CHAPTER II

LITERATURE REVIEW

The Chemokines

In 1995, basic research in AIDS pathogenesis, the study of how HIV causes disease, led to the creation of a new field of biochemical research: the study of chemokines. The word "*chemokine*" is a new generic name given to a family of pro-inflammatory activation–inducible cytokines. A chemokine is a soluble factor that attracts white blood cells to places where they are needed (e.g. sites of inflammation or infection).[1,23] Chemokines are small, with molecular weight in the range of 8 to 12 kD., and show approximately 20-50 percent sequence homology among each other at the protein level.[1,23,24] Chemokines are secreted by various types of immune system cells. [1] The targets of chemokine are receptors, specialized docking areas on the surface of white blood cell such as monocytes, lymphocytes, basophils, and eosinophils. Receptors are tailored to accept only specific shapes, and a chemokine must be linked with its appropriate, cognate receptor.[24,25] When a chemokine successfully binds to a receptor, a cascade of events begins at the surface and within the cell. This process is referred to as signaling or signal transduction.[1,23,24]

Chemokine Families

Chemokines are divided into families based on structural differences. All chemokines are structurally similar, having at least three beta (β)-pleated sheets (designated as β_1, β_2, β_3) and a Carboxyl (C) terminal alpha (α) helix (Figure I). Most chemokines also have at least four cysteines in conserved positions. One major chemokine subfamily is called "CXC" or α-chemokine because the two cysteines nearest the amino (N) terminal of protein is separated by single amino acid. This is in contrast to the other major subfamily, which are called "CC" or β-chemokines because these two cysteines are directly adjacent (Figure I). According the chromosomal locations of individual genes, member of CXC chemokine are referred to also as the 4q

chemokine family because the genes encoding members of this family map to human chromosome 4q12 to 4q21 (excepting stromal derived factor-1 (SDF-1) whose genes map to chromosome 10).[1,24] Some members of the human CXC chemokine are defined by the conserved ELR sequence motif (glutamic acid-leucine-arginine) immediately preceding the first cysteine residue near the N-terminus. Chemokines with an ELR sequence motif have been found to primarily chemoattract and activate neutrophils. Chemokines without the ELR sequence motif appear to chemoattract and activate monocytes, dendritic cells, T-cells, NK cells, B-lymphocytes, basophils, and eosinophils.[1,24] In addition, the members of the CC chemokine or β chemokine or 17 q chemokine family map to human chromosome 17q11 to 17q32.[1,24,25]

Two other minor subfamily of chemokine have been described i) the C chemokine or gamma (γ) chemokines, which differ from other chemokines by the absence of a cysteine residue (Figure I) and ii) the CX_3C chemokines or delta (δ) chemokines which have three amino acids intervening between the first two cysteines (Figure I).[1]

Chemokine Function

Chemokines are essential mediators for normal leukocyte trafficking. Chemokines are multipotent cytokines that localize and enhance inflammation by inducing chemotaxis and cell activation of the different types of inflammatory cell typically present at an inflammation site. CXC chemokines, for example, appear to attract neutrophils but not macrophages (Table I), while CC chemokines preferentially induce migration of macrophages (Table II). Some chemokines have also been shown to induce selective migration of leukocyte subsets. [1,24,25]

Chemokine Receptors

Chemokines mediate their activities by binding to target cell surface chemokine receptors that belong to the large family of G protein-coupled, seven transmembrane domain (7 TM) receptors. Based on the receptor nomenclature established at the 1996

Gordon Research Conference on chemotactic cytokines, the chemokine receptors that bind CXC chemokines are designated CXCRs and the receptors that bind CC chemokines are designated CCRs. To date, five CXC chemokine receptors (CXCR1 through CXCR5) and ten CC chemokines receptors (CCR1 through CCR10) have been reported.[1,25,26] In addition, the duffy blood group antigen (DARC) has been shown to be an erythrocyte chemokine receptor that can bind selected CXC receptor, as well as CC chemokines. [27]

Two virally encoded chemokine receptors, a CC chemokine receptor encoded by a cytomegalovirus open reading frame (CMV US28),[25] and a CXC chemokine receptor encoded by herpes saimiri virus open reading frame (HSV ECRF3),[25] have also been described. Leukocytes have generally been found to express more than one receptor type. The various CXCRs and CCRs are known to exhibit overlapping ligand specificity.

i) CXC Receptors

CXCR-1 and CXCR-2, previously known as IL-8RA (or type I IL-8 receptor) and IL-8RB (or type II IL-8 receptor), respectively, have been shown to share approximately 77% amino acid sequence identity. IL-8 binds to both receptors with high affinity and induces rapid elevation of cytosolic calcium (Ca^{++}) levels.[25] Whereas CXCR-1 is highly specific for IL-8, CXCR-2 has broad specificity and has been shown to bind with high affinity to other ELR motif-containing α chemokines, including GRO-α, GRO-β, GRO-γ (Growth Related Oncogene alpha, beta and gamma), NAP-2 (Neutrophil Actvating peptide 2), and ENA-78 (Epithelial Cell-derived Neutrophil Activating protein-78). In contrast, PF4 (Platelet Factor 4) and IP-10 (IFN-γ Inducible Protein 10), two α chemokines that lack the ELR motif, have been shown to lack binding affinity for CXCR-2. CXCR-1 and CXCR-2 are expressed by neutrophils, and T-lymphocytes but not B-lymphocytes.

CXCR-3, also known as the IP-10/Mig receptor, is a cloned chemokine receptor that shares approximately 40% protein sequence identity with CXCR-1 and CXCR-2,

and 34.2-36.9% amino acid sequence with five other known CC chemokine receptors. [1,24,26,29] CXCR-3 is highly expressed by IL-2-activated T-lymphocytes (but not by resting T-lymphocytes), B-lymphocytes, monocytes, and granulocytes. CXCR-3 binds IP-10 and Mig (Monokine Induced by IFN-γ), but not PF4, with high affinity and mediates Ca^{++} mobilization and chemotaxis. CXCR3 does not bind any of the CXC chemokines containing the ELR motif.

CXCR-4, also known as fusin or LESTR, [29,30] was originally discovered as an orphan receptor with structural similarity to chemokine receptors. CXCR-4 was subsequently identified as a necessary cofactor for entry of T cell tropic HIV virus into $CD4^+$ cells. [29] The CXC chemokine PBSF/SDF-1 has now been shown to be the ligand for CXCR4 and a powerful inhibitor of infection by T cell-tropic HIV-1 strains *in vitro*. [31,32] CXC chemokine receptors and their ligands are shown in Table III.

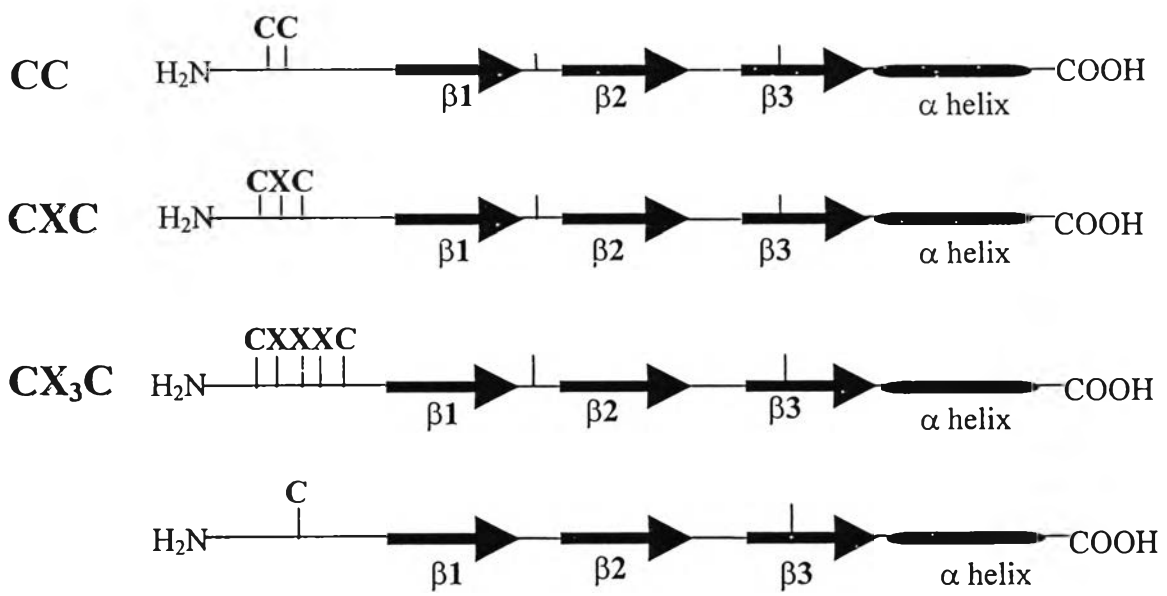
Table I. The CXC Chemokines.[1]

Name	Target cell
ELR	
IL-8	Neutrophils, T-lymphocytes, Basophils, endothelial cells
GRO-α (MGSA)	Neutrophils, Melanoma cells, endothelial cells
GRO-β (MIP-2α)	Neutrophils, endothelial cells
GRO-γ (MIP-2β)	Neutrophils, endothelial cells
ENA-78	Neutrophils
GCP-2 (Granulocyte chemotactic protein 2)	Neutrophils
Platelet basic protein	
CTAP III	Fibroblasts
β-Thromboglobulin	Fibroblasts
NAP-2	Neutrophils, Basophils
Non ELR	
Platelet factor 4	Fibroblasts, Endothelial cells, Activated T-lymphocytes, Tumor Infiltrating Lymphocytes (TIL)
IP-10	Endothelial, Natural killer (NK) cells
MIG	Activated T-lymphocytes, TIL, T-lymphocytes, CD43+ Progenitor cells, B-cells
SDF-1α	Lymphocytes

Table II. The CC chemokines[1]

Name	Target cell
Monocyte chemoattractant protein 1 (MCP-1)	Monocytes, Memory T-lymphocytes, Basophils, NK cells, Hematopoietic Progenitors, Dendritic cells
MCP-2	Monocytes, Memory and Naïve T-Lymphocytes, Eosinophils, Basophils, NK cells
MCP-3	Monocytes, Memory T-lymphocytes, Basophils, NK cells, Hematopoietic progenitors, Dendritic cells
MCP-4	Monocytes, T-lymphocytes, Eosinophils
MCP-5 (mouse only)	Monocytes, T-lymphocytes, Eosinophils
MIP-1α	Monocytes, T-lymphocytes, Basophils, Eosinophils, NK cells, Hematopoietic progenitors, Dendritic cells
MIP-1β	Monocytes, T-lymphocytes, NK cells, Hematopoietic Progenitors, Dendritic cells
MIP-1γ (mouse only)	Resting and Activated T-lymphocytes
RANTES	Memory T-lymphocytes, Basophils, NK cells, Dendritic cells
Eotaxin	Eosinophils
I309	Monocytes
HCC-1(Hemofilltrate CC chemokine1)	Monocytes, Hematopoietic Progenitors
TARC (Thymus and Activation –Regulated Chemokine)	T-Lymphocytes
C10 (mouse only)	?
CCF18 (mouse only)	T-lymphocytes, Hematopoietic progenitors
MIP-3α/LARC	?
MIP-3β	?

? = Unidentified



Family **Structure**

Modified from Barrett J.R., Blood 1997; 90(3): 909

Figure I. The structural classification of chemokines

ii) CC Receptors

CCR-1 is the first identified CC chemokine receptor and is expressed on monocytes, neutrophils, and eosinophils. CCR-1 binds MIP-1α, RANTES and MCP-3 with high affinity.[25,33] CCR-2A and CCR-2B (MCP-1RA and MCP-1RB) differ in their carboxy-termini and are probably derived from alternatively-spliced variants of a single RNA.[34]

CCR-2A and B specifically bind MCP-1 and MCP-3. The two receptors are expressed on monocytes but not on neutrophils or eosinophils.[23,25]

CCR-3 is high affinity receptor for eotaxin, an eosinophil-specific chemo-attractant. In humans, CCR-3 has been found to be expressed exclusively on eosinophils.[1,24]

CCR-4 was originally cloned from a human immature basophilic cell line[35] and has since been shown to be expressed in T-lymphocytes and IL-5-primed basophils. CCR-4 has been shown to mediate the biological activities of RANTES, MIP-1α and MCP-1.[35]

CCR-5 is the most recently discovered CC receptor and has 48-75% amino acid sequence identity to the other member of this family.[36] CCR5 is expressed in primary adherent monocytes, but not in neutrophils or eosinophils.[1,24] CCR5 mediates the activities of MIP-1α, MIP-1β and RANTES.[5] Recently CCR5 has also been shown to be a co-receptor on CD4+ target cells for infection with primary, monocyte-tropic HIV-1 viruses.[3,5,8,9] CC chemokines and their ligand were showed in Table IV.

Table III. CXC Chemokine receptors and their ligands [1,26]

Chemokine Receptors	Ligands
CXCR1	IL-8
CXCR2	IL-8, GRO-a, GRO-b, GRO-g, NAP-2, ENA-78
CXCR3	IP-10, MIG
CXCR4	SDF-1
CXCR5	BLC/BCA-1

Table IV. The CC Chemokine receptors[1,26,37]

Chemokine Receptors	Ligands
CCR-1	MIP-1α, RANTES, MCP-3
CCR-2	MCP-1, MCP-3, MCP-5
CCR-3	Eotaxin, RANTES, MCP-2, MCP-3, MCP-4
CCR-4	MIP-1α, RANTES, MCP-2, TRAC
CCR-5	MIP-1α, MIP-1β, RANTES
CCR-6	MIP-3α/LARC
CCR-7	MIP-3β/ELC
CCR-8	TRAC, I309, MIP-1β
CCR-9	TECK
CCR-10	MCP-1, MCP-3

TECK = Thymus expressed chemokine

Stromal cell-derived factor 1 (SDF-1)

The CXC chemokine SDF-1 occurs as two alternatively spliced variants, SDF-1α and SDF-1β, that were cloned from mouse bone marrow stromal cell lines.[38,39] Both SDF-1α and SDF1-β are encoded by single gene. Unlike other known CXC chemokine subfamily members that cluster on chromosome 4, the gene for SDF-1 has been localized to chromosome 10q11.[40] The nucleotide and amino acid sequence of SDF-1α and SDF-1β are highly conserved between species, showing only one amino acid substitution between the human and mouse proteins. The SDF-1 amino acid sequence also shows equal homology to both CC and CXC subfamily chemokines. SDF-1, unlike other chemokine family members, has been found to be constitutively expressed in a broad range of tissues.[24,39,40] SDF-1 stimulates the proliferation of B-cell progenitors and because of this was also originally termed pre-B cell growth stimulating factor (PBSF).[39,40] Murine SDF-1α was purified as a lymphocyte chemoattractant from a stromal cell culture supernatant.[41] Subsequent studies showed that synthetic human SDF-1 stimulates monocytes, neutrophils, and peripheral blood lymphocytes, as is indicated by Ca^{++} changes and chemotaxis.[32,41] SDF-1 binds to CXCR-4 and induces Ca^{++} mobilization in Chinese hamster ovary (CHO) cells that stabley express this receptor. [31,32] No cross-desensitization is observed with other chemokines, which underlines the selectivity of binding to CXCR-4. In transfected cell lines coexpressing CXCR4 and CD4 and in blood lymphocytes, SDF-1 is a powerful HIV-1 suppressive factor.[31]

CC Chemokine Receptor 2 (CCR-2)

CC Chemokine receptor 2 (CCR-2) belongs to the CC chemokine receptor family, and is a member of the G protein-coupled, 7 TM receptor family. The gene that encodes CCR-2 maps to the human chromosome 3q21.[42] CCR-2 occurs in two forms, CCR-2A and CCR-2B, that are the results of alternative RNA spicing.[34] CCR-2A and CCR-2B differ only in their C-terminal intracellular tails, hence their ligand binding specificities defined to date (Table IV) are identical. [1,24] Although the messenger RNA (mRNAs) for both receptors are expressed at nearly equivalent levels in monocytic

cells, the C-terminal tail of CCR-2B is rich in serine and threonine residues and is homologous to the C-terminal tail of CCR1.[40] CCR2 has also been implicated as an HIV-1 co-receptor on certain cell types.[4,13,43]

Acquired Immunodeficiency Syndrome

The acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV). The first type of this virus, namely HIV-1, to be identified was isolated by Montagnier's group at the Institute Pasteur, Paris, in 1983 from a patient with lymphadenopathy syndrome, and was thus named lymphadenopathy-associated virus (LAV).[44] It was more fully characterized by the same group and by Gallo et al. in a report published in 1984, and named by Gallo as T-lymphotropic virus type III. (HTLV-III) [45] and by Levy et al., in San Francisco, AIDS-associated retrovirus (ARV).[46] A second virus, HIV-2, was isolated from West African patients with AIDS or AIDS related complex (ARC) by Montagnier's group in 1986.[47]

HIV-1 Biology

HIV-1 is classified in the Family Retroviridae, Subfamily Lentiviridae. It has a complex genomic organization. Like all retroviruses, HIV-1 is a single-stranded plus-sense RNA virus, an icosahedral sphere with a diameter of approximately 100 nm. The outer coat of the virus, known as the viral envelope, is composed of two layers of fatty molecules called lipid, taken from the bi-lipid membrane when a newly formed virus particle buds from a human cell. Embedded in the viral envelope are proteins from the host cell. This envelope is studded by characteristic knobs that represent oligomeric structures (tetramers or trimers) of the virally encoded envelope glycoproteins gp120 and gp41. The gp120 subunit comprises the extracellular portion of the viral envelope, while gp41 portion spans the membrane and anchors the glycoprotein complex to the surface of the virion.

Within the envelope of a mature HIV particle is a bullet-shaped core or capsid, made of approximately 2000 copies of another viral protein, p24. The capsid surrounds

two single strands of HIV RNA, each of which has a copy of the virus's nine genes. Three of these *gag, pol*, and *env,* contain information needed to make structural proteins for new virus particles. The *env* gene, for example, codes for a protein called gp160 that is broken down by an enzyme to form gp120 and gp41, the components of envelope. [47,48,49,50] Three regulatory genes, *tat*, *rev*, and *nef*, and three auxiliary genes, *vif*, *vpr*, and *vpu*, contain information necessary for the production of proteins that control the ability of HIV to infect a cell, produce new copies of virus and cause disease. The protein encoded by *nef*, for instance, appears necessary for the virus to replicate efficiently [51], and the *vpu*-encoded protein influences the release of new virus particles from infected cells.[52]

The two ends of each strand of HIV RNA contain a sequence called the long terminal repeat (LTR).[53] Regions in the LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or the host cells.[51] The core of HIV also includes protein p7, the HIV nucleocapsid protein; and three enzymes that carry out later step in the virus's life cycle: reverse trancriptase, integrase and protease. Another HIV protein called p17, or the HIV matrix protein, lies between the viral core and the viral envelope.[48,49]

The HIV Life Cycle

Transmission

Transmission of HIV usually requires transfer of bodily fluids. The most important of these are blood, semen, and vaginal secretions that contain the virus, or may permit the transfer of cells, especially macrophages, containing virus.[54] In general, HIV is transmitted between humans in three ways.[55]

1. Sexual transmission: sexual contact with an infected person
   - male to male
   - male to female
   - female to male

2. Blood-borne transmission: exposure to infected blood or blood component from an HIV-infected donor
   - Blood transfusion
   - Shared injection equipment
3. Vertical transmission
   - perinatally from an infected mother to her child
   - postpartum from nursing mother (presumably through breast feeding)

Sexual transmission accounts for the majority (75%) of cases of HIV infection worldwide. The number of unprotected sexual contacts, the stage of infection (which may dictate the viral load) and the existence of genital ulceration may all increase the risk of transmission and thus play important role in the sexual spread of HIV.

Entry of HIV into cells

Infection typically begins when an HIV particle encounters a cell with a surface molecule called cluster designation 4 (CD4). Cells with this molecule are known as CD4 positive (CD4+) cells. One or more of the virus gp120 molecules binds tightly to CD4 molecule(s) on the cell surface.[56,57] The membrane of the virus and cell fuse, a process that probably involves both gp41 and a second "fusion cofactor" molecule on the cell surface, known as chemokine receptors.[3-5] Although CD4+ T cells appear to be the main target of HIV, other immune system cells with CD4 molecules on their surface are infected as well. These include monocytes, macrophages, Langerhans cells of the skin, follicular dendritic cells in the lymph nodes, alveolar macrophages in the lung, retinal cells, and cells of the uterine cervix. In addition, HIV may infect microgial cells in the brain which may not bear CD4 surface proteins.[58-61] Cell-to-cell spread of HIV also can occur through the CD4-mediated fusion of an infected cell with an uninfected cell. Following fusion, the HIV RNA, protein, and enzymes are released into the target cell.

Replication

In the cytoplasm of the cell, HIV reverse trancriptase converts viral RNA into DNA, the nucleic acid form in which the cell carries its genes. Six of the nine

antiretroviral drugs approved in the United States for the treatment of people with HIV infection (AZT, ddC, ddI, d4T, 3TC, and nevirapine) work by interfering with this stage of the viral life cycle.[61] The newly made HIV DNA moves to the cell nucleus, where it is spliced into the host DNA with the help of HIV integrase. Once incorporated into the cell genome, HIV DNA is called a "provirus." [48,62]

For a provirus to produce new viruses, the special LTR sequence contains the appropriate promotor, enhancer, and other signals required for transcription of genes by the host RNA polymerase II.[62,63] Some of the RNA will be used to form the genetic material of new viral particles and some will be used to direct the translation of HIV structural proteins or regulatory proteins, which work to control viral replication. The new virions are produced from multiple copies of the viral proteins. These proteins are formed as large precursors - long chain protein molecules, which are then specifically cleaved to become the enzymes and structural proteins of the new virions.

The assembly of a new virus particle begins with two of precursor proteins collecting at the edge of the cell, where they join together and attach themselves to the host cell membrane. They begin to form a spherical structure, which bulges outwards from the cell membrane and draws two strands of viral RNA into it. An enzyme called protease, which is contained on one of the precursor protein molecules, then carries out the final steps of protein cleavage as follows: first, it cuts itself free from the polyprotein molecule; then, the protease works to cleave all the other viral components from the protein chain. Drugs called protease inhibitors interfere with this step of the viral life cycle. Four such drugs (saquinavir, ritronavir, indinavir, and nelfinavir) have been approved.[61] The remaining protein segments make up the protein coat that surrounds the RNA and the viral enzymes, forming the inner 'capsid', at the core of the virus particle. A third structural protein, the envelope protein, is made and transported to the cell surface independently. It contains the envelope glycoproteins, which together with elements from the host cell membrane, totally enclose the new virus particle, which leaves the cell in process known as budding.[62]

M-tropic and T-tropic HIV

Tropism refers to which cells an organism such as HIV prefers to infect. The type of HIV that infects monocytes and macrophages is called macrophage (M) tropic virus, and uses CCR5 as the major coreceptor. M-tropic HIV replicates in peripheral blood lymphocytes, but does not usually form syncytia in in vitro culture. For this reason, M-tropic HIV strains are often referred to as Non-syncytia-inducing (NSI) strains, although the terms are not interchangeable.

Strains of HIV that infect T-cells and T-cell lines are referred to as T-cell line tropic. T-tropic HIV isolates are frequently syncytia-inducing (SI). The major coreceptor for T-tropic virus is CXCR4. NSI strain of the virus are considered to be less virulent than syncytia-inducing (SI) strain. The presence of SI virus is associated rapid progression and an unfavorable prognosis, and they tend to dominate in the later stages of HIV disease. [43,44,46,47,64]

The Immunopathogenesis of HIV Infection

The rate of progression of HIV disease may be substantially different among HIV-infected individuals. Following infection of the host with any virus, the delicate balance between virus replication and immune response to the virus determines both the outcome of the infection, i.e. the persistence versus elimination of the virus, and different rates of disease progression.[65-67]

i) Clinical Course of HIV Infection

The clinical course of HIV infection generally includes three phases or stages: (a) primary infection, (b) clinical latency, and (c) AIDS. Such a course of infection is characteristic of the so-called typical progressors who represent the majority of HIV-infected individuals. The median time from initial infection to progression to AIDS in typical progressors is eight to ten years

ii) Primary Infection

Approximately three to six weeks after initial infection, 50-70% of HIV–infected individuals develop an acute mononucleosis-like syndrome. This period is associated with high levels of viremia, and within one week to three months there is an antibodies response to HIV. This immunity is apparently inadequate to suppress viral replication completely, since HIV expression persists in lymph nodes even when plasma viremia is difficult to detect. Detectable viremia declines markedly or disappears weeks to months after the acute syndrome subsides. Although a substantial percentage of patients with HIV infection do not have a clinically recognizable acute syndrome after primary infection, the events described above probably occur even in the absence of symptoms.

iii) Clinical Latency

Most patients have a period of "clinical latency" that lasts for years after primary infection, viral distribution, the appearance of HIV-specific immunity, and the apparent curtailment of viral replication. During this period virtually all patients have a gradual deterioration of their immune system, manifested particularly by the depletion of CD4+ T cells. Although this depletion may occur even without large increases in plasma concentrations of virus (as manifested by p24 antigenemia, viral RNA levels or culturable virus), viral replication in lymphoid organs, together with the spectrum of immunologic events that are directly or indirectly triggered by the virus, may contribute to it. Thus, HIV disease is clearly progressive during the so-called latent period.

iv) AIDS-defining Illness

AIDS–defining illness or clinically apparent disease is the inevitable outcome of the progressive deterioration of the immune system that occurs in most patients with HIV infection. Exceptions to the direct correlation between deteriorating immune function and clinically apparent disease are the progressive generalized

lymphadenopathy; Kaposi's sarcoma, which can occur before the onset of severe immunosuppression; and neurologic disease that may reflect direct or indirect effects of the virus or its products on neurons. The profound immunosuppression that occurs during this phase of HIV infection is the end stage of the immunopathogenic events that began at the time of primary infection, and continued for years through the clinically latent but microbiologically active stages of infection.

v) Typical Progressors

The majority (70-80%) of HIV-infected individuals belong to the group of typical progressors. Following primary infection, as mentioned above, typical progressors experience a long period (up to six to eight years) of clinical latency. Despite the lack of symptoms, HIV disease is active as is indicated by the persistent replication of virus and by the progressive loss of CD4+ T cells. Individuals with CD4+ T- cell counts > 500 per $cumm^3$ generally remain free of symptoms, whereas the appearance of constitutional symptoms is generally more frequent in individuals with CD4+ T-cell counts below 500 per $cumm^3$. Exceptions to this paradigm are subjects with CD4+ T-cell counts higher than 500 per $cumm^3$ who develop progressive generalized lymphoadenopathy, Kaposi's sarcoma, or neurologic diseases. Progression to clinically apparent disease or AIDS-defining illness generally occurs within eight to ten years in typical progressors. When CD4+ T-cell counts are below 200 per $cumm^3$, the clinical picture may be characterized by severe and persistent constitutional signs and symptoms; at this level of CD4+ T cells, there is an increased susceptibility to opportunistic infections or neoplasms.

vi) Rapid Progressors

A significant percentage (10-12%) of HIV- infected individuals experience an unusually rapid progression to AIDS with two to three years of primary infection. Rapid progressors may experience a prolonged acute viral syndrome and the period of true clinical latency may be absent or very brief. Downregulation of the initial burst of viremia may not be very efficient in rapid progressors; even after the initial decrease,

the levels of viremia may rise rapidly. Inefficient control of the initial burst of viremia and rapid rise in viremia within the first or second year after primary infection reflect a poor control of HIV infection by the immune system. In this regard, a delay in the appearance of the primary immune response or a rapid disappearance if certain immune functions during the early stages of the chronic phase of infection may be detected in rapid progressors.

vii) Long-Term Nonprogressors

A small percentage (less than 5 % on the basis of different cohorts) of HIV-infected individuals do not experience progression of disease for an extended period of time. Long-term nonprogressors by some definitions have CD4+ T-cell counts that are within the normal range and are stable over time; in addition, they generally have low levels of virologic parameters and preservation of lymphoid tissue architecture and immune function. From a clinical standpoint, long-term nonprogressors are asymptomatic; it seems that in these individuals, HIV infection has been arrested with regard to disease progression. It is unknown whether long-term nonprogressors have experienced a primary infection similar to that of other groups of HIV- infected individuals, i.e. associated with an acute viral syndrome and burst of viremia.

viii) Long-Term Survivors

In a small percentage of subjects who experience progression of HIV disease within a period of time similar to typical progressors, both clinical and laboratory parameters, although abnormal, remain stable for an extended period of time. The mechanisms, either virologic or immunologic, that are responsible for preventing further progression of HIV disease are unclear at present; the possibility that changes in virus genotype and/or phenotype, as well as the possibility that preservation of certain HIV-specific immune responses are involved, is being investigated.

Mechanisms of CD4 T Lymphocyte Dysfunction

Researchers are studying how HIV destroys or disables CD4+ T cells, and many think that a number of mechanisms may occur simultaneously in an HIV infection individual. Recent data suggest that billions of CD4+ T cell may destroyed very day, eventually overwhelming the immune system's regenerative capacity.[68,69,70]

Direct Cell Killing

HIV may be directly involved in destruction of CD4 T cells, the budding of HIV from the cell surface disrupts cellular integrity and the intracellular interactions between gp120 and CD4 may interfere with normal cellular metabolism

Syncytia Formation

The formation of syncytia involves fusion of the cell membrane of the infected CD4 cells, which results in giant multinucleated cells. Large syncytia can form rendering the targeted cells non-functional and susceptible to lysis. Patients infected with SI strains have a more rapid progression to AIDS.

HIV-specific Immune Responses

Both humoral and cellular immune responses contribute to antiviral immunity. Antibodies directed against some regions of the envelope of HIV may gave an additional protective function related to their ability to mediate antibody-dependent cellular cytotoxicity (ADCC) after binding to NK cells, leading to the killing of HIV-infected cells. HIV-specific cytotoxic T-lymphocytes (CTL) may play an important part in the immune response against HIV.

Autoimmune Mechanisms and Molecular Mimicry

Non-polymorphism determinants of major-histocompatibility-complex (MHC) class II molecules share some degree of structural homology with the gp120 and gp41 proteins of HIV, and antibodies to these HIV proteins could therefore cross-react with HLA class II molecules. These antibodies could prevent interaction between CD4 and class II molecules expressed on the antigen-presenting cells, thus impairing the cellular interaction required for efficient antigen presentation and inhibiting antigen-specific functions mediated by helper CD4 T cells.

Anergy

Several soluble HIV proteins appear to be capable of preventing the T cells from proliferating after contact with antigen, thereby reducing the capacity for clonal expansion and replacement of the T cell pool.

Superantigens

The superantigen hypothesis regarding HIV infection stems from the observation that endogenous or exogenous retrovirus-encoded superantigens stimulate murine CD4 T cells *in vivo*, leading to the anergy or deletion of a substantial percentage of CD4 T cells that have the specific variable β-regions. However, rather than that cause deletions of specific subgroups of T cells, it more likely that superantigens serve as potent activators of T cells, rendering them more susceptible to infection with the virus.

Apoptosis

There has been speculation that cross-linking of the CD4 molecule by HIV gp120 or gp120-anti-gp120 immune complexes prepares the cell for programmed death, or apoptosis, which occurs when an MHC class II molecule in complex with an antigen

binds to the T-cell antigen receptor. Apoptosis, like the superantigens, would help each depleted cell be infected with HIV.

The Host Response to HIV Infection

Primary HIV-1 infection is characterized by high levels of infectious HIV-1 in plasma and peripheral blood mononuclear cells (PBMC) during the first few weeks of infection; in one study levels of 1000-10000 tissue-culture-infective doses per millilitre of plasma and 100-10000 infective doses per million PBMC were found.[71] The short and intense period of viral replication is followed in the ensuing weeks by a rapid decline in peripheral blood viral load (at least 100-fold) and concurrent resolution of the acute illness. Viral clearance might be due either to a progressive lack of susceptible target cells or, more likely, to the emergence of an effective host mechanism for viral clearance. The mechanisms of this partial viral clearance during primary HIV-1 infection are not fully delineated, but the effect of humoral, cellular, and cytokine responses to HIV, is certainly far greater and more sustained than that produced by current antiretroviral agents. [71,72]

Humoral Response

Neutralizing antibodies to gp41 and gp120 may contribute to viral clearance, although a direct correlation between decline in viral load and development of neutralizing antibody has not yet been demonstrated. These antibodies tend to develop after resolution of primary infection, suggesting that they are not the main mechanism of viral control. Neutralizing antibodies directed against the initial infecting quasi-species tend to persist for years, but do not evolve to subsequent variants, a possible factor in disease progression.[73] Antibodies that inhibit syncytium formation and antibodies that mediate antibody-dependent cellular cytotoxicity (ADCC) against virally infected cells also develop soon after infection. Their *in vivo* significance is unclear. Antibodies develop to all major HIV proteins, although with disease progression, antibodies to p24 tend to reduce in titer.[73]

Cellular Response

An appreciable CD8+ lymphocytosis occurs during primary HIV-1 infection, generally beginning in the second week after onset of illness. Unlike the development of neutralizing antibodies, the increase in the number of CD8+cells during primary HIV-1 infection occurs concomitant with resolution of clinical symptoms and a decrease in the detectable levels of serum p24 antigen, suggesting that the CD8+ cell response to primary HIV-1 infection has a part in controlling viral replication *in vivo* as it has been shown to have *in vitro*.[74] These CD8+ cells represent HLA-restricted, HIV-specific cytotoxic T cells, and the HIV epitopes are gradually being characterized. Autologous CD8+ cells have been found to inhibit HIV replication *in vitro* by both cell-cell contact and by secretion of cytokines.[73] However, it is possible that some cytotoxic T-lymphocytes (CTLs) are detrimental to the host, as they could recognize and attack an uninfected cell presenting HIV antigens such as gp120 on its surface. Recently, it has been shown that restricted usage of T-cell receptor VB genes at the time of primary infection may correlate with a poor outcome as opposed to those subjects who generate a greater response using several VB genes. As HIV infection becomes chronic the CD8+ CTL response can become paucicIonal and directed towards a few immunodominant epitopes. Variants of the dominant HIV epitopes expressed by quasi-species within the infected host can specifically antagonize recognition of the parental epitope thwarting the ability of HIV-specific CTL to control the infection.[75,76,77]

Cytokines and HIV Disease

Cytokines have a highly complex network to regulate the immune system. This network is redundant and pleiotropic, and operates in an autocrine and paracrine manner to stimulate or suppress cellular proliferation and differentiation, and to modulate immune function.[78,79] Chronic immune activation induced by HIV infection results in dysregulation of the cytokine network. Alteration of cytokine production contributes to HIV pathogenesis by further stimulating viral replication, suppressing the ability of the immune system to mount a strong antiviral response, and inducing cytokine-mediated cytopathic effects.[80,81,82,83]

HIV infection is associated with increased expression of proinflammatory cytokines, especially during the later stages of disease. The high levels of TNF-α, IL-1β, and IL-6 are secreted by peripheral blood mononuclear cells (PBMC) and macrophages from HIV-infected subjects.[80,81] These cytokines are also found at elevated levels in the serum, cerebrospinal fluid, and tissue, particularly in lymphoid tissue, a major site of HIV replication throughout the course of disease.[80]

Proinflammatory cytokines, particularly TNF-α, are considered the most potent HIV-inducing cytokines. Both TNF-α and IL-1β activate the cellular transcription factor nuclear factor (NF) κB, a strong inducer of HIV long terminal repeat (LTR)-mediated transcription. IL-6 alone appears to increase HIV expression primarily by a post-transcription mechanism. The role of endogenous proinflammatory cytokines in the regulation of HIV replication has been demonstrated in several cellular systems *in vitro*. In cultures of HIV-infected macrophages, the viral suppressive activity of several cytokines, such as IL-10 and TGF-β, is attributable large to their ability to inhibit the secretion or activity of HIV-inducing proinflammatory cytokines.[81]

High circulating levels of these cytokines may cause some of the clinical manifestations of primary HIV-1 infection (e.g. fevers, chills, myalgia, headache, fatigue, leucopenia, and weight loss). Such early rises in cytokine levels occur before the development of HIV-specific antibodies and before the rise in CD8+ cells, suggesting that it is a first line of defense against HIV-1 infection; the precise source of each of these cytokines is unclear.

Another major disruption in the cytokine pattern observed in HIV disease is a progressive loss in the ability to produce immunoregulatory cytokines, such as IL-2 and IL-12. Both cytokines are critical for effective cell-mediated immune responses, as they stimulate proliferation and lytic activity of cytotoxic T-lymphocytes (CTL) and NK cells. These cell-mediated immune effectors represent the primary mechanism whereby most viral infections are cleared. In addition, IL-12 is essential for stimulating the production of T helper (Th) 1-type cytokines, including IL-2 and IFN-γ, which favor the

development of cell-mediated immune responses. While it is clear that the Th1 limb of cellular immune responses is impaired during the course of HIV infection, controversy surrounds the proposed dominant of Th2-like response (i.e. secretion of IL-4, IL-5, and IL-10) during progression of HIV disease, although not all data support this hypothesis. This change may determine whether the primary response to HIV is cellular or antibody in nature. However, as cellular responses appear to be the more important, this Th1-Th2 switch may be detrimental to the patient, the reasons for such a switch are unclear. (81,84,85)

Following initial clearance of circulating virus, there is a clinical state of symptomless infection during which HIV can often not be isolated from the circulation and which may last from only a few months to over 10 years. However, during this period of relative clinical latency, HIV accumulates and replicates in lymphoid tissue despite a low viral burden in peripheral blood. Because of its high replicative and mutative capacity, with time a multitude of quasi-species appears in a given individual. HIV appears to be retained within lymphoid tissue by follicular dendritic cells which may present HIV to local immune cells. Concurrently, there is a gradual reappearance of HIV in blood as well as in non-lymphoid tissue coupled with a reduced load in lymphoid tissue. Dissemination of HIV may be a result of the immune system failing to develop effective immunological response to the initial infecting strain may be well preserved.

Recent research suggests that HIV undergoes rapid replication after the initial infection, and destroys many T cells but that it is met with a vigorous response by the immune system, and the viral load drops after the primary illness. However, over time the immune system often, but not always, fails to keep the virus under control, and HIV becomes ascendant in the struggle, the immune system fails, and full-blown AIDS develops. Nowak and Mc Michael[86] put forward the suggestion that the HIV virus continually evolves and in doing so produces such a plethora of new epitopes that the immune system loses its way and fails to keep up with the new targets. However if the initial immune response to conserved epitopes is strong the immune system defense will not be influenced by mutation in other epitopes, and the body should control the virus indefinitely if the response is directed more to non-conserved epitopes the HIV levels

should rise as there is the emergence of mutants that escape recognition and because of increase diversity of viral epitopes.

Chemokines and HIV Disease

When HIV binds to the surface of a T cell or macrophage, a process is initiated whereby the virus is pulled inside the cell. For many years, it was known that HIV could enter human immune system cells by binding to a receptor called CD4.[56] However, other animals whose cells also have CD4 receptors did not become infected with HIV [87] There therefore had to be another necessary binding step.. Recent research has identified two missing-link transport receptors that are found on the cell surfaces of the immune system cells most commonly infected by HIV.[3,4,5,29] Monocytes and macrophages have a different transport receptor than do CD4 T-cells. Both of the receptors and several of the chemokines that stimulate them were identified, and the study of chemokines in HIV disease was born. The new field of chemokine research has progressed remarkably quickly. It has recently been discovered that the congenital absence of a specific co-receptor effectively protects some individuals from HIV infection.

Cell Antiviral Factor

Since 1986, Jay Levy had maintained that CD8 cells (cells that have a CD8 receptor on their surface) secrete a soluble factor, which he called cell antiviral factor (CAF) that inhibits HIV replication in infected cells.[88,89] CAF works independently of the usual CD8 strategy for controlling virally infected cells, which is direct destruction (cytolysis). Indirect evidence for the existence of such a factor or factors was described in studies of long-term non-progressors, and researchers began a lengthy process of eliminating potential candidates. What human factor or factors account for the fact that, in some people, HIV disease progression is very slow or even absent? In December 1995, it was shown that 3 chemokines, RANTES, MIP-1α and MIP-1β, were made by

CD8 cells. All 3 chemokines were able to inhibit HIV replication in test tube studies.[6] RANTES, MIP-1α and MIP-1β were isolated. These chemokines were able to inhibit growth of the HIV taken from infected individuals, but not the laboratory strain of HIV that was commonly studied (HIVIIIB).

Chemokine Receptors as HIV Coreceptors.

Although CD4 was identified as the primary receptor for HIV-1, it was evident that an additional co-receptor was necessary for infection.[29,87] Since CD4 had been found to be necessary but not sufficient for viral entry in model systems, an expression cloning strategy to confer an HIV-1 susceptible phenotype in nonpermissive cells expressing human CD4 was pursued. In 1996, Yu Feng, and colleagues titrated genetic material from HIV-susceptible cell in to mouse CD4 cells so as to discovery precisely what substance conferred susceptibility.[29] Eventually the effort identified a huge membrane spanning protein that they are call fusin (since remained CXCR4). It was chemokine receptor. At about the same time, the receptor for the chemokine that Cocci, and colleagues had found to block HIV infection was identified as CKR5 (now called CCR5).[6,64,90,91] Chemokine and their receptors became a matter of intense interest among AIDS researchers. Many studies have now confirmed CCR5 to be a co-receptor utilized by M-tropic HIV-1 to enter macrophages, as the T-tropic HIV-1 uses CXCR4 to enter T-cells.[3,4,5,29,92] Many strains of HIV-1 have been shown to use other chemokine receptors as co-receptors in *in vitro* studies, including CCR2, CCR3, CCR8, and CCR9.[3,4,5,7,8,91]

Genetic Polymorphisms that Delay AIDS Progression

Fortunately, several relatively large, well-characterized and well-managed cohorts of seroconvertors were initiated during the early years of HIV-1 epidemic. So far four coreceptors/chemokine genetic polymorphism have been identified and correlated with delayed HIV-1 disease progression rate using theses cohorts: CCR5Δ32.[9,10,11]CCR5m303,[11]CCR2-64I,[12,13,14,15] and SDF1-3'A.[12,16,17] Several polymorphism in the gene encoding CXCR4 have been found, but none has proven informative.[9]

CCR5Δ32 and CCR5m303 Polymorphisms

The most studies of importance in host genetic resistant to HIV infection is a 32 nucleotide deletion in the CCR5 gene, CCR5Δ32, that results in truncation of the CCR5 protein and abrogation of its HIV co-receptor. Individuals homozygous for this mutation are highly resistant to HIV infection.[9,10,17] Population surveys of this allele estimate a frequency of approximately 10% in Caucasian population; but have found it to be absent or present at very low levels in Asian and African populations.[18] CCR5-m303 is an independent mutation in the CCR5 gene; a single nucleotide polymorphism, Thymidine (T) to Adenine(A) substitution at position 303, that also lead to lack of CCR5 on the cell surface in homozygotes.[11]

CCR2-64I Polymorphism

CCR2 is a co-receptor for only few HIV strains[4,5,8] CCR2-64I is a point mutation in CCR2 gene , the mutation place guanine (G) instead of adenine (A) at position 190 (counting from the ATG start codon), causing the amino acid isoleucine to substitute for valine at position 64 in protein (CCR2-64I), a conservative change located within the first transmembrane domain of the CCR2 receptor, a region that has complete amino acid sequence conservation with CCR5.[13,14] Analysis of 3,003 patients from five AIDS –study cohorts showed that the mutation had no effect on the incidence of HIV infection.[13] However, infection in persons homozygous or heterozygous for CCR2-64I progressed to AIDS a mean of two to four years later than infection in person with only normal alleles of this gene.[13,14,15] Unlike CCR5Δ32, CCR2-64I is a chemically conservative mutation, one that specifies a full-length CCR2 receptor and permits it to be expressed at normal levels.[15] The only alteration is in the first of the receptor's seven transmembrane region. How then does CCR2-64I delay progression to AIDS?. Although the answer remains uncertain, there are at least three possible explanations. One is that the mutation, though a change in function of CCR2, indeed slows the kinetics of HIV proliferation and spread in its human host. The hypothesis gain support from finding that CCR2 can substitute for CCR5 as a co-receptor for some HIV strain.

(Physiologically, however, CCR2 binds different chemokines than does CCR5. In particular, it binds MCP-1, MCP-2, and MCP-3, whereas CCR5 binds RANTES, MIP-1α, and MIP1-β.) A second idea is that CCR2-64I had no effect but travels with another mutation, perhaps in the CCR5 regulatory region, that more directly affects HIV. A third possibility is that CCR2-64I acts though crosstalk between CCR2 and other host molecules important to HIV infection. So far, an ongoing search has failed to identify any large differences in cellular function corresponding to different CCR2 genotypes. There is, however, early evidence that the presence of mutation in CCR2 slightly diminishes the amount of CXCR4 in cells expressing both receptors.[15] The allele is found in all racial groups tested at the following frequencies: Caucasians, 10%; African Americans, 15%; Hispanics, 17%; and Asians, 26%.[13,14,9,20]

SDF1-3'A Polymorphism

A stromal-derived factor 1 (SDF-1) is the principal ligand for CXCR4, [1,24,40,41,93] a co-receptor with CD4 for T-tropic HIV-1. Cheryl Winkler and colleagues [16] searched for a polymorphism in SDF-1 structural gene variants that might influence HIV-1 transmission or pathogenesis. They screened 1354 of 3526 base pairs (bp) in human SDF-1β transcripts with series of polymerase chain reaction (PCR) primers and a single-stand conformation polymorphism (SSCP) heteroduplex assay in a subgroup of 144 patients enrolled in five epidemiologic cohorts assembled to monitor HIV-1 infection and AIDS. Sequence analysis of common variants revealed a G to A transition at position 801 (counting from the ATG start codon) in the 3' untranslated region (3'UTR) of the reference sequence (Genbank accession number L36033). The polymorphism designated SDF1-3'UTR-801G-A and abbreviated SDF1-3'A, is represented in the SDF-1β transcript but not in the SDF-1α transcript. Because this variant eliminated an *Msp I* restriction sites, a PCR-restriction fragment length polymorphism (RFLP) assay was used for rapid detection of genotyping.[16] SDF1-3'A allele frequency ranges widely across ethnic groups from 3-71%.[12,16,19,20,21,22] It occurs at 16-36 % allele frequency in Caucasians, Hispanic and Asians.[12,16,19,20,21] The highest frequency is observed in Oceanians, including New Guinean, Melanesian and Australia Aboriginal populations (53.6-71.4%), and the lowest frequencies in African Americans

(5.7%) and Africans (3%).[16,22] A increasing cline of the SDF1-3'A frequency form north to south was observed in East Asia.[94]

Like CCR2-64I, the mutation showed no effect on a person's risk of HIV infection. [16] But like CCR2-64I and also like CCR5Δ32, it proved beneficial in delaying progression to AIDS. The benefit, however, was genetically recessive. In 639 HIV-positive persons whose dates of seroconversion could be estimated precisely, those with wild type alleles showed no difference in rate of progression to AIDS from those heterozygous for the mutation. By contrast, those homozygous for SDF1-3'A showed a marked slowing in progression. Indeed, for delaying AIDS, homozygosity was twice as effective as the genetically dominant influence of mutations in CCR5 or CCR2. The effect, moreover, was additive. That is, infected patients with protective genotypes of both SDF1 and a CCR gene appeared to avoid AIDS longer than those with genetic protection of only one kind or the other. The 3' untranslated region of SDF1 is highly conserved in sequence, with 69% identity between its DNA in the human gene and in the homologous mouse gene. Such a level of conservation suggests that natural selection has had a role in preserving the sequence, acting as a constraint against evolutionary divergence. The implication is that the region, despite being untranslated into protein, may have an important genomic function. For example, it might be a target for factors affecting the gene's expression. The simplest idea is that SDF1-3'A upregulates the biosynthesis of SDF-1, making the protein more highly available to complete with HIV for binding to CXCR4, and thereby blocking the emergence of HIV strains reliant on that receptor.

HIV/AIDS Epidemic

As of the end of 1999, an estimated 33.6 million people worldwide (32.4 million adults and 1.2 million children younger than 15 years) are living with HIV/AIDS. More than 69 percent of these people (23.3 million) live in Sub-Saharan Africa; another 18 percent (6 million) live in South and Southeast Asia. Worldwide, approximately one in every 100 adults aged 15 to 49 is HIV-infected. Approximately 46 percent of the 32.4 million adults living with HIV/AIDS worldwide are women; this proportion is growing.

An estimated 5.6 million new HIV infections occurred worldwide during 1999. More than 95 percent of these new infections occurred in developing countries. Through 1999, cumulative HIV/AIDS-associated deaths worldwide numbered approximately 16.3 million –12.7 million adults and 3.6 million children.[95]