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AN IL-15 DEPENDENT CD8 T CELL RESPONSE TO SELECTED HIV EPITOPES IS RELATED TO VIRAL CONTROL IN EARLY-TREATED HIV-INFECTED SUBJECTS

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In some early-treated HIV$^+$ patients, Structured Treatment Interruption (STI) is associated to spontaneous control of viral rebound. Thus, in this clinical setting, we analyzed the immunological parameters associated to viral control. Two groups of early treated patients who underwent STI were retrospectively defined, according to the ability to spontaneously control HIV replication (Controller and Non-controller). Plasma cytokine levels were analyzed by multiplex analysis. CD8 T cell differentiation was determined by polychromatic flow cytometry. Antigen-specific IFN-γ production was analyzed by ELISpot and intracellular staining after stimulation with HIV-peptides. Long-term Elispot assays were performed in the presence or absence of IL-15. Plasma IL-15 was found decreased over a period of time in Non-Controller patients, whereas a restricted response to Gag (aa.167-202 and 265-279) and Nef (aa.86-100 and 111-138) immunodominant epitopes was more frequently observed in Controller patients. Interestingly, in two Non-Controller patients the CD8-mediated T cells response to immunodominant epitopes could be restored *in vitro* by IL-15, suggesting a major role of cytokine homeostasis on the generation of protective immunity. In early-treated HIV$^+$ patients undergoing STI, HIV replication control was associated to CD8 T cell maturation and sustained IL-15 levels, leading to HIV-specific CD8 T cell responses against selected Gag and Nef epitopes.

Several studies indicate that HIV-1 infection rapidly induces virus-specific CD8$^+$ T responses (1), and much evidence, as reviewed by A.J. McMichael et al (2), suggests that these responses play a relevant role in the control of acute and early viral replication. Moreover, robust and persistent HIV-specific CD8$^+$ responses were observed in an HIV$^+$ population known as "long term non-progressors" (LTnP) (3). The most convincing evidence for direct CD8-mediated T cell control of HIV replication came from CD8 depletion experiments, demonstrating the functional role of these cells in regulating viremia in SIV-infected rhesus macaques (4). Differently, dysfunctional virus-specific CD8 T cells with impaired maturation were found in chronic HIV infection (5). Several strategies have been proposed to boost CD8$^+$ T cell response in chronically infected HIV patients, including structured treatment

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interruption (STI) (6). However, HIV-specific $CD8^+$ T lymphocytes are detectable at high frequencies after treatment interruption but fail to control virus replication, indicating that the quality but not the quantity of HIV-specific $CD8^+$ T lymphocytes may determine the outcome of the antiviral response (7). Differently, a critical view of STI as a therapeutic scenario has been suggested by several data in chronically HIV-infected patients (7-8). However, in the acute phase of HIV and SIV infection, a number of reports have shown a complete or transient containment of viremia after STI (9-12).

In HIV infection, changes in cytokines milieu may deeply influence immune response (13-14). In addition, secretion of type 1 cytokines, namely IL-2, IL-12 and interferon (IFN) γ, is generally decreased, while production of both type 2 (including IL-4, IL-5, IL-13) and proinflammatory cytokines (e.g. IL-1, IL-6, IL-8, TNF-α) is increased. Such changes correlate with disease stage and immune depletion (13, 15). Specifically, IL-15, a cytokine that plays a major role in $CD8^+$ T cell maturation and survival (16) was shown to play a crucial role in the ability to control HIV replication in chronically infected patients (17-18). Nevertheless, both CD8 T cell response and cytokine networks are deeply affected during chronic HIV infection.

In some early-treated HIV^+ patients, Structured Treatment Interruption (STI) is associated to spontaneous control of viral rebound (12). Thus, we analyzed the immunological parameters associated to spontaneous viral control in early treated HIV patients undergoing STI.

MATERIALS AND METHODS

Study Population

A randomized controlled trial was carried out on 16 patients who started HAART during the early phase of HIV infection. Two different STI protocols (8 patients on 4 weeks off/8 weeks on HAART; 8 patients on intermittent therapy guided by plasma HIV-RNA levels) were performed over twelve months. No differences in immunological parameters, clinical status or virological outcome were found between the two STI protocols.
During the first year of the study, one patient dropped out because of insufficient compliance. At follow-up, the patients resumed treatment only when necessary in accordance with *pro-tempore* guidelines (19) (CD4<350 cells/μl or plasma HIV-RNA>50,000 copies/ml), and were monitored for viral and immunological parameters until month 24. The immunological criteria for inclusion in this study were: CD4 cell counts ≥500 cells/μl and repeatedly undetectable plasma HIV-RNA. All 16 patients were male; immunological and virological parameters before initial HAART are reported in Table I where treatment regimens and lengths are also shown. The Ethics Committee of the Institute approved this study, and signed informed consent was obtained from enrolled patients. Clinical and immunological follow-up were performed at the time of suspension of HAART (t0) and one month after the conclusion of STI protocols (t13), by analyzing the response of HIV-specific CD8/CD4, naïve/memory T lymphocytes dynamics and the capacity of inducing a selective response to HIV CD8 epitopes. In order to investigate whether any immunological parameter could be associated to a "drug-free" control of HIV replication, at month 24 we decided to retrospectively assign patients to two different outcome groups. Therefore, patients able to remain HIV treatment-free from month 12 to 24 were retrospectively associated to "Controller" group; on the contrary, patients not able to remain HIV treatment-free from month 12 to 24 were retrospectively associated to "Non-Controller" group. Patient characteristics are summarized in Table II.

Cell preparation, stimulation and ELISpot assay for IFN-γ producing cells

PBMC were separated by density gradient centrifugation (Ficoll-Hypaque, Pharmacia Biotech, Norway). Enzyme-linked immunospot (ELISpot) assay was used to identify antigen-specific T cells. 2 x 10^5 PBMC were plated in duplicates in complete medium, in 96 well plates (MAIPS45; Millipore, Sunnyvale, CA) pre-coated with IFN-γ capture antibody (M-700A, Endogen, Woburn, MA), and incubated for 48 hrs at 37°C, 5% CO_2. Gag, Nef and Tat peptides were used as antigens both as whole pools and as peptide matrix as described below. Spots were counted by an automated ELISA-Spot assay video analysis system (AELVIS, Hannover, Germany). The total number of antigen-specific T cells was calculated after subtracting the mean of the background number of Spot Forming Cells (SFCs) in the negative control from the mean of the spot number in the sample.

In long-term ELISpot assays (20-21), 10 x 10^6 freshly isolated PBMC in complete medium were stimulated with HIV peptides (1 μg/ml/peptide) at 37°C under 5% CO_2 atmosphere. After 3-d incubation period, 5 ml of the cell culture supernatant were removed and replaced with complete medium containing recombinant IL-15 (50 ng/ml). On day 7 culture medium was changed; on day 8, cells were transferred to duplicate wells of ELISpot plate

and stimulated with HIV peptides as described below.

Antibodies and peptides

The following anti-human monoclonal antibodies were obtained from BD Pharmingen, San Diego, CA: anti-perforin FITC (fluorescein isothiocyanate) (clone 27-35), anti-CD45RA FITC (clone L48), anti-CD27 PE (phycoerythrin) (clone M-T271), anti-CD4 CyChrome (clone RPA-T4), anti-CD8 APC (allophycocyanin) (clone RPA-T8).

MHC Class-I-binding (CD8) peptides of 15 amino acids in length were used, including selected regions from the consensus sequence (clade 1B) of HIV-1 Gag and Nef proteins. These peptides were obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (catalogue numbers 8117, 5189), or purchased from Sigma-Genosys (Cambridge, UK), and were previously shown to be able to bind promiscuously most HLA haplotypes (22). Lyophilized peptides were re-suspended in DMSO at stock concentrations of 10 mg/ml for each peptide; Gag, Nef and Tat peptide pools were made at a final concentration of 1 μg/ml/peptide. A matrix was built in such a way that each peptide was present in two different peptide pools, allowing the identification of the response to the single peptide (23). The frequency of responding subjects (i.e. showing a significant peptide-specific response) was calculated for each peptide and expressed as net increase vs. T0. Significant response was defined when it was higher than the lower one-tail (95%) confidence value.

Analysis of surface markers and intracellular cytokine (IFN-γ) expression

5 x 10^5 PBMC were washed in PBS containing 1% BSA and 0.1% sodium azide and were incubated for 15 min. at 4°C with the indicated FITC-, PE-, CyChrome-, and APC-conjugated mAbs. Samples were fixed in 1% paraformaldehyde in PBS. Perforin production was detected after incubation with anti-perforin mAb diluted in PBS, 1% BSA, 0.1% saponin. Each sample was acquired with FACSCalibur flow cytometer (BD Biosciences) for 20,000 events in the lymphocyte gate, and analyzed using CellQuest software (BD Biosciences).

Analysis of cytokines plasma levels

Plasma IL-2, IL-4, IL-5, IL-8, IL-10, IL-12(p70), IL-13, TNFα and IFNγ levels were determined by Human TH1/TH2 cytokine array 1 (SearchLight™ Multiplex assay, Pierce, Woburn, MA) using a pre-spotted plate with 9 capture antibodies per well, followed by ELISA procedure amplified by streptavidin-HRP and SuperSignal® substrate generating a chemiluminescent signal imaged by a 16-bit cooled CCD camera and analyzed by ArrayVision Software for calculation of spots within each well. Plasma IL-15 was measured by colorimetric sandwich Quantikine ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Sensitivity of all assays was below 1 pg/ml, and reference standards for calculating the results were provided by the manufacturers.

Statistical analysis

Statistical analysis was performed by Prism 4 software (GraphPad, San Diego, Ca., USA). To evaluate differences between t0 (before STI) and t13 (one month after the end of STI protocols) in Controller versus Non-Controller patients, Wilcoxon matched-pair non-parametric test was used. Values of $p < 0.05$ were considered significant.

RESULTS

Plasma cytokine levels and T-cell maturative patterns in HIV⁺ early-treated Non-Controller and Controller patients

Plasma IL-2, IL-4, IL-5, IL-8, IL-10, IL-12(p70), IL-13, IL-15, TNFα and IFNγ levels were determined at t0 and t13. By comparing Controller to Non-Controller patients, no differences were found for IL-2, IL-4, IL-10, IL-12(p70), TNFα and IFNγ values (data not shown). Differently, in Non-Controller patients significant decreases of IL-8 and IL-15 plasma levels were found at t13 in respect to t0 (Fig. 1, panel A), whereas in Controller patients significant increases of IL-5 and IL-13 plasma levels at t13 in respect to t0 were observed (Fig. 1, panel D), suggesting that a complex modulation of cytokine network may be associated to the control HIV replication after treatment suspension.

According to the T cell maturative pathway (24), different T cell subsets could be identified by analyzing the expression of CD27 and CD45RA molecules. Specifically, Naïve (CD27+, CD45RA+), Central-Memory (CD27+, CD45RA-), Effector-Memory (CD27-, CD45RA-) and Terminally Differentiated (CD27-, CD45RA+) T cell subsets were determined at t0 (before STI) and at t13 (one month after the end of STI). By multiplex staining, CD4 and CD8 T differentiation pathways were analyzed among Controller and Non-Controller patients (Fig. 1, panels E-H). No difference was found in CD4 T cell maturation at t0 and t13 in Non-Controller patients (Fig. 1, panel E) with some variability among Central Memory cells that was not statistically significant. Similarly, no difference

was found in CD4 T cell maturation at t0 and t13 in Controller patients (Fig. 1, panel F). Moreover, no differences in CD8 T cell maturative pathways were found in Non-Controller patients (Fig. 1, panel G). Interestingly, significant increases of Central-Memory (t0 median 143 cells/μl, IQR 72-202; t13 median 218 cells/μl, IQR 203-377, p=0.078) and Effector-Memory (t0 median 97 cells/μl, IQR 27-126; t13 median 225 cells/μl, IQR 110-344, p=0.0391) $CD8^+$ T cell subsets were found in Controller patients at t13 vs. t0 (panel H). Since a reduction in perforin content was previously associated with disease progression in chronic HIV infected patients (5, 7), we analyzed whether a similar pattern was associated to treatment interruptions in our cohort of early treated HIV^+ patients. An increase of perforin content was observed in all patients at t13 along $CD8^+$ T cell differentiation pathway (naïve T cells: mean+/-SD MFI 12.6+/-7.0; Central Memory T cells: MFI 24.1+/-16.1; Effector-Memory T cells: MFI 120.0+/-98.6 and Terminally-Differentiated T cells: MFI 119.7+/-73.7), but when data were divided among Non-Controller and Controller groups, no significant differences were found between the groups at both t0 and t13 (data not shown). As expected, the perforin content among $CD8^+$ T cell subsets increased in the presence of viral replication after treatment interruption, but no differences were detectable between the two groups of patients. These observations indicate that the different $CD8^+$ T cell profile of the Controller group is related to cytokine-producing effector cells, rather than to perforin-containing cytolytic effectors.

HIV-specific CD8 T cell responses to Gag and Nef peptide pools

To evaluate HIV-specific antiviral response, PBMCs from Non-Controller and Controller patients were stimulated *in vitro* with CD8-specific peptide pools from Gag, and Nef HIV proteins. A representative flow cytometry analysis for IFN-γ production by $CD8^+$ and $CD4^+$ T cells is shown in Fig. 2 (Panels A-B). The peptide pools were selectively able to trigger $CD8^+$ T cells, as shown in Fig. 2 (Panel A), since most of the IFN-γ producing cells after stimulation were indeed $CD8^+$ T cells. The number of IFNγ-producing cells per 10^6 PBMC evaluated by ELISpot, was compared between t0 and t13 in Non-Controller and Controller patients. No difference was found in HIV-specific responses when comparing Non-Controller to Controller groups both at t0 and at t13. As shown in Fig. 2, no difference was found by comparing t0 to t13 responses by Gag-, and Nef-specific $CD8^+$ T cells within the Non-Controller group (panel C). Interestingly, an increase in responses to Gag (p=0.015) and Nef (p=0.031) was found in Controller patients when comparing t0 to t13 (panel D). Thus, the improved effector/memory profile among $CD8^+$ T cells in the Controller patients was associated with an increased HIV-specific cytokine response.

HIV-specific CD8 T cell responses to Gag and Nef epitopes and influence of IL-15

To investigate the $CD8^+$ T cell epitopes that were involved in this response, the selective response against different pools of Gag and Nef peptides was analyzed by ELISpot. The proportion of patients showing a significant HIV-specific response was calculated for each peptide. The results confirmed the involvement of a specific response against HIV Gag and Nef proteins. As shown in Fig. 3 (Panel A), the response to four Gag-specific peptides (aa.167-181, 184-198, 188-202 and 265-279) and to four Nef-specific peptides (aa.86-100, 111-122, 113-125 and 126-138) increased in Controller in comparison to Non-Controller groups. Thus, an increased response to Gag and Nef immunodominant epitopes was associated to the control of HIV replication.

Since IL-15 is known to influence antigen-specific T cell responses (25-27), PBMC from two representative Non-Controller patients were cultured *in vitro* with recombinant IL-15 (50 ng/ml) to check whether IL-15 may restore CD8 T cell responses to Gag and Nef peptides. As shown in Fig. 3 (Panel B), specific responses to selected Gag (aa.167-181, 184-198, 188-202 and 265-279) and Nef (aa.86-100) peptides were recovered by cytokine co-culture.

DISCUSSION

Structured Treatment Interruption (STI) may alleviate some of the problems associated with long-term anti-retroviral therapy (28). Moreover, STI has been proposed to boost the antiviral immunity in HIV-infected subjects. However, the emergence

Table I. ***Patients baseline characteristics.*** *The clinical parameters of the patients before initial treatment, the therapeutic regimen and the length of treatment before STI are shown. Azt: Zidovudine; 3tc: Lamivudine; nfv: Nelfinavir; efv: Efavirenz; idv: Indinavir; d4t: Stavudine; lop: Lopinavir; rit: Ritonavir.*

Patient	Age	CD4	HIV-RNA	Therapy	Months of
		(cells/μl)	(copies/ml)		therapy before STI
01	35	1103	310,000	Azt, 3tc, nfv	30
02	27	307	1,300,000	Azt, 3tc, efv	10
03	19	405	1,300	Azt, 3tc, idv	24
04	40	711	75,000	Azt, 3tc, nfv	28
05	19	653	800,000	d4t, 3tc, idv	52
06	24	882	41,000	Azt, 3tc, idv	46
07	57	322	4, 900,000	Azt, 3tc, efv	27
08	35	568	1,300,000	Azt, 3tc, efv	15
09	39	302	192,000	Azt, 3tc, efv	24
10	52	545	39,000	Azt, 3tc, efv	24
11	34	390	780,000	Azt, 3tc, idv	19
12	30	1038	150,000	Azt, 3tc, idv	21
13	40	444	1,000,000	Azt, 3tc, idv	42
14	26	768	500,000	Azt, 3tc, efv	14
15	29	224	27,000	Azt, 3tc, efv	24
16	27	341	189,000	Azt, 3tc, lop/rit	20

of pre-terminally differentiated CTL suggests a critical view of STI in chronic HIV-infected patients as a possible clinical-therapeutic scenario (7). Differently, a number of reports have shown a complete or transient containment of viremia after STI in the acute phase of retroviral infection (9-12, 29). Nevertheless, the preservation of IFN-γ and IL-2 production by HIV-specific $CD4^+$ T cells was found to be induced by an early antiretroviral treatment, but it was not correlated with a successful control of HIV replication during STI (30). Thus, data to justify STI as a safe and effective approach aimed at improving antiviral immunity remain controversial (28, 31). In our study, we evaluate the immune correlates of viral control in early-treated HIV-infected patients undergoing STI.

After one-year of STI, opposite changes were observed in Controller and non-Controller subjects regarding IL-15 and IL-8 plasma levels. IL-15 has been previously shown to correlate with

Table II. ***Clinical and laboratory characteristics after STI.*** *STI protocols (4 weeks off/8 weeks on HAART or intermittent therapy guided by plasma HIV-RNA levels) were performed over one year. The viral-immunological parameters were analyzed before STI (t0) and one month after the end of the STI protocols (t13). At month 24, the patients were retrospectively assigned to Controller (A) and non-Controller (B) groups according to the ability to control HIV replication from month 12 to 24 in absence of antiretroviral treatment.*

A. *HIV-controller patients.*

Patient	CD4(t0)	HIV-RNA(t0)	STI Protocol	CD4(t13)	HIV-RNA(t13)	Therapy at t24
	(cells/μl)	(copies/ml)		(cells/μl)	(copies/ml)	
01	741	<50	4wks off/8wks on	1241	1767	Off therapy
04	1102	<50	4wks off/8wks on	803	13354	Off therapy
10	1038	<50	4wks off/8wks on	901	<50	Off therapy
13	867	<50	4wks off/8wks on	690	6132	Off therapy
06	754	<50	HIV-RNA guided	1150	715	Off therapy
07	1154	<50	HIV-RNA guided	596	23011	Off therapy
08	1326	<50	HIV-RNA guided	1053	327	Off therapy
12	2097	<50	HIV-RNA guided	1136	22182	Off therapy

B. *HIV-non controller patients.*

Patient	CD4 (t0)	HIV-RNA(t0)	STI Protocol	CD4(t13)	HIV-RNA(t13)	Therapy resumption
	(cells/μl)	(copies/ml)		(cells/μl)	(copies/ml)	
03	458	<50	4wks off/8wks on	774	1208	t20
09	919	<50	4wks off/8wks on	878	1848	t15
14	1451	<50	4wks off/8wks on	853	89592	t14
02	872	<50	HIV-RNA guided	759	45571	t16
11	513	<50	HIV-RNA guided	428	4281	t19
15	638	<50	HIV-RNA guided	416	22785	t15
16	594	<50	HIV-RNA guided	455	29429	t20

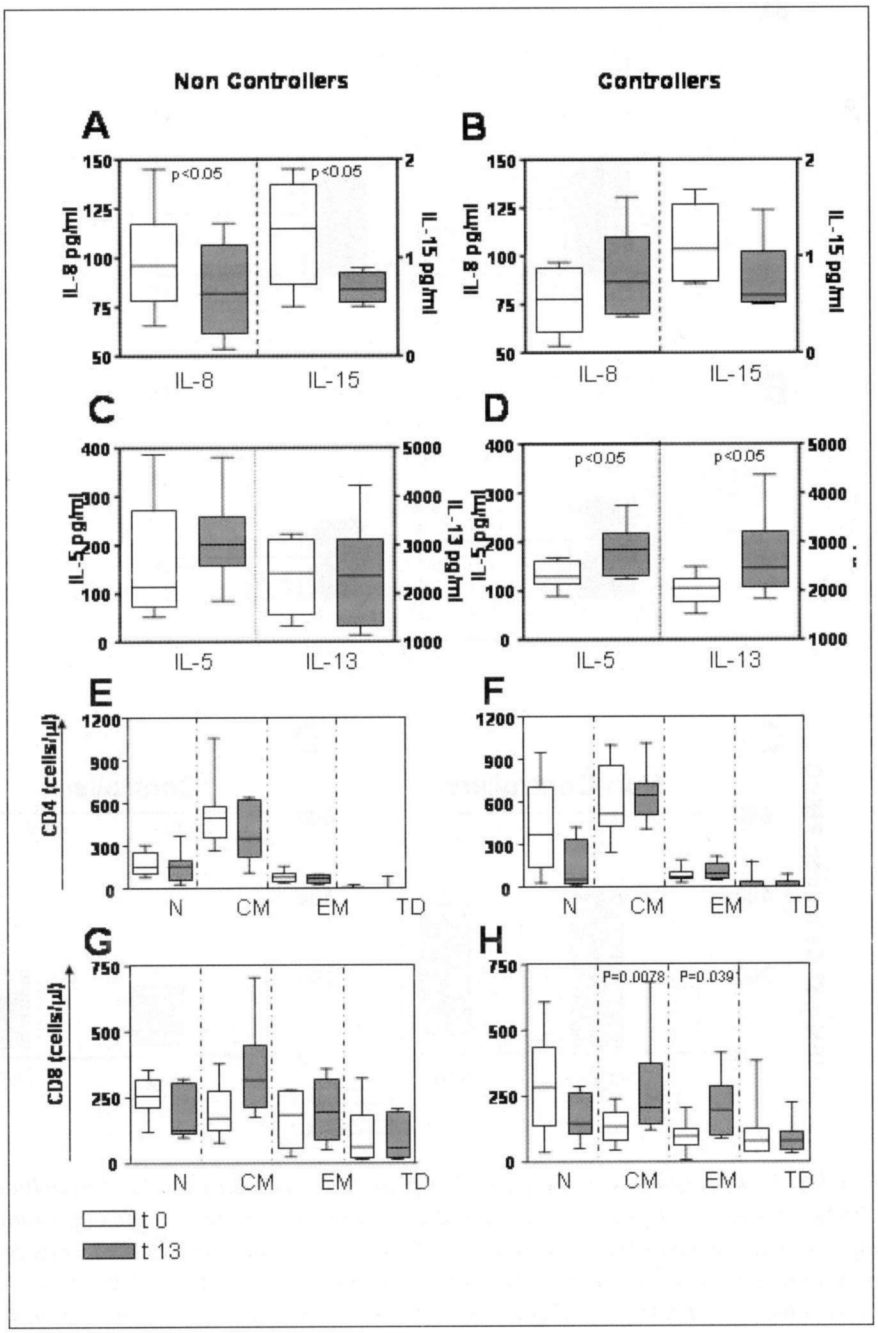

Fig. 1. *Ex vivo plasma cytokine-profiles and T cells maturative pattern kinetics in Controller and Non-Controller groups of early treated HIV+ patients. Plasma cytokine levels were determined at t0 and t13 in Non-Controller (A,C) and Controller patients (B,D). Longitudinal assessment of maturative patterns was analyzed among CD4⁺ (E, F) and CD8⁺ T cells (G, H). CD4⁺ and CD8⁺ T cells were expressed as cells/µl. We compared the T cell phenotypes in the peripheral blood (Naïve, Central-Memory, Effector-Memory, Terminally-Differentiated cells) before STI, t0 (white boxes), and one month after the end of STI protocols, t13 (grey boxes), in Non-Controller (E, G) and Controller (F, H) patients. The bars encompass the middle 50% of the individual measurements and the horizontal bar-dividing line indicates the median value. The vertical lines span the range of the lowest and highest measurements, and the boxes indicate Inter-Quartile Ranges (IQR). Significance refers to non-parametric Wilcoxon matched (t13 vs. t0) test. Values of $p < 0.05$ were considered significant.*

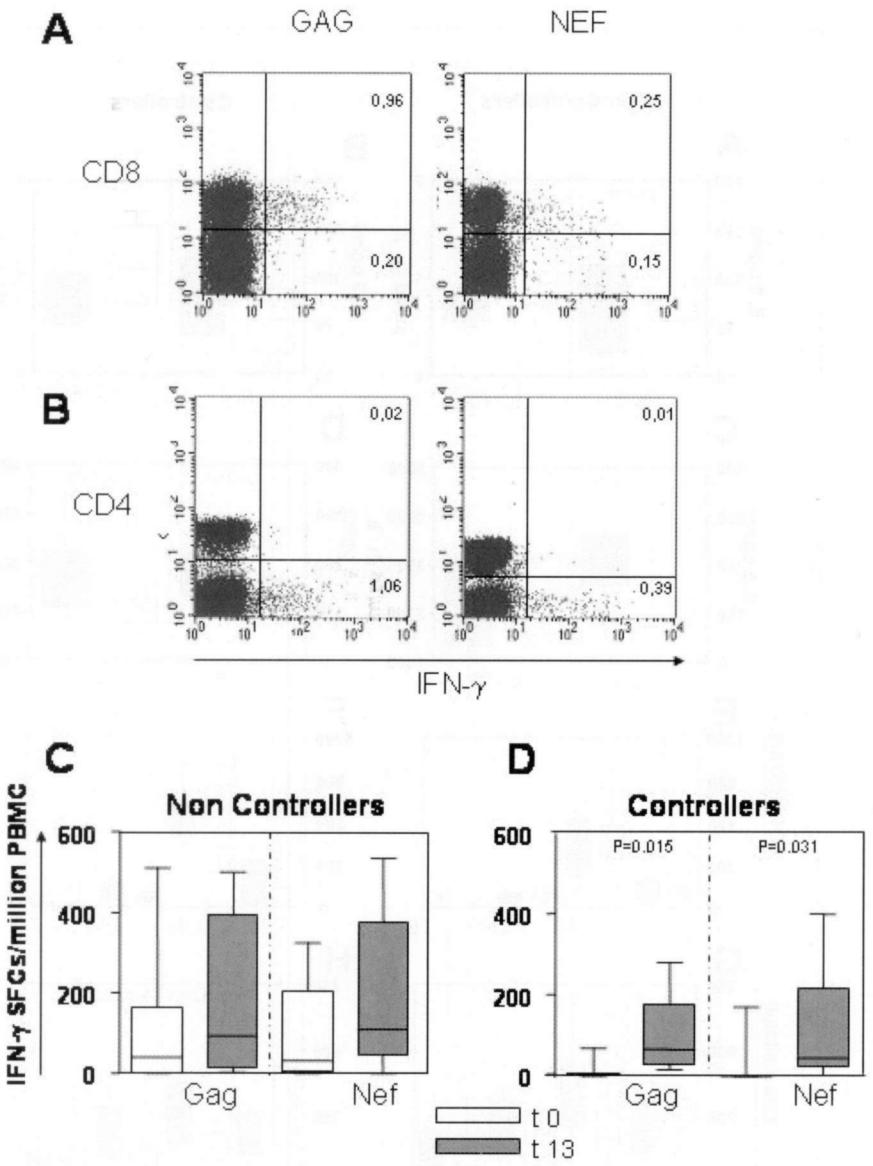

Fig. 2. *HIV-specific CD8 T cell responses to Gag and Nef peptide pools measured by intracellular cytokine staining and EliSpot assay.PBMC cytokines response were evaluated by intracellular flow cytometry assay after CD8-specific stimulation with Gag, Nef and Tat peptides. In particular, IFN-γ production among CD8⁺ T cells (panel A) and CD4⁺ T cells (panel B) was determined. Flow cytometry panels are exemplificative of a typical response from an HIV⁺ subject. IFN-γ production was evaluated in PBMC by EliSpot assay after CD8-specific peptide stimulation with Gag, Nef and Tat HIV proteins. We compared IFN-γ production among Non-Controller (panel C) and Controller (panel D) patients before STI, t0 (white boxes), and one month after the end of STI protocols, t13 (grey boxes). The bars encompass the middle 50% of the individual measurements and the horizontal bar-dividing line indicates the median value. The vertical lines span the range of the lowest and highest measurements, and the boxes indicate IQR. Significance refers to non-parametric Wilcoxon matched (t13 vs. t0) test. Values of $p<0.05$ were considered as significant.*

disease progression and to predict the response to STI (17-18). A decrease of IL-15 concentration is conceivably associated with failure to control HIV replication after STI, as confirmed in our study, whereas maintenance of production of IL-15/IL-8 ensures a vigorous cell mediate antiviral response. IL-15 has been reported to be a chemo-attractant for T lymphocytes and NK cells, enhancing immune

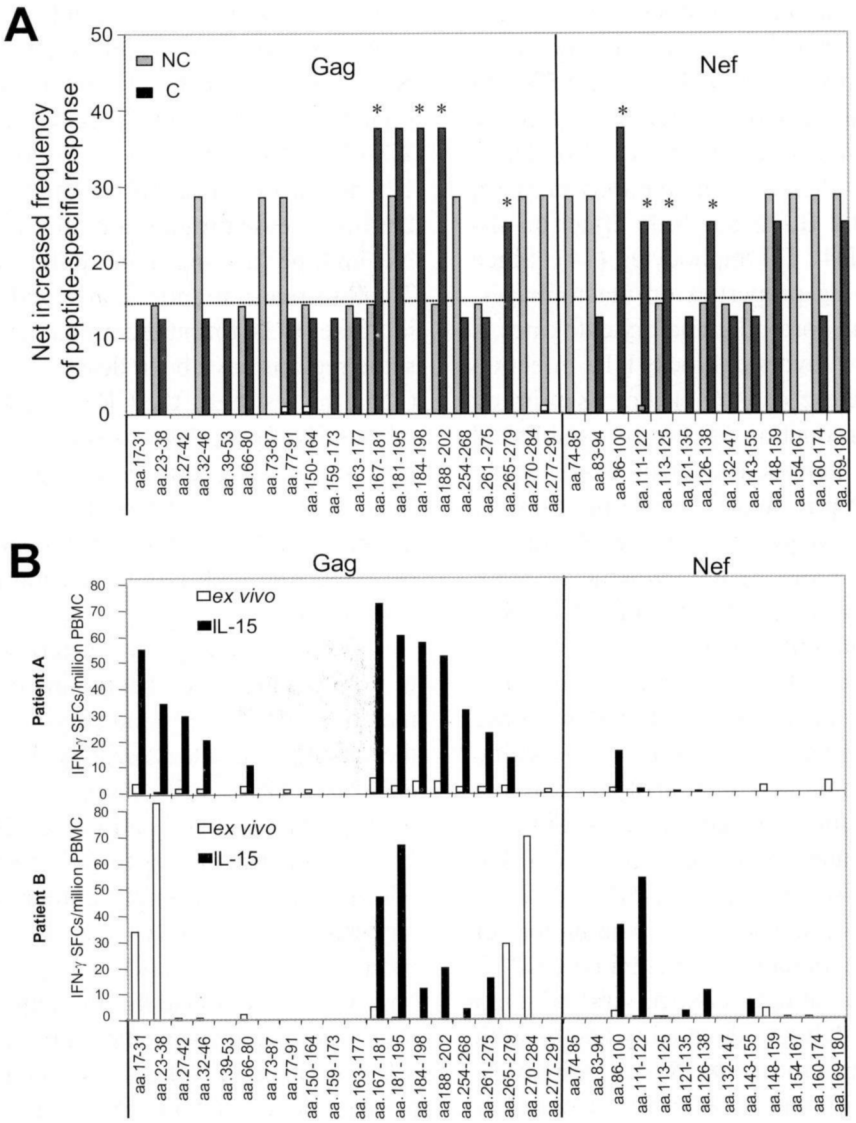

Fig. 3. *IFN-γ production in response to Gag and Nef selected epitopes and influence of IL-15 cytokine in vitro. In panel A, the comparison of frequency of peptide-specific response between Non-Controller and Controller patients is shown. The frequency of responding subjects (i.e. showing a significant peptide-specific response) was calculated for each peptide and expressed as net increase vs. T0. Significant response was defined as higher than the lower one-tail (95%) confidence value. Net increased frequency of peptide-specific response was compared between Non-Controller (grey bars) and Controller (filled bars) patients. 4 Gag-specific peptides and 4 Nef-specific peptides (*) showed an increase in Controller patients in comparison to Non-Controller. In panel B, representative Non-Controller patients were studied for antigen-specific response ex vivo (white bars) and after stimulation in the presence of IL-15 (black bars). IL-15 was able to induce a recovery of net specific responses to selected Gag and Nef epitopes.*

functions during HIV-infection and being able to promote the generation of cytolytic effectors (14-32). IL-15 may contribute to leukocyte recruitment to tissues not only by direct chemotaxis of T cells, but they also act as a proinflammatory cytokine that induces monocytes to secrete IL-8 (33). Specifically, IL-15 stimulates monocytes to express IL-8 at both the mRNA and protein levels in the same concentration range that is effective in neutrophil activation. Thus, the IL-15/IL-8 production in HIV-infected subjects may improve the natural response to intracellular pathogens (34). IL-5 and

IL-13, both increased in controller patients, are type 2 cytokines described as negative regulators of cytotoxic responses (35), even if IL-13 decreases in progressive HIV disease and is restored by effective antiviral treatment, together with IFNγ (36). There is scant reported evidence of interactions between IL-15 and IL-13, and IL-15 has little effect on IL-4 production by $CD4^+$ T lymphocytes (14). Since IL-13 has deactivating properties on macrophages, down-regulating many proinflammatory cytokines, it is disputed whether it favors or blocks HIV infection and replication in these cells (37), but its priming effect for IL-12 production is firmly established (38). Therefore an increased level of IL-13 might carry beneficial implications for restoration of antiviral responses. Altogether, our results indicate that complex modulations of the cytokine network were found to be associated to the control of HIV replication after treatment suspension.

Individuals with chronic progressive HIV infection have an impaired $CD8^+$ T cell response (39). In addition, patients with a strong HIV-specific CTL response show a rapid reduction of HIV viremia compared with patients that have a weak immune response and are unable to control viremia (1). We have previously shown that chronic HIV^+ patients undergoing STI have a specific accumulation of non-functional pre-terminally differentiated $CD8^+$ T cells, making them unable to control viral rebound (7). With the aim of defining the quality and breadth of protective HIV-specific $CD8^+$ T cell responses, we studied the differences between Controller and Non-Controller groups in early treated HIV^+ patients undergoing STI. A successful control of HIV replication was linked to a preserved cytokine balance, finally leading to the expansion of virus-specific $CD8^+$ T cells with well-differentiated central memory and effector phenotypes.

It was previously shown that acutely and chronically infected patients have different HIV-specific $CD8^+$ T cells response repertoires (40-42). During early HIV infection, specific $CD8^+$ T cells are restricted, and directed preferentially toward high entropy peptides, while chronic infected patients targeted conserved peptides with lower entropy (43-44). In our study, Controller and Non-Controller patients showed different $CD8^+$ response repertoires: a protective response was associated with a skewed response to immunodominant Gag and Nef epitopes, in comparison to a wider and weaker response in Non-Controller patients. Interestingly, we found both Gag (aa.167-181, 184-198, 188-202 and 265-279) and Nef epitopes (aa.86-100, 111-122, 113-125 and 126-138) are associated with viral control. During disease progression, the response to variable Nef epitopes declines longitudinally, while the $CD8^+$ T cell response to more conserved Gag/Pol epitopes increases (43). Immunodominant Nef epitopes of the same regions have been described in acute infection (45). Interestingly, the $CD8^+$ T cell response to one of these Nef peptides was related to a better clinical outcome in long-term non progressor HIV-patients (46). Moreover, the $CD8^+$ T cell reactivity to a Nef dominant epitope in HLA B13-positive patients may be associated with a more favorable course of HIV infection (47).

Previous studies have demonstrated that IL-15 is able to stimulate the proliferation of naïve and memory $CD8^+$ T cells and plays an important role in the maintenance of these cells *in vivo* (48). The survival effect on $CD8^+$ T cell subsets are associated with the up-regulation of Bcl-2 and Bcl-xl (25, 49). Moreover, IL-15 stimulates the expansion of HIV-specific T cell effectors (26). Interestingly, immune response to recall antigens or HIV-peptides was shown to be strikingly enhanced by IL-15 (14). In agreement with these observations, we found that the addition of IL-15 could restore the peptide-specific $CD8^+$ T cell response to selected Gag and Nef epitopes. Our data confirm the pivotal role of cytokines able to modulate lymphocyte activity and lifespan such as IL-15, suggesting a possible role in immunotherapy (18).

Continuous replacement and maintenance of cells endowed with antiviral activity need a finely tuned cytokine environment, which may account for HIV control after HAART discontinuation. By analyzing several plasma cytokines we were able to show a complex modulation of cytokine network that was associated to the control HIV replication after treatment suspension; these changes correlated with the preservation of $CD8^+$ T cell function. Patients able to spontaneously control HIV replication were intrinsically able to mount a restricted and effective $CD8^+$ T cell response. Since response profiling was associated with successful treatment interruption in early treated patients, monitoring $CD8^+$ T cell

responses to selected HIV epitopes may allow determining time off-therapy.

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