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Role of IL-15 in HIV-1-associated hypergammaglobulinaemia

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SUMMARY

IL-15 is a novel cytokine, produced by monocytes/macrophages, with biological activities similar to IL-2 but with no significant sequence homology. IL-15 also stimulates human B cells to proliferation and immunoglobulin secretion. We measured serum levels of IL-15 in 84 HIV-1-infected individuals at different stages of disease in reference to 41 healthy blood donors. Our results show a marked elevation of IL-15 serum levels during HIV-1 infection. Moreover, we found that this increase correlated with serum levels of IgG ($r = 0{\cdot}376, P < 0{\cdot}0001$), and partly with serum IgM ($r = 0{\cdot}265, P = 0{\cdot}015$). A significant increase of IL-15 production by cultured peripheral blood mononuclear cells (PBMC) and purified monocytes in the presence of HIV-1 virus suggests that monocytes/macrophages may be a source of higher IL-15 serum levels in HIV-1-infected individuals. These findings indicate a participation of IL-15 in the hypergammaglobulinaemia frequently associated with HIV-1 infection.

Keywords hypergammaglobulinaemia IL-15 serum IgG serum IgM HIV-1

INTRODUCTION

Infection with HIV-1 is known to cause an intense polyclonal activation of B cells, as manifested by hypergammaglobulinaemia, elevated serum levels of immune complexes and autoantibodies, increased numbers of spontaneous immunoglobulin-secreting cells, and an elevated frequency of B cell lymphomas [1–3]. The HIV-1 envelope glycoprotein gp120 has been identified to bind and induce immunoglobulin secretion via superantigen interaction by a subpopulation of B cells, expressing the V_H3 family of immunoglobulin on their surface [4]. Recently, peptide epitopes that block binding of gp120 to immunoglobulin have been determined [5].

The induction of B cell differentiation is a complex process that is regulated by T lymphocytes and cytokines. Several mechanisms for the excessive B cell activation in HIV-induced hypergammaglobulinaemia have been suggested, including T cell contact-dependent interactions [6,7], IL-6 and tumour necrosis factor-alpha (TNF-α) stimulation by monocytes [3,8–10] and IL-10 secretion by B cells [11].

To shed more light on HIV-induced hypergammaglobulinaemia, we investigated the role of a novel cytokine, IL-15, which shares many of the stimulatory activities associated with IL-2 [12,13].

IL-15 is a 14–15-kD protein that stimulates proliferation of the CTLL cell line, phytohaemagglutinin (PHA)-activated blasts [12], activation of natural killer (NK) cells [14] and secretion of IgM, IgG and IgA by B cells [15] to the same extent and with similar potency as IL-2. Expression of IL-15 mRNA has been detected in several human tissues such as heart, lung, liver, kidney, placenta, skeletal muscle and epithelial cell lines, but the highest level of secretion has been seen in monocytes/macrophages [12,16]. Although it has no sequence homology with IL-2, IL-15 uses IL-2 receptor (IL-2R) β- and γ-chains for binding and signal transduction to the cell [14,17,18].

The primary purpose for our investigations was to find an explanation for hypergammaglobulinaemia in late stages of HIV infection, when $CD4^+$ T cells are diminished and no longer able to provide a stimulatory signal for broad B cell activation. In our study we measured IL-15 serum levels in 84 HIV-1^+ individuals at different stages of the disease (WR I to WR VI) in comparison with 41 HIV-1^- blood donors. Our results show a correlation between IL-15 and immunoglobulin levels in serum of HIV-1^+ patients. Moreover, we observed an increase in IL-15 secretion from monocytes cultivated in the presence of HIV-1_{IIIB} virus isolate (HIV-1_{IIIB}).

MATERIALS AND METHODS

Samples

Serum samples were kindly provided by Dr R. Zangerle (Universitäts-Klinik für Dermatologie, Innsbruck, Austria). The study population comprised 84 HIV-1^+ individuals and 41 healthy blood donors (HIV-1^-). HIV-1^+ subjects were classified according to the Walter Reed (WR) staging classification with 38 in WR I or II, 16 in WR III or IV and 30 in WR V or VI. Serum samples were stored at -70°C.

Detection of IL-15 in serum samples

Various dilutions (undiluted samples and samples diluted 1 : 1,

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Table 1. Determination of IL-15 serum level in HIV-1$^+$ and HIV-1$^-$ individuals

HIV-1 infection stage	IL-15 serum level	
	Detectable/not detectable	%
WR I–II ($n = 38$)	15/23	39
WR III–IV ($n = 16$)	8/8	50
WR V–VI ($n = 30$)	15/15	50
Healthy blood donors ($n = 41$)	12/29	29

1 : 2, 1 : 4) of serum samples were assayed by an ELISA kit for the specific quantitative determination of human IL-15 (Genzyme, Cambridge, MA) according to the manufacturer's instructions. The kit is specific for native or recombinant human IL-15 (detection limit 10 pg/ml) with no detectable cross-reaction with other cytokines and serum proteins.

Detection of IgM and IgG

IgM and IgG levels in serum were measured by routine sandwich ELISA using polyclonal rabbit anti-IgM or anti-IgG as capture antibody and polyclonal goat anti-IgM or anti-IgG antibodies, conjugated to horseradish peroxidase (HRP; Dako A/S, Glostrup, Denmark) for antigen detection.

Mononuclear cells and monocytes

Peripheral blood mononuclear cells (PBMC) were obtained from heparinized peripheral blood of healthy donors by centrifugation on a Ficoll–Hypaque density gradient. Monocytes were separated from PBMC by adherence on gelatin-coated Petri dishes as described previously [19]. Briefly, 10 ml of PBMC suspension ($3–6 \times 10^6$ cells/ml) in RPMI 1640 + 10% fetal calf serum (FCS) +3% autologous human serum were incubated on gelatin-coated (2% gelatin in water) Petri dishes for 40 min at 37°C. Non-adherent cells were aspirated and dishes washed three times with prewarmed RPMI 1640 + 10% FCS medium. Adherent cells (92–97% CD14$^+$ monocytes) were incubated for 10 min in RPMI 1640 + 10% FCS medium with 5 mM ethylenediaminotetraacetate (EDTA). Detached monocytes were aspirated, washed and resuspended in RPMI 1640 + 10% FCS medium.

Cocultivation of PBMC and monocytes with HIV-1_{IIIB} isolate

HIV-1_{IIIB} virus isolate was used in all experiments. Virus stock was prepared by propagation in PBMC from healthy HIV-1$^-$ donors. Virus was concentrated by ultracentrifugation and quantified by capture ELISA for HIV-1 p24 antigen. Heat-inactivated (56°C for 30 min) HIV-1_{IIIB} isolate (iHIV-1_{IIIB}) was used in some experiments. PBMC and monocytes (1×10^5 cells/well, 100 μl) were incubated in RPMI 1640 + 10% FCS medium with different HIV-1_{IIIB} concentrations for 7 days. One hundred microlitres of 1 : 1 diluted supernatants were used immediately for IL-15 determination as described above.

Statistical analysis

Results are expressed as mean ± s.e.m. of the data obtained from two independent measurements of each serum sample performed in duplicate. Non-parametric Mann–Whitney test for unpaired data was carried out for statistical comparison between HIV-1$^+$ and HIV-1$^-$ serum samples. Spearman's non-parametric test was used for correlations between IL-15 and IgG or IgM serum levels. Two-tailed Student's *t*-test for unpaired data was applied for comparison of IL-15 levels in supernatants from PBMC and monocytes.

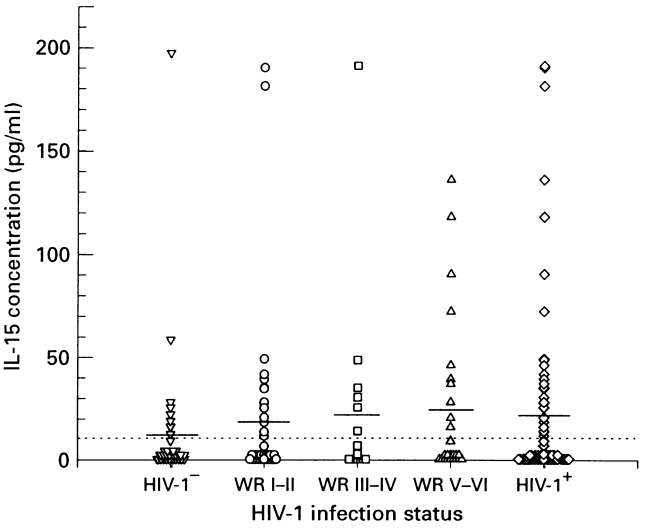

Fig. 1. Determination of IL-15 serum levels in 41 healthy donors and 84 HIV-1$^+$ individuals. HIV-1$^+$ individuals were divided in three groups according to the Walter Reed Classification (WR I–II, WR III–IV, WR V–VI). HIV-1$^-$, healthy donors; horizontal bars, mean values of each group of subjects; dotted line, detection limit.

RESULTS

Serum levels of IL-15 in HIV-1-infected subjects

We were able to detect serum levels of IL-15 only in parts of HIV-1$^+$ and HIV-1$^-$ subjects (Table 1). Only 29% of HIV-1$^-$ serum samples contained measurable levels of IL-15. With progression of disease the number of IL-15-positive individuals increased to 50% (WR III–VI).

The mean values of serum IL-15 showed a significant (Mann–Whitney test; $P = 0{\cdot}0034$) increase in 84 HIV-1$^+$ individuals compared with 41 HIV-1$^-$ donors ($20{\cdot}83 \pm 4{\cdot}47$ pg/ml *versus* $10{\cdot}78 \pm 5{\cdot}01$ pg/ml) (Fig. 1). Among HIV-1$^+$ individuals, IL-15 serum levels were higher in subjects at later stages (WR III–IV $21{\cdot}99 \pm 11{\cdot}91$ pg/ml and WR V–VI $22{\cdot}65 \pm 6{\cdot}65$ pg/ml) than in HIV-1$^+$ individuals at earlier stages (WR I–II $18{\cdot}91 \pm 6{\cdot}88$ pg/ml) of the disease. After increase during WR III–IV stages, the IL-15 serum level in ARC/AIDS patients (WR V–VI) remained stable. In all groups, no significant difference was found for serum IL-15 ($P > 0{\cdot}05$).

Correlation between serum levels of IL-15 and gammaglobulins

The hypergammaglobulinaemia in HIV-1$^+$ subjects was mainly due to an increase in IgG, although IgM were also elevated. Our results show that, among HIV-1$^+$ carriers, the mean IgM and IgG level is higher in patients with detectable serum levels of IL-15 than in those with undetectable levels of IL-15 (for IgM $403{\cdot}2 \pm 69{\cdot}4$ mg/100 ml *versus* $247{\cdot}3 \pm 30{\cdot}6$ mg/100 ml; for IgG $2191{\cdot}4 \pm 79{\cdot}1$ mg/100 ml *versus* $1811{\cdot}1 \pm 34{\cdot}1$ mg/100 ml) (Fig. 2a,b). In addition, the difference in serum levels of IgG was statistically significant (for IgG $P < 0{\cdot}0001$, for IgM $P = 0{\cdot}1351$).

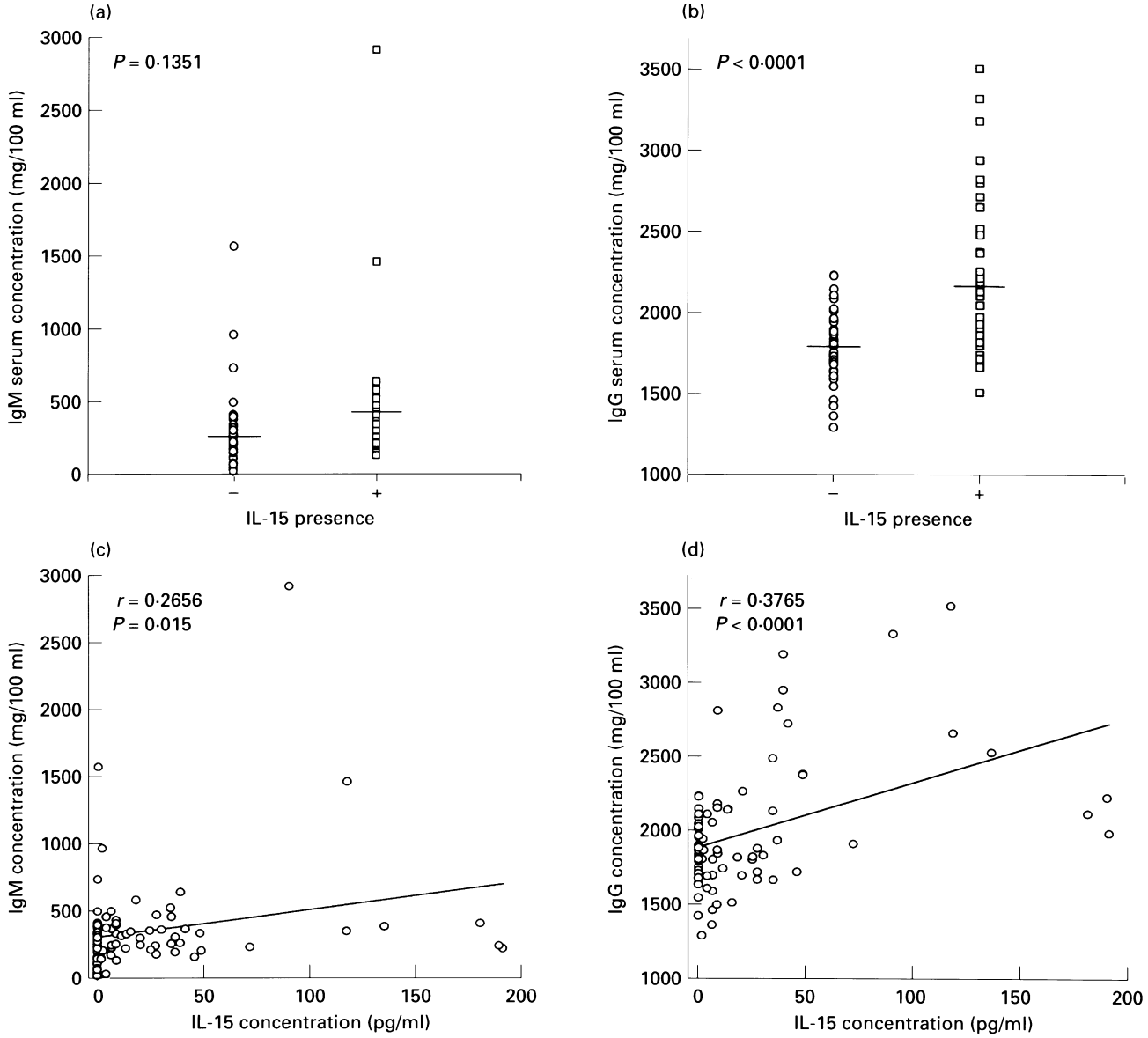

Fig. 2. Correlation between IL-15 and immunoglobulin serum levels in HIV-1$^+$ individuals. IgM (a) and IgG (b) serum levels in HIV-1$^+$ individuals with undetectable (○) or detectable (□) levels of serum IL-15. Horizontal bars, mean values of each group of subjects; *P* value, non-parametric Mann–Whitney test for unpaired data. Correlations of IgM (c) and IgG (d) levels in sera with serum IL-15. *r* and *P* value, non-parametric Spearman's test for correlation.

A moderate, but significant correlation was observed between IL-15 and IgM serum levels ($r = 0{\cdot}2656$; $P = 0{\cdot}015$) in HIV-1$^+$ individuals (Fig. 2c). Moreover, we actually found a significant correlation between serum levels of IL-15 and IgG ($r = 0{\cdot}3765$; $P < 0{\cdot}0001$) (Fig. 2d).

IL-15 production by monocytes and PBMC

The production of IL-15 by cultured monocytes and PBMC was examined to prove relevance of increased IL-15 serum levels in HIV-1$^+$ individuals (Fig. 3). Monocytes and PBMC obtained from healthy donors showed a spontaneous production of IL-15 which was increased after stimulation with HIV-1_{IIIB} or iHIV-1_{IIIB} isolate. We detected maximum two-fold increase against mock control in IL-15 production by stimulation with 10 ng/ml of HIV-1 p24 antigen (monocytes + iHIV-1_{IIIB} 139·54 pg/ml *versus* 69·24 pg/ml, monocytes + HIV-1_{IIIB} 154·34 pg/ml *versus* 68·33 pg/ml; PBMC + iHIV-1_{IIIB} 76·31 pg/ml *versus* 40·17 pg/ml, PBMC + HIV-1_{IIIB} 88·93 pg/ml *versus* 39·74 pg/ml). In addition, a significant increase of produced IL-15 was observed by cultivation of monocytes and PBMC with HIV-1_{IIIB} isolate at the concentration 1 ng/ml and 10 ng/ml of HIV-1 p24 antigen ($P < 0{\cdot}01$; Student's *t*-test). IL-15 production from purified monocytes was much higher than that observed from PBMC, thus confirming that monocytes are the main cell type involved in IL-15 production.

DISCUSSION

B lymphocyte dysfunctions, frequently observed during HIV infection, include hypergammaglobulinaemia, circulating activated B cells, spontaneous immunoglobulin secretion and the presence of autoantibodies [1,20]. Several mechanisms have been postulated to explain this abnormal B cell function. Some

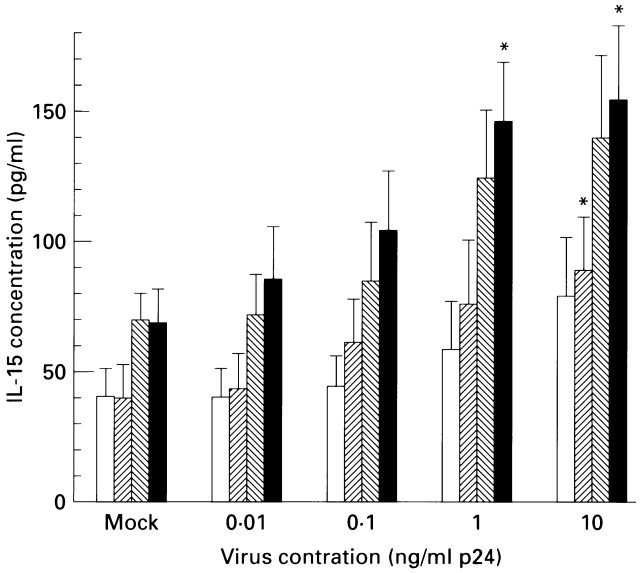

Fig. 3. Determination of IL-15 levels in culture supernatants of monocytes and peripheral blood mononuclear cells (PBMC) (1×10^6 cells/ml) after 7 days incubation in presence or absence of HIV-1_{IIIB} and iHIV-1_{IIIB}. Data are the mean ± s.e.m. of six separate experiments performed in duplicate. □, PBMC + iHIV-1; ▨, PBMC + HIV-1; ▧, monocytes + iHIV-1; ■, monocytes + HIV-1. *$P < 0{\cdot}01$.

groups [6,7] described T cell contact-dependent activation of B cells by HIV infection. Nevertheless, none of these mechanisms clearly explained hypergammaglobulinaemia at all stages of HIV infection, even in ARC/AIDS patients that exhibit CD4$^+$ T cell dysfunction. Unlike CD4$^+$ T cells, which are rapidly depleted in HIV-1$^+$ individuals, monocytes/macrophages withstand prolonged periods of infection by HIV with significantly less cytopathology and cell death, show altered cytokine production and have an activated phenotype [21]. Therefore, we propose that a monocytes/macrophages-mediated cosignalling pathway rather than T cell contact-dependent interactions is involved in HIV-1-associated hypergammaglobulinaemia.

In the present study we investigated serum levels of IL-15 in 84 HIV-1$^+$ individuals at the different stages of disease. Fifty-five percent of HIV-1$^+$ and 71% of HIV-1$^-$ subjects in our study showed undetectable levels of serum IL-15. However, similar detectability was previously described for IL-6 serum levels in HIV-1$^-$ and HIV-1$^+$ individuals [8].

We observed a significant increase in serum levels of IL-15 in HIV-1$^+$ carriers compared with healthy controls. This elevation was found to correlate with disease progression. In particular, the IL-15 level rose during WR III–IV stages, but stabilized and remained unchanged in ARC/AIDS patients (WR V–VI). A similar tendency has been previously described for IgG serum levels [8]. Therefore, we analysed serum levels of IgG and IgM in HIV-1$^+$ individuals in a further set of experiments. We observed significant increases of IgG levels in the group of HIV-1$^+$ individuals with detectable levels of serum IL-15 compared with HIV-1$^+$ individuals with undetectable levels of serum IL-15. Although the difference in concentrations appears to be of no clinical importance, we conclude that IL-15 may play a role in inducing hypergammaglobulinaemia associated with HIV-1 infection.

Numerous cytokines are able to support proliferation and/or differentiation of activated B cells. Among them, IL-6, IL-4 and TNF-α were considered to induce B cell hyperactivity [8,22,23]. Some authors have ascribed the hypergammaglobulinaemia of HIV-1-infected individuals to an increased production of IL-6 by cells of the monocyte-macrophage system as a consequence of HIV-1 infection [22,24]. Nevertheless, hypergammaglobulinaemia in HIV-1-infected persons does not clearly correlate with plasma levels of IL-6 [8]. We demonstrate that IL-15 levels correlate with serum IgG in HIV-1$^+$ individuals ($r = 0{\cdot}376$; $P < 0{\cdot}0001$), but do not clearly correlate with serum IgM ($r = 0{\cdot}265$; $P = 0{\cdot}015$). This is the first report of serological analysis that describes a correlation between HIV-1-associated hypergammaglobulinaemia and immunological factors.

Since increased production of IL-15 by lipopolysaccharide (LPS)-activated murine macrophages has been reported [16], in further experiments we analysed IL-15 production by purified monocytes and PBMC from healthy donors. We detected a two-fold, significant increase in the amount of IL-15 released from monocytes and PBMC stimulated with HIV-1_{IIIB} virus isolate. These results suggest that HIV-1 virus exerts direct influence on IL-15 production in monocytes and therefore also on IL-15 serum levels.

In conclusion, we determined serum levels of IL-15 in HIV-1$^+$ persons at different stages of disease in comparison with healthy controls. We demonstrate that IL-15 levels are significantly elevated in HIV-1-infected individuals. Moreover, as investigated in the present study, we have found correlation between IL-15 and immunoglobulin serum levels, indicating that IL-15 may contribute to the pathogenesis of HIV-1-associated hypergammaglobulinaemia, even in the later stages of infection. IL-15, secreted by activated monocytes/macrophages, costimulates proliferation and differentiation of activated B cells in a manner similar to IL-2 [15]. Because of its broad stimulatory activities, we suggest that this interleukin is a useful parameter for HIV-disease and can provide more precise assessment of the immune status.

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