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Chemokine Deregulation in HIV Infection: Role of Interferon Gamma Induced Th1-Chemokine Signaling

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Abstract

One of the hallmarks of AIDS is the progressive decline in CD4+ T cells in peripheral blood of HIV-infected individuals. This review focuses on how HIV-1 modulates inflammatory molecules, especially C-X-C chemokine ligand 10 (CXCL10), a Th1-chemoattractant chemokine to establish infection. HIV-infected T-cells, lead by Th1-agonist chemokines, gain access to the LNs where they die upon activation. The resultant T-cell death positively correlates with severe immunodeficiency and deteriorating HIV-1-infected patient's health. CXCL10 is produced by wide range of cells (macrophages, neutrophils, endothelial cells, and astrocytes) in response to inflammation and attracts T-lymphocytes and NK cells to the site of infection. Increased level of CXCL10 is found in body fluids (Serum, and cerebrospinal fluid) of HIV-1 infected subjects and it correlates with disease severity. Furthermore, HIV-induced high levels of Th1 chemokines are accounted for failed taxing of effector T cells from lymphoid organs to the site of infection. Thus, the ability of effector T cells to combat viral infection comes to halt, failing 'adaptive immunity'. CXCL10 is considered a positive indicator of the onset of HIV-associated neurocognitive disease in HIV-1 infected individuals. This review summarizes the current understanding of CXCL10 impact on development of central nervous system (CNS) abnormality. We hypothesize that drugs targeting chemotaxis of immune cells into brain might prove useful in the treatment of HIV-associated neurocognitive disorders (HAND). Further research in deciphering the role of chemotaxis will prove useful for better understanding of HIV pathogenesis both in periphery and brain.

Keywords: HIV-1; Chemokine signaling; CXCL10; IP-10; CXCR3; Interferon gamma; Inflammation; Gene regulation; Neuronal dysfunction; miRNA

Abbreviations: AIDS: Acquired Immunodeficiency Syndrome; HIV-1: Human Immunodeficiency Virus type-1; CXCL10: C-X-C Chemokine Ligand 10; CXCR3: C-X-C Chemokine Receptor 3; CSF: Cerebrospinal Fluid; CNS: Central Nervous System; NK cells: Natural Killer cells; HAND: HIV- Associated Neurocognitive Disorders; IFN-γ: Interferon gamma; MAPKs: Mitogen Activated Protein Kinases; ISRE: IFN-Stimulated Response Element; TRAF2: TNF-α Receptor Associated Factor-2; STAT-1, Signal Transducer and Activator of Transcription; ERK: Extracellular Signal-Regulated Kinases; JNK: c-Jun N-terminal Kinase; PI3K: Phosphoinositide 3 Kinase; LNs: Lymph Nodes; PDGF: Platelet Derived Growth Factor; pDCs: plasmacytoid Dendritic Cells; HCV: Hepatitis C Virus; LPS: Lipopolysaccharide; TLR: Toll Like Receptor

Introduction

Massive $CD4^+$ T-cells depletion in the peripheral blood of HIV-infected subjects is attributed for the immunodeficiency and AIDS. Both 'direct viral toxicity' and 'aberrant immune activation' are considered a root cause of apoptosis in $CD4^+$ T cells. A group of patients termed 'long term non-progressors' support the latter notion that host immune system play an important role in maintaining 'disease-free' status of HIV-infected individuals. These patients harbor HIV, but are able to resist T-cell depletion and ensuing disease pathology. Number of studies focus on understanding 'host-immune factors' that protect $CD4^+$ T-cells. Therefore, it is extremely important to understand that how does T-cell depletion occurs during HIV infection? This could be because of following: (1) Low production of $CD4^+$ T-cells in bone marrow, (2) increased degradation of $CD4^+$ T cells in periphery and lymphoid organs, and (3) increased trafficking of T cells to the lymphoid organs [1]. Published reports favor the latter two reasons and suggest an important role of host chemokine's in regulating HIV pathogenesis. Macrophages and Dendritic cells are among first antigen presenting cells (APCs) that respond to chemotactic signals and reach to the site of infection. Ability of macrophages to resist HIV-mediated apoptosis and migrate to lymphoid organs makes them excellent vehicles to spread infection. HIV-infected macrophages produce chemo-attractants for T-cells and NK cells which are activated and express CXCR3+ receptors. As a result, Th1 cells migrate towards lymph nodes (LNs) in response to Th1 chemokines. In HIV-infected patients, persistent migration of CXCR3+ cells result in their accumulation into the LN follicles and associate with the disease status. Once in the LNs, cells expressing CXCR3+ receptors are activated via inflammatory signals. Priming via type-1 interferons and IL-12 promote differentiation into effector Th1 cells. Priming environment depends on route of infection, viral dose and organ or cell types targeted. The newly differentiated Th1 cells (effector cells) produce a large amount of IFN-γ, which on one hand activate macrophages to produce chemokine, and on the other hand it suppress IL4 production (Th2 response). Upon Th1 cell interaction with macrophage, macrophages produce IFN-γ in self-amplifying loop and release CXCL10. Thus, CXCR3+ Th1 cells suppress Th2 cells (CCR3+) and maintain homeostasis between Th1 and Th2 cells. The

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resultant chemokine dysregulation influences the disease progression to AIDS in HIV-infected individuals [2-4].

Chemokines constitute a group of low-molecular weight secretory-proteins (~8-10 kDa) that regulate immune activation, leukocyte migration, and inflammation. Based on the arrangement of cysteine residues that form disulfide bonds, chemokines are classified into four families: CXC, CC, CX_3C, and C ('X' = any amino acid). CXC (α-chemokines) and CC (β- chemokines) are the two widely studied chemokine families that have 20 to 40% homology [5,6]; members of α- and β-chemokine subfamilies share 25-70% intra-molecular sequence homology. Interferon gamma (IFN-γ) induces three CXC chemokines (CXCL-9, -10 and -11), which are strongly associated with Th1 mediated immune response. Chemokines are either homeostatic (constitutively produced and secreted), or produced by cells upon pathogenic infection. Inflammatory chemokines recruit specific leukocytes to the site of infection by binding to their cognate receptors on target leukocytes, which transduce intracellular signaling cascades in order to counteract pathogen. However, many pathogens have evolved to modulate the host cellular immune system for their advantage. HIV-1 infection is a classical example of how a pathogen can successfully control the chemokine network for its own benefits [2,7-13]. At the onset of infection, HIV-1 binds to CD4 and CXCR4 or CCR5 co-receptors for its entry into the immune cells. At the same time, it controls chemokines-induced signaling pathways, via STAT-1, and NF-κB, to facilitate its replication and immune evasion. During HIV-1 infection both pro- and anti-inflammatory chemokine molecules are differentially regulated in different cell types [7,8,14].

Chemokine ligand 10 (CXCL10) is a member of chemotactic and immunomodulatory cytokine family, that share sequence similarity with platelet basic proteins, platelet factor 4 and β-thromboglobulin [15]. CXCL10 was identified nearly three decades ago as an, 'interferon-gamma inducible protein-10 (IP-10)', an immediate early gene induced in response to interferon gamma (IFN-γ) [15,16]. CXCL10, in addition to chemoattractant, is also a potent proinflammatory cytokine released as Th1 cell response. Serum levels of CXCL10 are enhanced as a result of inflammatory immune responses against a pathogen. CXCL10 functions by binding to CXCR3 and activates GPCR pathway followed by MAPKs via activation of adenylate cyclase. The resulting biological effect include: (1) chemokine activity, (2) Actin cytoskeleton reorganization that enforces effective antiviral response, (3) cell migration, and (4) immunity and inflammation (Figure 2). Role of CXCL10 has been implicated in multiple viral infections, including Rhinovirus, HBV, HCV, Coxsackievirus, Dengue, and Respiratory syncytial virus [17-27]. Immune cells including leukocytes, neutrophils, monocytes, macrophages, microglial cells (resident macrophages in CNS) and astrocytes, secrete CXCL10 in response to inflammation. Non-immune cells such as endothelial, stromal, fibroblasts and particularly epithelial cells also secrete CXCL10 [15,17,28,29]. Thus, CXCL10 guides specific effector cells including T-lymphocytes, NK cells, monocytes, and mast cells at the site of infection/inflammation and evokes an antiviral response [25,30]. It is remains to be studied if CXCL10 expressed by non-immune cells differ in its functional activity relative to immune cell derived CXCL10.

Molecular Structure of CXCL10

Structurally-related 'C-X-C chemokines' are divided into two classes based on the presence of glutamate-leucine-arginine (ELR) motif in the N-terminus region. Member of this family CXCL-9, -10, and -11 are produced in response to IFN-γ and they share the common receptor, CXCR3. CXCL10 belongs in a class devoid of ELR motif that renders its chemoattractant property for lymphocytes (T and B cells) [31]. The gene encoding CXCL10 is localized on chromosome 4, clustered in the region q21 [32], and comprises four exons and three introns [33,34] (Figure 1). CXCL10 promoter contains conserved regulatory motifs including IFN-stimulated response element (ISRE)

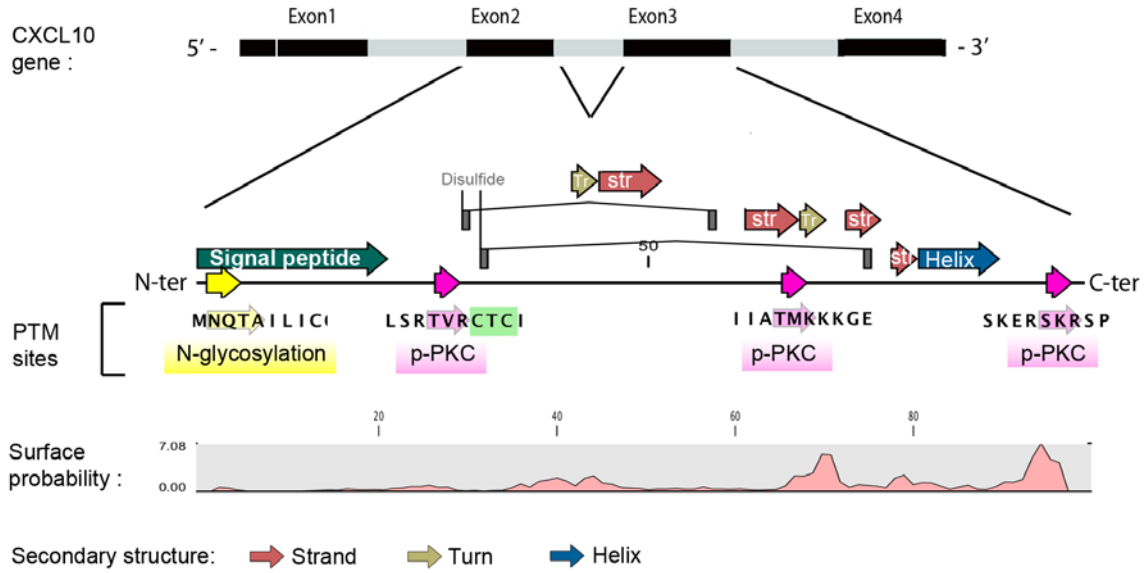

Figure 1: Schematic diagram of CXCL10 gene and protein. The gene encoding CXCL10 (top) contain four exons. Exons 1 and 4 are un-translated regions; exon 2 and 3 encode CXCL10 protein. 98-amino-acid long precursor protein containing signal peptide of 21 amino acids is cleaved leaving behind 77 amino acids mature protein. Posttranslational modifications (PTM) are highlighted. Three domains with putative 'Protein kinase C (PKC)' phosphorylation site are highlighted with pink arrows: '27TVR29, 65TMK67, 94SKR96' (gene information adapted form NCBI protein database accession # P02778). Predicted high surface probability suggests the likely regions of CXCL10 outside in secondary or tertiary structure.

and NF-κB/ RelA-p65 binding sequences. The spliced gene encodes 98-amino-acid precursor protein with an N-terminal 21 amino acid signal peptide leaving behind 77 amino acids long protein [16]. Further maturation of CXCL10 requires cleavage of four residues (KRSP) at the c-terminus by furin (between R75-S76) and carbopeptidase (between K74-R75) [35]. CXCL10 protein consists of a turn of 3-10 helix at the N-terminus, followed by three antiparallel β-strands packed against a C-terminal helix as revealed by NMR analysis [36]. Three dimensional crystal structure of CXCL10 exhibits a conventional β-sheet dimers with distinct tetrameric assembly thought to be promoted by binding of glycosaminoglycans [37].

Presence of R-C-X-C motif (Arg-Cys-Thr-Cys), in CXCL10 at 67-70 amino acids position is a general requirement for binding to CXC receptors. Cysteine residues in 'CTC domain' take part in the formation of two disulfide bridges between β1-β2 and β2-β3 strands. The resulting loop confirmation imparts specificity of binding to their receptors. In addition, N-terminus, GPH motif (Gly-Pro-His), N-loop, 30s-loop, and residues next to disulfides and in the α-helix are functionally important for receptor binding specificity. Loops between β1-β2 and β2-β3 strands may be involved in glycosaminoglycan binding/activation mechanisms. Compared to β- chemokines (e.g. eotaxin), CXCL10 structure shows unusual distortion of the second β-strand that is thought to impart CXCL10's ability to bind CCR3 [36]. N-terminus domain also contains a putative N-glycosylation site- '2NQTA5'. CXCL10 protein contains three putative 'Protein kinase C (PKC)' phosphorylation sites (Figure 1). Although, it is not known if these modifications help in better secretion or they modify CXCL10 functions.

Induction of CXCL10 and its Receptor CXCR3 by HIV-1

HIV enhances CXCL10 expression by both IFN-γ dependent and independent mechanisms. HIV-1 accessory proteins, gp120, Nef, and Tat, have been known to stimulate CXCL10 production and succeeding inflammation [10,13,38]. Nef transgenic mice develop an AIDS-like disease [39]. Nef induces inflammatory signaling involving transcirption factors- NF-κB, MAPKs, and IRF-3 [40]. Nef binds directly to TNF-α receptor associated factor-2 (TRAF2) and phosphorylate both subunits of STAT-1 alpha and beta. TRAF6 also appear to be involved indirectly in inducing inflammatory response by Nef [41]. Macrophages either treated with recombinant Nef protein or infected with *nef* expressing virus activate STAT-1 alpha and beta. Tat and gp120 induce CXCL10 expression by ERK1/2 pathway [8,38,42]. Tat activates CXCL10 production in IFN-γ- and TNF-α-treated human astrocytes via activation of NADPH oxidase. Respiratory outburst following activation of NADPH oxidase, and activation of kinase pathway (via p38, ERK1/2, JNK, and Akt) results in overall induction of CXCL10 [10,43]. HIV-1 gp120 directly induces CXCL10 expression via STAT1 [7,38]. STAT1 activation and functional impairment of monocytic cells relates to chronic immune activation in HIV-1 infected patients [44]. Majority of studies rely upon the use of recombinant HIV proteins to show induction of CXCL10. However, their relevance to the clinical setting remains to be determined. Nevertheless, the ability of multiple HIV proteins to induce CXCL10 expression results in localized infiltration of Th1 cells and affects cell migration to LNs. The resultant increase in inflammation directly enhances viral transcription and replication.

At the cellular level, multiple cytokines synergize inflammatory response in target cells by promoting CXCL10 secretion. Macrophage derived inflammatory cytokines including TNF-α, IFN-γ, and platelet derived growth factor (PDGF)-B chain are the classical examples, which induce CXCL10 production in infiltrating macrophages by multiple signaling pathways [12,45]. Notably, combination of IFN-γ and TNF-α promote CXCL10 production via p38, Akt, JNK and downstream transcription factors JAK2/STAT-1α and NF-κB activation [46-48] (Figure 2). CXCL10 production is enhanced by IFN-γ, while TNF-α mainly promotes migration of CXCR3 expressing cells to the site of inflammation. However, TNF-α and IFN-γ do not confer synergistic effect on transcription factors binding to the CXCL10 promoter, rather they recruit CREB binding protein (CBP) to CXCL10 promoter, accompanied by higher expression of RNA polymerase II [46].

CXCL10 specifically binds with high affinity to its receptor, CXCR3 (a G-protein coupled, seven-transmembrane receptor) and transduces signals into intracellular compartment via trimeric G protein cytoplasmic tail. This results in activation of multiple intracellular signals effecting: cytoskeletal reorganization, integrin activation along with chemotactic migration, leukocyte recruitment, and inflammation [49]. Cytoplasmic changes brought by signaling events facilitate HIV-1 infection and disease progression either directly and/or indirectly. For instance, activation of CXCL10-induced transcription factors via MAPK pathway results in enhanced HIV-1 transcription [50]. Furthermore, integrin activation and effector leukocyte recruitment result in viral spread by providing new host cells for infection [51]. HIV-1 viral entry and viral replication result in IFN production, which leads to CXCL10 and enhanced CXCR3 receptor expression on peripheral blood lymphocytes [51]. Thus, CXCL10 further stimulate HIV-1 replication in HIV-1 infected lymphocytes. Blocking of CXCR3 can be effective in preventing viral replication [52]. In response to HIV mediated inflammation, target cells such as pDCs and activated T-lymphocytes exhibit increased CXCR3 surface expression. As a result, migration of targets cells towards the CXCL10 concentration gradient directs them towards distant lymph nodes and further enhances viral dissemination [51,53-55].

A wide range of cells both in periphery and brain (endothelial, macrophages, T cells, astrocytes, microglia, oligodendrocytes, pDCs) exhibits CXCR3 receptors in response to inflammation. These cells either directly or indirectly involved in HIV-1 infection and pathology. Alternate splicing in these cells generates CXCR3 variants (differing in their Gα regulatory subunit): CXCR3-A, -B, and –alt [56,57]. Binding of CXCL10 to CXCR3-A or -B stimulates antagonistic signaling pathways. The relative expression of these variant determines the phenotypic effect on cell proliferation and migration. Activation of CXCR3A receptor increases intracellular calcium and inflammation via activation of ERK1/2, p38/MAPK, JNK, and PI3K/Akt [57-59]. In contrast, activation of CXCR3-B stimulates cell proliferation and migration [57,60,61]. CXCR3A is the most common variant while CXCR3-alt is co-expressed with CXCR3-A in low levels [57,62,63]. CXCR3A was induced selectively in brain of HAD patients, while no distinction was found in expression of CXCR3B in HIV-infected normal patients' brain compared to HIV-infected demented patients' brain [64]. Further, less information is available about how HIV-1 modulates transcription of these variants in other HIV-infected target cells.

To achieve robust phenotypic effects, distinct chemokine receptors work in conjunction with each other [55]. When simultaneously engaged with CXCR4-induced signaling, CXCL10- CXCR3 mediate signaling potentiates an effective pDC migration [65,66]. CXCR3 is

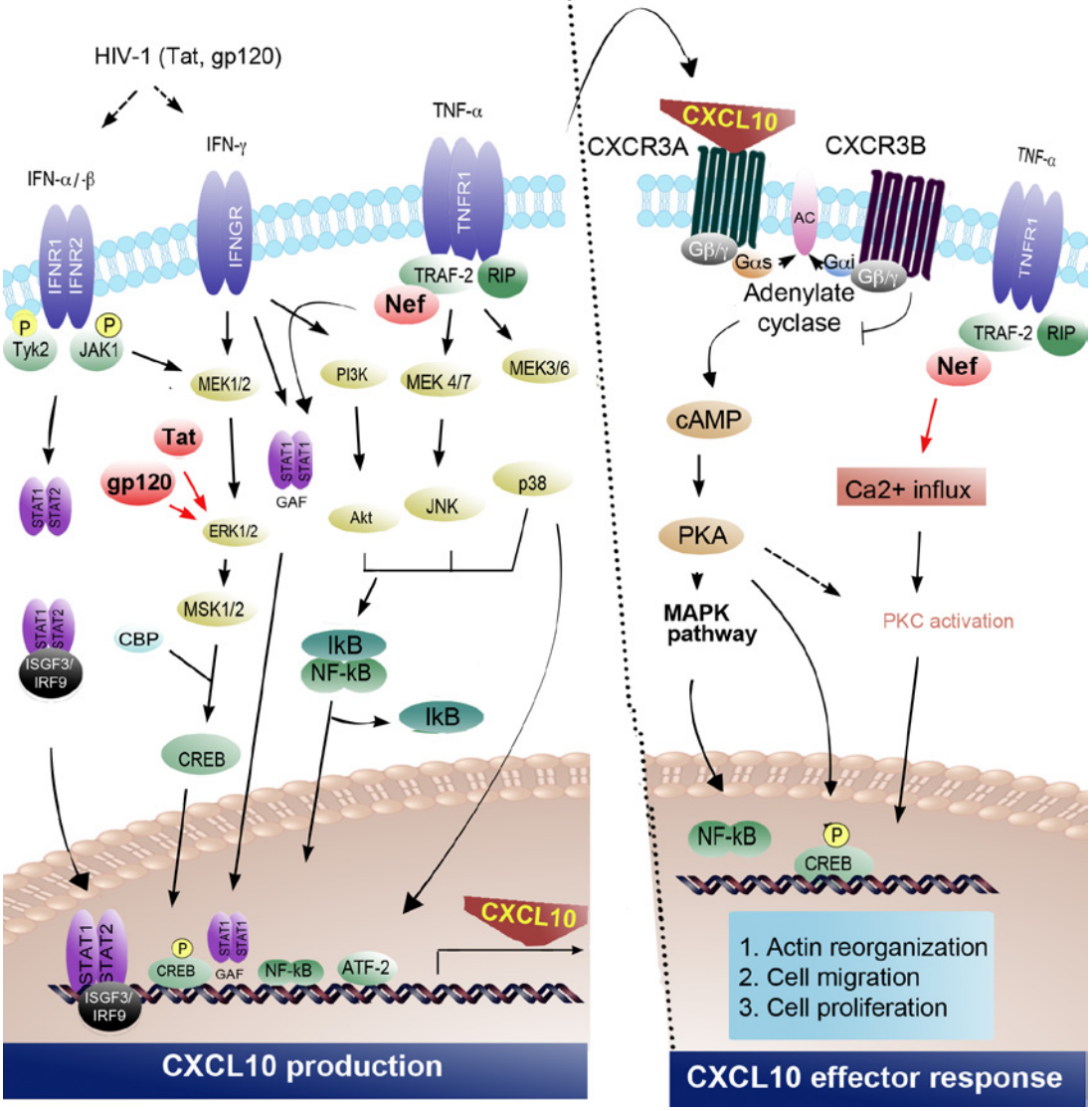

Figure 2: Intracellular CXCL10-CXCR3 signaling events in HIV-1 infected cells. Left: HIV infection result in enhanced CXCL10 production. Tat and gp120 directly and/or indirectly induce CXCL10 production. Right: CXCL10 acts on effector cells. The cytoplasmic tail of CXCR3 receptor contains trimeric G protein subunits: Gα constitute regulatory subunit; Gβ and Gγ constitute catalytic subunits. CXCR3A variants cytoplasmic tail contains Gαi, an adenylate cyclase inhibitory subunit. This increases intracellular calcium level and inflammation via activation of ERK1/2, p38/MAPK, JNK, and PI3K/Akt. Activation of CXCR3B Gαs, adenylate cyclase stimulatory subunit, induces cell proliferation and migration.

expressed on $CD4^+$ Th1 cells (CXCR3/CCR5 positive) and functions as a potential receptor for other two structurally homologous chemokines: CXCL9 and CXCL11 that may enhance Th1 cell recruitment in HIV-1 infected sites. During HIV infection immune activated T cells and natural killer (NK) cells secrete IFN-γ that in turn induces CXCL9, -10 and -11 and result in robust Th1 response.

Effect of CXCL10 and Downstream Events on HIV-1 Pathogenesis

CXCL10 signaling is involved in establishment of HIV-1 infection at the very onset of the virus encounter. In case of sexual transmission, interaction of HIV-1 envelope with the cervical epithelium triggers production of CXCL10. Therefore, the virus encounters newly recruited CXCR3 positive activated immune cells in intraepithelial layer and establishes new round of infection. Activated Th1–effector response leads to production of IFN-γ that activates macrophages to produce chemokines CXCL-9, -10, and -11. In response to inflammation, expression of CXCR3+ receptor is upregulated on activated lymphocytes and monocytes that migrate to LNs. Upon infection-induced inflammation, naïve T cells traffic to lymphoid tissues guided by CCL19 and CCL21 by binding to their receptor CCR7. On the other hand, effector $CD8^+$ CTLs exits lymphoid tissues in response to CXCL10 to the site of infection/inflammation. This however does not happen during HIV infection. HIV directly targets Naïve T cells and replicate in lymphoid organs. Production of HIV proteins thus promotes CXCL10 and restricts CXCR3+ effector T cells to the lymphoid organs. Therefore, chemokine deregulation limit the effector T cells trafficking that helps to establish HIV infection. Cells producing CXCL10 were moderately associated with the viral load in periphery, while strongly associated in lymph nodes [67]. Further, during HIV-1

infection production of CXC chemokines suppresses Th2 chemokine proliferation resulting in Th cell polarization in the LNs.

CXCL10-CXCR3 interaction induces both apoptotic, and survival signaling in T-lymphocytes through p38/MAPK, and PI3K/Akt activation pathways, respectively [68]. Dominance of signaling events appears to depend upon the stage of viral infection, CD4 T-cell count, viral load and subsequent concentration of the transcription factors that are deregulated during HIV-1 infection. CXCL10-CXCR3 mediated cytoplasmic signaling triggers effector functions like cell migration, and proliferation that are mediated through Ras/ERK, Src, and PI3K/Akt pathways (Figure 2) [69,70].

During HIV infection, multiple confounding factors correlate with CXCL10 production and accelerate disease pathology. Intestinal epithelial cells produce high levels of CXCL10 in HIV-1 co-infection with cryptosporidiosis [71]. HCV co-infection, owing to common blood borne transmission route, occurs in 25% of HIV-infected individuals, and associates with faster disease progression [72-76]. CXCL10 plasma level was proposed as biomarker for disease severity and prospective treatment in HIV-HCV co-infection [77,78]. Interestingly, levels of CXCL10 below 150 pg/ml in HIV-HCV co-infected patients are positive indicators of successful treatment against HCV [23,77]. Cells (macrophages or T) treated by a combination of Morphine and Tat dramatically enhance production of CXCL10 and other inflammatory cytokines [79]. Similarly, cocaine upregulates HIV-1 transcription in macrophages via NF-κB activation, and enhances expression of CXCL10. Thus, high serum levels of CXCL10 are indicative of overall severity of disease.

HIV-1-Mediated Brain Encephalopathy via CXCL10 Deregulation

Chemokines are instrumental in maintaining normal neurophysiology and brain development by engaging healthy glial cells in the brain [80,81]. CXCL10-CXCR3 signaling critically affects brain pathology in non-infectious and infectious diseases, and deserves special attention. CXCL10 dysregulation is found in multiple CNS diseases like Multiple Sclerosis (MS), ischemic infarcts, astrocytic neoplasmas, and HAND [82]. In fact, much of our understanding about CXCL10 signaling in brain came from other neurodegenerative diseases, including MS and Alzheimer's disease. Activated astrocytes/oligodendrocytes secrete high levels of CXCL10 in autocrine or paracrine manner in MS lesions [83-85] that coordinate with upregulation of CXCR3 surface expression [82,83]. This results in increased trafficking of myelin-laden macrophages in the CNS compartment that express high levels of CCR7 and CXCR3 and migrate towards CCL21 and CXCL10 [86]. The outcome of chemoattraction by CXCL10 varies in different infections. Interestingly, CXCL10 is elevated in cerebrospinal fluid during late stage of trypanosomiasis and considered as a serum biomarker for predicting mortality in cerebral malaria [87,88]. In contrast, CXCL10 helps clear viruses such as West Nile and Herpes simplex virus via recruitment of $CD8^+$ T cells in the brain [89-91].

Studies have shown that there is a correlation between CXCL10/CXCR3 expression and neurological dysfunction and progression of the HIV-1-induced CNS disease [9,11]. HIV- associated neurocognitive impairment is the result of neuronal damage and loss of neurites in the brain and correlates with the formation of multinucleated giant cells (MNGC), microglial nodules, and astrocytosis. In HAND, elevated level of CXCL10 was associated with senile plaques with coordinated up regulation of MIP-1β [86]. In HIV-induced encephalopathy, CXCL10 not only takes part in immune cell migration towards CNS compartment, but also directly enhance inflammation and neuronal damage. Neurons constitutively express low levels of CXCL10 in the absence of neuronal injury/stress. Upon injury, injured neurons secrete large amount of CXCL10 and trigger a chemotaxis of CXCR3+ target cells. Increase in CXCL10 level correlates with increased CXCR3 expression in activated glial cells (CD11b+ GFAP+) indicating homing of glial cells at the site of infection [92]. CXCR3 expression concomitantly increases in the brain of HIV-1 infected patients and associate with severity of HAND [93]. Among glial cells, microglia (resident macrophages in CNS) expresses high levels of CXCR3, while astrocytes produce moderate levels [94]. Microglia detects neuronal injury at early stage and starts migrating towards the zone of axonal degeneration in response to CXCL10 (produced by damaged neurons) [63].

Neuronal damage occurs as a result of either direct neuronal insult by HIV-1 or indirectly via chemokine deregulation in cells surrounding neurons. CXCL10 enhances ERK1/2 pathway in mouse cortical neurons [95], which is associated with proliferation in glioma cells [96]. However, sustained high level of localized CXCL10 production by migrated cells those surround neurons contributes to neuronal damage by multiple mechanisms. In microglia, MAPK cascade- MKK3, MKK6 and TGF-β activated kinase-1 (TAK1) stimulate promoter activity of CXCL10 gene [97]; whereas, astrocytes secrete CXCL10 in response to 'excitotoxicity' by NMDA [98]. CXCL10 production via JAK-STAT pathway recruits more lymphocytes, and generate inflammatory response in CNS of demented patients [99]. Once CXCL10 is produced from inflamed cells in brain, it acts on neurons expressing CXCR3 receptors [100,101]. CXCL10-CXCR3 signaling differentially affects NMDA-induced neuronal death in mouse hippocampus that affects specific regions of brain. Neuronal death was specifically increased in DG region in response to NMDA [102]. Binding of CXCL10 to CXCR3 receptors on neurons increase intracellular calcium and leads to an increase in neuronal activity (both spontaneous and evoked electrical activity; neuronal firing) [103]. CXCL10-CXCR3 signaling in neurons cytoplasmic Ca^{2+} accumulation following release from endoplasmic reticulum [104]; it was associated with higher mitochondrial membrane permeabilization and cytochrome C release form mitochondria. Thus, CXCL10 results in neuronal apoptosis via cytochrome C-dependent activation of initiator caspase-9 and effector caspase-3 [104-106].

Regulation of CXCL10-CXCR3 Signaling Pathway

CXCL10 induction is regulated by multiple mechanisms. CXCL10 produced at the site of infection not only recruits effector T cells but also regulatory T cells ($CD4^+CD25^+Foxp3^+$). Increase in Treg derived anti-inflammatory cytokines (IL-10, IL-2), relay intracellular signals to suppress CXCL10, either directly, or indirectly via inhibition of TNF-α and IFN-γ [107]. Th2 derived cytokines, or those that promote differentiation of naïve T-cells into Th2 cells (e.g. IL4), also suppress CXCL10 production and reduce inflammation at the site of infection [108]. Maingat and coworkers found that chronic LPS-conditioning of CNS down-regulated CXCL10 expression in CNS via Th2 cytokine, IL-10. In their experiments, CXCL10-suppression resulted in neuroprotection against FIV (feline lentiviral model of HIV) by reduced leukocyte infiltration, neurotoxins [109]. At the molecular level, CXCL10 promoter contains binding site for different transcription factors- RelA-p65, JunD, NF-E2 p45, SRF, Sp1, NF-YA, -YB, E2F, STAT1 and RNA pol-II. Transcription factors including BCL6, ErbB1 suppress CXCL10 expression. Although, limited studies

have been done to study transcription factors regulating CXCL10 production and how upstream factors regulate these transcription factors. When CXCL10 is synthesized, functional form of native CXCL10 is secreted only after adequate post-translational processing in the cytoplasm. CXCL10 contains putative domains for posttranslational modifications: an N-terminus glycosylation domain at 2-5 amino acids (Glycosaminoglycan binding site), three phosphorylation, and one citrullination site. Peptidases released from cells are thought to alter structure and function of multiple cytokines. Loos and coworkers encountered a modified CXCL10 secreted in synovial fluid from arthritis patients [110], however, similar modifications have not been reported in HIV infection. Deamination by peptidylarginine deaminase modifies R5 position of CXCL10 to citrullin resulting in dramatic reduction in CXCL10 induced chemotaxis without affecting CXCL10-CXCR3 binding affinity [110]. Neurtophil collagenase (MMP8) degrade CXCL9 and cleaves CXCL10 at 2 positions while gelatinase (MMP9) degrade CXCL10 and cleaves CXCL9 at 3 positions. Interestingly, CXCL9 and CXCL10 were found in inverse correlation and thought to maintain homeostasis in both periphery and LNs [67].

Regulation of CXCL10-downstream effects is also modified by other cytokines directed towards CXCL10-target receptor- 'CXCR3'. Cytokines may regulate CXCL10 activity by internalizing its receptor, CXCR3, such that it is not available for interaction. For instance, Brain derived neurotrophic factor (BDNF) is suggested to induce internalization of CXCR3 receptors [111]. Cleavage product of CXCL12 (5-67 amino acids) engages and activates CXCR3 receptors, and induces neuro-toxicity by suppression of neuronal autophagy pathway via CXCR3 [64]. CXCR-3A and -3B alternate splice variants may also be involved in differential signaling.

Recently, microRNA, a non-coding, 21-24 nucleotides, regulatory RNA species has received attention in regulating translation. MicroRNAs bind to the 3'-untranslated region (3'-UTR) of target mRNAs and suppress gene expression by 'post transcriptional gene silencing (PTGS)'. Based on full or partial sequence complementarity, they either degrade the target mRNA or suppress mRNA translation respectively (reviewed in [112,113]). A handful of studies implicates miRNAs in regulation of CXCL10 signaling (Table 1). miR-15b and miR-155 are directly linked to CXCL10 signaling. miR-15b is a positive regulator of CXCL10 production [114]. miR-155 is present in monocytes, macrophages and dendritic cells [115], and targets over 25 genes affecting different inflammatory reactions acting as key players in innate and adaptive immune response. It is upregulated in mDCs matured by LPS and IFN-γ stimulus, and correlates with upregulation of Th1 chemokine profile (CXCL9, CXCL10, CXCL11, and CCL5) but not Treg attractants (CCL22 and CXCL12) and proposed as biomarker for mature DCs potency [116]. miR-155 was also found up regulated in dermal infiltrates of $CD4^+$, $CD8^+$ and $FOXP3^+$ cells in T cell mediated chronic inflammatory skin disorder [117]. It has also been implicated in multiple DNA viral infections [115]. It can be induced by bacterial LPS, IFN-β, poly inocinic:cytidylic acid, or TNF-α in monocytes and macrophages [115]. It is induced during Th1 activation and helps in Th1 cell differentiation by inhibiting IFN-γ signaling in CD4 T cells. Overexpression of TNFα and IFN-γ synergistically enhance miR155 expression [118], which further negatively regulates CXCL10 production.

Perspective

Great deal of information has been accumulated in the last three decades in understanding the functions of Th1-attracting chemokines. In HIV infection, deregulation of CXCL10-CXCR3 pathway contributes significantly to HIV-disease progression. Given the dynamic nature of CXCL10 levels distributed between periphery and lymph nodes. It is suggested that CXCL10 levels in vivo should measure both CXCL10 in periphery and other organs simultaneously for better interpretation. CXCL10 signaling plays a critical role in developing HIV-induced neurodegeneration. CXCL10 in brain functions as a chemoattractant for microglia, macrophages and lymphocytes to the site of infection. Albeit, astrocytes and neurons are not 'productively infected', HIV-1 RNA is found in the brain of HIV-demented individuals, and thought to primarily affect neurons. In addition, persistent activation of CXCL10-mediated signaling directly induces neuronal apoptosis. Transfection of synthetic double-stranded RNA into epithelial cells showed robust induction of CXCL10 via Toll-like receptor 3 (TLR3) signaling [27]. It is not known if TLR signaling is also important in stimulating CXCL10 in glial cells. Further, CXCL10 functions specifically in recruitment

	Target	microRNA	Comments	References
Direct interaction	CXCL10	miR-497	Regulate neuronal cell death through Bcl2 and cyclin D2	[129]
		miR-15a, 15b	Suppress CXCL10 expression by targeting CXCL10 3'-UTR	[114]
		miR-155	Regulate CXCL10 via TAB2 and NF-κB expression in response to TNF-α and IFN-γ Mimic inhibits TAB2 expression	[115]
		miR-424, miR-16, miR-195	Suppress CXCL10 expression by targeting CXCL10 3'-UTR	Target Scan
	CXCR3	miR-1254, miR-3116, miR-4739, miR-1321, miR-4756-5p, miR-4739, miR-473-5p, miR-4673, miR-4645-5p, miR-3154, miR-4725-3p, miR-4487, miR-4663, miR-4503	Regulate CXCL10 through its receptor CXCR3	Target Scan
Indirect interaction	NF-κB	miR-210, 125a, b	Induces proinflammatory cytokines production	[130,131]
	NF-κB, MEK-1/2, JNK-1/2	miR-146a, b	Regulate cytokine expression through MAPK	[132]

Table1: miRNAs involved in regulation of CXCL10-CXCR3 signaling.

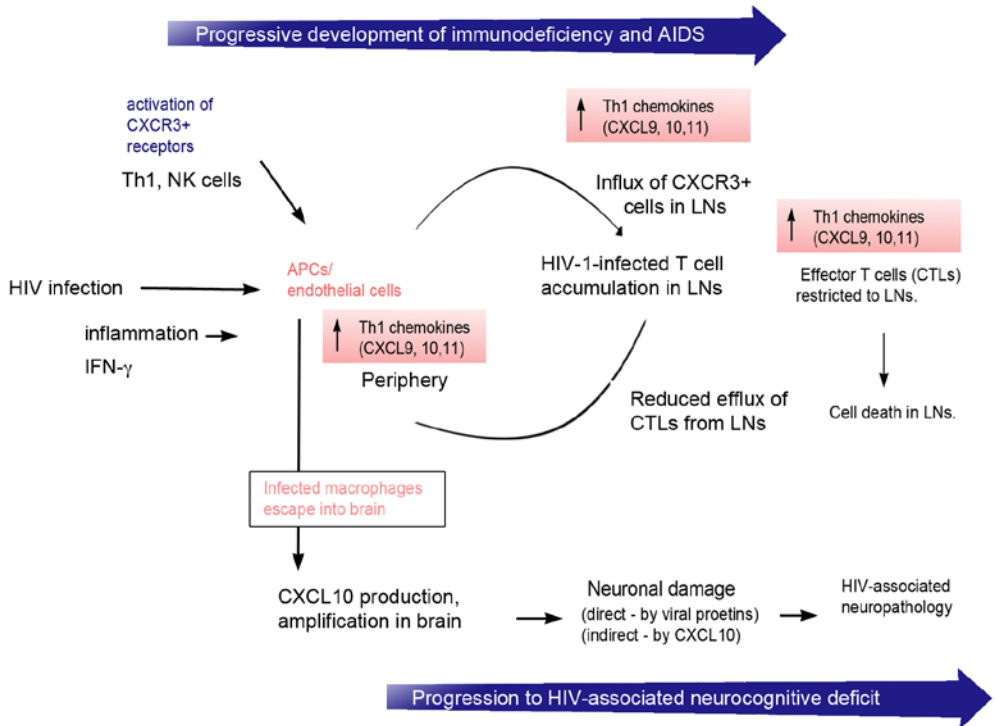

Figure 3: Effect of Th1-chemokines on HIV-1 pathogenesis. HIV-1 infected cells generate inflammatory cytokines. Activation of CXCL10 signaling and upregulation of CXCR3 receptors in target NK and T cells results in their recruitment at the site of infection. Further, HIV-1 infected APCs migrate to to LNs and present viral peptides to Th1 cells. CXCL10 signaling chemoattracts activated Th1-lymphocytes (CXCR3+) to LNs and concomitantly suppress Th2 cell migration in response to CCR3 ligand. Deregulation of CXCL10 signaling by HIV-1 can contribute to polarization of T-cell recruitment and immunodeficiency. CXCL10 induction in brain leads to neuronal damage and progression to HAND.

of effector T cells. Therefore, high level of CXCL10 in brain suggests effector T cell infiltration. However, little is known about role of effector T cells in relation to neurodegeneration.

Therapeutic strategies targeting the production of CXCL10 or enhancing anti- inflammatory cytokines production like IL-10 can prove valuable for HIV-1-associated inflammatory complications. Direct blocking of CXCL10 signaling will prevent homing in LNs and may have diverse outcomes. (1) It will impair clearance of HIV by immune system by preventing clearance of CXCR3+ T cells from periphery or, (2) Prevent severe inflammation and restoration of immune function, loss of which is observed during AIDS [67]. Treatment with decoy chemokine receptor plasmid DNA (encoding binding sites of CXCR3 and CCR2) in murine model suppressed the development of chronic relapsing–experimental autoimmune encephalitis (CR-EAE) [119]. Interestingly, anti-retroviral treatment with didanosine (ddI) and zidovudine prevents microglial activation and protects synaptic proteins in feline immunodeficiency virus (FIV) infected cats indicating that the effect may be because of the consequence of reduced systemic viral burden. Supplementation with anti-inflammatory drugs such as naturally occurring polyphenol compounds, may prove effective in treatment of HIV associated inflammatory complications. This could be either directly as anti-inflammatory effects, or indirectly by reducing cell surface co-receptors that blocks the inflammatory signals transducing across the cell membranes [8]. CXCR3 antagonists (e.g. TAK-779,) and cholesterol lowering drugs called 'Statins (Atovastatin, lovastatin and simvastatin, fluvastatin)', with anti-inflammatory effects, are effective in reducing CXCL10 levels in Crohn's disease, MS, and allergic asthma respectively [120-125]. Neutralizing antibodies against CXCR3 receptors may have implication as potential therapeutic against HIV-1 progression. Blocking of CXCL10 pathway showed suppression/attenuated inflammatory colitis, cerebral malaria and EAE (autoimmune encephalomyelitis) [126-128]. Passive transfer of neutralizing antibodies against CXCL10 reduced recruitment of inflammatory lymphocytes across the blood brain barrier [128].

Recent understanding of PTGS by miRNAs showed potential as therapeutic molecules to reduce chemotaxis and inflammation. Since miRNAs target multiple RNA molecules based on sequence homology, a tailor-made miRNA sequence targeting different steps of the inflammatory pathway remains to be achieved. Another idea is to use drug molecules that could target single or multiple miRNAs to achieve therapeutic effects. Chinese herbal medicine Genseng derivative suppresses CXCL10 expression via restoration of miR-15b levels in human endothelial cells against H9N2/G1 mediated apoptosis [114]. Although, it is an interesting concept, the utility of this in context to HIV-1 has not yet explored.

Given that multiple HIV proteins enhance CXCL10 production (eg. Extracellular Tat, Nef and gp120), the outburst of CXCL10 expression along with other inflammatory cytokines will increase localized Th1 cell presence and impede the retreat of these cells to LNs (Figure 3). The result is establishment, persistent infection and enhanced inflammation. In conclusion, targeting CXCL10-CXCR3 pathway may offer powerful approach to suppress inflammatory signaling cascades and ensuing HIV-1 associated neuropathogenesis as well as inflammation induced tissue damage in periphery. Therefore, monitoring CXCL10 levels can serve as a marker for decision-making point for either intensifying therapy or supplementing with anti-inflammatory drugs.

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