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ROLE OF INTERFERON-GAMMA (IFN-γ) IN IMMUNE RESPONSE REGULATION IN HIV-1 AND HIV-1 + *MYCOBACTERIUM TUBERCULOSIS* (TB) INFECTED PATIENTS

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The aim of this research was to investigate the role of IFN-γ in interaction between IL-10, IL-18, IL-1b, CD4 cell counts and HIV-1 RNA viral load in the development of HIV-1 in patients co-infected with Mycobacterium tuberculosis *(TB). The study was conducted by Rīga East Clinical University Hospital with data from the HIV-1 register, in collaboration with the RSU Joint Laboratory of Clinical Immunology and Immunogenetics. 200 HIV-1 infected patients and 184 HIV-1 with TB co-infection patients divided in four groups were included in the study. IFN-γ, IL-10, IL-18, IL-1b levels were measured in serum with commercially enzyme-linked immunosorbent assay (ELISA Vector-Best Corporation, Novosibirsk, Russia). CD4 cell counts were measured by flow Partec IVD cytometry (USA). HIV-1 RNA quantification was performed using the COBAS AmpliPrep/COBAS Taqman HIV-1 Test (Germany). All groups were compared with each another. IFN-γ production was significantly lower, and IL-10 and CD4 cell counts were significantly higher, in HIV-1 patients without TB compared with the other groups. The group with HIV-1 and TB had significantly elevated IL-18 production. HIV patients with primary TB had significantly elevated IFN-γ production and HIV-1 RNA viral load and significantly lower IL-10 production.*

Key words: *IFN-γ, cytokines,* Mycobacterium tuberculosis*, HIV-1.*

INTRODUCTION

Mycobacterium tuberculosis (TB) is globally the most common opportunistic infection affecting HIV-1-seropositive individuals, and it remains the most common cause of death in patients with AIDS (Pawlowski *et al*., 2012). *Mycobacterium tuberculosis* and HIV-1 act in synergy, creating a global public health problem by accelerating the decline of immunological functions and leading to subsequent death if untreated. The mechanisms behind the breakdown of the immune defence of the co-infected individual are not well known. It is known that host protective immune response against this pathogen is mediated by cellular immunity in which specific cytokines and Th1 cells have a critical role (Chen and Kolls, 2013). Understanding the mechanisms involved in this response, and in particular the function of the cytokine network involved in this disease, is of significant relevance in making advances in the development of effective control and prevention. An important aspect associated with the production of cytokines in TB infection is the activation of macrophages in response to interferon-γ (IFN-γ) signalling, and also promoting the migration of immune cells to the infection site, which controls disease progression. The regulation of the innate and the adaptive immune responses are extensively intertwined and tightly regulated. Ag-driven immune responses that are modulated by immune complexes (ICs) are known to inhibit IFN-γ-dependent MHC class II expression (Boekhoudt *et al*., 2015). Past research has led to the general conclusion that IFN-γ is much more than an interferon, in that it has broader effects on the various arms of the immune system than most any other lymphokines or cytokines. In review (Young and Hardy, 1995) discussed the effects of IFN-γ on the various cell lineages of the immune system, focusing on the biology of its actions. IFN-γ plays various roles in the pathogenesis of HIV-1/AIDS (Young and Hardy, 1995). In HIV-1 infected individuals, the production of IFN-γ is detected early in the acute phase. Results from both *in vitro* and *ex vivo* studies show that IFN-γ can promote HIV-1 virus replication and is associated with disease development. On the

other hand, IFN-γ has been shown to promote cytotoxic T lymphocyte and NK cell activities against HIV-1 infected cells, which are important in controlling HIV-1 replication.

Both TB and HIV-1 have profound effects on the immune system, as they have the ability to disarm the host's immune responses, through mechanisms that are not fully understood. HIV-1 co-infection is the most powerful known risk factor for progression of *M. tuberculosis* infection; it increases the risk of reactivation of latent TB by 20 times (Selwyn *et al.,* 1989; Getahun *et al.,* 2010). Likewise, TB has been reported to exacerbate HIV-1 infection (Whalen *et al.,* 1995; Modjarrad and Vermund, 2010). Various lines of evidence indicate that inborn errors of immunity, as well as genetic polymorphisms, have an impact on susceptibility to TB and HIV-1 (Moller and Hoal, 2010).

The aim of this study was to investigate the role of IFN-γ in interaction between IL-10, IL-18, IL-1b, and CD4 cell counts and HIV-1 RNA viral load in the development of HIV-1 in patients co-infected with *Mycobacterium tuberculosis*.

MATERIALS AND METHODS

This study was conducted in the Rīga East Clinical University Hospital "Latvian Center of Infectology", Latvia (LCI) and Rīga Stradiņš University, Joint Laboratory of Clinical Immunology and Immunogenetics. Medical documentation (ambulatory cards) were examined for 600 patients in the period from 2004 to 2014, and based on criteria for inclusion and exclusion, 384 HIV-1 positive patients with AIDS stages were included in our study, and 184 of all HIV-1 positive patients were co-infected with TB. All patients were acquainted with the document "Information for patients" and signed the "Patient Agreement Statement". The 184 HIV-1 with TB co-infection patients were separated into three groups: HIV-1 patients with primary TB (n = 80), HIV-1 patients with secondary TB (n = 12), and HIV-1 patients with TB of more than five years duration (HIV-1+ TB) (n = 92). HIV-1 patients without TB were used as a control group (n = 200). Clinical characteristics of the study population are shown in Table.1

IFN-γ, IL-10, IL-18, IL-1b levels were measured in serum with commercially enzyme-linked immunosorbent assay (ELISA Vector-best corporation, Novosibirsk, Russia) in Rīga Stradiņš University, Joint Laboratory of Clinical Immunology and Immunogenetics. The measurement ranges were 0 to 1000 pg/ml for IFN-γ, 0 to 500 pg/ml for IL-10, 0 to 1000 pg/ml for IL-18, and 0 to 250 pg/ml for IL-1b. Concentrations of cytokines were determined using an absorbance microplate reader HiPo (Biosan, Latvia) and microplate data collection and analysis software (Biosan, Latvia). CD4 cell counts were estimated by flow cytometry (FastTrack; Becton Dickinson, San Jose, CA, USA). For HIV-1 RNA quantification, 100 μl of plasma was suspended in 900 mkl phosphate buffered saline (PBS), and the samples were analysed using the COBAS AmpliPrep/ COBAS Taqman HIV-1 Test 2.0 (Roche diagnostics, GmbH, Mannheim, Germany), in accordance with manufacturer's instructions. Determination of CD4 cell counts and HIV-1 RNA were performed at the LCI Laboratory.

Table 1

CLINICAL AND DEMOGRAPHICAL INFORMATION ON PATIENTS OF TOTAL RESEARCH GROUPS

Characteristic	Patient group			
	HIV-1 patients with primary TB, n = 80	HIV-1 patients with secondary TB, n = 12	HIV-1 +TB patients, n = 92	HIV-1 patients, n =200
Male	71	9	77	112
Female	9	3	15	62
Age, median years	39 (25–60)	55 (43–65)	52 (41–64)	39 (30–65)

Data are presented as median value. Nonparametric methods were performed using Microsoft Office Excel 2003 and DOS Stat Calc software (Anonymous, 2013).

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee No. 27.09.2012 and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

RESULTS

All groups were compared with each another. IL-1b production did not differ between the groups (Fig. 1).

The IFN-γ level was significantly lower in the HIV-1 patients group with secondary TB, with TB of more than five years duration (HIV-1+TB), and in the HIV-1 without TB group, compared with HIV-1/TB primary patients. The level of IL-18 was significantly higher in HIV-1 patients co-infected with TB, compared to the control group; IL-10 production was higher in the control group. In groups with higher levels of IL-18, CD4 cell counts were lower. The highest HIV-1 RNA viral load was observed in the HIV-1 group with primary TB infection and this group also had a higher IFN-γ level. The lowest HIV-1 RNA viral loads were observed in the control group. (Fig. 1)

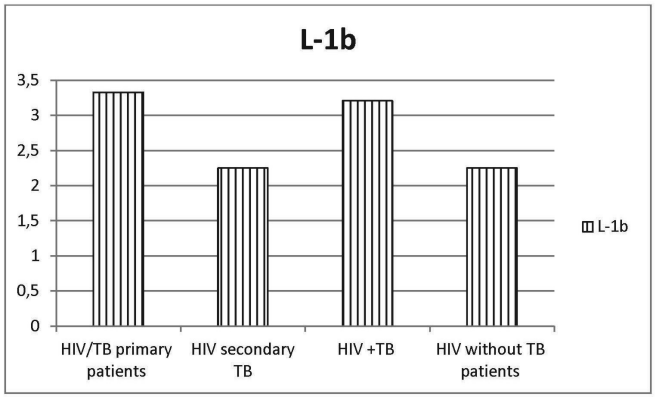

Fig.1. IL-1b levels (pg/ml) in patient groups.

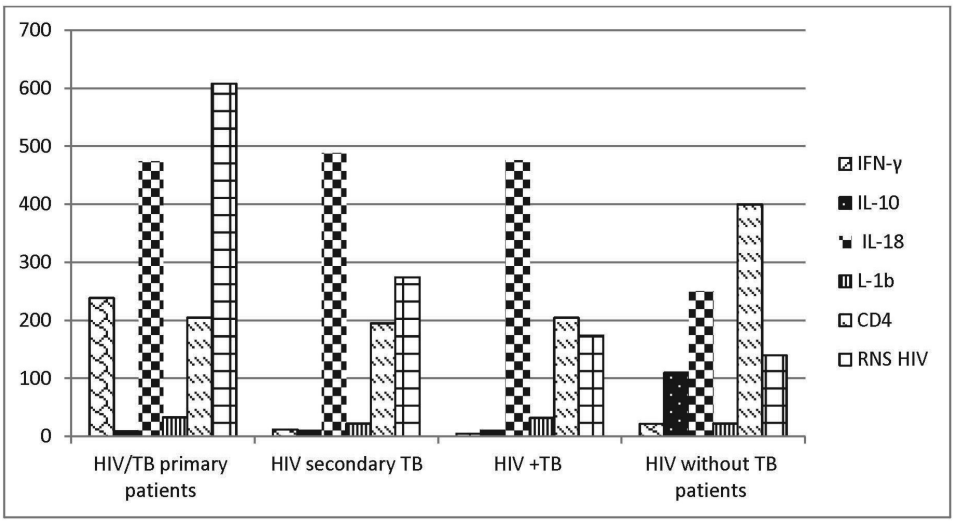

Fig. 2. Cytokine levels, CD4 cell counts and HIV-1RNA load in patient groups.

However, HIV-1/TB primary patients had significantly higher IFN-γ and IL-18 production, and HIV-1 RNA viral load, and lower IL-10 and CD4 cell counts. In addition, the production of IL-18 was correlated with CD4 cell counts in all groups. The highest levels of IFN-γ production and HIV-1 RNA viral load were associated with significantly lower CD4 cell counts (Fig. 2).

DISCUSSION

This study identified the interaction of IFN-γ with IL-10, IL-18, IL-1b, and CD4 cell count and HIV-1 RNA viral load in the development of HIV-1 in patients co-infected with *Mycobacterium tuberculosis*. We measured the serum levels of pro-inflammatory cytokines (IL-1b, IL-18, IFN-γ) and anti-inflammatory cytokine (IL-10). The main findings of this study were: 1) the serum IFN-γ level was significantly higher in the HIV-1 patient group with primary TB, 2) patients with primary HIV-1/TB had higher IFN-γ levels and HIV-1 RNA viral load, and lower IL-10 levels and CD4 cell counts, compared with the control group, 3) the level of IL-18 was significantly higher in HIV-1 patients co-infected with TB, 4) CD4 cell counts were lower in groups with higher levels of IL-18, and 5) HIV-1/TB co-infection did not seem to affect the median serum level of IL-1b in HIV-1 patients and there was no statistically significant difference between TB positive and TB negative HIV-1 patients.

In individuals infected with HIV-1, the normal Th1 response to viral infection is shifted to a Th2 response (Klein *et al.*, 1997; Osakwe *et al.*, 2010). Measurement of the serum cytokine levels of HIV-1 infected patients has shown an increase in Th2 cytokine levels and a decrease in Th1 cytokine levels. Our study showed that primary coinfection with *Mycobacterium tuberculosis* shifts Th2 response to Th1 (Klein *et al.*, 1997; Osakwe *et al.*, 2010).

In addition to the Th1 subset response mediation mentioned earlier, IFN-γ normally acts on APCs to enhance their expression of major histocompatibility complex II (MHC-II), thereby enhancing their antigen presentation ability (Li *et al.*, 2011). HIV-1 transactivator protein (TAT) interferes with the intracellular signalling normally performed by the IFN-γ bound IFN-γ receptor (Cheng *et al.*, 2009). In so doing, the TAT protein lowers the antigen presentation capacity of dendritic cells and macrophages, further limiting the immune response to the invading virus (Cheng *et al.*, 2009; Li *et al.*, 2011).

IFN-γ-producing CD4 T lymphocytes contribute to the generation of granulomas, besides being important co-stimulators to the adequate activation of CD8 T lymphocytes. The importance of CD4 T lymphocytes function is seen in patients with HIV, where the risk of TB increases with the decrease of cell counts (Spellberg and Edwards, 2001).

Clinical assessment of IFN-γ level in the serum of HIV-1+ TB in different clinical groups has been used to determine the importance of IFN-γ in the pathogenesis of HIV-1 with TB. The effects of IFN-γ are far-reaching, exhibiting polyfunctional effects on immune activation, proinflammatory responses, and immune modulation. IFN-γ has a major effect on the regulation of antigen presentation by macrophages and dendritic cells, and in induction of class switching of B cells (Roff *et al.*, 2013). As a proinflammatory cytokine, IFN-γ directly activates phagocytic cells and stimulates oxidative burst and the release of degradative enzymes, thereby supporting the host defence responses against intracellular pathogens (Roff *et al.*, 2013). Our study revealed the presence of a high level of IFN-γ in HIV-1 patients with primary TB infection.

IFN-γ stimulates the production of reactive nitrogen intermediates (RNIs), thus mediating the tuberculostatic function of macrophages, and also stimulates the migration of immune cells to the infection site, contributing to granuloma formation, which controls the disease progression. In chronic, stable disease, IFN-γ levels decline to a steady state that is often equivalent to healthy controls (Spellberg and Edwards, 2001; Roff *et al.*, 2013). The primary TB infection in HIV-1 patients has induced IFN-γ production that have reduced the adaptive immune responses to the development of HIV-1. If not appropriately controlled, such inflammatory activities can promote HIV-1 infection

and may cause a higher viral set point before T cell immunity can control the HIV-1 load.

CONCLUSION

Our findings showed that IFN-γ in a group of patients with HIV-1 and primary TB group had higher HIV-1 RNA viral load and CD4 cell counts, as well as shifted Th2 response to Th1. Increased IL-18 levels significantly lower CD4 cell counts in HIV-1 patients co-infected with TB. Also, the observed significantly higher levels of IFN-γ in the serum of HIV-1 patients may reflect an active phase and latent *M. tuberculosis* infection, and may be a potential marker for improving the targeting of preventative therapy to HIV-positive individuals with latent tuberculosis infection and in HIV-positive individuals with high sensitivity TB.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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INTERFERONA-GAMMA (IFN-γ) LOMA IMŪNĀS ATBILDES REGULĒŠANĀ PACIENTIEM AR HIV-1 UN HIV-1 + *MYCOBACTERIUM TUBERCULOSIS*

Raksta mērķis bija izpētīt IFN-γ lomu mijiedarbībā starp IL-10, IL-18, IL-1b un CD4 šūnu skaitu un HIV-1 RNS vīrusu kopiju slodzes skaitu HIV-1 infekcijas attīstībā pacientiem ar *Mycobacterium tuberculosis* (TB) dubultinfekciju. Pētījums veikts Rīgas Austrumu klīniskās universitātes slimnīcas stacionārā "Latvijas Infektoloģijas centrs" un Rīgas Stradiņa universitātes Klīniskās imunoloģijas un imunoģenētikas starpkatedru laboratorijā. Izanalizēti 200 ar HIV-1 inficētie pacienti un 184 HIV-1 pacienti ar TB dubultinfekciju. IFN-γ, IL-10, IL-18, IL-1b līmeņi seruma paraugos noteikti, izmantojot ELISA tehnoloģijas bāzēto testēšanas sistēmu (*ELISA Vector-best corporation,* Novosibirska, Krievija). CD4 šūnu skaits noteikts, izmantojot plūsmas citometriju metodi (PARTEC IVD ASV), un HIV-1 vīrusa RNS kopiju skaits plazmā — izmantojot *Cobas AmpliPrep /Cobas TaqMan HIV-1 Test* (Vācija). Mūsu iegūtie dati liecina, ka IFN-γ produkcija bija būtiski samazināta, turpretī IL-10 produkcija un CD4 šūnu skaits bija būtiski palielinājies HIV-1 pacientiem, salīdzinot ar HIV-1 pacientiem, kuri bija vienlaikus inficēti ar tuberkulozi. HIV-1 pacientiem, kuri bija vienlaikus inficēti ar tuberkulozi, bija būtiski palielināta IL-18 produkcija. HIV-1 pacientiem, vienlaikus pirmreizēji inficētiem ar tuberkulozi, IFN-γ produkcija un HIV-1 RNS vīrusu slodze bija būtiski palielināta, bet IL-10 produkcija samazināta, salīdzinot ar citam grupām.