Journal of
Immunology Research and Infectious Diseases

Research Article | Open Access

# A New Therapeutic Target Against HIV/AIDS Virion Proteins Stimulation by Cells Exposed

**Marie V Miller*, Engels Kwong**

Forum for Collaborative HIV Research, School of Public Health, University of California Berkeley, USA

***Correspondence:** Marie V Miller, Forum for Collaborative HIV Research, School of Public Health, University of California Berkeley, USA. E-mail: miller_v@berkeley.edu

Received: August 20, 2021; Accepted: September 21, 2021; Published: September 30, 2021



## Abstract

**Background:** The pathologic process and response to a chronic infection is varied particularly for HIV infection. The impact of HIV on the general immune environment of the infected individual is profound and triggered by multiple stimuli. whereas replication competent infection induces an oversized spectrum of immune responses, like protein unharness, we tend to be targeted on what happens once cells are exposed to replication incompetent virions and their parts.

**Results:** observation the cytokines free from lymph cell lines exposed to HIV particle parts, we tend to find many cytokines were upregulated with IL-16 being the foremost upregulated. HIV particle parts evoked Caspase-3 activation that is needed for IL-16 unharness and this IL-16 unharness relies on macromolecule parts inside the HIV particle.

**Conclusion:** Overall, this work permits United States to envision that replication incompetent HIV virions have a bearing on the immune landscape of an individual infected by HIV. specifically, we tend to see that exposure of T cells to HIV particle proteins ends up in Caspase-3 activation and ensuant IL-16 unharness.

**Keywords:** HIV; AIDS; Innate Immunity; Cytokines, Cytokine; Array; IL-16; Caspase-3

## INTRODUCTION

As of 2014, it's calculable that thirty six.9 million folks live with Human immunological disorder Virus (HIV) worldwide with concerning a pair of million freshly infected p.a. HIV is calculable to cause concerning one.2 million deaths among adults and youngsters annually. though there has been abundant improvement in each interference and treatment, realization of fully effective therapies, cures or vaccines needs a more robust understanding of the pathophysiology and medical specialty consequences of HIV infection.

In specific, as an outbreak with system reaction, HIV induces broad, system-level changes in protein levels in a private infected with HIV [1,2]. whereas bound major cytokines are well studied, as well as those related to mortality from HIV, few studies have checked out comprehensive protein analysis once exposure to HIV [3].

HIV-1 infects CD4+ T cells. previous analysis on protein response to HIV-1 has shown that HIV-1 causes a decrease in Th1 cytokines (IL-2, IFN-gamma) however causes a rise in Th2 and inflammatory cytokines (IL-4/IL-10 and tumor necrosis factor alpha respectively). Reconstituting Th1 protein response has been incontestable to boost CD8+ T cells and management of the virus [4]. alternative cytokines like IL-16 associate degreed IL-2 have a repressive impact on HIV-1 and are instructed as doable therapeutic modalities [5]. throughout any infection, a high level of defective virions is free into the native microenvironment as exemplified within the evolution of contagion virus [6]. These defective virions are usually structurally enough to realize entry into cells.

The goal of this work is {to investigate to research to associate decreolize} however exposure to HIV particle parts modulates traditional protein unharness mistreatment an unbiased approach. we tend to be found that exposure to HIV particle parts induce the discharge of many cytokines with IL-16 being most evoked. we tend

to additional targeted on IL-16 unharness since levels of IL-16 are shown to be exaggerated within the initial stages of HIV-1 infection, followed by a decrease as HIV infection progresses [7,8]. This powerfully implies that IL-16 is concerned in initial response to the HIV. Here we tend to conjointly show however HIV exposure induces IL-16 production.

## MATERIALS AND STRATEGIES

CEM and Jurkat, each leukemic CD4+ lymph cell lines, were civilized in RPMI + five-hitter craniate Bovine liquid body substance (FBS) + I Chronicles Penicillin/ antibiotic (P/S). we tend to use five-hitter FBS to limit innate immune sign. Cells were incubated at thirty-seven °C and five-hitter carbon dioxide and maintained by diluting to 1x105 cells/mL each 2-3 days to permit growth. Cells were used at a operating concentration between 2x105 and 5x105 cells/mL. HEK 293T cells, a reworked human embryonic urinary organ animal tissue cells line that expresses the massive T matter from SV40, was full-grown in DMEM + five-hitter FBS + I Chronicles Penicillin/Streptomycin and maintained at 20-70% confluence. All cell lines were purchased from ATCC.

### Transfection of HIV Lysate

Cells were transfected with sublimate HIV particle Lysates (ABI) with Lipofectamine 2000 (Invitrogen, Corp) transfection chemical agent in line with a changed manufacturer's protocol as delineated below. Jurkat and CEM cells were transferred to RPMI + five-hitter FBS with no P/S at cell concentration of either 2x105 or 5x105 cells/mL in 12-well dishes. To four hundred four hundred of RPMI, twelve of Lipofectamine 2000 chemical agent was another and allowed to incubate for five minutes at temperature. once the incubation, fifty of the RPMI/ Lipofectamine 2000 chemical agent combine was another to tubes with zero (vehicle), 1 µL, 3 µL, 6 µL, or nine nine of HIV lysate and allowed to incubate for quarter-hour at temperature. once incubation, these mixtures were another to the acceptable cells. Transfected cells were full-grown for 24-48 hours underneath the conditions as delineated higher than.

### Cytokine Array Assay

Changes in protein production were assessed with Human protein Array Panel A (R&D Systems) in line with the manufacturer's directions. In brief, nitro cotton membranes in an exceedingly 4-well multi-dish, noticed with antibodies for a panel of cytokines, were blocked to forestall nonspecific binding. Signal made by the protein array was detected and quantitated by a computer-aided gel documentation system (Bio-Rad).

### Enzymatic Treatment of HIV particle Lysates

HIV particle Lysates were treated with 1µl of transferase A for twenty minutes at thirty seven o C, with one unit (0.5 µl) of RNase H for twenty minutes at thirty seven o C, ten of proteolytic enzyme K at fifty o C for half-hour or mock treated. Enzymes were inactivated at sixty-five o C for twenty minutes. Either one metric capacity unit or ten metric capacity unit of treated lysates were transfected into CEM cells for thirty-six hours and supernatants were analyzed by assay for IL-16 production, as delineated higher than.

## RESULTS

### Exposure to HIV particle parts Induces Modulation of Specific Cytokines

To better perceive however CD4+ T cells square measure plagued by exposure to HIV virions we tend to use an individual's protein Array to interrogate the modulation of various cytokines. we tend to use CEM CD4+ lymphocyte lines and transfected a lysate from refined HIV virions into the cells. during this manner we tend to mimic exposure of the CD4+ T cells to defective virions. Supernatants from the transfected CEM cells were analyzed by an individual's protein Array to find levels of protein with or while not exposure to HIV parts (Figure 1).

The protein array measures thirty-six completely different cytokines and that we compared cytokines levels in CEM cells transfected with particle lysates versus CEM cells exposed to vehicle alone. As shown in Figure 1, some cytokines were upregulated whereas others

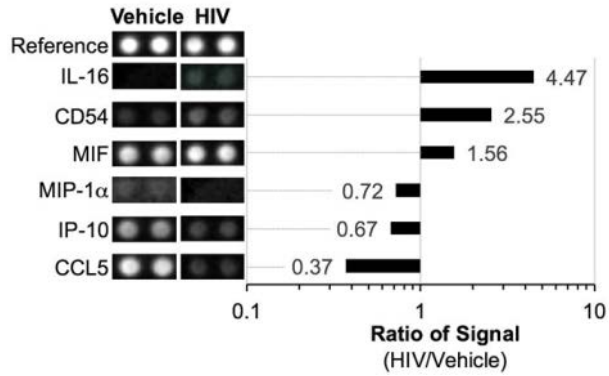


**Figure 1:** Exposure to HIV Virion Components Modulates Cytokines Levels in CD4+ T cell Lines

downregulated. every try of spots like the various cytokines on the Human protein Array were digitally quantitated and reference spots were accustomed normalize every of the cytokines. we tend to premeditate ratios of signal for every protein as viron-exposed cells compared to vehicle alone cells. many cytokines had increase in expression, most notably IL-16. On the opposite hand, many cytokines had a decrease in their expression, with CCL5 showing the largest decrease in expression. several different cytokines on the panel had no expression by CEM CD4+ T cells and everyone array information is conferred in Table one.

## IL-16 is free upon Exposure to HIV particle parts

IL-16 has been we tend toll-known to limit HIV replication thus we were intrigued by the high induction seen once we exposed our samples to HIV particle lysates [9]. to substantiate the results from the protein array, we tend to be exposed CEM and Jurkat CD4+ lymphocyte lines to HIV particle lysate and quantitated the amount of IL-16 made mistreatment assay. 2 separate cell concentrations of CEM CD4+ T cells were transfected with completely different amounts of HIV particle lysate, whereas Jurkat cells were transfected at one concentration of cells. Supernatants of the cells were assayed to quantitate the extent of IL-16 made.

CEM cells incontestable increasing unleash of IL-16 in response to increasing concentrations of HIV particle Lysate at completely different cell concentrations (Figure 2). Jurkat cells incontestable increasing unleash of IL-16 in response to increasing concentrations of HIV particle Lysate at one cell concentrations. Transfection of HEK 293T cells, that don't seem to be CD4+ T cells, showed no production of IL-16 (Data not shown). additionally, transfection of CEM cells with HIV particle Lysate showed no production of the protein IFN-gamma (as previous studies have found attenuate expression), confirming the info from the Human protein Array (Data not shown) [10].

## Exposure to HIV Induces Protease Activation

Since IL-16 unleash depends on activation of protease three, we tend to monitor activation of protease three upon exposure of CEM cells to HIV Lysate [11,12]. protease three activation is monitored because of the chemical action cleavage of protease three into a smaller kind. to ascertain this, we tend to use western blotted for the assembly of the smaller sort of protease three throughout exposure to HIV Lysate. Exposure of CEM cells to HIV Lysate iatrogenic a smaller sort of protease three as cells square measure exposed.

## Virion Proteins, however, not particle polymer, induces IL-16

Cytokines square measure free upon initiation of cellular communication at intervals cells. In an endeavor to higher perceive what kind of immune communication is being initiated, we tend to treat particle lysates with enzymes to get rid of completely different molecular parts. Specifically, we tend to pre-treated particle lysates with RNaseA to get rid of polymer, RNaseH to get rid of polymerase intermediates or ProteinaseK to get rid of macromolecule parts of the virions. The treated virions were then exposed to CEM cells and therefore the levels of IL-16 within the supernatants were assessed by assay. the assembly of IL-16 was below the limit of detection for the supernatant from CD4+ T cells transfected with particle lysates treated with ProteinaseK. The supernatant from CD4 T cells treated with RNaseA and RNaseH still showed detectable levels of IL-16 production in a very dose-dependent manner (Figure 3).

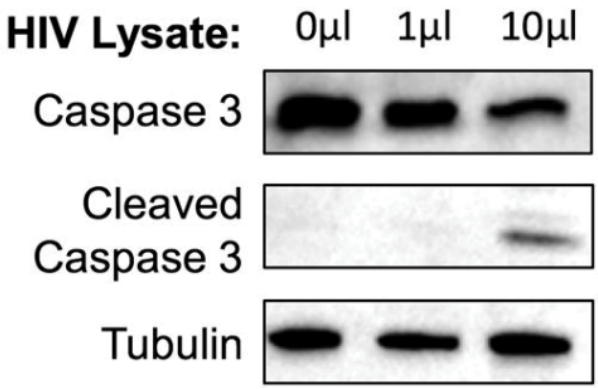


**Figure 2:** Caspase 3 is activated upon exposure to HIV Lysate

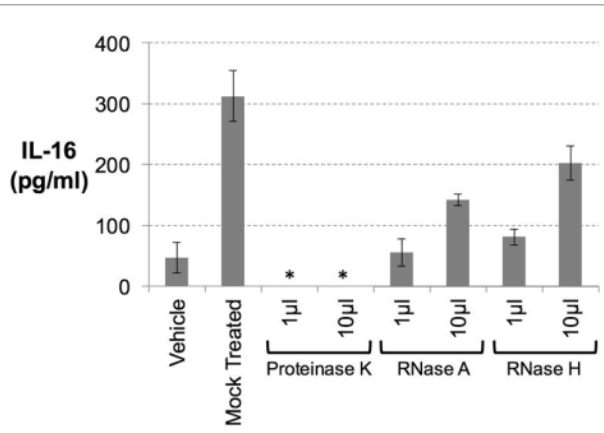


**Figure 3:** IL-16 release in cells exposed to HIV is dependent on virion protein components

## DISCUSSION

HIV has evolved to with efficiency move someone because of its ability to elaborately manipulate the host immunologic response moreover as its infectious agent proteins [13,14]. HIV seems to control the fragile balance of cytokines essential in traditional immune perform towards its goal of immune evasion and a heightened ability to unfold [14]. To model this method, we tend to transfect CD4+ T cells with lysates from sublimate HIV virions. we tend to use Human protein Arrays to interrogate a group of cytokines and the way they're stricken by exposure to HIV virions. The HIV particle Lysate transfected CEM cells yield each induced and blocked expression of cytokines.

### Induced Cytokines

We saw that levels of IL-16, a chemoattractant for immune cells, were greatly accrued when CD4+ T cells were exposed to HIV particle Lysate [15]. Previous studies have found that IL-16 will induce human T-cells to be proof against HIV infection [16,17]. because the HIV particle Lysate represents the elements of HIV virions, and not functionally-replicating virus, AN actively-replicating virus might not induce IL-16, or might induce it at lower levels, allowing replication. There could also be a mechanism, presumably attached obstruction cleavage of the pro-IL-16 kind, to suppress the conventional unleash of IL-16 in patients living with HIV infection and will be any studied. However, this might additionally indicate AN initial induction of IL-16, as has been incontestable in early infection of a cohort of patients living with HIV infection, with blockage of IL-16 occurring at later stages of the infection [18].

These temporal aspects of IL-16 unleash could also be a fitting target for future therapies. we tend to additionally be discovered that sICAM-1 (a soluble kind of AN living thing adhesion marker), that plays a task in system activation, was accrued. This trend has additionally been incontestable in patients living with HIV infection [19]. moreover, we tend to saw a rise in MIF (macrophage migration repressing factor) once exposed to HIV particle elements. this can be discovered in patients with HIV, wherever MIF activates communication pathways that aid in HIV-1 replication [20].

### Down-Regulated Cytokines

Cytokines like CCL5 (RANTES), that inhibits HIV-1 by binding and obstruction the CCR5 HIV-1 co-receptor, were cut within the HIV particle Lysate transfected samples [21]. in addition, IP-10 (CXCL-10), that is secreted in response to IFN-gamma and aids in enlisting of cytotoxic T-cells, additionally cut [22]. this might facilitate limit the cytotoxic T-cell enlisting and killing of HIV-infected cells.

### Mechanism of IL-16 Unleash

Our experiments show a correlation between increasing HIV lysate concentrations and IL-16 production levels. with no HIV lysate, IL-16 productions levels were minimal-to-undetectable in our management cells. Therefore, area unit able to} conclude that once CD4+ T cells are transfected with HIV particle Lysate, they increase IL-16 production.

When we enzymatically removed the super molecule elements of HIV particle Lysate, IL-16 production levels (of the CD4+ lymphocyte supernatant) fell below the detectable vary. Eliminating the other a part of the HIV particle Lysate didn't considerably decrease the amount of induced IL-16. this might indicate that CD4+ T cells acknowledge a super molecule section of HIV-1 and that we conclude that HIV proteins stimulate CD4+ T cells to supply IL-16.

IL-16 is expressed in high abundance in CD4+ and CD8+ T cells, moreover as in a very larger precursor super molecule in some vegetative cell cells. In CD4+ T cells, substance or agent has been shown to stimulate the interpretation of the pro-IL-16 super molecule, that is then cleaved by active proteolytic enzyme three to the active IL-16 kind. The pro-IL-16 molecule is translated and positioned within the perinuclear space of the protoplasm in lymphocytes. In response to stimulation, proteolytic enzyme three cleaves this molecule cathartic the C-terminal portion (including a PDZ domain) as active IL-16. The N-terminal portion includes a nuclear translocation signal that directs it into the nucleus wherever it afterward induces arrest of cell cycle in G0/G1. There seems to exist some unknown mechanism to forestall localization of the pro-IL-16 molecule to the nucleus before cleavage therefore preventing premature cell cycle arrest. This maybe is expounded to the PDZ domain within the C-terminal portion or presumably associated with the dimensions of the pro-molecule preventing localization to the nucleus. Induction of IL-16 changes communication among the cell and seems to own antiviral mechanisms that area unit each intra- and animate thing [12,23-25].

Located in each the protoplasm and nucleus of T cells as a pro-molecule, IL-16 has several functions. within the protoplasm, Pro-IL-16 is cleaved by Caspase-3 and therefore the activated IL-16 includes a chemoattractant property for preponderantly Th1 T cells (having binding properties to CD4 receptors and triggering secretion of IL-2). additionally, IL-16 has been found to safeguard CD4+ T cells from activation-induced necrobiosis. IL-16 has multiple restrictive properties on mast cells and basophils as well as creating them less vulnerable to M/R5-tropic strains of HIV-1. The protein additionally has repressing properties of Th2 T cells, direct repressing properties to HIV, and therefore the nuclear cleaved product of pro-IL-16 induces cell cycle arrest, as mentioned on top. These activities might by the rationale why IL-16 levels are shown to be cut in progression to AIDS because the infectious agent levels increase however- any studies have incontestable elevation of IL-16 levels in correlation with anti-protease activity (protease matter therapy) and a few HIV-1 long-run nonprogress or patients. in contrast to antiviral medical aid, IL-16 levels are discovered to stay grossly unchanged when structured interruption of therapy. it's doable that HIV should inhibit IL-16 to propagate, to ultimately cause AIDS.

IL-16 has been shown to extend within the initial stages of HIV-1 infection and will represent Associate in Nursing adequate response to inhibit the virus. At later stages, because the infectious agent load will increase, IL-16 levels sharply decline. However, some long-run non-progressors (without symptoms or detectable viremia) fail to point out a rise in IL-16 levels, as they will lack the initial information for IL-16 levels to extend, presumably infectious agent load [18].

IL-16, as antecedently mentioned, has properties of a CD4 substance, inhibiting HIV-1 replication and entry into CD4+ T cells, monocyte derived macrophages, and nerve fiber cells. IL-16 is additionally a chemoattractant for T cells, monocytes and eosinophils. In parallel to its result on IL-16, HIV-1 infection causes a decrease in secretion of Th1 cytokines like IL-2, IFN-gamma, however causes a rise in secretion of Th2 cytokines like IL-4, IL-10, and inflammatory cytokines like TNF-alpha, IL-1, IL-6, IL-8. As a result, cytotoxic T cells don't get activated, as would usually be seen {in a|during a|in Associate in Nursing exceedingly|in a very} Th1-mediated response to a living thing virus. HIV-1 causes this inexplicable infectious agent response cytokines whereas limiting the effectiveness of cell-mediated immunity.

Of additional interest is that the ability of IL-16 to boost the IL-2 receptor alpha and HLA-DR expression, further as suppress HIV-1 promoter activity in CD4+ T cells. humor levels of IL-16 ab initio stay traditional or slightly inflated in well patients living with HIV, however humor levels of IL-16 then undergo vital depletion as HIV infection progresses. IL-2 treatment for 2 weeks has been shown to dramatically increase the amount of IL-16 in patients living with HIV infection [21]. IL-16 stimulates CD4+ T cells to reply to IL-2 by up-regulating expression of the IL-2 receptor, CD25. Co-treatment of PBMCs with IL-16 and IL-2 expands the CD4+ T cell population [5].

## CONCLUSION

In conclusion, exposure of CD4+ T cells to HIV particle Lysate was found to induce IL-16 unleash - what is more, a super molecule part of the lysate was found to be the active part that elicited this protein unleash. though IL-16 levels are found to be elevated in early stages of HIV-1 in vivo infection, IL-16 levels decrease as infectious agent load will increase. Induction of IL-16, a familiar HIV-1 substance, seems to run contrary to sustained HIV-1 infection. this might indicate that HIV-1 includes a mechanism of block sustained IL-16 unleash to avoid the repressive result of upper levels of the protein. distinctive this unknown super molecule further as discovering the mechanism by that HIV-1 evades the IL-16 response is crucial. additional studies searching for this mechanism is of preponderating, further as exploration of administration of IL-16 as a therapeutic approach to inhibition of HIV-1 in patients. to boot, understanding the mechanisms and patterns of protein dysregulation might cause targeted therapies that embrace administration or inhibition of bound cytokines to maximize the traditional response to HIV-1. Also, previous analysis has shown progressive destruction of CD4+ T cells throughout HIV infection, causes loss of associated cytokines, however the extent of overall protein dysregulation is unclear and warrants additional investigation. As additional analysis is additionally trying into victimization therapy and therapeutic vaccines to combat HIV, a mixture approach with targeted protein modification might be useful as a multi-prong treatment. Examination HIV-caused protein dysregulation to the response in alternative infectious agent infections, like dysregulation by West river Virus, might hold a clue to augmenting cell-mediated immunity to HIV-1.

## CONFLICT OF INTEREST

None.

## REFERENCES

1. Selimova LM, Kalnina LB, Serebrovskaya LV, Ivanova LA, Gulyaeva AN, Nosik DN. [CYTOKINES DURING THE HUMAN IMMUNODEFICIENCY VIRUS INFECTION TYPE 1(HIV-1)]. Vopr Virusol. 2016;61(1):39-41.
2. Alfano M, Poli G. Cytokine and chemokine based control of HIV infection and replication. Curr Pharm Des. 2001;7(11):993-1013.
3. French MA, Cozzi-Lepri A, Arduino RC, Johnson M, Achhra AC, Landay A, et al. Plasma levels of cytokines and chemokines and the risk of mortality in HIV-infected individuals: a case-control analysis nested in a large clinical trial. AIDS. 2015;29(7):847-851.
4. Snell LM, Osokine I, Yamada DH, De la Fuente JR, Elsaesser HJ, Brooks DG. Overcoming CD4 Th1 Cell Fate Restrictions to Sustain Antiviral CD8 T Cells and Control Persistent Virus Infection. Cell Rep. 2016;16(12):3286-3296.
5. Kornfeld H, Cruikshank WW. Prospects for IL-16 in the treatment of AIDS. Expert Opin Biol Ther. 2001;1(3):425-432.
6. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. Microbiol Rev. 1992;56(1):152-179.
7. Scala E, D'Offizi G, Rosso R, Turriziani O, Ferrara R, Mazzone AM, et al. C-C chemokines, IL-16, and soluble antiviral factor activity are increased in cloned T cells from subjects with long-term nonprogressive HIV infection. J Immunol. 1997;158(9):4485-4492.
8. Amiel C, Darcissac E, Truong MJ, Truong MJ, Truong MJ, Mouton Y, et al. Interleukin-16 (IL-16) inhibits human immunodeficiency virus replication in cells from infected subjects, and serum IL-16 levels drop with disease progression. J Infect Dis. 1999;179(1):83-91.
9. Zhou P, Goldstein S, Devadas K, Tewari D, Notkins AL. Human CD4+ cells transfected with IL-16 cDNA are resistant to HIV-1 infection: inhibition of mRNA expression. Nat Med. 1997;3(6):659-664.
10. Meyaard L, Hovenkamp E, Keet IP, Hooibrink B, de Jong IH, Otto SA, et al. Single cell analysis of IL-4 and IFN-gamma production by T cells from HIV-infected individuals: decreased IFN-gamma in the presence of preserved IL-4 production. J Immunol. 1996;157(6):2712-2718.
11. Zhang Y, Center DM, Wu DM, Cruikshank WW, Yuan J, Andrews DW, et al. Processing and activation of pro-interleukin-16 by caspase-3. J Biol Chem. 1998;273(2):1144-1149.
12. Zhang Y, Kornfeld H, Cruikshank WW, Kim S, Reardon CC, Center DM. Nuclear translocation of the N-terminal prodomain of interleukin-16. J Biol Chem. 2001;276(2):1299-1303.
13. Wood N, Bhattacharya T, Keele BF, Giorgi E, Liu M, Gaschen B, et al. HIV evolution in early infection: selection pressures, patterns of insertion and deletion, and the impact of APOBEC. PLoS Pathog. 2009;5(5):e1000414.
14. McMichael AJ, Rowland-Jones SL. Cellular immune responses to HIV. Nature. 2001;410(6831):980-987.
15. Cruikshank WW, Kornfeld H, Center DM. Interleukin-16. J Leukoc Biol. 2000;67(6):757-66.
16. Baier M, Werner A, Bannert N, Metzner K, Kurth R. HIV suppression by interleukin-16. Nature. 1995;378(6557):563.
17. Baier M, Kurth R. Interleukin-16 for the gene therapy of HIV infection. Expert Opin Investig Drugs. 1997 ;6(12):1879-1881.
18. Bader A, Brockmeyer N, Schnaitmann E, Mertins L, Otteken A, Kurth R, et al. Interleukin-16 serum levels during the course of HIV-1 infection. AIDS. 2001;15(4):528-529.
19. Baker J, Ayenew W, Quick H, Hullsiek KH, Tracy R, Henry K, et al. High-density lipoprotein particles and markers of inflammation and thrombotic activity in patients with untreated HIV infection. J Infect Dis. 2010;201(2):285-292.
20. Regis EG, Barreto-de-Souza V, Morgado MG, Bozza MT, Leng L, Bucala R, et al. Elevated levels of macrophage migration inhibitory factor (MIF) in the plasma of HIV-1-infected patients and in HIV-1-infected cell cultures: a relevant role on viral replication. Virology. 2010;399(1):31-38.
21. Kedzierska K, Crowe SM. Cytokines and HIV-1: interactions and clinical implications. Antivir Chem Chemother. 2001;12(3):133-150.
22. Dufour JH, Dziejman M, Liu MT, Leung JH, Lane TE, Luster AD. IFN-gamma-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. J Immunol. 2002;168(7):3195-3204.
23. Mashikian MV, Ryan TC, Seman A, Brazer W, Center DM, Cruikshank WW. Reciprocal desensitization of CCR5 and CD4 is mediated by IL-16 and macrophage-inflammatory protein-1 beta, respectively. J Immunol. 1999;163(6):3123-3130.
24. Zhou P, Devadas K, Tewari D, Jegorow A, Notkins AL. Processing, secretion, and anti-HIV-1 activity of IL-16 with or without a signal peptide in CD4+ T cells. J Immunol. 1999;163(2):906-912.
25. Skundric DS, Cai J, Cruikshank WW, Gveric D. Production of IL-16 correlates with CD4+ Th1 inflammation and phosphorylation of axonal cytoskeleton in multiple sclerosis lesions. J Neuroinflammation. 2006;3:13.