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IL-31 expression in HIV-infected patients with different routes of disease transmission

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Abstract

Acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV). AIDS is characterized by an impaired immune system and low cellular immunity. The main manifestation of AIDS is a reduction in the number of CD4+ T cells and alteration in cytokine concentration. The present work aimed to explore the expression of IL-31 in HIV infection and disease progression.

Serum samples were collected from HIV-infected patients with different routes of disease transmission. The subjects included 24 patients who were infected with HIV upon blood transmission and 36 patients who had acquired the disease through sexual transmission (21 cases of homosexual transmission and 15 cases of heterosexual transmission). In addition, 20 normal healthy individuals were included to serve as the control group. The levels of IL-31 in the collected serum samples were estimated using the human IL-31 Platinum ELISA kit.

The serum analysis results revealed that the concentration of IL-31 in the serum samples for the blood transmission, sexually transmission, and normal group patients was 4.07 ± 1.63 pg/L, 7.43 ± 1.15 pg/L, and 2.87 ± 1.04 pg/L, respectively. The statistical analysis revealed that the concentration of IL-31 in HIV-1 infection was higher than that in the normal control. In addition, the expression of IL-31 was significantly higher in the sexual transmission group compared to the blood transmission group ($P < .05$).

IL-31 could have an important role in HIV infection, although the role of IL-31 in disease progression in HIV-infected individuals requires further research.

Abbreviations: AIDS = acquired immunodeficiency syndrome, ART = antiretroviral therapy, DC = dendritic cell, HIV = human immunodeficiency virus, HIV-1 = human immunodeficiency virus type 1, HSC = hematopoietic stem cell, MAPK = mitogen-activated protein kinase.

Keywords: cytokine, HIV, IL-31, serum

1. Introduction

Infection with human immunodeficiency virus type 1 (HIV-1) impairs the immune system of the body,[1] which manifests as low cellular immunity, reduced number of CD4+ cells, and altered cytokine concentration.[2] Moreover, prolonged immune system activation is a typical characteristic of HIV infection.[3] In addition, HIV induces widespread inflammation, massive depletion of CD4+ T-cells, and release of inflammatory cytokines in huge quantities.[4]

Systemic immune hyperactivation and loss of CD4+ T cells are generally considered the initial markers of HIV infection.[5] Currently, antiretroviral therapy (ART) is considered effective in the control of HIV replication, although the number of CD4+ T cells may not be recovered completely even in the cases where

effective virus control is achieved.[6] Moreover, long-term treatment of HIV infection may lead to problems such as adverse reactions, drug resistance, or decreased adherence to medication regimens.[7] On the contrary, it might be beneficial to inhibit the abnormally activated immune system in HIV-1-infected patients. Persistent immune system activation is harmful as it gradually increases the viral load and generates protective immune responses toward HIV.[8] In order to overcome these issues, several clinical trials have been conducted in recent years to evaluate various adjuvant treatments, including immunomodulation.

IL-31 is a recently discovered helical cytokine that belongs to the gp130/IL-6 family of cytokines.[9] IL-31 is released mostly from activated CD4+ T cells. In particular, among the Th2 cells, the CD45RO+ (memory) T cells exhibit extensive expression of IL-32.[10] IL-31 is involved in maintaining the homeostasis of hematopoietic stem cells (HSCs) and the induction of pro-inflammatory activities for activated macrophages and monocytes.[12] Previous studies have reported the elevated expression of IL-31 in association with the advancement of atopic dermatitis. In addition to its itch-promoting effect, IL-31 is reported to play a pro-inflammatory role through the upregulation of the expression of pro-inflammatory genes in T cells.[7] The pro-inflammatory function of IL-32 has also been observed in various chronic autoimmune skin diseases and psoriasis.[13]

IL-31/IL-31 receptor may activate several pathways, including the Janus tyrosine kinase–Signal transducer and activator of transcription (JAK-STAT) pathway, the Akt pathway, the NF-κB pathway,[14,15] the mitogen-activated protein kinase (MAPK) pathway, and the PI3K signaling pathways.[16,17] Moreover, IL-31/IL-31 receptor is reportedly hyperactivated in HIV infection, in which it promotes viral replication.[18–20] When HIV attacks the CD4 lymphocytes, systemic inflammation is induced. Owing to the critical role of IL-6 in CD4 T cell responses, the elevation of IL-6 expression is adopted as a predictor of death or risk of non-AIDS-defining complications in subjects with chronic HIV infection.[21] While IL-31 is a four-helix cytokine mainly released from the CD4+ Th2 cells, its role in HIV infection has not been studied much.

2. Materials and methods

2.1. Ethical statement

The present research was approved by the Ethics Review Committee of the First Affiliated Hospital of Harbin Medical University. All participants were thoroughly instructed regarding the study protocol. Written informed consent for participation was obtained from each patient prior to the study.

2.2. Samples

The sample population for the present study included the HIV-1-infected patients who visited the Outpatient Department of the First Affiliated Clinical Hospital, Harbin Medical University, between January 2016 and January 2017. Serum samples were collected from untreated patients who were admitted to the hospital, and patients with significant chronic diseases, tumors, skin diseases, and autoimmune diseases were excluded from participation in the study. Serum samples were collected from 60 HIV-1-infected people with different disease transmission routes, among which 24 cases had been infected through blood transmission, while 36 patients were infected through sexual transmission. Among these 36 patients, there were 21 and 15 cases of homosexual and heterosexual transmission, respectively. Disease confirmation in each of the included 60 HIV-infected subjects was provided by the AIDS confirmation Laboratory of Harbin Center for Disease Control and Prevention. In addition, serum samples from 20 healthy individuals (physically examined at the outpatient department) were collected to serve as the control group samples.

2.3. CD4+ T cell counts and quantification of serum IL-31 levels

The peripheral blood samples were collected from HIV-infected patients and mixed with EDTA to prevent coagulation. The whole blood samples were then subjected to evaluation of the CD4+ T cell count using the BD FACS Calibur platform (BD Biosciences, San Jose, CA) with anti-CD4 (RPA-T4, BD Biosciences). The serum levels of IL-31 were determined using the human IL-31 Platinum ELISA kit (E-EL-H5469c, Elabscience, China) strictly in accordance with the manufacturer's instructions. The double antibody sandwich method was used for the accurate determination of the cytokine concentration. The absorbance values at 450 nm were determined, and a standard curve was plotted to obtain the cytokine concentration in the test samples.

2.4. Statistical analysis

Data were statistically analyzed using GraphPad Prism 8 software and expressed as mean ± SD. The statistical significance was evaluated using a t test. The P-value of $<.05$ indicated statistical significance. Since both of the variables used in the present study were continuous, Pearson's correlation analysis was adopted, and $P < .05$ was considered the threshold of statistically significant correlation.

3. Results

The HIV-infected patients were divided into two groups based on the mode of transmission of the disease: the blood transmission group and the sexual transmission group. Thereafter, serum IL-31 levels were quantified using samples from both the groups of patients and healthy controls.

The serum IL-31 levels in the HIV patients infected due to blood transmission were determined to be 4.07 ± 1.63 pg/L, while the levels in HIV patients infected through sexual transmission were 7.43 ± 1.15 pg/L. In the healthy control group, the serum IL-31 levels were 2.87 ± 1.04 pg/L (Fig. 1A). The concentration of IL-31 in HIV-infected individuals, due to both blood and sexual transmission, was markedly increased relative to that in the healthy controls ($P = .0015$ and $P < .0001$, respectively). Moreover, the serum IL-31 levels of the HIV patients infected through sexual transmission were evidently higher than those of the patients infected due to blood transmission.

The sexual transmission group of HIV patients was further categorized into the homosexual transmission group and the heterosexual transmission group. Both of these groups exhibited evidently elevated ($P < .0001$) levels of serum IL-31 relative to normal controls (Fig. 1B).
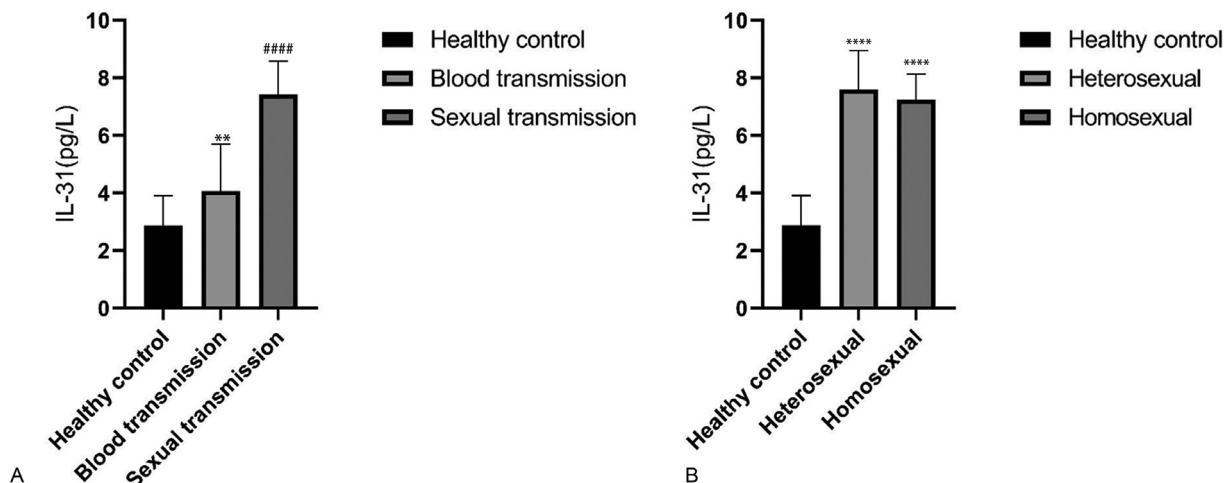
Figure 1. Serum IL-31 levels in patients. (A) Serum IL-31 levels in patients with different transmission routes and healthy controls. The HIV-infected patients were divided into two groups: the blood transmission group and the sexual transmission group. Left to right: healthy controls, blood transmission group of patients, and sexual transmission group of patients. In comparison to the control group, the other two groups presented significantly higher levels of IL-31 ($P = .0015$ and $P < .0001$, respectively). (B) Serum IL-31 levels in patients in the homosexual transmission group, heterosexual transmission group, and healthy control group. Left to right: control group, heterosexual transmission group, and homosexual transmission group.

CD4 count is one of the important predicting factors of HIV-1 progression. The association of serum IL-31 levels with CD4 count was investigated for all HIV-infected patients to decipher the role of IL-31 in AIDS disease progression. The results revealed a correlation ($P = .025$) between IL-31 and CD4 in HIV-1-infected patients (Fig. 2A). Further correlation analysis was performed between CD4$^+$ cells and IL-31 levels in the blood transmission and sexual transmission groups of patients. The
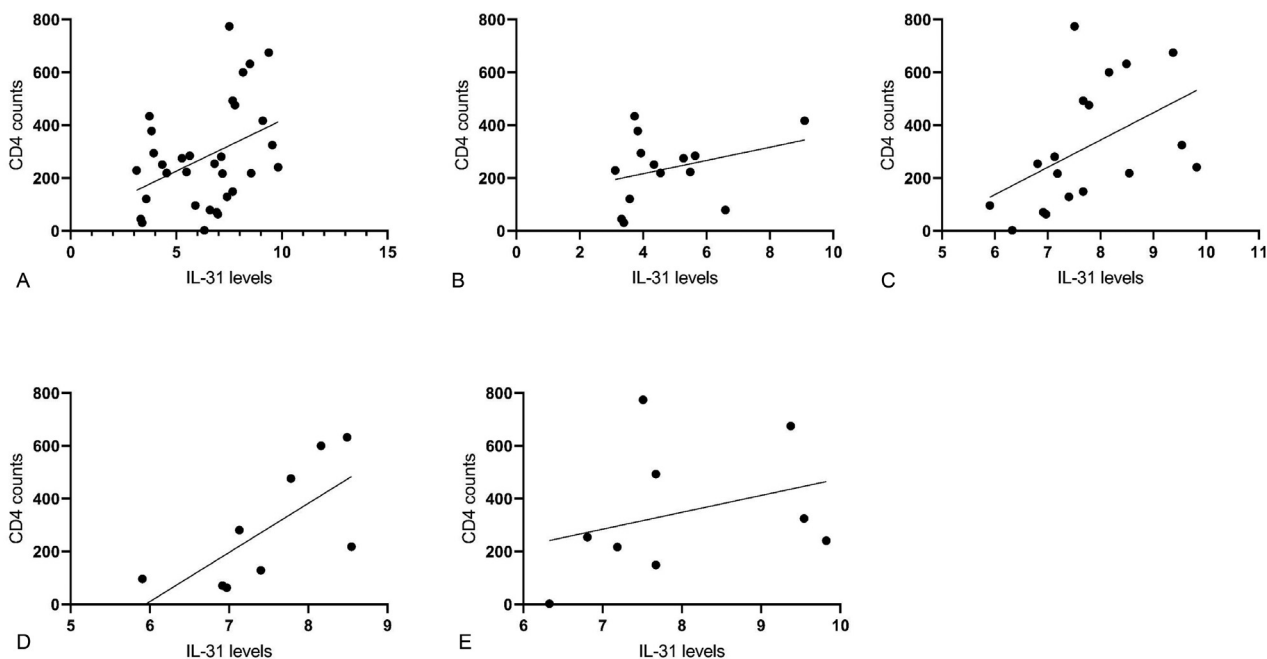
Figure 2. Correlation analysis between IL-31 levels and CD4$^+$ cell counts. (A) Results for the correlation analysis between IL-31 levels and CD4$^+$ cell counts in HIV-1-infected patients. The horizontal axis indicates the serum IL-31 levels, while the vertical axis indicates the CD4$^+$ cell counts; the line represents the correlation ($P = .025$). (B) Results for the correlation analysis between IL-31 levels and CD4$^+$ cell counts in patients infected due to blood transmission. The horizontal axis presents the serum IL-31 levels, while the vertical axis presents the CD4$^+$ cell counts; the line represents the correlation ($P = .27$). No correlation was observed between IL-31 levels and CD4$^+$ cell count in the blood transmission group. (C) Results for the correlation analysis between IL-31 levels and CD4$^+$ cell counts in patients infected through sexual transmission. A correlation ($P = .046$) was observed between IL-31 levels and CD4$^+$ cell count in the sexual transmission group patients. (D) Results for the correlation analysis between IL-31 levels and CD4$^+$ cell counts in patients infected through homosexual transmission. Among all subgroups, the homosexual transmission group presented the strongest correlation ($P = .036$) between serum IL-31 levels and CD4$^+$ cell counts. (E) Results for the correlation analysis between IL-31 levels and CD4$^+$ cell counts in patients infected with heterosexual transmission of HIV. No association was observed between CD4$^+$ cell counts and IL-31 levels in the heterosexual transmission group ($P = .398$).

results revealed that while no correlation existed between CD4+ cells and IL-31 levels in the blood transmission group ($P = .27$), a significant correlation ($P = .046$) was observed between CD4+ cells and IL-31 levels in the sexual transmission group (Fig. 2B and C). Since the CD4+ cell count was strongly correlated to IL-31 levels in the sexual transmission group, this group was divided into the homosexual transmission group and heterosexual transmission group for further analysis of the correlation between CD4+ cells and IL-31 levels.

It was revealed that a strong correlation existed between serum IL-31 levels and CD4+ cells in the homosexual transmission group ($P = .036$), while there was no correlation ($P = .398$) between the two variables in the heterosexual transmission group (Fig. 2D and E, respectively).

4. Discussion

The present study revealed the association of HIV-infected patients with elevated serum levels of IL-31. In addition, the study revealed that the IL-31 levels are elevated in both blood-transmitted and sexually-transmitted cases of HIV. Previous studies have reported elevated levels of pro-inflammatory cytokines in the plasma, including IL-2, IL-6, IFN-γ, and TNF-α, in association with HIV.[22] IL-31 is reported to promote the production of pro-inflammatory cytokines, such as IL-6 and IL-1β, in different cell types.[12] These increased levels of pro-inflammatory cytokines may, in turn, promote the production of IL31 along with the corresponding receptor complex. Moreover, TNF-α, IFN-γ, and IL-1β are also reported to increase the levels of OSMR, IL31, and IL31Rα genes,[23,24] indicating the possible role of IL-31 in activating the immune system in HIV-infection cases.

The present study also revealed significantly higher levels of IL-31 in patients infected through the sexual transmission of HIV compared to those infected through blood transmission ($P < .01$). This could be because the mucosal immune system is the storehouse of activated CD4+ cells,[25] where HIV replicates further efficiently.[26,27] The CD4+ cells harbor IL-31, leading to increased cytokine concentrations in these cells. This could account for the increased levels of IL-31 in patients infected through sexual transmission compared to those infected due to blood transmission of HIV.

Subsequent analysis revealed a correlation between CD4+ cells and IL-31 levels in HIV-infected patients. Further correlation analysis conducted for the blood transmission, sexual transmission, homosexual transmission, and heterosexual transmission groups revealed a significant correlation between IL-31 and CD4+ cells in the sexual transmission and homosexual transmission groups, while no correlation was observed between the two variables in the blood transmission and heterosexual transmission groups. As indicated by the above results, IL-31 might have an important role in sexually-transmitted HIV infection, as opposed to blood-transmitted HIV infection. HIV is known to be transmitted mainly via skin and mucous membranes. Recently published studies investigating IL-31 in allergic diseases, spontaneous asthma, inflammatory bowel disease, and chronic urticaria have suggested a potential role of IL-31 in mucosal tissue immunity.[28,29] However, in the correlation analysis, differences were observed in the results obtained in the present study between the blood transmission group and the sexual transmission group. According to the literature, similar to IL-13 and IL-17,[30] the receptor complex of IL-31 has also been detected in non-hematopoietic tissues, such

as skin and trachea, which suggests a regulatory function of IL-31 in multiple tissue responses.[31] The findings of the present study suggest that IL-31 possibly plays a tissue-specific functional role. Studies have indicated dendritic cells (DCs) as a source of IL-31.[32] The fact that the IL-31 released from dendritic cells attracts lymphocytes into the epidermis validated the findings of the present study. Moreover, it has been hypothesized that the cytokines originating from DCs recruit and bring CD4+ cells to the epidermis, which consequently affects disease progression. This hypothesis has been supported by evidence in previous reports in the literature.[33] The findings of the present study may, therefore, suggest that IL-31 plays a significant role in sexual transmission of HIV, rather than in the blood transmission of HIV, mainly via skin and mucous membranes. This inference is supported by the findings of most of the recent studies, which have reported the association of IL-31 levels with allergic diseases, spontaneous asthma, inflammatory bowel disease, and chronic urticarial.[25,26,34]

CD4+ cells serve as important indicators of disease progression and treatment response in HIV-infected patients. These cells are also the most important cell type for IL-31 production. In the present study, all groups of patients presented varying degrees of decrease in the CD4+ cells, although the overall levels of IL-31 in all groups of HIV-infected patients were higher than those in the healthy controls. Although experimental limitations did not allow deciphering whether there exists a link between IL-31 levels and HIV RNA, it may be speculated that IL-31 plays a role in immune activation during HIV infection.

The present study is a pioneer in reporting that HIV-1-infected patients have higher levels of IL-31 in the serum. IL-31 is, therefore, speculated to play a vital role in the pathogenic mechanism of HIV-1 and also in mucosal immune system responses. However, the small number of clinical samples used in the present study and the limited diversity in the patient background could have influenced the outcomes of the analysis. Therefore, further studies elucidating the effects of IL-31 on HIV infection and its progression are warranted. Nonetheless, despite the small sample size of the present study, its findings represent valuable evidence in this relatively unexplored field.

Author contributions

Changxin Yan and HZ contributed to the conception of the study.

Changxin Yan, Huafeng Xu, and Haizhou Zhou contributed significantly to analysis and manuscript preparation;

Chunli Rong and Meilin Cao performed the data analyses and wrote the manuscript;

Zhuo Miao helped perform the analysis with constructive discussions.

All authors approved the manuscripts.

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References

[1] Eron JJJr, Ashby MA, Giordano MF, et al. Randomised trial of MNrgp120 HIV-1 vaccine in symptomless HIV-1 infection. Lancet 1996;348:1547–51.

[2] Lepej SZ, Begovac J, Vince A. Changes in T-cell subpopulations during four years of suppression of HIV-1 replication in patients with advanced disease. FEMS Immunol Med Microbiol 2006;46:351–9.

[3] Perdomo-Celis F, Feria MG, Taborda NA, Rugeles MT. A low frequency of IL-17-producing CD8(+) T-cells is associated with persistent immune activation in people living with HIV despite HAART-induced viral suppression. Front Immunol 2018;9:2502.

[4] Wang X, Mbondji-Wonje C, Zhao J, Hewlett I. IL-1beta and IL-18 inhibition of HIV-1 replication in Jurkat cells and PBMCs. Biochem Biophys Res Commun 2016;473:926–30.

[5] Gaardbo JC, Hartling HJ, Gerstoft J, Nielsen SD. Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. Clin Dev Immunol 2012;2012:670957.

[6] Zanzibar Wizara ya Afya . Mradi wa Kupambana na UKIMWI (Zanzibar): 2013 Epidemiologic Profile-Zanzibar: Integrated Epidemiologic Profile for HIV and AIDS Prevention, Care and Treatment, and Strategic Information. Zanzibar: Ministry of Health, Zanzibar AIDS Control Programme; 2014.

[7] Velu V, Shetty RD, Larsson M, Shankar EM. Role of PD-1 co-inhibitory pathway in HIV infection and potential therapeutic options. Retrovirology 2015;12:14.

[8] Morris K. HAART and host: balancing the response to HIV-1. Highly active antiretroviral therapy. Lancet 1998;352:1686.

[9] Jones SA, Jenkins BJ. Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer. Nat Rev Immunol 2018;18:773–89.

[10] Castellani ML, Felaco P, Galzio RJ, et al. IL-31 a Th2 cytokine involved in immunity and inflammation. Int J Immunopathol Pharmacol 2010;23:709–13.

[11] Raap U, Gehring M, Kleiner S, et al. Human basophils are a source of-and are differentially activated by-IL-31. Clin Exp Allergy 2017;47:499–508.

[12] Ginaldi L, De Martinis M, Ciccarelli F, et al. Increased levels of interleukin 31 (IL-31) in osteoporosis. BMC Immunol 2015;16:60.

[13] Narbutt J, Olejniczak I, Sobolewska-Sztychny D, et al. Narrow band ultraviolet B irradiations cause alteration in interleukin-31 serum level in psoriatic patients. Arch Dermatol Res 2013;305:191–5.

[14] Zhang Q, Putheti P, Zhou Q, Liu Q, Gao W. Structures and biological functions of IL-31 and IL-31 receptors. Cytokine Growth Factor Rev 2008;19:347–56.

[15] Dambacher J, Beigel F, Seiderer J, et al. Interleukin 31 mediates MAP kinase and STAT1/3 activation in intestinal epithelial cells and its expression is upregulated in inflammatory bowel disease. Gut 2007;56:1257–65.

[16] Lee CH, Hong CH, Yu WT, et al. Mechanistic correlations between two itch biomarkers, cytokine interleukin-31 and neuropeptide β-endorphin, via STAT3/calcium axis in atopic dermatitis. Br J Dermatol 2012;167:794–803.

[17] Park K, Park JH, Yang WJ, Lee JJ, Song MJ, Kim HP. Transcriptional activation of the IL31 gene by NFAT and STAT6. J Leukoc Biol 2012;91:245–57.

[18] Chovatiya R, Paller AS. JAK inhibitors in the treatment of atopic dermatitis. J Allergy Clin Immunol 2021;148:927–40.

[19] Zhou N, Luo Z, Luo J, et al. Structural and functional characterization of human CXCR4 as a chemokine receptor and HIV-1 co-receptor by mutagenesis and molecular modeling studies. J Biol Chem 2001;276:42826–33.

[20] Lai T, Wu D, Li W, et al. Interleukin-31 expression and relation to disease severity in human asthma. Sci Rep 2016;6:22835.

[21] Manion M, Hullsiek KH, Wilson EMP, et al. Vitamin D deficiency is associated with IL-6 levels and monocyte activation in HIV-infected persons. PLoS One 2017;12:e0175517.

[22] Kisuya J, Chemtai A, Raballah E, Keter A, Ouma C. The diagnostic accuracy of Th1 (IFN-γ, TNF-α, and IL-2) and Th2 (IL-4, IL-6 and IL-10) cytokines response in AFB microscopy smear negative PTB-HIV co-infected patients. Sci Rep 2019;9:

[23] Dillon SR, Sprecher C, Hammond A, et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. Nat Immunol 2004;5:752–60.

[24] Kasraie S, Niebuhr M, Werfel T. Interleukin (IL)-31 induces pro-inflammatory cytokines in human monocytes and macrophages following stimulation with staphylococcal exotoxins. Allergy 2010;65:712–21.

[25] Veazey R, Lackner A. The mucosal immune system and HIV-1 infection. AIDS Rev 2003;5:245–52.

[26] McDougal JS, Mawle A, Cort SP, et al. Cellular tropism of the human retrovirus HTLV-III/LAV I. Role of T cell activation and expression of the T4 antigen. J Immunol 1985;135:3151–62.

[27] van Noesel CJ, Gruters RA, Terpstra FG, Schellekens PT, van Lier RA, Miedema F. Functional and phenotypic evidence for a selective loss of memory T cells in asymptomatic human immunodeficiency virus-infected men. J Clin Invest 1990;86:293–9.

[28] Di Salvo E, Ventura-Spagnolo E, Casciaro M, Navarra M, Gangemi S. IL-33/IL-31 Axis: a potential inflammatory pathway. Mediators Inflamm 2018;2018:3858032.

[29] Gibbs BF, Patsinakidis N, Raap U. Role of the pruritic cytokine IL-31 in autoimmune skin diseases. Front Immunol 2019;10:1383.

[30] Gaffen SL. Structure and signalling in the IL-17 receptor family. Nat Rev Immunol 2009;9:556–67.

[31] Stott B, Lavender P, Lehmann S, Pennino D, Durham S, Schmidt-Weber CB. Human IL-31 is induced by IL-4 and promotes TH2-driven inflammation. J Allergy Clin Immunol 2013;132:446–54. e445.

[32] Cornelissen C, Brans R, Czaja K, et al. Ultraviolet B radiation and reactive oxygen species modulate interleukin-31 expression in T lymphocytes, monocytes and dendritic cells. Br J Dermatol 2011;165:966–75.

[33] Malek M, Glen J, Rebala K, et al. Il-31 does not correlate to pruritus related to early stage cutaneous T-cell lymphomas but is involved in pathogenesis of the disease. Acta Derm Venereol 2015;95:283–8.

[34] Spina CA, Prince HE, Richman DD. Preferential replication of HIV-1 in the CD45RO memory cell subset of primary CD4 lymphocytes in vitro. J Clin Invest 1997;99:1774–85.