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The spreading of HIV-1 infection in the human organism is caused by fractalkine trafficking of the infected lymphocytes—a review, hypothesis and implications for treatment

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Abstract The reviews on HIV-1/AIDS [1–8] highlighted the mechanism by which HIV-1 virions utilize dendritic cells (DCs) for transport from the genitals, the portal of virus infection, to the draining lymph nodes where DCs carry HIV-1 virions and present viral antigens by HLA class I and II to $CD4^+$ T cells. Interaction of the T cells with viral antigens presented by HLA class II molecules polarizes them to become Th2 cells, the targets of HIV-1 infection and producers of HIV-1 progeny virions. The T cells which interact with viral antigen presented by HLA class I polarize to become Th1 cells, which stimulate the $CD8^+$ T cell precursors to develop into antiviral cytotoxic T cells. In addition, HIV-1 virions shed gp120 glycoprotein molecules which bind to IgE immunoglobulin molecules bound to FCεRI+ innate system cells (basophils, mast cells and monocytes) and induce them to release large amounts of Th2 cytokines (IL-4, IL-5, IL-10, IL-13), thereby creating an allergy-like condition. The present review attempts to define the role of chemokine receptors like CCR5 and CXCR4, and especially fractalkine receptor CX3CR1 in the trafficking of lymphocytes in healthy individuals and HIV-1/AIDS patients. The role of chemokine receptors as co-receptors for HIV-1 virion gp120 glycoprotein has been defined, but the role of fractalkine and fractalkine receptor has been clarified only recently [9–19]. In healthy individuals fractalkine is expressed by blood vessel endothelial cells and the CX3CR1 receptors are expressed on leukocytes that migrate in the peripheral blood in the direction of increased fractalkine concentration. In HIV-1/AIDS patients the virus-infected $CD4^+$ Th2 cells migrate to organs that harbor the adaptive immune system cells in the thymus, genitals, gastrointestinal tract, and to the brain. A most significant finding which revealed the importance of the human CX3CR1 gene expression to the progression of the infection to the stage of AIDS was recently reported by Faure and collaborators [20, 21] who showed that the delayed or rapid progression to AIDS was affected in HIV-1-infected individuals who had inherited a fractalkine receptor gene with the polymorphisms V249I or T280M, respectively, located in the sixth and seventh transmembrane domains of CX3CR1 protein. The T280M mutation in the CX3CR1 gene caused a rapid progression to AIDS, while in patients with the V249I mutation progression to AIDS was much slower. These studies led to the idea that it might be possible to slow or prevent HIV-1/AIDS progression in HIV-1 patients by treating them with fractalkine antagonists that will bind to and inhibit the activity of the fractalkine receptor. It is hypothesized that treatment of HIV-1/AIDS patients with a combination of fractalkine antagonists, IL-4 antagonist IL-4δ2 and the adjuvant CpG ODN induced release of type I IFN from PDF, and may inhibit HIV-1 infection, especially in HAART-treated patients infected with drug-resistant HIV-1 mutants due to prevention of the availability of immune cells needed for the viral evasion of the immune response. The hypothesis implies that the advantage of the suggested mode of treatment of HIV-1-infected people is prevention of cellular processes that are used by the viral protein to cause immunodeficiency, and prevention of HIV-1 replication without induction of resistant mutants.

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Introduction

Antiviral drugs to treat HIV-1/AIDS patients and prolong their lives have been developed, but attempts to develop vaccines to immunize and protect large human populations, with the hope of stopping the spread of pandemic HIV-1, have not yet been successful. While anti-HIV-1 drugs targeted against virus-coded enzymes (e.g. reverse transcriptase and protease) have been developed, and HIV-1 patients are treated with combinations of several anti-HIV-1 drugs (HAART) that reduce HIV-1 load in the blood, the effect of the antiviral drugs diminishes due to the gradual increase of multidrug-resistant HIV-1 mutants. Recently, the first anti-HIV-1 drug, Enfuvirtide, which inhibits the fusion of HIV-1 virion membrane with the $CD4^+$ Th2 cell membrane and reduces the HIV-1 load in the blood of patients by $1.96\log_{10}$ was approved by the USA Food and Drug Administration (FDA) for treatment of HIV-1 patients [22].

Most of the experimental vaccines that were tested in humans contained the HIV-1 gp120 glycoprotein and additional viral proteins as antigens. Unfortunately, these vaccines failed to induce a protective antiviral adaptive immune response in healthy individuals, and some of the vaccinated individuals were infected with HIV-1 during the vaccination period. Such negative findings indicated that the viral proteins in the vaccine, either the glycosylated gp120 or the additional viral proteins, are able to inhibit the antiviral adaptive immune response in the healthy vaccinees due to an increase in the level of Th2 cytokines and inhibition of the adaptive immune response.

In allergic individuals exogenous and endogenous allergens skew the normal Th1/Th2 cytokine balance toward a marked increase of Th2 cytokines (IL-4, IL-5, IL-10, IL-13) leading to a marked increase in the level of anti-allergen IgE in the patient's blood [2]. I therefore searched the literature for publications following the 1982–1983 discovery of HIV-1 that reported increased levels of Th2 cytokines and IgE, markers of allergy, in the blood of HIV-1/AIDS patients. Indeed, several research groups reported that increased IL-4 and IgE are biomarkers for HIV-1 infection. These reports, and additional evidence for the way HIV-1 causes AIDS, were reviewed in the first paper of my series on HIV-1/AIDS [3].

The properties of the viral gp120 glycoprotein as an allergen and its ability to skew the Th2 cytokines, and the role of IL-4 in the up-regulation of anti-allergen IgE levels in HIV-1/AIDS patients were also reviewed [4]. The reported studies on HIV-1 gp120 revealed that its binding to IgE/FcεRI$^+$ hematopoietic cells (mast cells, basophils and monocytes, cells of the innate system) induce these cells, which contain Th2 cytokines in cytoplasmic granules, to release large amounts of IL-4, IL-5, IL-10 and IL-13.

The fate of HIV-1 following infection of cells at the portal of virus entry and the engagement of the innate system dendritic cells (DCs) with the virions was reviewed [5]. It was reported that the DC DC-SIGN receptors bind the virion gp120 molecules and mobilize the virions through the lymphatics to the draining lymph nodes, the site of viral antigen presentation by DC HLA class I and II molecules to the $CD4^+$T cells, which then polarize and differentiate to become $CD4^+$ Th1 cells and $CD4^+$ Th2 cells, respectively. The latter cells are subsequently infected by HIV-1 [6]. This study presents the sequence of events by which HIV-1 causes AIDS in humans. Based on the above information two additional papers were published, dealing with two major questions: how to stop the AIDS epidemic [7] and the reason why some individuals that are exposed to multiple HIV-1 infection remain resistant to infection for many years [8]. The collected, reviewed findings revealed that HIV-1 replication in $CD4^+$ Th2 cells causes T cell depletion and increased virus load in the infected individual. The shed virion gp120 molecules induce IgE/FcεRI$^+$ hematopoietic mast cells, basophils and monocytes to release large amounts of IL-4, IL-5, IL-10 and IL-13 cytokines that skew the Th1/Th2 balance toward Th2 cytokines. IL-4 inhibits the polarized Th1 cells from releasing the Th1 cytokines IL-2, IL-12 and interferon (IFN) gamma necessary for activation of $CD8^+$ CTL precursors. IL-4 also inhibits the synthesis of IgG and IgA by B cells and induces in these cells a switch to IgE synthesis. Under these conditions the patient's adaptive immune response is gradually inhibited.

It was hypothesized that treatment of HIV-1/AIDS patients needs to be supplemented with treatment with the IL-4 antagonists IL-4δ2 [8] and the adjuvant CpG ODN [7], inducer of plasmacytoid DCs to release large amounts of type I IFN, inhibitors of HIV-1 replication and reactivators of cytokine synthesis by the inhibited Th1 cells.

The present review deals with (1) the impact of the chemokine genes coding for CX3CL1 fractalkine

(FKN) and its receptor CX3CR1 (FKNR1) on HIV-1/AIDS infection and disease progression; and (2) the role of the expressed proteins in the distribution of HIV-1-infected $CD4^+$ Th2 lymphocytes to the thymus, lymph nodes and the immune system compartments in the gastrointestinal tract, and into the CNS, causing brain dementia. The possible usefulness of fractalkine antagonists in the treatment of HIV-1-infection and AIDS patients will be discussed as a hypothesis.

The human genes coding for fractalkine (CX3C chemokine) and its receptor CX3CR1

(i) The CX3CL1 chemokine fractalkine and its receptor CX3CR1

Bazan et al. [9] reported the identification of a fourth human chemokine family, derived from non-hematopoietic cells, bearing a new CX3C chemokine fingerprint, in addition to the three chemokine families with cysteine motifs of CXC, CC and XC. The human CX3C chemokine motif is part of a 373-amino acid protein that carries the chemokine domain on top of an extended mucin-like stalk. This chemokine molecule exists in two forms: a 95kD membrane-anchored glycoprotein or a shed 95kD glycoprotein; the latter is a potent chemoattractant for T cells and monocytes. The former is induced in activated primary endothelial cells and promotes strong adhesion of leukocytes. The authors cloned the CX3C chemokine cDNA into a mammalian expression vector and transfected human embryonic kidney 293 (HEK293) cells using three plasmids: (1) a truncated CX3C chemokine cDNA (nucleotides 1–700) encoding a 180 aa protein devoid of the transmembrane coding segment; (2) a full-length (1.7 kb) CX3C chemokine cDNA coding for the complete CX3C chemokine protein; and (3) a control expression vector without the chemokine cDNA. The authors named the CX3C chemokine 'fractalkine' and concluded that ''the structure of fractalkine and its inducible expression by epithelial cells suggest that it could function on endothelium''.

Imai et al. [10] identified a high-affinity functional receptor for fractalkine, CX3CR1, and reported on the way fractalkine and CX3CR1 mediate the adhesion and migration of leukocytes such as monocytes and $CD16^+$ NK cells, and how $CD3^+$ $CD8^+$ T cells that express CX3CR1 on the cell membrane migrate efficiently toward a soluble fractalkine gradient. The authors concluded that ''at the critical interface between leukocyte cell surface and endothelium, fractalkine and CX3CR1 blend adhesion and chemotactic properties at the molecular and functional levels''.

Jung et al. [11] generated a mouse mutant that lacks the fractalkine receptor gene and reported that this mutant enabled them to assign murine CX3CR1 expression to monocytes, subsets of NK cells, dendritic cells (DCs) and brain microglia. The authors suggested that the prominent response of CX3CR1-deficient microglia to peripheral nerve injury may indicate unimpaired neuronal-glial cross talk in the absence of CX3CR1.

(ii) Mapping chemokine receptors CCXCR1, CX3CR1, CCBP2, CCR8 and CCR9 to the CCR cluster within the human chromosome 3p21.3 region

Maho et al. [12] reported that a main cluster of six genes (CCR1, CCR3, CCRL2, CCR5, CCR2 and CCXCR1) mapped to chromosome 3p21.3. Five other genes (CCR9, CCBP2, CX3CR1, CCR8 and CCR4) were found to be spread over a relatively large region between this main cluster and the chromosome 3p telomere.

Devries et al. [13] described the genomic organization and evolutionary history of two chemokine receptor genes encoding CCR8 and CX3CR1. The latter gene has three promoters which transcribe three separate exons that are spliced, with a fourth exon that contains the coding region. CCR8 has two promoters, one of which produces a transcript of two spliced exons, while the other transcribes an exon containing the coding region and lacks introns. Examination of the CX3CR1 and CCR8 genes and the surrounding genomic regions indicated that these genes resulted from duplication of an ancestral gene prior to the divergence of teleost fish and mammals. The CX3CR1 receptor appears to have undergone subsequent evolution in the human, leading to the addition of two non-coding exons and the loss of P2 promoter in mice. In humans alone, polymorphisms of the receptor lead to altered phenotypes and changes in disease susceptibility (see below).

(iii) The structure and dynamics of the fractalkine CX3C chemokine domain and its binding to the N-terminal fragment of CX3CR1

Mizoue et al. [14] employed heteronuclear NMR methods to determine the solution structure of the CX3C domain (residues 1–76) of fractalkine. The fractalkine CX3C module is monomeric, in contrast

to many chemokines which form homodimers. There is a bulge formed by the CX3C motif, the relative orientation of the N-terminus and the 30's loop (residues 30–38) and the conformation of the N-loop (residues 9–19). The authors titrated ^{15}N-labeled protein with a peptide from the N-terminus of the receptor CX3CR1 and confirmed that this region of the receptor contacts the CX3C chemokine domain. Hoover et al. [15] employed x-ray crystallography to solve the structure of fractalkine CX3C domain at 2.0Å resolution. The chemokine monomers form a dimer through an intramolecular β-sheet and a novel quaternary structure relative to other chemokines. Harrison et al. [16] identified specific residues within the amino acid sequence of CX3CL1 that are critical for CX3CL1-CX3CR1 interactions. The authors identified residues Lys7 and Arg47 as important determinants in mediating the CX3CL1-CX3CR1 interaction. Using two fractalkine (FKN) mutants, FKN-K7A and FKN-R46A, a 30–60-fold decrease in affinity for CX3CR1 was exhibited and CX3CR1-expressing cells were not arrested efficiently under physiological flow conditions. The FKN-K7A mutant acted as an equipotent partial agonist, whereas the FKN-R47A mutant had marked decreased potency and efficacy in chemotactic activity.

(iv) Fractalkine receptors are potent HIV-1 co-receptors

Combadiere et al. [17] reported that the recombinant 76-aa chemokine domain of fractalkine chemotactic agonist for a human orphan receptor (chemokine beta receptor-like 1) named V28x, was expressed in neutrophils, monocytes, T lymphocytes and several solid organs, including brain. The authors found that CX3CR1 can serve as a functional co-receptor for HIV-1 and fractalkine can block the fusion of virions with T cells.

Garin et al. [18] identified two novel isoforms of CX3CR1 which were produced by alternative splicing and had N-terminal regions extended by 7 and 32 aa. The expression of the messenger RNAs coding for these isoforms is high in CD4$^+$ T cells and lower in monocytes and NK cells. It was found that all three isoforms bound CX3CL1 with similar affinity, but in kinetic binding studies the binding was significantly greater for the extended CX3CR1 isoforms. In signaling studies all three CX3CR1 isoforms mediated agonist-dependent calcium mobilization. The role of CX3CR1 in fusion and infection by HIV-1 was studied and the longer isoforms were found to be more efficient than the classic form for CCR5, CXCR4 and for X4R5 HIV-1 strains.

(v) Genetic polymorphism in CX3CR1 and risk of HIV-1 disease

(a) Two mutations in the human fractalkine receptor gene change the course of HIV-1/AIDS

Moatti et al. [19] identified in the open reading frame of CX3CR1 two common single-nucleotide polymorphisms which are non-synonymous substitutions, each causing relatively conservative amino acid changes, V249I and T280M, in the CX3CR1 protein. The two polymorphisms are in strong linkage disequilibrium, forming a common I249M280 haplotype. The authors reported that functional CX3CR1 analysis showed that fractalkine binding to the receptor was reduced in peripheral blood mononuclear cells (PBMCs) from HIV-1 patients who were homozygous for the I249M280 haplotype [20]. The authors reported that the V249I allele is associated with a reduced risk of acute coronary artery disease as well as altered CX3CR1 expression and ligand affinity binding. The V249I and T280M polymorphisms, located in the sixth and seventh transmembrane domains of the CX3CR1 protein, respectively, are the first genetic tool for studying the specific role of CX3CR1 in human disease. Examination of CX3CL1 binding to CX3CR1 revealed that FKN binding-site density of PBMCs from individuals carrying the VI genotype (either VI-TT or VI-TM) was approximately 40% lower than on PBMCs from individuals bearing the reference genotype VV-TT. The authors concluded that there is a potential mechanism for the reduced risk of acute coronary events associated with this genotype. The authors also indicated that the binding phenotype of PBMCs from VI heterozygotes is consistent with their study [19] of four HIV-1$^+$ II homozygotes (II-MM compound genotype), in whom fractalkine binding-site density on PBMCs was only 20% of that of HIV$^+$ VV-TT controls. It was concluded that ''HIV-1-infected patients homozygous for CX3CR1-I249M280 progressed to AIDS more rapidly than those with other haplotypes''. Faure et al. [21] reported that HIV-1-infected individuals homozygous for the specific mutation (M280) in CX3CR1 progressed to AIDS more rapidly than those with other genotypes. The authors tested the hypothesis that the deleterious effect predicts depletion of M280 carriers in a cohort of prevalent HIV-1/AIDS patients because these patients would have died prior to recruitment. The authors reported that in the French SEROCO cohort of HIV-1/AIDS patients homozygous for M280 allele were indeed rare among the seroprevalent group.

McDermott et al. [23] studied the role of the chemokine receptor CX3CR1 M280 mutation in the pathogenesis of atherosclerosis cardiovascular disease (CVD). The authors showed that FKN-dependent cell–cell adhesion is severely reduced in cells expressing CX3CR1-M280, which was associated with markedly reduced kinetics of CX3CL1 binding to the mutated receptor that reduced fractalkine-induced chemotaxis of primary leukocytes from donors homozygous for the mutant CX3CR1-M280. The authors also showed that CX3CR1-M280 is independently associated with a lower risk of CVD in the offspring cohort of the Framingham Heart Study, a long-term prospective study of the risks and natural history of this disease. It was suggested that CX3CR1-M280 is a reduced genetic risk factor for CDV. The authors wrote that the ''data suggest that the chemokine receptor mutation CX3CR1-M280 in heterozygous form is independently and causally associated with reduced risk of CVD in humans''. McDermott et al. [24] reported their analysis on the role of CXCR1 receptor mutations I249T280 and V249M280 in three North American (NA) cohorts of HIV-1 seroconverters: (1) the D.C. Gay (DCG) Cohort, (2) the Multicenter AIDS Cohort Study of homosexual men (MACS) and (3) the Multicenter Hemophilia Cohort Study (MHCS). It was reported that the V249T280 mutation was the common allele in all racial groups and was similar in frequency between Caucasian random blood donors from North America (NA), at 72.2%, and the SEROCO cohort from France, at 74.3% [19]. The M280 allele was present in 20.2% of NA Caucasian blood donors, compared to 13.5% of the French cohort. The 7.7% of NA blood donors possessing I249M280 mutations were compared to 12.2% from France. The authors did not find significant differences between HIV-1-exposed but uninfected ($n = 109$) and HIV-1-infected ($n = 573$) Caucasian participants of the MACS cohort, a finding that failed to support a role for CX3CR1 mutation in HIV transmission among homosexual men. The M280 heterozygocity was weakly associated with a 1.5-year delay in median time to both AIDS and all-cause death in the combined NA cohorts. This delay in disease progression was not seen in the French SEROCO cohort. However, consistent with delayed disease progression the receptor encoded by CX3CR1 allele M280 had 15–50% activity, compared to the reference receptor V249T280, as coreceptor for three envelope gp120 glycoproteins of HIV-1 strains 89.6, ADA, SF-162 in a standard HIV fusion assay. The authors concluded that ''at present, the results from this study and from that of Faure et al. [20], taken together, do not support a clear and consistent role for CX3CR1 mutations in HIV-1 pathogenesis''. Hendel et al. [25] studied the CX3CR1 polymorphisms in 244 AIDS non-progressors (NP) and 80 rapid progressors (RPs) to AIDS from the largest case control cohort known today, the GRIV cohort. Surprisingly, the genetic frequencies found were identical for both groups under all genetic models. The authors hypothesized that their use of a limited number of non-progressor (NP) subjects in ALT ($n = 63$) required reanalysis of the data previously collected on GRIV for over 100 different genetic polymorphisms. The authors observed that genetic frequencies of some genetic polymorphisms could vary by as much as 10% when computing them on the first NP, or the first 100, or on all NP patients tested ($n = 240$). However, the authors concluded that ''the association of CX3CR1 polymorphism with progression seems quite significant in Kaplan–Meier analysis of the SEROCO cohort ($n = 426$) and the difference observed with the GRIV Cohort might be explained by a delayed effect of polymorphisms on disease.

(vi) Expression of the fractalkine and fractalkine receptor complex in HIV-1-infected patients

Foussat et al. [26] reported that, in contrast to HIV-uninfected individuals, a large number of cells expressed fractalkine in T cell zones in lymph nodes from HIV-1-infected patients. In healthy individuals CX3CL1 is constitutively expressed by neurons and blood vessel endothelial cells, but no CX3CL1 expression has been found in the gut Peyer's patches. In reactive lymph nodes, high endothelial venule cells, dendritic cells (DCs), follicular DCs and a few germinal center lymphocytes express CX3CL1.

The fractalkine receptor (CX3CR1) is expressed on monocytes, microglial cells, natural killer (NK) cells, and subpopulations of T cells (see below). The authors [26] studied fractalkine expression in tissues from HIV-infected patients and reported that CX3CL1 expression is up-regulated in lymph node DCs and plasma cells and also in the gut-associated lymphoid tissue (see below). The production of fractalkine in lymph nodes of four HIV-infected patients was tested by immunohistochemistry, immunofluorescence and in situ hybridization. Fractalkine was detected in all lymph nodes, expressed by cells in high endothelial venules (HEV), lymphatic sinuses, and germinal centers that did not differ between HIV-infected patients and uninfected controls. The density of CX3CL1-expressing cells in T-cell zones was higher and more clustered in HIV-infected patients than in controls. The CX3CL1-expressing cells had the morphology of

plasma cells and DCs that were identified by double-labeling with CD83 and fractalkine. It was noted that only a few $CD83^+$ DCs in uninfected controls expressed CX3CL1, while $CD83^+$ DC-expressing cells were abundant in HIV-infected patients. The plasma cells were identified by immunofluorescence to study CX3CL1expression by $CD79alpha^+$ B lymphocytes and $CD123^+$ plasmacytoid DCs. It was reported that both cell types expressed fractalkine. It was determined that the density of $CD83^+$ DCs expressing CX3CL1was similar in HIV-1-infected patients and controls. Yet, the density of $CD123^+$ plasmacytoid DCs and $CD79alpha^+$ plasma cells was much higher in T-cell zones from HIV-infected patients than from controls.

The authors measured the plasma concentrations of soluble CX3CL1and found identical concentrations in HIV-1-infected patients and healthy individuals. The testing for CX3CR1 expression in T helper (Th) cells revealed that in controls few Th cells expressed CX3CL1, but the fraction of $CD4^+$ T cells expressing CX3CL1 in HIV-infection was higher, as measured by flow cytometry.

Fractalkine and CX3CR1 expression by T cells, polarized Th1 cells, $CD8^+$ CTLs and mast cells and their role in leukocyte migration

(i) Leukocyte recruitment from the circulation into tissues in healthy individuals

Foussat et al. [27] studied the expression and function of CX3CR1 by T lymphocyte subpopulations and reported that in $CD8^+$ T lymphocytes, CX3CR1 was expressed in both $CD45RO^-$ and $CD45RO^+$ cells. In $CD4^+$ T lymphocytes, CX3CR1 was expressed by $CD45RO^+$ cells, and almost exclusively by activated $HLA\text{-}DR^+$ T lymphocytes. This group also detected fractalkine in endothelial cells of normal lung and thymus by in situ hybridization and immunohistochemistry. In hyperplastic lymph nodes, fractalkine was expressed by endothelial cells of high endothelial venules (HEV) and of subcapsular blood vessels, by follicular DCs (FDCs) and by some other follicular lymphocytes. The authors indicated that (1) naïve normal B lymphocytes do not express the fractalkine gene, but that expression is rapidly induced following activation. (2) T lymphocytes in germinal centers have the phenotype of memory T cells: they are antigen-specific, and are activated to produce cytokines and CX3CR1 expression. B cells also produce CXCL1. (3) Fractalkine was expressed in hyperplastic lymph nodes and also in the thymus. The authors suggested that ''the selective recruitment of activated T lymphocytes to the thymus may be explained by restricted expression of CX3CR1 by this cell population, combined with the constitutive expression of fractalkine by endothelial cells of the thymus''. (4) Fractalkine production by endothelial cells in the normal lung tissue samples participates in the trafficking of immune cells through lungs in healthy individuals. (5) No fractalkine was detected by the authors in the gastrointestinal (GI) tract, suggesting that in healthy individuals, fractalkine plays no role in the homing of immune cells to the uninflamed gut.

Fong et al. [28] reported that fractalkine molecules on blood vessel endothelium interacted with CX3CR1 on leukocytes and mediated the initial capture, firm adhesion and activation of circulating leukocytes. The authors concluded that the ''novel fractalkine-mediated pathway may be particularly relevant to the recruitment of monocytes, $CD8^+$ T cells and NK cells to sites of inflammation.

(ii) The role of fractalkine in polarized Th1, CTLs and polarized Th2 cells

Fraticelli et al. [29] examined the role of fractalkine, a membrane-bound CX3C chemokine induced by primary proinflammatory signals in endothelial cells (ECs), and in polarized Th1 and Th2 cells. It was reported that proinflammatory signals by LPS, IL-1, TNF, CD40 ligand and interferon gamma induce fractalkine synthesis. However, IL-4 and IL-13 did not stimulate the expression of fractalkine and markedly reduced its induction by TNF and interferon gamma and inhibited the release of soluble fractalkine. The authors studied the interaction of fractalkine with NK cells and polarized T-cell populations and reported that NK cells expressed high levels of CX3CR1 and responded to fractalkine. CX3CR1 was also expressed by Th1 cells compared to Th2 cells. It was indicated that endothelial cells (ECs) expressed CX3CL1 in patients with psoriasis, a Th1-dominated skin disorder, but not in Th2-driven atopic dermatitis. It was concluded that ''regulated expression of fractalkine in ECs participates in an amplification circuit of polarized Th1 responses''. The authors also concluded from their findings that: (1) the CX3CL1/CX3CR1 are part of the chemokine-based amplification and orientation circuits of polarized Th1 responses and may present a valuable target for blocking Th1-mediated responses (e.g. acute allograft rejection), and (2) explanation of the effects of mutations in CX3CR1 I249M280 allele that showed rapid progression to AIDS [20]. The CX3CR1 I249M280 mutations are associated with reduced

affinity and surface expression of the receptor. Based on the protective role of polarized Th1 responses against many pathogens including HIV-1, the authors speculated that ''the rapid progression to AIDS of the I249M280 homozygous individuals might depend on defective capacity to mount fully effective type I responses''.

Nishimura et al. [30] demonstrated that fractalkine and its receptor play dual roles in recruitment of effector lymphocytes with cytotoxic activity. CX3CR1 is a highly selective surface marker for terminally differentiated T_c cells (cytotoxic effector cells).

(iii) CX3CR1 controls homing and antiviral potencies of $CD8^+$ effector memory T cells in HIV-1-infected patients

Combadiere et al. [31] reported an increased frequency of $CD8^+$ T cells expressing CX3CR1, compared to healthy individuals, that correlated with disease progression in HIV-infected patients. The CX3CR1 was expressed mainly on activated CCR7-CD45RA7 memory lymphocytes. The $CD8^+$ $CX3CR1^+$ lymphocytes were engaged in the cytotoxic lineage ($perforin^+$, $CD27^-$, $CD57^+$).

Ancuta et al. [32] studied the transepithelial migration of $CD16^+$ monocytes in response to fractalkine under constitutive and inflammatory conditions. The $CD16^+$ monocytes represent 5–10% of circulating monocytes in healthy individuals but are expanded in pathological conditions like HIV-1-associated AIDS and AIDS dementia. $CD16^+$ monocytes produce high levels of cytokines and neurotoxic factors and are recruited into the CNS and other peripheral tissues in response to locally produced chemokines, a critical event in immune surveillance. The authors investigated the ability of $CD16^+$ monocytes to undergo transendothelial migration (TEM) under constitutive and inflammatory conditions. It was reported that $CD16^+$ monocytes underwent TEM across unstimulated human umbilical vascular and brain microvascular endothelial cell monolayers in response to sCX3CL1. Stimulation with tumor necrosis factor (TNF) induced high expression of membrane-bound CX3CL1 and IFN gamma induced a low level of expression of fractalkine and prevented TEM of $CD16^+$ monocytes in response to CX3CL1. The authors concluded that ''understanding molecular mechanisms of $CD16^+$ monocyte trafficking under inflammatory conditions will help to develop new therapeutic strategies to prevent deleterious consequences of monocyte infiltration at sites of chronic inflammation in vivo''.

(iv) Response of mast cells to membrane-bound fractalkine constitutively expressed by skin cells

Papadopoulos et al. [33] studied the molecular mechanisms which direct the innate system mast cells (MC) to localize near nerves and blood vessels in the skin and gastrointestinal tract and in cutaneous tumors like neurofibroma. CX3CL1 is constitutively expressed by skin blood vessel endothelial cells, dermal dendrocytes and neurofibroma cells and CX3CR1 is expressed by murine cultured bone marrow-derived MCs of both connective tissues and mucosal phenotypes. In chemotaxis assays, CX3CL1 attracted MCs with maximal migration occurring at concentrations between 25 and 125 ng/ml. Bone-marrow-derived MCs were not stimulated to release cytokines in the presence of fractalkine.

(v) Fractalkine induces chemotaxis and actin polymerization in human dendritic cells (DCs)

Papodopoulos et al. [34] reported that fractalkine synthesis is associated with DCs in skin epidermis and lymphoid organs. $CD83^+$ $CD1a^+$ skin Langerhans cells (LCs) express fractalkine mRNA and protein.

Dichmann et al. [35] studied the chemotactic activity and intracellular signaling of fractalkine. The authors reported CX3CR1 mRNA expression in immature and mature DCs and that fractalkine elicited actin polymerization and chemotaxis in a dose-dependent manner in DCs, independent of their state of maturation.

(vi) CX3CR1 tyrosine sulfation enhances fractalkine-induced cell adhesion

Fong et al. [36] tested the role of tyrosine sulfation in CX3CR1 on its function in cell adhesion. Tyrosine residues 14 and 22 in the N terminus of CX3CR1 were mutated to phenylalanine and were stably expressed on H56L cells. In static binding assays, CX3CR1-Y14F mutants had a 2–4-fold decreased affinity to fractalkine compared with wild-type CX3CR1, but were competent in signal transduction but defective in capture and firm adhesion. By plasmon resonance measurements of fractalkine binding to biosensor chips in immobilized cell membranes, CX3CR1-Y14F mutants had a 100-fold decreased affinity to fractalkine. It was also suggested that synthesized sulfated N-terminal CX3CR1 peptides immobilized on biosensor chips showed a high affinity for fractalkine than non-sulfated peptides.

Trafficking of leukocytes from the thymus to organs in healthy individuals and in HIV-1/AIDS patients and the impact on $CD4^+$ T cells and $CD8^+$ T cell differentiation

(i) The thymus and the lymphoid progenitor cells

Kyewski and Debrinski [37] reviewed the role of the thymus in the tolerance induction of bone marrow-derived T cells to self antigens from all organs of the human body that are specifically expressed by thymic epithelial cells and abundant blood-borne self antigens. The experimental demonstration of acquired self-tolerance and the clonal deletion of self-reactive lymphocytes led to the understanding of self-tolerance in the adaptive immune system cells and the self-non-self discrimination by the innate immune mechanisms [38]. The authors indicated that ''the two arms of the immune response maintain a state of non-reactivity to self in different ways''. The innate immune response is based on the recognition of microbial components by limited number of germline-encoded receptors, while the adaptive immune response is based on stringent selection of highly diverse, somatically generated antigen-specific receptors. The medullary thymic epithelial cells (mTECs) promiscuously overexpress cellular genes which are present in all tissues and organs: ''The pool of genes promiscuously expressed consists of genes of diverse ontology, represents most, if not all parenchymal genes and is estimated to include up to 3000 (or 5–10%) genes of all currently known genes''.

Takahama [39] reviewed the lympho–stromal interactions in multiple microenvironments within the thymus that have a crucial role in the regulation of T-cell development and selection. The author indicated that thymocyte development involves stringent repertoire selection in which only 1–3% of thymocytes survive and export from the thymus. Most of the thymocytes are not able to complete their journey through the thymus and their cross-talk with the stromal cells of the thymus that produce chemokines in individual microenvironment. The developing thymocytes sequentially express different chemokine receptors to guide them to the different microenvironments of the thymus.

Entry into the thymus of T-progenitor cells occurs by the vasculature-independent pathway before vascularization of the thymus and later by the vasculature-dependent pathway as early as embryonic day 11.5 (E11.5) in mice and the eighth week of gestation in humans. Two chemokine ligands CCL21 and CCL25 have a role in the early stage of fetal thymus colonization. In the post-natal thymus, lymphoid progenitor cells that enter the thymic parenchyma are found close to the cortico-medullary junction where the vasculature is well developed, transmigrating from the blood to the thymus parenchyma. The entry of lymphoid progenitors occurs in waves during embryogenesis and adulthood. During embryogenesis, distinct progenies give rise to gamma/delta T cells. In the adult thymus, the lineage is alpha/beta T cells. The developmental pathway to become T cells starts with the expression of CD25 and CD44. The outward migration of $CD4^-$ $CD8^-$ thymocytes to the capsule is regulated by CXCR4 and CCR7 receptors, and to the subcapsular region by CCR9 chemokine receptor. The $CD4^+$ $CD8^+$ thymocytes generated in the outer cortex interact with stromal cells localized in the cortex for positive and negative selection. The positively selected $CD4^+$ $CD8^+$ thymocytes survive and differentiate into $CD4^+$ or $CD8^+$ thymocytes with increased expression of CCR7, through which they are directed to the medulla cells expressing CCR7 ligand. In the medulla there is further deletion of tissue-specific, antigen-reactive T cells and generation of regulatory T cells. The mature T cells express sphingosine-1 phosphate receptor 1 through which the cells are attracted back into the circulation that contains a high concentration of sphingosine-1 phosphate.

(ii) The effect of HIV-1 infection on thymocyte proliferation

Geenen et al. [40] reported a new tool to estimate thymic function based on the generation of T cell receptor (TCR) diversity of thymocytes through recombination of gene segments encoding variable parts of TCR alpha and beta chains. During the process, by-products of the rearrangements are generated in the form of TCR excision circles (TRECs). As these molecules are lost upon further cell division, their quantification is a valuable tool to estimate thymic function.

Dion et al. [41] used the TRECs technique to study the supply of naïve T cells by the thymus which requires precursor T cell proliferation within the thymus. Their study was aimed at understanding the reasons for depletion of both naïve and memory T cells during HIV-1 infection by using the T cell receptor excision circles (TRECs) as the quantitative index of intrathymic proliferation. These parameters were studied in cell populations of the peripheral blood. The authors reported on the effect of HIV-1 infection on intrathymic precursor T cell proliferation by longitudinal analysis of PMBCs from recently infected individuals. The findings revealed a substantial reduction in intrathymic proliferation that indicated the existence

of a compensatory mechanism acting to sustain the number of recent thymic emigrants in the periphery. The authors concluded that ''primary HIV infection rapidly imposes, mainly in younger patients, a lasting thymic dysfunction characterized by impaired thymocyte proliferation''.

In an analysis of the Dion et al. study [41] Douek [42] wrote ''The major finding of Dion et al. study..., indicating a significant reduction of intrathymic proliferation and consequently thymic output...Increased frequencies of beta TRECs within peripheral T cells suggest that direct killing of proliferating lymphocytes by HIV is unlikely to account for their decreased proliferation''.

HIV-1 infection in the human genital tract

The portal of HIV-1 entry during heterosexual intercourse activity with an infected partner is the genital tract of the healthy partner. The dendritic cells in the genital tract epithelium migrate to the site of virus infection and carry the virus to the afferent lymph nodes, the site of HIV-1 infection of polarized Th2 cells [5]. Recent studies investigated the type of HIV-1 strains that reside in the genital tract and in the peripheral blood.

(i) HIV-1 in the secretion from the genital tract of infected women

Andrealetti et al. [43] assessed the HIV-1 RNA and proviral DNA loads in paired blood and cervico-vaginal samples of clinically asymptomatic, treatment-naïve HIV-1-infected women. The study population comprised 213 women (mean age 27 year; age range 18–49 year) enrolled during June–October 1998 at the Centre National de Référence des Maladies, Sexuellement Transmissibles et du SOA, in Bangui, the capital city of the Central African Republic. The authors detected and quantified HIV-1 RNA and DNA in plasma and in genital compartments of 30 clinically asymptomatic, treatment-naïve women, and reported that cell-free virus RNA was detected in cervico-vaginal lavage supernatants from 22 (73%) women and in the plasma samples from 25 women (83%). Five women did not have detectable viral RNA in the plasma. Proviral DNA was detected in the cellular pellet of cervico-vaginal lavage of 20 (67%) women and in the peripheral blood cell samples from 26 (87%) women. The levels of HIV-1 RNA ranged from <20 to 820,654 copies/ml in plasma versus <50–110,830 copies/ml in cervico-vaginal secretions. The authors concluded that ''these findings support the hypothesis that cell-associated HIV-1 DNA in the female genital tract originates not only from the genital reservoire of HIV but also from migrating blood cells recruited from the cervicovaginal submucosa''.

Tirado et al. [44] reported on the differential evolution of HIV-1 in blood and the genital tract of infected women, suggesting distinct replication compartments for HIV-1 that may serve as virus reservoir. Paired blood and vaginal swabs were collected from forty-four HIV-1-positive females, most of them receiving antiretroviral therapy at several clinics in Puerto Rico. The mean age of the study population was 40 year (range 19–55). The majority of individuals (39/44: 88.6%) reported heterosexual contact as a risk factor: 12.8% reported contact with IV drug users and 38.4% had contact with known HIV-1-infected males. Only two females reported IV drug use and one had received a blood transfusion. Twenty percent of the study population was on two nucleoside analogs (NRTI) or a combination of NRTI and a non-nucleoside analog (NNRTI). Another group of 60% females was on NRTI or NNRTI, and the remaining individuals were naïve HIV-1 cases or were not receiving any treatment. In this study the authors determined the drug-resistance pattern of the virus from vaginal secretions and plasma of HIV-1-infected women. Nine subjects (20%) had no detectable viral loads in either plasma or vaginal secretions. Fourteen women (31%) had virus in plasma, but not in vaginal secretions, 21 females (46.6%) had detectable viruses in both compartments. The viral load was 1000 RNA copies Eq/ml in 19 (42.2%) plasma samples and 31 (68.8%) in vaginal samples.

Of the 44 HIV-1-infected females, 29 plasma samples were examined for drug-resistant variants of the virus. The other 15 samples could not be analyzed due to a low viral load. About 21 (72.4%) women showed resistance to one or another class of drugs, and nine women (31%) showed resistance to only one class of ART (20.6% NRTI, 6.9% NNRTI, and 3.5% PI). An additional nine women (28.5%) also showed double resistance, but triple resistance was least common. The authors concluded that their study demonstrated the presence of unique HIV-1 drug-resistant mutants in the vaginal compartment, suggesting that the vaginal tract may serve as a virus reservoir and may contribute to the transmission of drug-resistant virus strains.

Berlier et al. [45] studied the selective sequestration of X4 isolates of HIV-1 by human genital epithelial cells and the implications during sexual transmission. Although both X4 and R5 HIV-1 are present in the semen of HIV-1-infected men, only R5 isolates are preferentially transmitted. The authors studied the role

of human epithelial cells in virus selection, using three cervical cell lines and a normal human vaginal cell line from HIV-1-negative donor. The cells were infected with X4 and R5 laboratory-adapted HIV-1 strains or primary virus isolates. The three cell lines expressed CXCR4 receptor and Gall-Cermide molecules, but did not express CD4 and CCR5. The normal vaginal cells were permissive to X4 HIV-1 strains. The authors concluded that human vaginal epithelial cells are permissive to CXCR4 (X4) HIV strain entry. Such a phenomenon may explain the selective spread of R5 HIV-1 isolates towards other permissive cells such as immature DCs present in the female genital tract.

(ii) HIV-1 in the seminal plasma and blood of HIV-1-infected individuals

Delwart et al. [46] noted the outgrowth of HIV-1 viruses with macrophage-tropic phenotype, CCR5 non-syncytium-inducing (NSI) HIV-1 strains, after transmission of HIV-1, despite the presence of CXCR4 syncytium-inducing (SI) virus phenotype in the blood of many donors. The authors examined the proviruses present in PBMC and in non-spermatozoa semen mononuclear cells of five HIV-1-infected individuals. It was reported that the distribution of the two virus variants in the two compartments was not consistent and it was concluded that ''it is therefore unlikely that restriction of X4 syncytium-forming (SI) HIV-1 variants from the male genital tract accounts for the observed NSI transmission bias''.

Nunnari et al. [47] studied the effect of HAART treatment on the levels of HIV-1 virions in blood and seminal plasma of HIV-1-infected patients. Three patients with fewer than 50 HIV-1 RNA copies/ml of plasma were enrolled in this eradication protocol. The two major viral isolates in semen lymphocytes were macrophage-tropic (R5) and dual-tropic R5X4 strains, the same HIV-1 viruses that were present in the blood. A rebound was observed six months later, with a shift toward dual-tropism (R5X4) virus in semen cell-associated proviral DNA, with the first appearance of X4 provirus in the semen compartment.

HIV-1 infection of $CD4^+$ lymphocytes in the effector sites of the gut immune system

(i) The organization of the gut and its adaptive immune system

HIV-1 infection occurs in compartments of the adaptive immune system of the gut. Cheroutre and Madakamutil [48] reviewed the organization of the gut epithelium in the villi and in the immune system structures below and inside the normal gut epithelium. The epithelial cells of the gut epithelium constitute the brush border of the gut that is covered by the mucus glycocalyx layer. Lymphocytes are present between the epithelial cells of the villi and in the crypts, although there are tight junctions between the epithelial cells. Lymphocytes are present inside the villi in the lamina propria regions. A special feature of the gut epithelium is the presence of M cells, situated above the lymph follicles that are connected to the mesenteric lymph node in a structure named Peyer's patches. The basement membrane is present underneath the Peyer's patches and the gut epithelium. The authors indicated that the lymphocyte compartments in the gut tissue may be divided into two main immune function-based compartments. The first, the initiation compartment-organized, gut-associated lymphoid tissue, includes the mesenteric lymph nodes, Peyer's patches of the small intestine and follicular aggregates present in the wall of the large intestine, appendix and cecum. The second main effector compartment consists of the lamina propria and the T cells that are interspersed between the intestinal epithelium. These T cells are $CD8^+$ T cells, and are similar to effector $CD8^+$ T memory cells that have an immediate cytolytic activity.

(ii) Antigen uptake by the intestinal M cells and dendritic cells (DCs)

Two cell types are employed to transfer foreign microbial antigens from the lumen of the gut to the gut immune system. The M cells, which reside in the apical side of the gut epithelium, serve as entry portals for active uptake of foreign antigens from the intestinal lumen which are transported to DCs of the Peyer's patches. The epithelial cells take up foreign antigens and present their peptides via HLA class I or class II molecules to mucosal DCs.

(iii) Fractalkine is an epithelial and endothelial cell-derived chemoattractant for intraepithelial lymphocytes in the small intestine mucosa, and mediates DC access to the intestinal lumen

Muehlhoefer et al. [49] studied the expression and function of fractalkine and CX3CR1 in the human small intestine in health and during inflammation. The authors reported that the basal expression of fractalkine mRNA and protein in the intestinal epithelial cell line T-84 in vitro was controlled by IL-1β and that fractalkine was shed from these cells. Also, human

intestinal intraepithelial $CD8^+$ T lymphocytes expressed fractalkine receptor and migrated along fractalkine gradients after activation with IL-2. Using immunohistochemistry the authors reported fractalkine expression in intestinal epithelial cells and endothelial cells in the normal small intestine. The intraepithelial $CD8^+$ T lymphocytes function as CTLs and secrete the cytokines IL2, IL-3, IL-5, IFN-γ, TNF-α and TGF-β. The intestinal epithelial cells express HLA class I and HLA class II and can act as antigen-presenting cells (APC) to DCs.

Niess et al. [50] reported that the $CX3CR1^+$ mouse myeloid-derived mucosal DCs, which populate the entire lamina propria of the small intestine, were found to depend on their CX3CR1 receptors to form trans-epithelial dendrites. Such dendrites allow the DCs to interact with antigens in the intestinal lumen. The authors reported that fractalkine receptors control the clearance of entero-invasive pathogens by DCs and studied the effect of CX3CR1 deficiency in mice, in which both copies of the fractalkine receptor gene were replaced by the gene coding for green fluorescence protein (GFP) reporter gene. Homozygous mice ($CX3CR1^{GFP/GFP}$) that are fractalkine receptor-deficient have defective lamina propria DCs with impaired sampling of bacteria from the intestinal lumen that impedes their ability to take up invasive pathogens. The sampling of foreign antigens from the intestinal lumen occurs by globular structures formed at the end of the DC transepithelial dendrites.

(iv) HIV-1 infection is associated with depletion of $CD4^+$ T lymphocytes from effector sites in the gut immune system

Mehandru et al. [51] investigated the reasons for the susceptibility of gastrointestinal mucosal CCR5-expressing $CD4^+$ T lymphocyte to HIV-1 infection. The authors aimed at defining whether a preferential depletion of mucosal $CD4^+$ T cells would be observed in HIV-1-infected individuals during the primary infection period, and determining the anatomic compartment from which these cells were depleted. It was reported that a significant and preferential depletion of mucosal $CD4^+$ T cells relative to peripheral blood $CD4^+$ T cells is seen during primary HIV-1 infection. The depleted $CD4^+$ T cells predominated in the effector compartment of the gastrointestinal mucosa, in contrast to the inductive compartment where HIV-1 RNA was present.

Brenchrey et al. [52] studied the effect of HIV-1 infection on the activation and depletion of defined subsets of $CD4^+$ and $CD8^+$ T cells in the blood, gastrointestinal tract (GI) and lymph nodes. The authors reported that the GI tract had the most substantial $CCR5^+$ $CD4^+$ T cell depletion at all stages of the disease. The authors also reported that HIV-associated immune activation resulted in abnormal accumulation of effector-type T cells in lymph nodes and concluded that ''the substantial depletion of GI tract $CCR5^+$ $CD4^+$ cells implies that total body $CD4^+$ T cell numbers are severely reduced, even in very early infection''.

Veazey and Lackner [53] evaluated the findings that HIV-1 infection caused a marked depletion of intestinal $CD4^+$ T cells in SIV-infected macaques [54] and the findings of CCR5+ CD4+ T cell depletion in HIV-1-infected individuals. The authors indicated that the intestinal tract contains both inductive (Peyer's patches, lymphoid follicules) and effector (diffuse lamina propria) lymphoid tissues, with $CCR5^+$ $CD4^+$ effector T cells residing in the effector lymphoid tissues distributed along the GI tract. After HIV-1 infection high levels of viral replication take place in the gut lamina propria, destroying the effector cells within 14 days, leaving intact the lymphocytes in the inductive lymphoid tissue. In a recently published study, Veazey and collaborators [55] reported that $CD4^+$ $CD8^+$ double-positive (DP) T cells had been described in several species including humans and are present in peripheral blood and in the effector regions (e.g. lamina propria) of the gastrointestinal tract. The existence of these DP lymphocytes in the peripheral blood led to the suggestion that these cells were prematurely released from the thymus. However, in HIV-1 and Epstein-Barr virus infections, the percentage of DP T cells may increase to 20% of the circulatory lymphocytes. The authors analyzed peripheral blood, lymph node and jejunum lamina propria DP T cells in normal macaques by eight-color flow cytometry. It was reported that the DP T cells are the major component of the intestinal lamina propria lymphocyte population and that they have the phenotype of memory T cells that produce higher levels of cytokines compared to single positive (SP) lymphocytes. The authors concluded that ''the intestinal DP T cells are terminally differentiated effector cells rather than being immature T cells precursors like the DP cells in the thymus''. These DP cells are target cells for HIV-1 infection.

HIV-1/AIDS neuropathogenesis—role of HIV-1 and the shed viral gp120 in brain dementia

(i) Neuropathogenesis of AIDS

Gonzalez-Scarano and Martin-Garcia [56] reviewed the current knowledge of HIV-1 entry into the brain

compartment and replication in several cell types—astrocytes, oligodendrocytes, neurons, perivascular macrophages and microglia—that are susceptible to lentiviruses, with the last two cell types also susceptible to HIV-1. The perivascular macrophages and microglia arise from bone marrow-derived cells that settle in the CNS at various times during embryonic development and throughout adult life. HIV-1 enters the brain early after systemic infection, possibly as passengers in cells that are trafficking to the brain through the blood-brain barrier (BBB), the selectively permeable-impermeable endothelium of micro-blood vessels in the brain. It was also noted that HIV-1 isolates from HIV-1-infected CNS are more closely related to each other than to virus isolates from blood, lymph nodes or the spleen of the same person. Since there is no marked neuronal infection by HIV-1 during HIV-associated dementia, other mechanisms may be the cause of the neuropathologic damage of AIDS.

Studies on the effect of monomeric gp120 glycoproteins derived from the CXCR4 pathogenic, syncytium-inducing (SI) HIV-1 strains show damage to CNS neurons. The viral-shed gp120 glycopeptides arrive in the CNS by the bloodstream to the blood-brain barrier (BBB) and attach to the endothelial cell DC-SIGN receptors. After internalization into the endothelial cells, the gp120 glycoproteins are transferred into the CNS [5]. In addition, the viral Tat and Vpr proteins have been suggested to be potential effectors of HIV-associated dementia. Secreted Tat might cause direct injury to neurons, but its presence in serum and body fluids has not been reported since HIV replication in the CNS is limited. It was reported that Tat alters the tight-junction protein and the BBB function and up-regulates the expression of many inflammatory regulators that promote monocytic infiltration into the brain. Both intracellular and extracellular HIV-1 Vpr induce apoptosis of neuronal precursor cells (NT2 cell line) in the CNS and mature neurons through a caspase-8-dependent mechanism. The authors concluded their review by stating that ''although the incidence of HIV-associated dementia (HAD) has diminished considerably after HAART treatment, there is still a population of patients that, despite therapy, are more prone to developing minor cognitive motor disorder (MCMD), potentially with negative consequences''.

(ii) Role of CX3CR1 fractalkine receptor in neuronal survival after HIV-1 gp120 neurotoxicity and dementia

Meucci et al. [57] reported that hippocampal neurons express CX3CR1 which is activated by soluble fractalkine that induces the activation of the protein kinase Akt, a major component of prosurvival signaling pathways, and the nuclear translocation factor NF-κB, a downstream effector of Akt. Fractalkine protects hippocampal neurons from the neurotoxicity induced by HIV-1 $gp120_{IIIB}$ glycoprotein, an effect blocked by anti-CX3CR1 antibodies. The authors reported that fractalkine inhibited the $gp120_{IIIB}$ neurotoxicity due to interaction with the neuronal CX3CR1 receptor and the subsequent activation of Akt kinase. In a subsequent study Meucci et al. [58] based their work on the findings that HIV-1 infection of glial cells results in overexpression of the envelope gp120 glycoprotein and that treatment of rat neurons with gp120 in vitro causes neuronal apoptotic death. The authors demonstrated that hippocampal neurons possess a wide variety of functional chemokine receptors whose activation affects neuronal signal transduction, neuronal survival and inhibition of gp120 neurotoxicity. The authors concluded that the ''binding of gp120 to neurons might initiate interference with normal effects of chemokines. These events may be directly neurotoxic or could enhance the sensitivity to other factors such as glutamate receptor activation''.

Harrison et al. [59] studied the use of fractalkine and its receptor system in the CNS. The authors found that neurons, widespread in the rat brain, produce fractalkine. The latter stimulated chemotaxis and elevated intracellular levels of microglia, responses that were blocked by anti-CX3CR1 antibodies.

Hatori et al. [60] demonstrated that complex regulatory patterns of mRNA for fractalkine and its receptor exist in neurons, astrocytes and microglia in the human CNS. It is possible that the autocrine effects of fractalkine protect neurons from damage by HIV-1 gp120 glycoprotein. The authors demonstrated that addition of fractalkine to microglia induced a three-fold increase in the number of glial cells, an increase that was blocked by anti-fractalkine antibodies. The authors concluded that ''constitutive gene expression of fractalkine in human neurons and astrocytes and CX3CR1 in human neurons and microglia in the brain may indicate that fractalkine is involved in the fundamental processes of communication between neurons and microglia and also between astrocytes and microglia in the human CNS in health and disease''.

Erichsen et al. [61] investigated HIV-1-infected patients suffering from acute systemic or opportunistic infections, and each had been subjected to a battery of neuropsychological tests. The patients were divided into two groups, 16 neurocognitively impaired subjects and eight neurocognitively unimpaired subjects. The authors determined the levels of fractalkine in

cerebrospinal fluid (CSF) samples collected from the 24 tested subjects. The fractalkine levels in the CSF of the 16 HIV-1-infected cognitively impaired patients were 219 ± 41 pg/ml, in contrast to 122 ± 29 pg/ml in the infected patients without cognitive impairment. The authors indicated that the incidence of HIV-associated dementia (HAD) and HIV-1 encephalitis dropped after active antiretroviral therapy (HAART) from 20% in adults and 50% in children to less than 10% for all infected subjects who suffer neurological dysfunction.

Kanmogne et al. [62] investigated the breakdown of the BBB, despite the lack of productive infection of brain endothelium, in patients with HIV-associated dementia. The objective of the study was to determine the effects of gp120 glycoprotein on brain endothelium cell permeability and junction protein expression. The authors treated cultured human brain blood vessel endothelial cells with gp120 for 24 h and reported that the viral gp120 increased the permeability of the endothelial monolayer under in vitro conditions. The viral gp120 glycoprotein caused disruption and down-regulation of the tight junction proteins ZO-1, ZO-2, and Occludin in these cells, while Claudin-1, Claudin-5 and other junctional proteins were not affected. The authors concluded that ''gp120 glycoproteins are likely to play an important role in BBB damage in infected patients and in the pathogenesis of HIV-associated dementia''.

Oh et al. [63] reported that cultured rat dorsal root ganglia (DRG) neurons express a variety of chemokine receptors, CX3CR1, CXCR4, CCR4 and CCR5 mRNAs, and their respective chemokines. HIV-1 gp120 produced excitatory effects on DRG neurons, stimulating the release of substance P, suggesting that ''stimulation of chemokine receptors on nociceptors can produce pain. Chemokines release from leukocytes in inflammatory infiltrates could be directly responsible for some of the heightened pain sensitivity observed in cases of inflammation. If this is so, then it is likely that chemokine receptor antagonists may constitute a novel class of analgesics for the treatment of inflammatoary pain''.

Walsh et al. [64] studied the anti-oxidant protection by ascorbate-2-O-phosphate (ASC-P) against the pathogenesis of HIV-1 gp120-associated neuroglial toxicity. Human CNS cultures were derived from 16–18-week gestation post-mortem fetal brain, and incubated with 400 μM ASC-P or vehicle for 18 h. They were then exposed to 1 nM gp120 for 24 h and the expression of inducible nitric oxide synthase (iNOS), an important source of NO, was determined. It was reported that exposure to gp120 up-regulated iNOS by astrocytes. Astrocytic hypertrophy and neuronal injury caused by gp120 were prevented by preincubation with ascorbate.

Chemokine and cytokine antagonists are needed to stop HIV-1 replication and the trafficking of infected and uninfected lymphocytes: hypothesis and implications

(i) The need to stop HIV-1 replication and the trafficking of infected lymphocytes

The above studies reveal that HIV-1 infection, contrary to infection by many other viruses, starts in one organ, but spreads rapidly from the portal of virus infection, carried by DCs to the draining lymph nodes. The virus infects polarized Th2 cells and replicates to produce the virus progeny, and sheds the gp120 glycoprotein molecules into the blood. Detailed analyses of the stages of HIV-1 infection were published [1–8]. The present review focuses on the role of soluble fractalkine in the trafficking of lymphocytes from the bone marrow to the thymus. After stringent regulation, development and selection the naïve T cells migrate from the thymus to the lymph nodes and through the blood vessels to the immune cell compartments in the gastrointestinal tract and to the brain. These findings suggested that the possible use of fractalkine antagonists that bind to the fractalkine receptor binding domain may prevent the trafficking of the lymphocytes in HIV-1-infected individuals and the use of CX3CR1 as a coreceptor for the virus.

(ii) Fractalkine antagonists

(a) Modification of the fractalkine protein molecules

Development of fractalkine antagonists requires information on the solution structure of the CX3C chemokine domain of fractalkine and its interaction with an N-terminus of its receptor CX3CR1. Mizoue et al. [65] studied the solution structure of the CX3C domain of FKN, aa residues 1-76, by the heteronuclear NMR method. The authors compared CX3C structure to that of CC and CXC chemokines and found differences relevant to receptor binding: there is a bulge formed by the CX3C motif, the relative orientation of the N-terminus 30's loop (aa30-38) and the conformation of the N-loop (aa9-19), regions of the protein that are dynamic. The CX3CR1 contacts the fractalkine CX3C domain that maps roughly to the regions of greatest flexibility and structural variability.

Inoue et al. [66] developed fractalkine analogs by truncating ≥4 aa from the N-terminus and reported that the truncated fractalkine failed to induce chemotaxis and calcium influx by CX3CR1-expressing cells. The most potent antagonists (FKN-AT) lacked the four N-terminal aa. The authors used the FKN-AT antagonist to inhibit fractalkine expression in the glomerular endothelial cells of 12-week-old MRL/lpr mice, suffering from the autoimmune disease systemic lupus erythematosus (SLE). MRL/lpr mice spontaneously develop a lethal glomerular disease with an increase in circulating immune complexes, autoantigen production and cytokine abnormalities. Fractalkine is expressed at a very low level by resting epithelial cells, but undergoes marked stimulation by cytokines like TNF-α and IL-1β. The authors reported that FKN-AT antagonists delayed the initiation of the disease and ameliorated the progression of murine lupus nephritis.

(b) The effects of NH2-terminal-truncated monocyte chemoattractant protein 1 (MCP-1/CCL2) or thymus and activation-regulated chemokine (TARC)/CCL17 analogs on the initiation and progression of lupus nephritis in MRL/lpr

Hasegawa [67] truncated the N-terminal aa from MCP1 and TARC antagonists and transfected the MRL/N1 cell line from an MRL/gld mouse which expresses and releases the chemokine antagonists. These cells were injected subcutaneously into 7-week-old MRL/lpr mice, before the onset of lupus nephritis, and 12-week-old mice, at an early state of the disease. After 8 weeks, mice bearing the MCP-1 antagonists showed markedly diminished infiltration of macrophages and T cells, glomerular hypercellularity and vasculitis compared to control mice. The authors suggested that the reason was the decreased production of IFN-γ and IL-2 in the kidney. In contrast, there was no significant difference in renal damage between mice bearing TARC antagonists and control mice. The authors concluded that ''MCP-1 antagonist ameliorated the initiation and progression of lupus nephritis and renal vasculitis, which might provide a new approach to the treatment of the disease''.

(c) vMIP-II virus encoded macrophage inflammatory protein—a chemokine receptor antagonist for CC, CXC and CX3C chemokines

Davis et al. [68] reported that the human herpesvirus-8 (HHV-8, latent in Kaposi's sarcoma cells) encodes the chemokine macrophage-infammatory protein -II (vMIP)-II, a non-selective chemokine receptor antagonist, also of CX3CR1, due to its structural similarity to fractalkine. The authors attempted to change the vMIP-II fractalkine CX3C domain to make the antagonist a specific inhibitor of the fractalkine receptor. Chimeric and insertional mutagenesis was used to generate mutants of both vMIP-II and fractalkine. The expressed recombinant proteins were evaluated for chemokine receptor CX3CR1 binding affinities and binding efficacy. The modification of the amino acids between the first conserved cysteine residues of fractalkine and vMIP-II revealed the role of the CX3C bulge of fractalkine in the affinity to CX3CR1. Substitution of vMIP-II N-terminus with that of fractalkine created an agonist that was as effective as fractalkine in binding CX3CR1. However, replacement of the fractalkine N-terminus with the same domain of vMIP-II disrupted the ability of the chimeric fractalkine to bind CX3CR1. The authors concluded that ''the development of specific chemokine receptor antagonists based upon virally encoded chemokine peptide offers the potential for improved strategies for targeting inflammatory mechanisms associated with human disease''.

This approach to developing fractalkine antagonists was based on the findings of Crump et al. [69] who studied the structure and function of human herpes virus-8 MIP-II (aa1-71) and the N-terminal segment (aa1-10), a broad range chemokine antagonist. The authors studied two N-terminal peptides, vMIP-II (aa1-10) and vMIP (aa1-11) dimer (dimerized through cysteine 11). Both peptides bind to CXCR4, the HIV-1 syncytium-inducing (SI) HIV-1 strains. vMIP-II (aa1-10) was $1.4x10^3$-fold less potent than the native protein, while vMIP-II (aa1-11) dimer was only 180-fold less potent. It was reported that the N-terminus of vMIP-II over aa5-8 is a turn-like structure. This feature was not observed during the application of standard NMR methods for solving the full protein structure and requires two-dimensional (2D) methods.

Chen et al. [70] reported that in addition to antagonistic activity against CC and CXC chemokines, vMIP-II has antagonistic activities against the CX3CR1 fractalkine receptor. The authors studied the anti-inflammatory activity of vMIP-II activity in experimental glomerulonephritis induced by antiglomerular basement membrane antibody. It was reported that fractalkine-induced chemotaxis of activated leukocytes isolated from nephritic glomeruli significantly reduced leukocyte infiltration to the glomeruli and markedly attenuated proteinuria.

(iii) The natural splice variant of IL-4, IL-4δ2, is an IL-4 antagonist, inhibitor of IL-4 receptor α on $CD4^+$ Th1, $CD4^+$ Th2 and B cells

In previous reviews on HIV-1 mechanisms of evasion of the host adaptive immune response [1–8] it was suggested that IL-4 antagonist IL-4δ2, or synthetic modified IL-4 may prevent HIV-1 replication in polarized Th2 cells and the ability of IL-4 to skew the Th1/Th2 cytokine balance towards Th2 cytokines and IgE synthesis. This hypothesis was stimulated by the findings of Demissie et al. [71] who reported that individuals who naturally produce the splice variant IL-4δ2 at a higher level than IL-4 resist the development of disease after exposure to *Mycobacterium tuberculosis* (TB). Sean et al. [72] studied PBMCs from healthy individuals and patients with atopic asthma and TB infection and reported that IL-4δ2 expression in leukocytes from asthmatic patients was 2.8 logs higher than from leukocytes of TB patients and 4.5 logs higher than healthy individuals. Avensa et al. [73] engineered a mutation, Tyr(124)Asp, in synthetic IL-4 molecules and reported that these synthetic antagonists prevented induction of IgE synthesis in human B cells by IL-4.

(iv) CpG oligonucleotide (ODN), inducer of type I interferon from plasmacytoid DCs

CpG ODN, a synthetic bacterial-like non-methylated DNA, is an inducer of plasmacytoid dendritic cells (PDCs) to release large amounts of type I interferons α and β, inhibitors of HIV-1 replication. CpG ODNs bind to Toll-like receptor 9 (TLR9) expressed on PDCs and induce the release of large amounts of type 1 interferon after binding of CpG ODN to TLR9.

In previous reviews [1–8] it was hypothesized that treatment of HIV-1-infected individuals with IL-4 antagonist and CpG ODN as an adjuvant may lead to the inhibition of the virus-induced inflammation and inhibition of virus replication and shedding of gp120.

A hypothesis on the use of the IL-4 antagonist IL-4δ2 and fractalkine antagonists, together with CpG ODN as treatment for HIV-1 patients that will reactiavate the adaptive immunity

The previous reviews [1–8] described the mechanisms by which the binding of HIV-1 gp120 to IgE/FcεRI$^+$ hematopoietic cells (basophils, mast cells and monocytes) induces the release of large amounts of Th2 cytokines (IL-4, IL-5, IL-10, IL-13). The increased level of IL-4 is responsible for inhibition of the antiviral cytotoxic $CD8^+$ T cells (CTLs) which require the Th1 cytokines (IL-2, IL-12 and IFNγ) for the activation of CTL precursors. IL-4 is responsible for inducing B cells to stop IgG synthesis and to switch to anti-gp120 IgE molecules that interact with the FcεRI$^+$ hematopoietic cells and continue the skewing of the Th1↔Th2 cytokine balance and cause immune deficiency. The initial hypothesis [1, 2] was that inhibition of IL-4 activity could be achieved by introducing as a drug the splice variant of IL-4δ2 which binds to IL-4Rα expressed on the target Th1, Th2 and B cells. It was implied that IL-4δ2 be used together with CpG ODN, an adjuvant that binds to Toll-like receptor 9^+ plasmacytoid DCs and induces the release of large amounts of type I IFNs α and β from cytoplasmic vesicles. It was reported that IFNs α and β inhibit the replication of HIV-1 in Th2 cells, and induce B cells to stop IgE synthesis and start the synthesis of anti-viral IgG.

The information in the present review describes the role of CX3CL1 fractalkine ligand and its receptor CX3CR1 in the progression of HIV-1 infection. The trafficking of uninfected and infected $CX3CR1^+$ lymphocytes to the thymus, genitals, lymph nodes and the gut to sites which contain immune system cells is directed by CX3CL1 produced by vascular endothelial cells. The resulting generalized HIV-1 infection, leading to the destruction of the adaptive and cellular immunity is the cause of AIDS and the shortened lifespan of untreated HIV patients. The present hypothesis extends the previous hypothesis by including the chemically modified CX3CL1 antagonist molecules that are able to bind to the receptor CX3CR1 molecules present on lymphocytes and DCs and prevent the ligand CX3CL1 from binding and activation of the GPCR signaling of the CX3CR1 receptor. It is implied that if the CX3CL1 antagonists are used for treatment of HIV-1-infected individuals early in the infection, the trafficking of infected lymphocytes released from lymph nodes into the blood will be limited and the infection of the gut immune system and other organs will be markedly reduced.

The hypothesis implies that the combination of IL-4δ2 and chemically modified CX3CL1 antagonist will prevent the skewing of the Th1↔Th2 balance toward Th2 cytokines. The cytokine and chemokine antagonists will protect the patients' cellular and adaptive immune responses. In addition, the trafficking of HIV-1-infected lymphocytes through the patient's vasculature will be prevented. The addition of CpG ODN as a treatment for HIV-1-infected individuals is designed to inhibit the replication of HIV-1 in Th2 cells. In

essence, the combined treatment will reactivate the antiviral adaptive and cellular immune responses in infected patients, and the trafficking of infected lymphocytes will be reduced, preventing brain dementia.

The present hypothesis provides a novel approach to the treatment of HIV-1/AIDS patients: instead of the use of antiviral drugs that inhibit the entry of the virus into Th2 cells and expression of the viral genes leading to selection of multidrug-resistant virus mutants, the use of cytokine and chemokine antagonists plus CpG ODN will inhibit the replication of HIV-1 by the CpG ODN-induced FDC release of IFNα and β, that will reactivate the IL-4-inhibited Th1 cells to synthesize and release IL-2, IL-12 and IFN-γ that will reactivate CTL precursors. IL-4δ2 will switch B cells to synthesize IgG instead of IgE and thus will reactivate the antiviral adaptive immune response. The fractalkine antagonists will prevent the trafficking of HIV-1-infected lymphocytes, as well as preventing virion binding to the CX3CR1 that serves as a co-receptor. The most important advantage of the suggested mode of treatment is the possibility of inhibiting HIV-1 replication by inhibiting the skewing of the Th1↔Th2 cytokine balance and inhibition of HIV-1 replication without targeting a specific viral enzyme or entry process. It is hypothesized that inhibition of the cellular processes that are directed by the infecting HIV-1 may lead HIV-1-infected people to suppress the infection, clear the virus by the cellular and adaptive immune system and become immunized against reinfections.

It is hoped that the hypothesis presented in this review and in previous publications [1–8] will be considered by the authorities responsible for fighting the continued transmission of HIV-1 in the world population.

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