Expression of Interleukin-15 and Interleukin-15R α in Monocytes of HIV Type 1-Infected Patients with Different Courses of Disease Progression

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Abstract

Interleukin-15 (IL-15) enhances the effector mechanisms of anti-HIV immune responses and thus is considered a potential adjuvant of HIV-1 vaccine. However, there are a lack of data concerning the relationships between IL-15 expression and regulation in HIV-1-infected patients and the course of disease progression. We found that IL-15, but not IL-15R α , is expressed at significantly higher levels in the CD14⁺ monocytes [stimulated or not with interferon (IFN)- γ] of long-term nonprogressors (LTNP) than in those of HIV-1 progressors or healthy controls. There was no between-group difference in the amounts of soluble IL-15 released from the cells. We also found that like the healthy controls, the LTNP expressed the IL-15 and IL-15R α genes in a more coordinated manner than the progressors. Our findings show that there are significant differences in IL-15 expression between patients with different courses of HIV infection, and that the coordinated expression of the IL-15 and IL-15R α genes is dysregulated in patients with progressive disease. They also provide important information concerning the mechanisms of infection and the potential use of IL-15 as a therapeutic agent.

Introduction

THE MAIN IMMUNOLOGICAL EFFECT of HIV-1 infection is the progressive depletion of CD4⁺ T lymphocytes and consequent immune deficiency. The number of circulating CD4⁺ T lymphocytes correlates inversely with the level of viral replication, and persistently high plasma viral loads predict a rapid disease onset.¹ In addition to the depletion of CD4⁺ T cells, CD8 T cells are also prone to be premature, and CD95/Fas-mediated apoptosis^{2–4} and the expression of CD38 have been found to be a strong predictor of AIDS after both short- and long-term HIV infection.⁵

T cell homeostasis is governed by various cytokines,^{6–8} including interleukin (IL)-15. What makes IL-15 different from the other cytokines in the same family is its prolonged availability. Although it can bind to the membrane of monocytes independently of IL-15R α ,⁹ together with it, it forms stable complexes that can be internalized and recycled to the cell membrane for efficient trans-presentation to interacting cells.^{10,11} The IL-15R α chain not only acts as a classical chap-

erone to the cell membrane, but it also helps regulate the expression of IL-15 by binding it within the intracellular compartment and transporting it through the Golgi apparatus.¹² Because of its high affinity binding to IL-15R α and cell recycling, IL-15 is hardly detectable in soluble form even after cell stimulation. It is expressed by monocytes, macrophages, dendritic cells, and a number of nonhematopoietic cells, and the expression of the IL-15 gene is increased after interferon IFN- γ ,⁹ lipopolysaccharide (LPS),¹³ or viral stimulation¹⁴ in human monocytes. These cells also have high IL-15 protein levels in the cytoplasm after LPS stimulation,¹³ or on the cell surface and in the cytoplasm after IFN- γ stimulation.⁹

Studies of IL-15-deficient¹⁵ and virus-infected IL-15 knockout mice¹⁶ have shown that IL-15 is important for the generation and survival of memory CD8 T cells.¹⁰ It also has important functions during HIV infection as it enhances IFN- γ production by HIV-specific CD8 T cells¹⁷ as well as their proliferation and degranulation.¹⁸

A minority (1–2%) of HIV-1-infected subjects are relatively resistant to the immunological deterioration caused by the

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infection even in the presence of detectable viremia. These long-term nonprogressors (LTNP)^{19,20} maintain stably "high" numbers of circulating CD4⁺ T lymphocytes (conventionally ≥ 500 cells/ μ l) and good health for many years, without any clinical signs of disease progression and without receiving antiretroviral therapy (ART).

It has been found that in comparison to LTNP, deficient CD8⁺ T cell activity significantly affects the course of HIV infection in patients experiencing disease progression, especially those possessing the HLA B5701 allele,^{21,22} although the role of IL-15 in such differences is unknown. However, given the fact that cytotoxic CD8⁺ T cells affect the course of disease and that IL-15 plays a crucial role in their homeostasis and activity, it is not surprising that IL-15 is considered to have therapeutic potential. Its application was shown to enhance the immune responses induced by DNA-based anti-HIV vaccines. In a murine model administration of IL-15 enhanced the cytolytic activity of anti-HIV-specific CD8⁺ T cells and the production of IFN- γ ,^{23–26} and its administration with IL-15R α boosts these responses to even higher levels.²⁷ Similar effects have also been observed in rhesus macaques vaccinated against simian immunodeficiency virus (SIV)^{28–30} or when immunized with HIV DNA vaccine.³¹ The use of IL-15 as an adjuvant together with an anti-HIV vaccine is currently being tested in clinical trials (www.Clinicaltrials.gov). Recent studies in humans confirm the adjuvant effects of IL-15 and demonstrate that in HIV-infected people IL-15 can overcome CD4 deficiency and enhance CD8 T cell responses.³²

Nevertheless, although the combination of IL-15 and anti-HIV vaccine may improve immune responses, the ultimate effect may depend on individual subjects' expression of IL-15 and its receptor, and their coordinated regulation before vaccination. Verifying whether there are differences in IL-15 and IL-15R expression and regulation in HIV-infected patients experiencing different disease courses may provide important information concerning the pathogenesis of HIV-1 and the use of vaccine-based therapy. The aim of this study was to analyze IL-15 expression in the monocytes of two groups of ART-naïve patients with differently progressing HIV-1 infection.

Materials and Methods

Patients

The study involved 36 ART-naïve patients with HIV-1 infection and different rates of disease progression: 20 LTNP who had had documented HIV-1 infection for at least 8 years without any history of AIDS-related symptoms and with

CD4⁺ T cell counts that had always been >500 cells/ μ l, and 16 progressors with CD4⁺ T cell counts of <500 cells/ μ l who had not experienced any AIDS-defining event. A control group consisted of 14 healthy volunteers with normal blood morphology (Table 1). The study was approved by the local Ethics Committee, and all of the patients gave their informed written consent.

Cell isolation

Within 1 h after draw of peripheral blood, mononuclear cells (PBMCs) were isolated by means of Ficoll-Paque gradient centrifugation (Euroclone, Pero, Italy) at 19°C for 30 min at 400 $\times$ g. The buffy coats were aspirated and washed twice in PBS, and the cells were counted in a hemocytometer chamber. The cells were analyzed immediately by means of direct flow cytometry or cultured overnight with or without IFN- γ .

Cell stimulation

PBMCs were seeded in 48-well plates (Corning, Pero, Italy) at a concentration of 2×10^6 cells/ml in complete culture medium consisting of RPMI 1640 supplemented with 10% heat inactivated fetal calf serum, penicillin, streptomycin, and L-glutamine (Euroclone, Pero, Italy), with or without stimulation with 1 μ g/ml IFN- γ (Peprotech, London, UK). After 18 h, the cells were collected, the wells were washed with ice-cold PBS without Mg²⁺ or Ca²⁺ ions, and the cells were scraped off the surface using a rubber policeman. After centrifugation, the supernatants were collected and stored at -80°C for further analysis. The cells were resuspended in buffer for antibody staining or suspended in an RNA-protect buffer (Qiagen, Jesi, Italy) and stored at -80°C for later gene expression analysis.

Flow cytometry

To analyze the expression of CD38 on CD8⁺ T cells, 0.5×10^6 cells were resuspended in 50 μ l of staining buffer [PBS+0.5% human serum albumin (HSA)] and stained for 30 min with anti-CD8 PE/Cy5 plus anti-CD38 FITC (Biollegend, Milan, Italy) antibodies. After washing with staining buffer (PBS+0.5% HSA+0.1% NaN₃), the cells were resuspended in 2% para-formaldehyde (PFA).

To analyze the surface expression of IL-15, 0.5×10^6 cells were preincubated for 20 min at room temperature with mouse serum (ebiosciences, Milan, Italy) to prevent any nonspecific reaction with antibodies used of murine origin. After washing, anti-IL-15 FITC or isotype control antibody (mouse IgG1, R&D Systems, Milan, Italy) were added and

TABLE 1. CHARACTERISTICS OF THE THREE GROUPS

	Healthy controls (n = 14)	Progressors (n = 16)	LTNP (n = 20)	Difference between HIV groups
Age (years)	35 (28–47)	39 (32–42)	43 (42–46)	NS
Duration of HIV infection (years)	0	2 (1–5)	15 (9–21)	$p < 0.001$
HIV RNA load	0	61,695 (140–263,054)	1,712 (0–8,481)	$p < 0.001$
Total number of CD4 T cells (Mean/ μ l)	860 (580–820)	362 ^a (171–436)	946 (501–1,837)	$p < 0.001$
CD4 T cells (Mean %)	38.4 (22–44.6)	21.5 ^a (10.5–44.3)	36.1 (8.4–60)	$p < 0.001$
CD38 CD8 T cells (Median %)	8.5 (2.94–16.08)	47.8 ^a (8.8–62.8)	15.1 (6.3–35.2)	$p < 0.005$

^aSignificantly different from the healthy control group.
LTNP, long-term nonprogressors.

incubated for 30 min on ice, after which the cells were washed and resuspended in PFA.

For the intracellular analysis of IL-15 expression, 0.5×10^6 cells were first incubated for 20 min at room temperature with anti-CD14-PE antibody (Biolegend, Milan, Italy), and then washed with staining buffer before being fixed and permeabilized for 30 min on ice with perm/fix buffer (Biolegend, Milan, Italy). After washing in permeabilization buffer (Biolegend, Milan, Italy), the cells were resuspended in 50 μ l of permeabilization buffer before the addition of IL-15 FITC (R&D Systems, Milan, Italy) or isotype control antibody (R&D Systems, Milan, Italy). After 30 min incubation on ice, the cells were washed in permeabilization buffer, resuspended in 400 μ l of staining buffer, and immediately analyzed.

Separate sample of cells were stained for IL-15R α detection by adding a purified mouse anti-IL-15R α IgG2b antibody (R&D Systems, Milan, Italy) to 0.5×10^6 cells in 50 μ l of staining buffer, and incubated for 30 min. After washing, secondary FITC-labeled antimouse IgG2b or FITC-labeled mouse IgG2b as a control antibody (Southern Biotechnology, Cambridge, UK), and anti-CD14 PE antibodies were added for another 30 min. After washing, the cells were resuspended in 2% PFA. Flow cytometry acquisitions were made using a Beckman-Coulter EPICS XL by gating on at least 10,000 live cells. The expression analyses were made using the manufacturer's software.

Real-time PCR

RNA was isolated using TRIzol Reagent (Invitrogen, San Giuliano Milanese, Italy) in accordance with the manufacturer's instructions, and cDNA was synthesized from 500 ng of total RNA using a SuperScript III First Strand synthesis kit (Invitrogen, San Giuliano Milanese, Italy). The expression of the genes for IL-15, IL-15R α chain, and β -actin was evaluated using dual-labeled probe real-time PCR and custom-synthesized primers and probes (Explera, Jesi, Italy). For IL-15: forward primer 5'-TCTGATCATCCTAGCAAACAAC-3', reverse primer 5'-CAATCAAGAAGTGTGATGAACAT-3', probe 5'-FAM-ATCTGGATGCAAAGAA TGTGAGGA-BHQ-1-3'. For the IL-15R α chain: forward primer 5'-GTCAA GAGCTACAGCTTGTA-3', reverse primer 5'-CTTGTTCAA CACGCACTC-3', probe 5'-FAM-TGTAAGTCTGGTTTCAAG CGTAAAG-BHQ-1-3'. For β -actin: forward primer 5'-CGT GACATTAAGGAGAAGCT-3', reverse primer 5'-TCAGG CAGCTCGTAGCTC-3', probe 5'-FAM-CTGGACTTCGAG CAAGAGATGG-BHQ-1-3'.

Real-time PCR was performed using mastermix FluoCycle Mix II for Probe (Euroclone, Pero, Italy) supplemented with 1 μ l cDNA, and a final concentration of 200 nM of primers and 100 nM of probe in a total volume of 25 μ l. The reactions were run in a thermocycler (Smartcyler, Capheid, Sunnyvale, CA) and the threshold cycle (*Ct*) at which the cDNA was amplified (10 times above background) was estimated against the second derivative. The efficiency of all of the genes was assessed using the Relative Expression Software Tool (REST 2008) program and included in a comparison of the relative gene expression analyses.³³

The delta values of the threshold cycles (ΔCt) for IL-15 and IL-15R α gene expression were obtained after correcting for the internal reference gene (β -actin) *Ct* value ($\Delta Ct = Ct$ of IL-15 or IL-15R α - *Ct* of β -actin). The ΔCt values were used to assess

the correlation between the expression of these genes in stimulated and unstimulated cells from all groups.

The fold-change ($Ct_{IFN/NS}$) in the expression of the IL-15 and IL-15R α genes after IFN- γ stimulation was derived from analyses made using Microsoft Excel-based REST spreadsheet. The fold-change values were calculated by dividing the ΔCt value of the IL-15 or IL-15R α gene after stimulation with IFN- γ by the ΔCt value of the same genes in unstimulated cells.

The calculations of ΔCt and $Ct_{IFN/NS}$ took into account the efficiency of gene amplification during RT-PCR, which was 1.97 for β -actin, 1.945 for IL-15, and 1.968 for IL-15R α .

ELISA

Interleukin 15 levels in the supernatants of cells stimulated or not with IFN- γ were estimated using an ultrasensitive chemiluminescent ELISA kit (minimum detectable dose 0.121 pg/ml; R&D Systems Europe, Milan, Italy) in accordance with the manufacturer's instructions. The samples were run in duplicate and the relative light units from each well were determined by means of a luminometer (Tecan, Milan, Italy) with a 1-min lag time and a 1 s/well read time. The IL-15 concentrations were read against a standard curve.

Statistical analyses

The data were statistically analyzed using the Mann-Whitney test, a paired *t* test, and correlation analysis. The calculations were made using GraphPad Prism and SPSS software.

Results

Enrolled patients

The study involved 36 ART-naïve patients with HIV-1 infection: 20 LTNP (defined as patients with documented HIV-1 infection for at least 8 years, no clinical symptoms of AIDS, and CD4 T cell counts always >500 cells/ μ l) and 16 progressors with CD4 T cell counts <500 cells/ μ l and no AIDS-defining event. It also involved a control group of 14 healthy volunteers. Table 1 shows the characteristics of the three groups.

As previously demonstrated, because the expression of CD38 on CD8 T cells in HIV-1-infected patients is a good indicator of the status of immune activation and can be used as a prognostic marker of disease progression,^{5,34} we characterized the patients further using flow cytometry. The groups were significantly different in terms of the duration of HIV infection ($p < 0.001$), CD4 T cell counts and percentages ($p < 0.001$), CD38 expression on CD8 T cells ($p < 0.005$), and viral load ($p < 0.001$). The percentage of CD38⁺CD8⁺ T cells, and CD4 cell counts and percentages were significantly different between progressors and healthy controls. There was no between-group difference in age (Table 1).

Ex vivo intracellular expression of IL-15, and the surface expression of IL-15 and IL-15R α on CD14⁺ monocytes of PBMCs

Flow cytometry was used to analyze the intracellular expression of IL-15 (icIL-15), and the surface expression of IL-15 and IL-15R α , in CD14⁺ monocytes. The surface expression of

FIG. 1. Intracellular interleukin (IL)-15 and surface IL-15R α expression in CD14⁺ monocytes. Flow cytometric analyses of peripheral blood mononuclear cells (PBMCs) isolated from HIV-1⁺ progressors ($n=16$), long-term nonprogressors (LTNP, $n=20$), and healthy controls ($n=14$). Intracellular IL-15 expression and IL-15R α were analyzed on CD14⁺ monocytes in separate cell samples but in a similar way as shown in the diagram. (A) Gating of monocytes based on SS and FS (left) and CD14⁺ monocytes based on CD14 and FS (right graph). (B) Histograms representing cells accumulated in gate B (according to CD14 and FS) were used to assess the intracellular IL-15 (IL15ic in white) or IL-15R α (in gray) expression by marking the region of positive staining (black line across the graph) outside the negative region of fluorescence obtained by use of control cells of the same patient stained with isotype control antibody (mouse IgG1 for icIL-15 or mouse IgG2b for IL-15R α). (C) IL-15ic and IL-15R α expression in CD14⁺ monocytes. The data were analyzed using the Mann-Whitney test: * $p < 0.05$.

IL-15 on CD14⁺ monocytes did not exceed 1.5% in any of the samples (data not shown). Figure 1A and B represents the mode of cell gating and acquisition of statistical data for surface expression of IL-15R α and the icIL-15. Figure 1B represents histograms of icIL-15 and IL-15R α . The frequency of monocytes

FIG. 2. Intracellular expression of IL-15 in CD14⁺ monocytes after *in vitro* culture with or without interferon (IFN)- γ stimulation. PBMCs isolated from HIV-1⁺ progressors ($n=15$), LTNP ($n=17$), and healthy controls ($n=14$) were stimulated overnight (IFN) or not (NS) with IFN- γ , and the expression of icIL-15 in CD14⁺ monocytes was analyzed by means of cytofluorimetry. The data were analyzed using a paired t test: ** $p < 0.005$.

expressing icIL-15 was significantly higher in the LTNP than in the progressors or healthy controls ($p < 0.05$) (Fig. 1C); the frequency of the surface expression of IL-15R α was also higher in the LTNP group than in the other two groups, but the difference was not statistically significant (Fig. 1B). Analyses of mean fluorescence intensity (MFI) for IL-15R α and for icIL-15 did not show significant differences between the groups (data not shown). IL-15R α and icIL-15 were expressed by fewer than 2% of CD8 T cells in all three groups (data not shown).

Expression of icIL-15 in and IL-15R α on CD14⁺ monocytes after *in vitro* stimulation with IFN- γ

The expression of IL-15 was tested in CD14⁺ PBMCs after 18 h of culture with or without IFN- γ (1 μ g/ml). Like the freshly isolated PBMCs, the cultured unstimulated monocytes of the LTNP showed significantly higher icIL-15 expression than those of the progressors ($p < 0.05$) or healthy controls ($p < 0.05$). IFN- γ stimulation caused a significantly greater increase in icIL-15 expression in the cells of the LTNP and healthy controls than in those of the progressors ($p < 0.005$) (Fig. 2), although it reduced IL-15 expression in the cells of three LTNP and two progressors whose cells showed a high level of expression before IFN- γ stimulation.

IFN- γ stimulation on average caused a significant increase (average before stimulation: 21.9–47% after IFN- γ stimulation, $p = 0.0176$) in expression of IL-15R α on CD14⁺ monocytes of the LTNP only; in spite of a similar effect in some cases of healthy controls, on average expression of this receptor did not change significantly (48.77–49.27, $p = 1.03$). Also cells of progressors responded to IFN- γ stimulation with no significant change in IL-15R α expression on CD14⁺ monocytes (17.5–14.9, $p = 0.52$) (Fig. 3).

FIG. 3. IL-15R α expression in CD14 $^{+}$ monocytes after IFN- γ stimulation. PBMCs isolated from HIV-1 $^{+}$ progressors ($n=14$), LTNP ($n=18$), and healthy controls ($n=12$) were stimulated overnight (IFN) or not (NS) with IFN- γ , and the expression of IL-15R α in CD14 $^{+}$ monocytes was analyzed by means of cytofluorimetry. The data were analyzed using a paired t test: * $p<0.05$.

Analyses of MFI for icIL-15 showed that IFN- γ stimulation did not significantly enhance the density expression of this cytokine in monocytes from LTNP (1.5 vs. 1.72, $p=0.062$) and healthy controls (1.55 vs. 1.84, $p=0.24$) but had no effect in case of progressors (1,709 vs. 1,704, $p=0.91$). In regard to IL-15R α , the MFI on monocytes of healthy controls did slightly increase after IFN- γ stimulation, although the change was not significant (1.9 vs. 2.15, $p=0.66$), but in the case of LTNP and progressors this parameter did not change at all (1.83 vs. 1.8 for LTNP and 1.86 vs. 1.83 for progressors) (data not shown).

Analyses of icIL-15 and surface expression of IL-15 and IL-15R α on CD8 T cells after IFN- γ stimulation did not reveal any significant changes in comparison with unstimulated cells, and there were no differences between the groups (data not shown).

IL-15 and IL-15R α gene expression in PBMCs stimulated or not with IFN- γ

There was no difference in IL-15 or IL-15R α gene expression between the unstimulated or stimulated PBMCs in the three groups, and the fold-increases in expression ($Ct_{IFN/NS}$) after IFN- γ stimulation were not significant in any of the groups (data not shown). The dependence of IL-15 on the IL-15R α chain for its expression and trans-presentation implies the existence of mechanisms that concomitantly control the activity of both genes. The expression of both genes was closely correlated in both the unstimulated and stimulated PBMCs of the healthy controls ($p<0.001$) and LTNP ($p<0.001$), but significantly correlated only in the unstimulated cells of the progressors ($p<0.05$) (Fig. 4).

IL-15 in vitro production

IL-15 levels were measured in the supernatants of cultured PBMCs by means of ELISA, and were never higher than

1.75 pg/ml in any of the samples, thus confirming that IL-15 is released in very small quantities even after IFN- γ stimulation. However, IFN- γ stimulation increased IL-15 production in all of the groups; this increase was more significant in the cells of the progressors (0.79–1.13 pg/ml, $p<0.001$) than in those of the LTNP (0.73–1.04 pg/ml, $p=0.002$) or healthy controls (0.82–1.104, $p=0.0039$) (Fig. 5).

Discussion

Given its role in lymphocyte homeostasis, especially in the case of CD8 T $^{10,15-17}$ and NK cells,^{35,36} the IL-15//IL-15R complex can significantly affect the course of HIV infection. It has been found that genetic polymorphisms in IL-15 and IL-15R are related to the recovery of CD4 T cell counts after antiretroviral therapy,³⁷ and that IL-15 greatly enhances the adaptive immune responses of HIV-specific CD8 T cells.^{17,18,38}

On the basis of these findings, IL-15 can be considered to have adjuvant potential to support DNA-based anti-HIV vaccines. This cytokine enhances the cytolytic activity of anti-HIV-specific CD8 T cells and increases the production of IFN- γ in animal models,²³⁻³⁰ and its use with anti-HIV vaccines is currently being tested in clinical trials (www.Clinicaltrials.gov). However, not all studies support the beneficial effect of IL-15. Application of this cytokine to simian immunodeficiency virus-infected macaques abrogated a vaccine-induced decrease in viral load.³⁹ IL-15 was also shown to increase the viral set point and cause an acceleration of simian AIDS despite mediating an increase in the number and activity of CD8 effector T cells and NK cells.^{40,41}

It is not known whether there are differences in IL-15/IL-15R expression and regulation in patients with HIV infection experiencing different disease courses, but answering this question may provide important information concerning the pathogenesis of HIV-1 and the potential use of IL-15 as a therapeutic agent.

IL-15 production is regulated at different levels and involves IL-15R α , which participates in recycling of the cytokine from the cell surface to lysosomal compartments and controls its release from the cell.^{10,12} Such close control of the production of IL-15 by the α -chain of the IL-15 receptor^{10,42} implies the coordinated expression of both genes and explains very limited extracellular release of this cytokine. Any dysregulation of this control may therefore lead to the excessive release and overexpression of IL-15, and thus contribute to the development of autoimmune or inflammatory diseases.⁴³⁻⁴⁵

Our data confirm the very limited cell release of IL-15 in healthy controls and patients with HIV-1 infection and although IL-15 concentrations were significantly increased after cell stimulation by IFN- γ , the effect was not different between the studied groups. Our analyses showed that the icIL-15 expression was higher in the *ex vivo* monocytes of LTNP than in those of progressors or healthy controls. Furthermore, the monocytes of the LTNP and healthy controls responded to IFN- γ stimulation with a significantly greater increase in icIL-15 expression and tended to express higher cell surface levels of IL-15R α . The changes described above in monocytes did not take place in or on concomitantly analyzed CD8 T cells (data not shown). Finally, unlike the progressors, there was a positive correlation between the levels of expression of IL-15 and IL-15R α genes in the PBMCs of the LTNP and healthy controls, especially after IFN- γ stimulation.

FIG. 4. Correlation analyses of IL-15 and IL-15R α gene expression. Correlations between the ΔC_t ($\Delta C_t = C_t$ of IL-15 or IL-15R α – C_t of β -actin) values of IL-15 and IL-15R α gene expression in unstimulated or IFN- γ -stimulated PBMCs from LTNP ($n=20$), progressors ($n=14$), and healthy controls ($n=14$). The data were analyzed by means of linear regression.

Although there are differences in IL-15 and IL-15R α expression and regulation between patients with different courses of HIV infection the mechanisms by which the cytokine contributes to protection from disease progression are not known. We speculate that the more coordinated expression of IL-15 and IL-15R α in LTNP may enhance the activity of HIV-specific CD8 T cells.^{17,22,46–48} Higher expression of IL-15 of LTNP compared to progressors may be a compensatory mechanism for insufficient IL-2 production and deficient IL-7 function that allows the maintenance of homeostasis of CD4 and CD8 T cells in this group of patients.^{49–51} Therefore, supplementation of anti-HIV vaccines with IL-15 constructs to develop more efficient immune responses is substantiated not

only by the adjuvant activity of this cytokine but also by its deficiency in the group of patients with disease progression.

The balanced and coordinated expression of IL-15 and its receptor in healthy controls and LTNP shows their close dependence, and so it is not surprising that the adjuvant activity of combined constructs is more efficient.²⁷ However, we cannot completely rule out that differences between studied groups in expression of icIL-15 are not the result of differential participation of the α -chain of IL-15R within the intracellular compartment. IL-15 is released only when bound to its private α -chain receptor, leaving other unbound forms behind.¹² Thus, our data cannot exclude the possibility that the increased number of monocytes of LTNP and healthy controls

FIG. 5. Analyses of IL-15 concentrations in the supernatants of cells stimulated or not with IFN- γ . PBMCs isolated from HIV-1⁺ progressors ($n=14$), LTNP ($n=14$), and healthy controls ($n=12$) were stimulated overnight (IFN) or not (NS) with IFN- γ , and the concentrations of IL-15 were assessed in the collected supernatants. The data were analyzed using a paired t test, and there were significant differences after IFN- γ stimulation in all of the groups but not between them: *** $p<0.001$; ** $p<0.005$.

containing icIL-15 in comparison to HIV progressors is not simply the effect of more stringent control mechanism, preventing IL-15 release, especially since in HIV progressors a higher release of IL-15 was observed. Although intracellular mechanisms of IL-15 recycling and participation of IL-15R α can affect our speculations, observations already made by us indicate that IL-15/IL-15R need to be considered as an important element determining the progression of HIV infection and should be considered in the design of therapeutic vaccines, especially those supplemented with IL-15 constructs.

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Author Disclosure Statement

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