia; Th1; Th2; Monocytes; Formil peptide receptor; LPS

## **Introduction**

IL-12 is a heterodimeric cytokine of 70 kD composed of two disulphide-linked protein subunits (p35 and p40) encoded by two different genes (Trinchieri, 1995). IL-12, mainly produced by phagocytes (monocytes/macrophages and neutrophils) and dendritic cells in response to microbial products, is pivotal in inducing IFN- $\gamma$  production by T lymphocytes and NK cells (Trinchieri, 2003). T lymphocytes can be distinguished in several functional subgroups, including T helper 1 (Th1) and Th2 based on IFN- $\gamma$  and IL-4 production, respectively. The Th1 pathway, characterized by IFN- $\gamma$  production, mediates cellular immunity against invading intracellular pathogens and helps B lymphocytes to produce certain immunoglobulin (Ig) classes (IgG). The Th2 pathway, mediated via IL-4 production, induces an immune response against extracellular pathogens, such as helminths, and provides help for other Ig classes (IgE) (Abbas et al., 1996).

A strong impairment of IL-12 production has been earlier observed in HIV-infected individuals, both asymptomatic and with AIDS (Chehimi et al., 1994) and in *in vitro* HIV-infected monocytes (Chougniet et al., 1996). Along this line, a switch

from Th1 to Th2 responses has been described in HIV infection and claimed as an unfavourable indicator of the immune deregulation occurring during the course of HIV infection (Clerici and Shearer 1993; Becker, 2004). Indeed, a number of studies reported that elevated levels of IgE are associated with disease progression (Mazza et al., 1995; Park et al., 2002) and are inversely related to the number of CD4+ T lymphocytes (Lin and Smith Jr, 1988; Agarwal and Marshall Jr, 1998).

Enfuvirtide (ENF), a synthetic peptide earlier named T-20 or DP178, is an HIV fusion/entry inhibitor (Matthews et al., 2004; Castagna et al., 2005) approved in combination with an optimized background therapy for use in the treatment of HIV-1 infected patients. ENF interferes with gp41-mediated fusion of the viral envelope with the host cell membrane; in particular, it binds to a structural intermediate of the fusion process preventing the transition of gp41 into a fusion-active state, thus blocking the final step of viral entry (Weiss, 2003).

It has earlier been reported that ENF can bind the formyl peptide receptor (FPR) (Su et al., 1999) expressed by human monocytes, neutrophils and several other cells (Becker et al., 1998). FPR, which is the receptor for the prototypic bacterial chemoattractant f-met-leu-phe (fMLP) (Panaro et al., 2006), is a member of the seven-transmembrane domain family which signals through a G-coupled heterotrimeric G-protein complex (GPCR). GPCR is a very large superfamily encompassing several receptorial families, including chemokine receptors. Of interest, binding of ENF/T-20 to FPR has been shown to lead to a selective inhibition of IL-12 production by human monocytes (Braun et al., 2001).

This study aims to verify the hypothesis that *in vivo* treatment with ENF could lead to a decrease of IL-12 production by autologous monocytes. We thus performed a retrospective study

**\*Corresponding author:** Priscilla Biswas, Ph.D., San Raffaele Scientific Institute, Hospital and University "Vita-Salute", Division of Immunology, Transplantation and Infectious Diseases, Via Olgettina 60, 20132 Milano, Italy, E-mail: [biswas.priscilla@hsr.it](mailto:biswas.priscilla@hsr.it)

**Received** September 11, 2009; **Accepted** October 12, 2009; **Published** October 13, 2009

**Citation:** Vecchi A, Hasson H, Galli A, Castagna A, Lazzarin A, et al. (2009) *In Vivo* Treatment with Fusion Inhibitor Enfuvirtide Leads to Increased IL-12 Production by Autologous *in Vitro* Activated Monocytes from HIV-infected Individuals. *J Antivir Antiretrovir* 1: 043-050. doi:10.4172/jaa.1000006

**Copyright:** © 2009 Vecchi A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

evaluating the *in vitro* production of IL-12 by stimulated monocytes derived from patients who underwent an ENF-containing antiretroviral regimen for at least 12 weeks.

At last, we have previously shown that addition of ENF to a failing antiretroviral regimen is accompanied by a selective increase of serum IgE in HIV-infected individuals at an advanced stage of disease (Hasson et al., 2006; Burastero et al., 2006), besides inducing a positive immunovirological response, i.e.: decrease of HIV viremia and increase of the CD4 count. For this reason the *ex-vivo* IL-12 production by the patients of this study was put in relationship to their CD4 counts, HIV-1 viremia and serum IgE levels.

## Methods

### Patients and samples

This study included White Caucasian individuals resistant to all clinically available antiretroviral drugs with a CD4 count of  $\leq 100$  cells/mm<sup>3</sup> who were failing their current antiretroviral regimen. A new antiretroviral regimen was thus introduced by their physicians, including enfuvirtide (Fuzeon®) as unique active drug.

Patients who introduced ENF were enrolled in the ENF Expanded Access Program. The protocol obtained the approval from the San Raffaele Ethical Committee and an informed consent was obtained for each patient. Our centre recruited 41

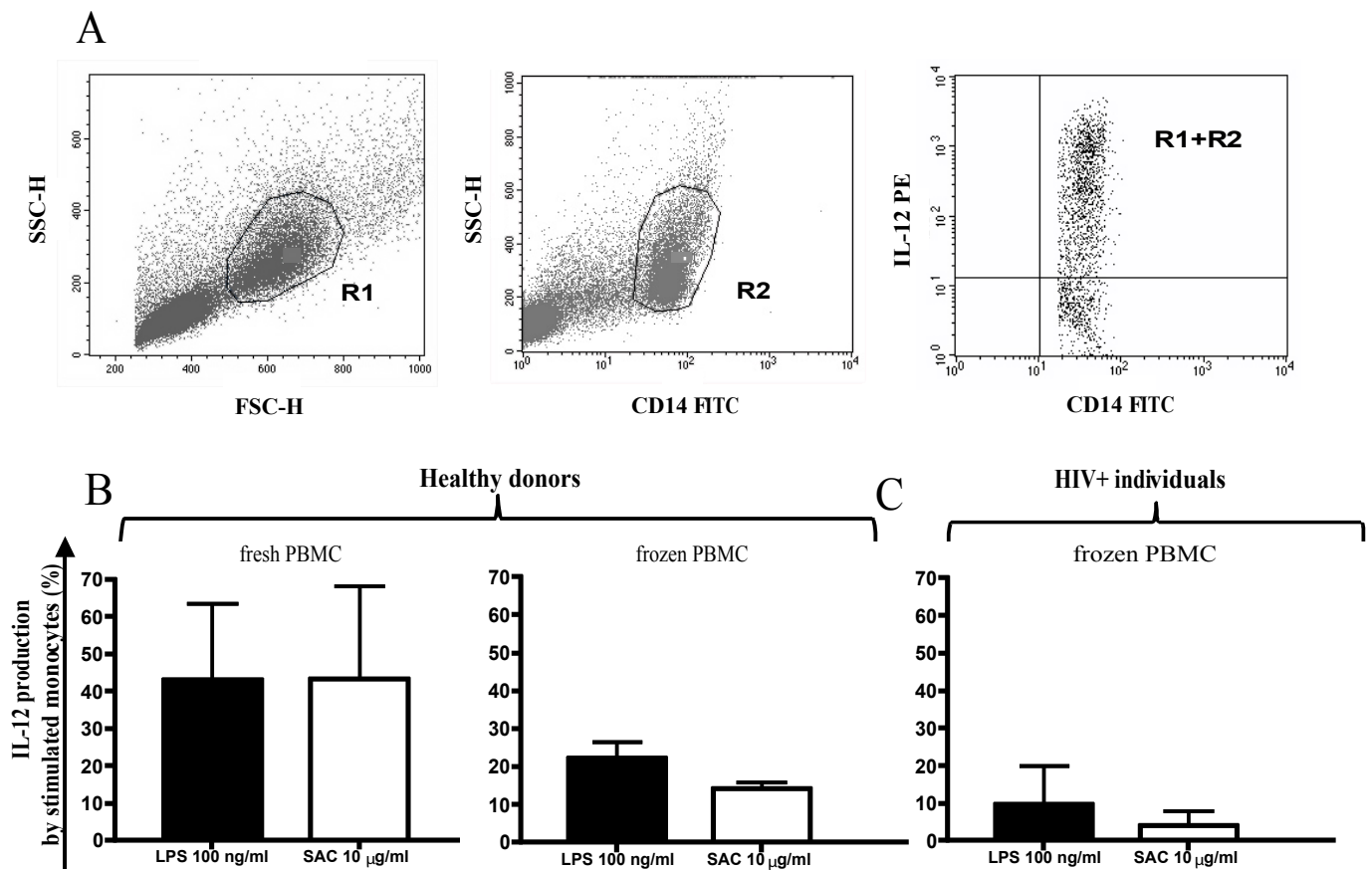
patients, but sequential PBMC samples were available only for thirteen patients (seven males, six females). For each of the latter the sequential set of PBMC was thawed on the same day and the cells immediately stimulated and assessed for flow cytometric analyses all together.

Whole blood was withdrawn in EDTA-containing tubes and were obtained by density gradient purification (Lymphoprep nycomed, Axis-Shield, Oslo, Norway). PBMC were frozen according to standard procedures.

PBMC from advanced stage HIV-infected individuals undergoing antiretroviral regimens not containing ENF (n=12) were also evaluated in this study, as well as fresh and thawed PBMC from healthy donors (n=5) (Figure 1).

### Stimulation conditions

PBMC were plated in flat-bottom 96-well microtiter plates at the concentration of  $5 \times 10^6$ /ml, 200  $\mu$ l/well at 37 °C in a 5% CO<sub>2</sub> incubator. PBMC were primed for 2 hours with interferon (IFN)- $\gamma$  (R&D Systems, Inc.) (Hayes et al., 1995) at 10 ng/ml, prior to activation with either lipopolysaccharide (LPS) (*E. coli* serotype O127:B8, Sigma) at 100 ng/ml or fixed *Staphylococcus aureus* (SAC, 10  $\mu$ g/ml, Calbiochem, San Diego, CA) plus IFN- $\gamma$  (10 ng/ml) in the presence of a protein transport inhibitor (2  $\mu$ M) (GolgiStop™, BD Biosciences Europe) for additional 18 hours. PBMC were kept on ice for 30 min to enhance detachment of cells prior to immunostaining.



**Figure 1:** Flow cytometric analyses of intracellular IL-12 production in different types of PBMC. A) The three dot plot panels show the gating strategy adopted for the evaluation of intracellular IL-12-production by stimulated monocytes (CD14+) within the PBMC. The following graphs depict the percentage of IL-12-producing monocytes in response to LPS and SAC derived from B) healthy donors' fresh and frozen PBMC or C) HIV-infected individuals' frozen PBMC.

## IL-12 production

IL-12 production by monocytes was evaluated by flow cytometry via intracellular staining of IL-12 and surface staining of CD14.

PBMC were harvested, stained with FITC-conjugated mouse anti-human CD14 monoclonal antibody (Mab)(BD, clone MΦ P9), fixed, permeabilized, and then stained with 0.125 µg/well of PE-conjugated mouse anti-human IL-12 Mab (BD clone C11.5) following PharMingen's staining protocol. According to the manufacturer this antibody recognizes the IL-12 p40 monomer and the p70 heterodimer, but not the p35 monomer.

## Flow cytometry

Flow cytometric analyses were carried out on a FACSCalibur instrument (BD) equipped with CELLQuestPro software (BD). In acquisition 5000 events were collected in a gate drawn on the monocyte morphologic parameters. In analysis an analogic gate was created integrating the morphologic gate with a fluorescence gate drawn on CD14-brightly positive cells. Data are expressed as percent of double positive (CD14 and IL-12) cells.

## Immunovirological parameters

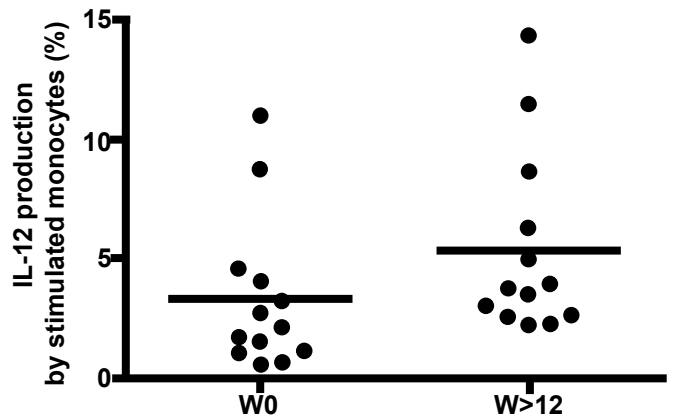
The following parameters were measured by licensed commercial laboratory assays. CD4 absolute counts are expressed as cells/mm<sup>3</sup>; IgE levels are expressed as IU/ml and the normal range is 1-100 in the adult population. The HIV RNA assay has a sensitivity threshold of 1.9 log<sub>10</sub> copies/ml (corresponding to 80 copies/ml).

## Results

### Experimental setting

In this study we measured IL-12 production via intracellular staining and flow cytometric analyses. Figure 1 A displays three representative dot plots showing the gating strategy adopted for the evaluation of intracellular IL-12 production within stimulated PBMC; CD14+IL-12+ cells are hereafter referred to as IL-12-producing monocytes. Unstimulated PBMC yielded irrelevant percentages of IL-12-producing monocytes (data not shown), therefore all following data refer to stimulated PBMC.

We first tested our experimental conditions with PBMC deriving from healthy donors (Figure 1B), comparing fresh (n=5) and frozen (n=5) samples. The reason for this comparison is that our retrospective study necessarily implied the use of frozen samples from ENF-treated patients. As expected, frozen PBMC yielded lower percentages (approximately half) of IL-12-producing monocytes as compared to fresh PBMC in response to both stimuli LPS and SAC. However, the data from the frozen samples were reproducible and of good quality. Since it is known that PBMC from HIV-infected individuals produce less IL-12, we also tested our experimental conditions with frozen PBMC deriving from HIV-infected individuals (n=12) under therapeutic regimens not containing ENF (Figure 1C). As expected, the percentage of IL-12 producing monocytes was lower than that of frozen PBMC from healthy donors in response to both stimuli. Mean percentage (±SD) of LPS-stimulated IL-12-producing monocytes from frozen samples was 22.3 (±4.1) and 9.6 (±10.4) in healthy and HIV-infected individuals, respectively (Figure 1B,C). Nevertheless, there was sufficient residual IL-12 production and



**Figure 2:** Percentage of LPS-stimulated IL-12-producing monocytes from thawed PBMC deriving from thirteen patients undergoing in vivo ENF treatment. The graph summarizes the sequential data of W0 and W>12, corresponding to a time point later than twelve weeks of in vivo ENF treatment.

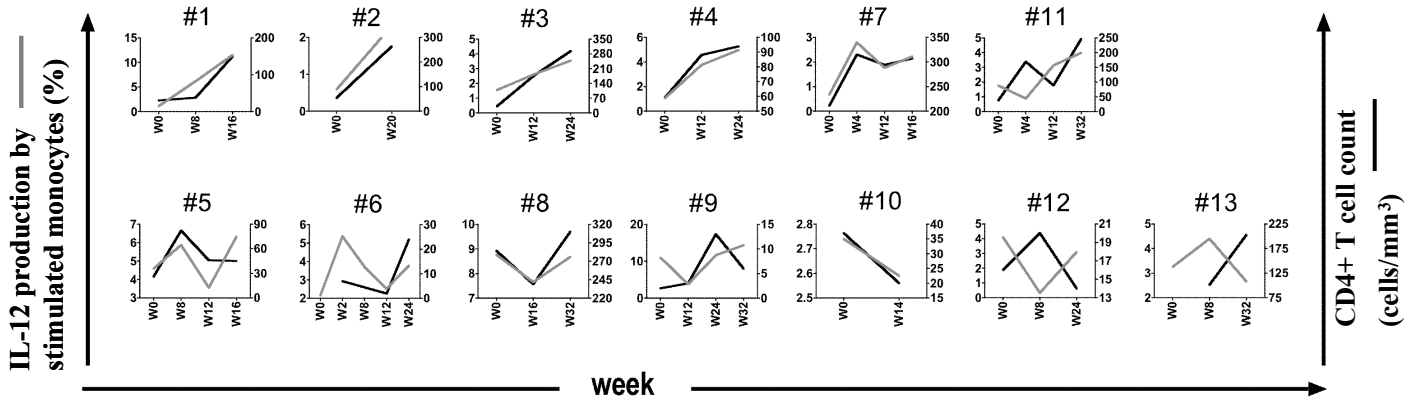
the data were reproducible and of acceptable quality in order to perform the study using frozen material from HIV-infected patients. Between the two tested stimuli, we chose LPS for the following experiments because SAC was less effective with frozen PBMC from both healthy and HIV-infected individuals (Figure 1B,C), also at higher concentrations (data not shown).

### Increase IL-12 production by “ex-vivo” activated monocytes

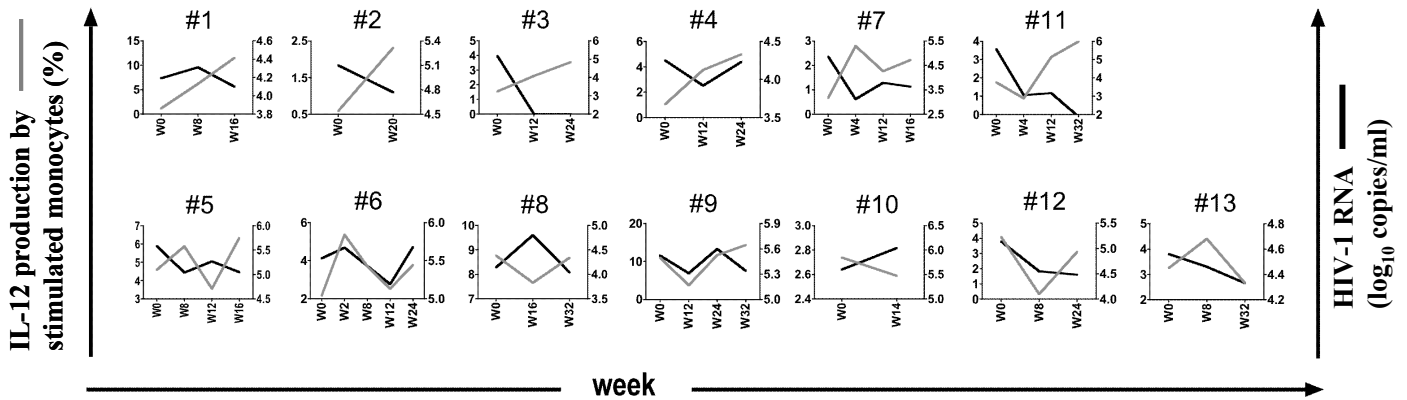
Forty-one patients were enrolled in the ENF Expanded Access Program; however, sequential frozen PBMC samples were available only for thirteen patients, which were the ones evaluated in the present study. All patients had a W0 corresponding to a time point prior to initiation of ENF and a time point after at least 12 weeks of *in vivo* ENF treatment. Of note, a modest but consistent increase of IL-12-producing monocytes was observed (Figure 2). The mean percentage (±SD) was 3.3 (±3.2) at W0 and 5.4 (±3.8) at W>12; the difference between W0 and W>12 was statistically significant (p=0.026, paired Student's t Test). This result was quite surprising, showing that also chronic, late-stage patients undergoing ENF-containing regimens were able to recover some ability to produce IL-12 *in vitro*. Indeed, unlike the published findings in chronic HIV infection, it has been reported that patients with acute/early HIV infection exhibited evidence of *in vivo* IL-12 production as well as maximal IL-12 production by their PBMC *ex vivo* (Byrnes et al., 2008). This suggests that this key immunologic function deteriorates with time after HIV infection, but is not completely lost even in very advanced patients as those included in our study.

### Comparison of IL-12 production with CD4 count and serum IgE

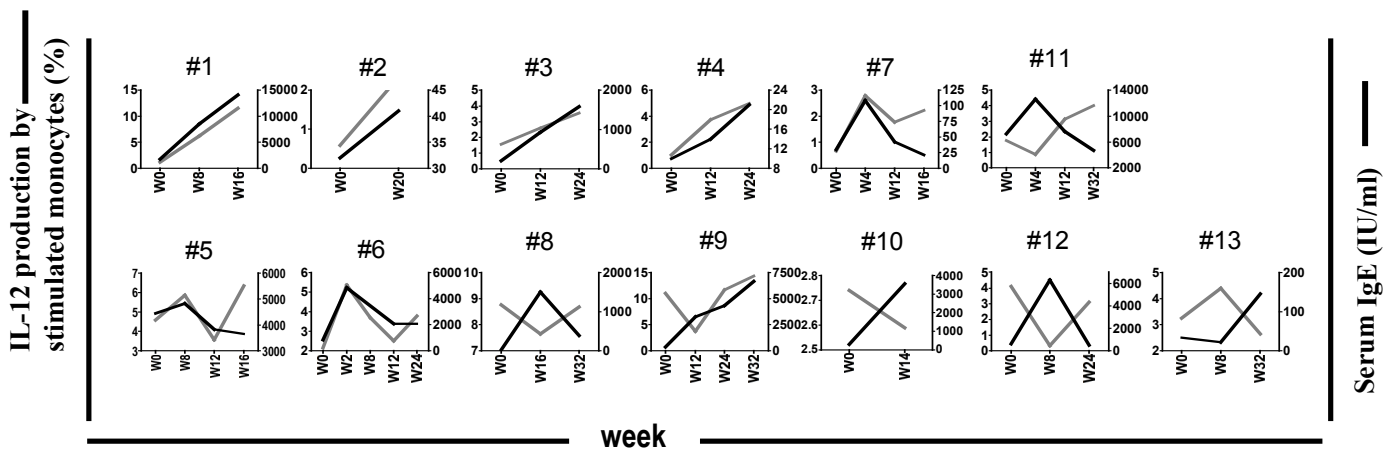
The two major immunovirological parameters considered to evaluate the response to antiretroviral regimens, including the addition of an active drug such as ENF in a salvage therapy, are CD4 counts and HIV-1 viremia. We thus proceeded in comparing the sequential IL-12 production with these two parameters. Figure 3 and Figure 4 display the percentage of IL-12-producing monocytes (gray line) on the left axis and either absolute CD4 counts (black line) or HIV-1 viremia (black line) on the right



**Figure 3:** Concomitant sequential evaluation of IL-12 and the immunological parameters CD4 in ENF-treated patients. Each panel summarizes the sequential data of a single patient. The percentage of LPS stimulated IL-12-producing monocytes is on the left y-axis (bold gray line), whereas the CD4 T lymphocyte absolute count, expressed as cells/mm<sup>3</sup> (bold black line) are on the right y-axes; the x-axis displays the time points, specified for each patient, in weeks after ENF treatment.



**Figure 4:** Concomitant sequential evaluation of IL-12 and HIV-1 viremia in ENF-treated patients. Each panel summarizes the sequential data of a single patient. The percentage of LPS stimulated IL-12-producing monocytes is on the left y-axis (bold gray line), whereas the HIV-1 viremia, expressed as log<sub>10</sub> copies/ml (bold black line), are on the right y-axes; the x-axis displays the time points, specified for each patient, in weeks after ENF treatment.



**Figure 5:** Concomitant sequential evaluation of IL-12 and IgE in ENF-treated patients. Each panel summarizes the sequential data of a single patient. The percentage of LPS-stimulated IL-12-producing monocytes is depicted on the left y-axis (bold gray line), whereas the IgE content (IU/ml) is depicted on the right y-axis (bold black line); the x-axis displays the time points, specified for each patient, in weeks after addition of ENF to the antiretroviral regimen.

axis, respectively. In six patients the increase of CD4 counts is paralleled by an increase of IL-12-producing monocytes (Figure 3, upper panels); in 4/6 of those same patients a decrease of HIV-1 viremia is clearly achieved (#2,3,7,11) (Figure 4, upper panels). In the remaining patients (lower panels) fluctuations occur in the percentage of IL-12-producing monocytes and either parameter, CD4 counts (Figure 3) or HIV-1 viremia (Figure 4).

To seek a putative link between IL-12 production and IgE levels, we plotted the data concerning percentage of IL-12-producing monocytes (left y-axis) against those of IgE (right y-axis) in a sequential manner for each of the thirteen ENF-treated patients (Figure 5). The serum IgE data derive from a previously published study which included all ENF-treated patients herein evaluated (Hasson et al., 2006). Five patients (#1,2,3,4,9) show an increase of both IL-12 production and serum IgE levels; of the remaining, three patients display substantially a concordant (#5,6,7) and five a discordant (#8,10,11,12,13) course between the two parameters. While the latter was the pattern expected considering IL-12 and IgE as opposite markers of Th1 and Th2 environments, quite surprisingly the pattern of IL-12 production paralleled that of serum IgE in 8/13 patients (61%). Patient 10 displayed the worst hypothetical pattern: increase of HIV viremia and IgE, decrease of CD4 count and IL-12 production.

#### Illustration summarizing the antiretroviral and immunologic effects of ENF

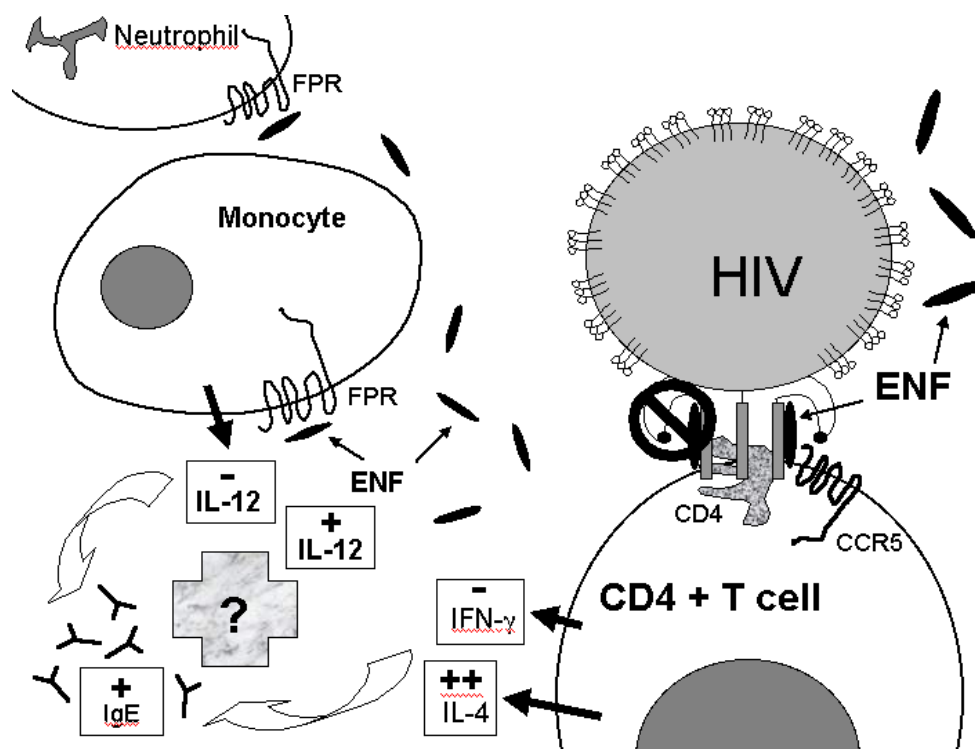
We provide a cartoon (Figure 6) that tries to integrate several pieces of information on ENF. The antiretroviral activity is summarized on the right side: ENF acts at a late stage of the multi-step process of HIV entry (Matthews et al., 2004; Weiss, 2003), hindering the zipping of the HR2 and HR1 regions of gp41, thus leading to a block of HIV fusion. The left side of the cartoon summarizes certain immunological effects of ENF

described by us and others. Decreased IFN- $\gamma$  and increased IL-4 production was observed by *ex vivo* CD4 lymphocytes from patients undergoing ENF treatment *in vivo* (Burastero et al., 2006); the latter also displayed an increase of serum IgE (Hasson et al., 2006). ENF/T20 peptide was shown to bind FPR present on monocytes and neutrophils (Su et al., 1999), and this induced a selective inhibition of IL-12 secretion *in vitro* (Braun et al., 2001). Thus, in the presence of ENF a putative cytokine milieu leading to increased IgE production could occur. Nevertheless, in this study we observed an increase of IL-12 production by *ex vivo* stimulated PBMC from ENF-treated patients, leaving an interesting but open question. Certainly, further larger, prospective, well planned and controlled studies are warranted to definitely understand the effect of *in vivo* ENF treatment on IL-12 production.

#### Discussion

ENF is the first member of a class of antiretroviral drugs, termed entry inhibitors, recently joined by the CCR5 inhibitor maraviroc (Biswas et al., 2007). Although ENF needs to be administered twice daily by subcutaneous injection, it is highly valuable for patients harbouring viruses broadly resistant to previous antiretroviral drugs and at an advanced stage of disease (Lalezari et al., 2003; Lazzarin et al., 2003).

To monitor the efficacy of the addition of an active drug in the antiretroviral regimen, clinicians refer not only to HIV-1 viremia, but also to increase of CD4+ T lymphocyte count, which at baseline was  $\leq 100$  cells/mm<sup>3</sup> in the individuals evaluated in this study. Increment of CD4 T cell count is a precious indicator of recovery of adaptive immunity especially in patients at such an advanced stage of disease; nevertheless, it does not directly address the contribution of innate immunity, which also is important in the complete restoration of an immune response.



**Figure 6:** Cartoon summarizing the antiretroviral activity of ENF (right side) and certain immunological effects of ENF (left side) discussed in this study.

We here sought to analyze this aspect, by measuring IL-12-producing monocytes as a quantifiable function of the innate response. We did observe an increase of IL-12 production by activated monocytes; although the number of patients we evaluated is relatively small, the increment resulted statistically significant. About 50% of the subjects displayed an increase of both parameters, IL-12-producing monocytes and CD4+ T lymphocyte count, as well as a decrease of HIV-1 viremia. This effect occurs after a considerably prolonged period of ENF treatment, equal to or more than three months. Accordingly, other ENF studies reported a prolonged increase of CD4 cell count, also after emergence of ENF resistance mutations (Aquaro et al., 2006) and in patients not achieving complete viral suppression (Saberri et al., 2008). This could be due not to the direct antiretroviral activity of ENF, but to indirect immune-mediated mechanisms leading to a general amelioration of the patients' immune system, including monocyte function (concepts recapitulated in Figure 6).

It was previously demonstrated that ENF/T-20 could bind to FPR (Su et al., 1999) present on monocytes and selectively inhibit *in vitro* production of IL-12 in response to known stimuli (Braun et al., 2001). Our present *ex vivo* study attempted to verify this hypothesis by measuring IL-12 production with intracellular staining and flow cytometric analyses. This approach was consistent with the previous finding by Braun, et al. (2001) that ENF inhibited not only secretion of IL-12 protein in culture supernatants, but also the mRNA for both IL-12 subunits, p35 and p40. Thus, an inhibition of intracellular IL-12 protein produced upon *in vitro* stimulation was plausible. Our results lead to the opposite finding: a prolonged *in vivo* treatment with an antiretroviral regimen including ENF as the active drug results in an increase of percentage of *in vitro* IL-12-producing monocytes. Importantly, this effect appears to be more pronounced in ENF *in vivo* treated individuals, than in patients who added *in vivo* as active drug the CCR5 inhibitor maraviroc (MVC) or the reverse transcriptase inhibitor tenofovir (TDF). Using the same experimental protocol with PBMC at T0 and T >12weeks only 3/8 of the TDF and 0/5 of the MVC treated individuals displayed an increase of IL-12-producing monocytes upon LPS stimulation (data not shown). It has been reported that the elevated plasma IL12p70 found in acute/early stages of HIV infection remained elevated also in those individuals who later underwent standard HAART, although the levels appear lower than in those individuals that refused HAART (Byrnes et al., 2008).

We have previously observed that treatment with ENF is associated with an increase of serum IgE, but not of other immunoglobulin classes (Hasson et al., 2006). A direct *in vitro* IgE-inducing effect of ENF on purified B lymphocytes was excluded (Burastero et al., 2006), whereas an increased IL-4 and a decreased IFN- $\gamma$  production by CD4+ T lymphocytes occurred by addition of ENF *in vitro* and *ex vivo* in selected ENF-treated patients (Burastero et al., 2006). This is relevant since several overlapping peptides covering the entire gp41 were unable to induce IL-4 (and histamine) release by FPR-positive basophils (de Paulis et al., 2002). Molecular mimicry with certain allergens has been postulated and indeed amino acid sequence of ENF resembles part of the allergenic domain of Asp f1 from *Aspergillus fumigatus* (Becker, 2007).

We here investigated an indirect mechanism that could contribute to the enhancement of IgE, i.e. decrease of IL-12 which is the major inducer of the counteracting Th1 cytokine IFN- $\gamma$ . By analyzing the kinetic relationship between IL-12 and IgE production, approximately 60% of patients presented an increase or concordant pattern of both, whereas 40% displayed a discordant course. A caveat is that the IL-12-specific antibody used in our study is claimed to recognize the active IL-12 heterodimer as well as the p40 monomer and p40 homodimers have been described to act as antagonists (Ling et al., 1995). There could be a chance that the antibody binds also p40 homodimers, since we are unable to discriminate between active p35/p40 heterodimers and antagonist p40 homodimers. We could hypothesize that concordance between IL-12 and IgE is observed in the presence of an excess of p40 homodimers, whereas when IL-12 and IgE are discordant more IL-12 heterodimers are produced than p40 homodimers. Further studies could address this particular aspect.

IL-12 represents a key cytokine in the orchestration of cell-mediated immunity against invading microbes. This is witnessed by the fact that several viruses, including HIV-1 (Ma and Montaner 2000), appear to elicit a number of strategies ultimately leading to impairment of IL-12 production in the infected host or cell. It has indeed been documented that infection of monocytes by RNA viruses, including measles (Karp et al., 1996) and HCV (Eisen-Vandervelde et al., 2004) and of dendritic cells by DNA viruses, such as HSV-1 (Pollara et al., 2003), CMV (Moutaftsi et al., 2002) and HHV-6 (Smith et al., 2005), results in a profound inhibition of IL-12 synthesis.

We here document that the majority of patients with highly advanced disease who underwent an *in vivo* ENF-containing antiretroviral regimen experienced an increase of IL-12-producing monocytes upon *in vitro* stimulation. This is a reassuring aspect for multidrug resistant patients at an advanced stage of disease with few therapeutic options left.

We also assessed that technically, following good laboratory procedures, this functional assay can be performed with long-term stored frozen PBMC deriving from advanced HIV-infected individuals. Further studies with a larger number of patients would be useful to assess whether this innate immunity parameter could complement the CD4 T lymphocyte count in the appraisal of responsiveness to antiretroviral treatment, especially in the case of discordant responses to treatment (reduction of viremia with no increase of CD4 counts or vice-versa).

## References

1. Abbas AK, Murphy KM, Sher A (1996) Functional diversity of helper T lymphocytes. *Nature* 383: 787-793. »CrossRef »PubMed »Google Scholar
2. Agarwal SK, Marshall GD Jr (1998) In vivo alteration in type-1 and type-2 cytokine balance: a possible mechanism for elevated total IgE in HIV-infected patients. *Hum Immunol* 59: 99-105. »CrossRef »PubMed »Google Scholar
3. Aquaro S, D'Arrigo R, Svicher V, Perri GD, Caputo SL, et al. (2006) Specific mutations in HIV-1 gp41 are associated with immunological success in HIV-1-infected patients receiving enfuvirtide treatment. *J Antimicrob Chemother* 58: 714-722. »CrossRef »PubMed »Google Scholar

4. Becker EL, Forouhar FA, Grunnet ML, Boulay F, Tardif M, et al. (1998) Broad immunocytochemical localization of the formylpeptide receptor in human organs, tissues, and cells. *Cell Tissue Res* 292: 129-135. »CrossRef »PubMed »Google Scholar
5. Becker Y (2004) The changes in the T helper 1 (Th1) and T helper 2 (Th2) cytokine balance during HIV-1 infection are indicative of an allergic response to viral proteins that may be reversed by Th2 cytokine inhibitors and immune response modifiers—a review and hypothesis. *Virus Genes* 28: 5-18. »CrossRef »PubMed »Google Scholar
6. Becker Y (2007) HIV-1 gp41 heptad repeat 2 (HR2) possesses an amino acid domain that resembles the allergen domain in *Aspergillus fumigatus* Asp f1 protein: review, hypothesis and implications. *Virus Genes* 34: 233-240. »CrossRef »PubMed »Google Scholar
7. Biswas P, Tambussi G, Lazzarin A (2007) Access denied? The status of co-receptor inhibition to counter HIV entry. *Expert Opin. Pharmacother* 8: 923-933. »CrossRef »PubMed »Google Scholar
8. Braun MC, Wang JM, Lahey E, Rabin RL, Kelsall BL (2001) Activation of the formyl peptide receptor by the HIV-derived peptide T-20 suppresses interleukin-12 p70 production by human monocytes. *Blood* 97: 3531-3536. »CrossRef »PubMed »Google Scholar
9. Burastero SE, Paolucci C, Breda D, Soldarini A, Dorigatti F, et al. (2006) Immunological basis for IgE hyper-production in enfuvirtide-treated HIV- positive patients. *J Clin Immunol* 26: 168-176. »CrossRef »PubMed »Google Scholar
10. Byrnes AA, Harris DM, Atabani SF, Sabundayo BP, Langan SJ, et al. (2008) Immune activation and IL-12 production during acute/early HIV infection in the presence and absence of highly active antiretroviral therapy. *J Leukoc Biol* 84: 1447-1453. »CrossRef »PubMed »Google Scholar
11. Castagna A, Biswas P, Beretta A, Lazzarin A (2005) From discovery of biological mechanisms to drug development: the appealing story of entry inhibitors. *Drugs* 65: 879-904. »CrossRef »PubMed »Google Scholar
12. Chehimi J, Starr SE, Frank I, D'Andrea A, Ma X, et al. (1994) Impaired interleukin 12 production in human immunodeficiency virus-infected patients. *J Exp Med* 179: 1361-1366. »CrossRef »PubMed »Google Scholar
13. Chougnet C, Wynn TA, Clerici M, Landay AL, Kessler HA, et al. (1996) Molecular analysis of decreased interleukin-12 production in persons infected with human immunodeficiency virus. *J Infect Dis* 174: 46-53. »CrossRef »PubMed »Google Scholar
14. Clerici M, Shearer GM (1993) A TH1—>TH2 switch is a critical step in the etiology of HIV infection. *Immunol Today* 14: 107-111. »CrossRef »PubMed »Google Scholar
15. de Paulis A, Florio G, Prevete N, Triggiani M, Fiorentino I, et al. (2002) HIV-1 envelope gp41 peptides promote migration of human Fc epsilon RI+ cells and inhibit IL-13 synthesis through interaction with formyl peptide receptors. *J Immunol* 169: 4559-4567. »CrossRef »PubMed »Google Scholar
16. Eisen-Vandervelde AL, Waggoner SN, Yao ZQ, Cale EM, Hahn CS, et al. (2004) Hepatitis C virus core selectively suppresses interleukin-12 synthesis in human macrophages by interfering with AP-1 activation. *J Biol Chem* 279: 43479-43486. »CrossRef »PubMed »Google Scholar
17. Ferrazzi M, De Rinaldis ML, Salotti A, Cirelli A (1993) Serum IgE levels in human immunodeficiency virus (HIV)-1 infected patients: correlation between IgE and CD4+ cells. *Riv Eur Sci Med Farmacol* 15: 67-70. »CrossRef »PubMed »Google Scholar
18. Hasson H, Biswas P, Galli L, Burastero SE, Danise A, et al. (2006) Selective increase in serum IgE following enfuvirtide administration in HIV-1 infected multidrug resistant patients. *New Microbiol* 29: 223-230. »CrossRef »PubMed »Google Scholar
19. Hayes MP, Wang J, Norcross MA (1995) Regulation of interleukin-12 expression in human monocytes: selective priming by interferon-gamma of lipopolysaccharide-inducible p35 and p40 genes. *Blood* 86: 646-650. »CrossRef »PubMed »Google Scholar
20. Karp CL, Wysocka M, Wahl LM, Ahearn JM, Cuomo PJ, et al. (1996) Mechanism of suppression of cell-mediated immunity by measles virus. *Science* 273: 228-231. »CrossRef »PubMed »Google Scholar
21. Lalezari JP, Henry K, O'Hearn M, Montaner JS, Piliero PJ, et al. (2003) Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. *N Engl J Med* 348: 2175-2185. »CrossRef »PubMed »Google Scholar
22. Lazzarin A, Clotet B, Cooper D, Reynes J, Arasteh K, et al. (2003) Efficacy of enfuvirtide in patients infected with drug-resistant HIV-1 in Europe and Australia. *N Engl J Med* 348: 2186-2195. »CrossRef »PubMed »Google Scholar
23. Ling P, Gately MK, Gubler U, Stern AS, Lin P, et al. (1995) Human IL-12 p40 homodimer binds to the IL-12 receptor but does not mediate biologic activity. *J Immunol* 154: 116-127. »CrossRef »PubMed »Google Scholar
24. Lin RY, Smith JK Jr (1988) Hyper-IgE and human immunodeficiency virus infection. *Ann Allergy* 61: 269-272. »CrossRef »PubMed »Google Scholar
25. Matthews T, Salgo M, Greenberg M, Chung J, DeMasi R, et al. (2004) Enfuvirtide: the first therapy to inhibit the entry of HIV-1 into host CD4 lymphocytes. *Nat Rev Drug Discov* 3: 215-225. »CrossRef »PubMed »Google Scholar
26. Ma X, Montaner LJ (2000) Proinflammatory response and IL-12 expression in HIV-1 infection. *J Leukoc Biol* 68: 383-390. »CrossRef »PubMed »Google Scholar
27. Mazza DS, Grieco MH, Reddy MM, Meriney D (1995) Serum IgE in patients with human immunodeficiency virus infection. *Ann. Allergy Asthma Immunol.* 74: 411-414. »CrossRef »PubMed »Google Scholar
28. Moutaftsi M, Mehl AM, Borysiewicz LK, Tabi Z (2002) Human cytomegalovirus inhibits maturation and impairs function of monocyte-derived dendritic cells. *Blood* 99: 2913-2921. »CrossRef »PubMed »Google Scholar
29. Panaro MA, Acquafredda A, Sisto M, Lisi S, Maffione AB, et al. (2006) Biological role of the N-formyl peptide receptors. *Immunopharmacol Immunotoxicol* 28: 103-127. »CrossRef »PubMed »Google Scholar
30. Park JH, Shin BC, Do BH, Oh JT, Lee JM, et al. (2002) Serum IgE levels in Korean patients with human

- immunodeficiency virus infection. Korean J Intern Med 17: 88-93. »CrossRef »PubMed »Google Scholar
31. Pollara G, Speidel K, Samady L, Rajpopat M, McGrath Y, et al. (2003) Herpes simplex virus infection of dendritic cells: balance among activation, inhibition, and immunity. J Infect Dis 187: 165-178. »CrossRef »PubMed »Google Scholar
32. Rancinan C, Morlat P, Chene G, Guez S, Baquey A, et al. (1998) IgE serum level: a prognostic marker for AIDS in HIV-infected adults. J Allergy Clin Immunol 102: 329-330. »CrossRef »PubMed »Google Scholar
33. Saberi P, Caswell NH, Gruta CI, Tokumoto JN, Dong BJ (2008) Immunologic benefits of enfuvirtide in patients enrolled in a drug assistance program. Ann Pharmacother 42: 621-626. »CrossRef »PubMed »Google Scholar
34. Smith AP, Paolucci C, Di Lullo G, Burastero SE, Santoro F, et al. (2005) Viral replication-independent blockade of dendritic cell maturation and interleukin-12 production by human herpesvirus 6. J Virol 79: 2807-2813. »CrossRef »PubMed »Google Scholar
35. Su SB, Gong WH, Gao JL, Shen WP, Grimm MC, et al. (1999) T20/DP178, an ectodomain peptide of human immunodeficiency virus type 1 gp41, is an activator of human phagocyte N-formyl peptide receptor. Blood 93: 3885-3892. »CrossRef »PubMed »Google Scholar
36. Trinchieri G (1995) Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol 13: 251-276. »CrossRef »PubMed »Google Scholar
37. Trinchieri G (2003) Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol 3: 133-146. »CrossRef »PubMed »Google Scholar
38. Weiss CD (2003) HIV-1 gp41: mediator of fusion and target for inhibition. AIDS Rev 5: 214-221. »CrossRef »PubMed »Google Scholar