Does HIV-1 Infection Always Cause AIDS?

Long-Term Survivors and Exposed-Uninfected Individuals

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Several key discoveries in HIV-1 pathogenesis and its treatment occurred in 1996: chemokines and their receptors were found to be critical components of infection with HIV-1 and the protease inhibitors were shown to dramatically reduce HIV-1 levels in blood. Perhaps the most remarkable finding was the confirmation of the long-felt suspicion that certain individuals are truly resistant to infection with HIV-1. In this review I will focus on the current understanding of HIV-1 pathogenesis especially as it relates to the contribution of host factors. Drawing from a large cohort of HIV-1 infected individuals at the Thomas Street Clinic, in Houston, we had the opportunity to study an interesting subgroup of individuals that developed a disease that phenotypically mimicked Sjögren's syndrome. These individuals were typically long-term nonprogressors, with high CD4 counts and a greatly expanded population of CD8 lymphocytes that were not only circulating, but also infiltrating many organs, resulting in the designation - the *diffuse infiltrative lymphocytosis syndrome* (DILS). Many intriguing issues emerged: Why did some individuals develop this interesting host response to infection with HIV-1? Were host factors like HLA involved? Did these patients acquire distinct strains of HIV-1? Did they really have delayed progression to AIDS? While some of these questions were answered by us [1] and by other investigators [2,3], new questions have arisen: What is the role of chemokines and their receptors in patients with DILS? Do their CD8 cells produce inhibitory chemokines? Are these patients heterozygous for mutant chemokine receptors?

Table 1

Rheumatologic Consequences of HIV-1 Infection	
Event in HIV-1 Infection	**Consequences for Rheumatic Diseases**
Selective CD4 Depletion	• Opportunistic Infections • Amelioration of CD4 Dependent Diseases • No Effect or Worsening of CD4 Independent Diseases
Chronic Immune Response to HIV-1 Antigens	• B Cell Hyperreactivity • Lymphocytic Infiltrative Symptoms • Inflammatory Myopathy • Vasculitides

Table 2

Immunopathologic Classification of Rheumatic Diseases in HIV-1 Infection		
	Reiter's Syndrome	**Rheumatoid Arthritis**
MHC Association	Class I (HLA-B27)	Class II (HLA-DR4)
Autoantibodies	Absent	Present
Response to Selective HIV-1 Immunosuppression	Unchanged or Worsened	Ameliorated
T-Cell Lineage Recognizing MHC-Antigen	CD8 (Cytotoxic)	CD4 (Help)
Implication for Immunopathogenesis	CD8 MHC Class I Driven	CD4 MHC Class II Driven

The Diffuse Infiltrative Lymphocytosis Syndrome (DILS)

In 1985, Solal-Celigny *et al.* [4] reported three cases from France of lymphoid interstitial pneumonitis in the setting of infection with HTLV III/LAV (HIV-1). All three patients had marked lymph node hyperplasia, one had bilateral parotid enlargement and a second case had sicca syndrome. Couderc *et al.* [5] in 1987 studied five French patients with the sicca complex by salivary gland scintigraphy and minor salivary gland biopsy; four patients had enlarged parotid glands but all five had lymphocytic infiltration of the salivary gland along with lymphocytic infiltration of extraglandular sites, including lung, liver, kidney and bone marrow. CD8 lymphocyte counts were > 1000 × 10^6/l cells in two patients. ANA, anti-Ro and anti-La antibodies were uniformly absent. From the United States, Ulirsch and Jaffe [6] also reported three similar cases in 1987. Tunkel *et al.* [7] reported five HIV-1-seropositive patients with bilateral parotid enlargement in 1989. Three of these patients underwent bilateral parotidectomies; all specimens had benign lymphoepithelial infiltration of the parotid tissue with cystic degeneration [7]. In 1989, Schiodt *et al.* [8] reported 12 cases and proposed the term HIV-associated salivary gland disease (HIV-SGD). Nine of their patients had parotid gland enlargement and three had xerostomia alone; labial salivary gland biopsy specimens showed a preponderance of CD8 lymphocytes [8].

Also in 1989 Itescu, Brancato and Winchester [2] reported 12 patients with sicca syndrome in the setting of HIV-1 infection and proposed the term diffuse infiltrative lymphocytosis syndrome (DILS) because of the striking increase in numbers of circulating CD8 lymphocytes. Eleven of these patients were African-American and 10 of the 11 expressed the MHC class II HLA-DR5 histocompatibility antigen, compared with 13 of the 45 matched controls. The estimated relative risk for DILS conferred by HLA-DR5 in African-Americans infected with HIV-1 was 16.9. The patients with DILS were followed for 304 patient-months with only one patient developing an opportunistic infection suggesting that DILS confers a survival advantage in HIV-1 infection. By 1990, Itescu *et al.* [3] had followed 17 patients for 544 months with only one patient developing an opportunistic infection. Lymphocyte phenotype analysis showed a significant expansion of the $CD8^+$ $CD29^+$ subset in patients with DILS when compared to patients with AIDS-related complex (ARC) [3]. Further studies by this group [9] expanded the clinical and immunogenetic spectrum of DILS to include VIIth cranial nerve paralysis, gastric CD8 lymphocyte infiltration, lymphocytic hepatitis, and mastitis. The rare HLA-DRB1*1102 (JVM) subtype of HLA-DR5, which is most common in African-Americans, was shown to be preferentially associated with DILS along with an allele of HLA-DR6 (HLA-DRB1*1301) [9]. These two HLA class II alleles encode HLA-DRB1 chains that share a critical amino acid sequence in positions 67-71, ILEDE, in the third diversity region. HLA-DRB1*1301 was also found in Caucasian patients with DILS along with HLA-DR7 [10], which differs by only one amino acid in position 71 (ILEDR). Furthermore DILS was noted to be negatively associated with HLA-B35, an HLA class I antigen, found to be associated with accelerated progression to AIDS [11]. Itescu *et al.* also detected certain HLA Class I antigens positively associated with DILS; namely, the structurally-related alleles HLA-B45, B49 and B50 were found in 45% of patients with DILS as compared to only 13% of asymptomatic HIV-1 positive controls [12,13].

Additional studies by Itescu *et al.* employing immunophenotyping of circulating and infiltrating lymphocytes [14] and salivary T cell receptor structure analysis [15] in patients with DILS, provided evidence that DILS represents an MHC-restricted, antigen driven oligoclonal selection of $CD8^+$ $CD29^+$ lymphocytes that express selective homing receptors and infiltrate the salivary glands, lung and other organs where they, by as yet undefined mechanisms, suppress HIV-1 replication. Further studies by Itescu *et al.*

[16] showed that the delayed progression to AIDS in patients with DILS was related to retardation of evolution of HIV-1 from the less aggressive M-tropic strain to the highly-replicating T-tropic strain by an effective CD8 lymphocyte response. These investigators were also able to demonstrate that amino acid residues 66-74 on the DRB1 chain of the HLA class II molecules DRB1*1102, 1301 were homologous to six residues in the C-terminal region of the HIV-1 envelope V3 loop on M-tropic strains, suggesting that DILS represents recognition of HIV-1 peptides by homologous self-DR restricted CD8 lymphocytes [17]. DNA sequencing of this highly variable V3 loop (which determines HIV-1 tropism) in patients with DILS and controls confirmed the molecular mimicry between the V3 loop of HIV-1 clones isolated from patients with DILS and the third diversity region of HLA-DRB1*1102 and 1301 [18,19]. This constraint in the V3 loop sequence was not noted in HIV-1 controls without DILS.

Our group studied 35 patients with DILS [1] and had similar findings. These are discussed in the sections below.

DILS-Epidemiology

Of the 32 males and three females, 21 (60%) were African-American, nine (26%) Caucasian, and five (14%) Mexican-American. Twenty-five (71%) were homosexual men; six (17%) had acquired HIV-1 through male-female contact and four (11%) were former parenteral drug users. The average age was 36.9 years (range 24 - 59 years). In comparison to other HIV-1 infected patients at Thomas Street Clinic, patients with DILS were more likely to be African-American (60% vs. 39%), odds ratio 2.32 (1.12-4.81), p = 0.02 and were also more likely to have acquired HIV-1 by male-male transmission (70% vs. 47%), odds ratio 2.82 (1.29-6.29), p = 0.007. The minimal prevalence of DILS in this population was 0.8%.

Table 3

Demographic Features of the DILS Cohort at TSC

	Proportion (%)		
Race	HIV-1-Infected (n = 4100)	DILS (n=35)	***P***
African-American	39	60	0.02
Caucasian	46	26	
Mexican-American	15	14	
Other	<1	0	
Mode of Transmission			
Male-Male	47%	71%	0.007
Parenteral	26%	11%	
Male-Female	24%	17%	
Other	3%	0%	

DILS-HIV-1 Stage

At the time of DILS diagnosis, the average duration of disease (defined by the time since the discovery of HIV-1 seropositivity) was 3.4 years (range 0-10 years). For three (9%) patients, DILS was the presenting manifestation of infection with HIV-1. The mean CD4 lymphocyte count was 342 × 10^6/l cells (range 44-847 × 10^6/l cells) and the mean CD8 lymphocyte count was 1639 ×10^6/l cells (range 560-4994 × 10^6/l cells). The mean CD4 to CD8 ratio was 0.23 (range 0.05-0.57). Only four (11%) patients had experienced an opportunistic infection or neoplasm (two patients each with pulmonary tuberculosis and Kaposi's sarcoma). Twenty-four (69%) patients were in stages A1, A2 or A3 and 13 (37%) met the 1993 CDC case-definition of AIDS (i.e. stage A3, B3, C1, C2, C3) [20].

DILS-Glandular Features

Bilateral parotid gland enlargement was present in all DILS patients, and was massive in 15 (43%). Sixteen (46%) patients also had submandibular gland enlargement and 11 (31%), lacrimal gland hypertrophy. Only two (6%) patients had no sicca symptoms; in an additional eleven (31%) sicca was mild (patients admitting to this symptom only upon direct inquiry). Nineteen (54%) patients had moderate sicca symptoms and admitted to frequent fluid intake and/or used artificial tears. In three (10%) patients sicca symptoms were particularly severe (sicca was one of the chief complaints).

Twenty-eight (80%) patients underwent minor salivary gland biopsies which were histologically graded by Daniels' criteria [21] ,a method in which a focus score is assigned to the histological findings. The focus score is the number of focal inflammatory cell aggregates containing 50 or more lymphocytes, plasma cells, or macrophages in a 4 mm^2 area of labial salivary gland; a focus score of 2 or greater is widely accepted as the threshold for diagnosis of the salivary component of Sjögren's syndrome [21]. The mean focus score was 4.25 (range 0-10). Three patients with a focus score of less than two and the seven patients who did not undergo a minor salivary gland biopsy either had positive Gallium-67 scintigrams and/or an extraglandular site of lymphocytic infiltration (one patient with VIIth nerve palsy) or had previously undergone a diagnostic parotid gland biopsy (two patients). Overall, seven patients underwent Gallium-67 scintigraphy, all showing intense uptake of the tracer in the salivary and lacrimal glands as well as in the nasal mucosa. Six patients developed unilateral cystic enlargement of a parotid gland; these resolved with aspiration but swelling recurred in two patients. Cytologic examination revealed benign lymphocytes in all cases.

DILS-Extraglandular Features

The most common extraglandular site of CD8 lymphocyte infiltration was the lung, manifested by lymphocytic interstitial pneumonitis in 11 (31%) patients. The most common presentation was dyspnea on moderate exertion and diffuse infiltrates on chest radiographs. Typically these patients had previously been extensively investigated for pulmonary infections and tumors. Eight patients (23%) had hepatomegaly and abnormal liver function tests not attributable to other causes. One patient underwent liver biopsy revealing lymphocytic infiltration. Another patient progressed to advanced hepatic failure in the absence of hepatitis B and C infection and developed terminal hepatic encephalopathy. A distal renal tubular acidosis was noted in two patients (6%). Six patients (17%) had persistent generalized lymphadenopathy. A symmetrical polyarthritis occurred in two (6%) patients. Three (9%) patients had an infranuclear (Bell's) palsy of the seventh cranial nerve (bilateral in one case).The mean serum creatine kinase (CK) level at 536.2 u/ml was positively skewed because of nine (26%) patients with CK elevations $\geq$ twice the upper limit of the normal range, two being > 2500 u/ml. Five of these nine patients had proximal muscle weakness. Skeletal muscle biopsy in four of these five patients showed inflammatory myositis. Only three patients had detectable ANA titres, all below 1:320. All patient sera tested negative for anti-Ro(SS-A) and anti-La(SS-B) antibodies.

Table 4

Clinical Features of 35 Patients with DILS					
Glandular			**Extraglandular**		
Glandular Enlargement			Lymphocytic Interstitial Pneumonitis	11	(31)
Parotid	35	(100)	Lymphocytic Hepatitis	8	(23)
Submandibular	16	(46)	Myopathy	9	(26)
Lacrimal	11	(31)	Persistent Generalized Lymphadenopathy	6	(17)
Sicca Symptoms	33	(94)	Peripheral VIIth Nerve Palsy	3	(9)
Salivary Gland Biopsy	28	(80)	Renal Tubular Acidosis	2	(6)
Focus Score	Mean 4.25		Symmetrical Polyarthritis	2	(6)
	Range 0-10				
Numbers in Parenthesis are percentages					

DILS-MHC Class II Alleles

Thirty-one (89%) patients underwent DNA typing for DRB1, DQA and DQB MHC class II alleles by sequence-specific oligotyping. Sixteen (52%) patients expressed the previously reported DRB1 alleles, DRB1*0701 (subtype of DR7), DRB1*1301, DRB1*1302, (both subtypes of DR5) DRB1*1102 (JVM subtype of DR5) [2,3,9,22] or expressed DRB1*1201 (subtype of DR5), DRB1*0803 (subtype of DR8) or DRB1*0103 (DR Br), all of which share a common amino acid sequence (ILEDE or ILEDR) in the third hypervariable region of the DRB1 chain, position 67-71. In addition, eleven patients expressed DRB1*1501 or 1503 (both subtypes of DR2).

Table 5

Comparison Between DILS and Sjögren's Syndrome		
	DILS	**Sjögren's Syndrome**
Age	Younger	Older
Sex	M>F	F>M
Race	African American	Caucasian
Glandular Enlargement	Massive	Minor
Sicca	Mild	Severe
Extraglandular Disease	Frequent	Rare
Infiltrating Cell Phenotype	$CD8^+$ Lymphocyte	$CD4^+$ Lymphocyte
Antinuclear Antibodies	<10% Low Titre	80% High Titre
Anti-Ro	Absent	50-80%
HLA Associations	DR5, DR6, DR7	DR2, DR3

HIV-1 Pathogenesis

Overview

AIDS is caused by a member of the lentivirus family, the human immunodeficiency virus, type 1 (HIV-1). A similar disease is caused by a closely related virus, HIV-2. When AIDS was first recognized, its preferential replication in $CD4^+$ cells suggested that CD4 antigen was the receptor for viral infection. Subsequent work showed that viral surface glycoprotein (gp120) binds to CD4 via its third hypervariable domain, the V3 loop [23]. CD4 antigen is expressed on a variety of cells including T lymphocytes and macrophages both of which are susceptible to infection with HIV-1. Interestingly, small variations in the V3 loop were associated with distinct cellular tropisms of HIV-1 [24,25], leading to the recognition of distinct macrophage tropic (M-tropic) and T lymphocyte tropic (T-tropic) strains of HIV-1 [26]. Moreover, it became apparent that M-tropic strains initiate new infection [27,28] and that T-tropic strains subsequently evolve by mutation of the initially established M-tropic strains [29]. As HIV-1 infection progresses to AIDS, increasing numbers of T-tropic strains are detected, correlating with disease stage [30]. While some investigators dismissed this phenotypic switch from M-tropic to T-tropic strains as an *in vitro* phenomenon [31,32], recent discoveries of the role of chemokines and their receptors in HIV-1 pathogenesis [33] have provided firm evidence that such a switch does indeed occur *in vivo*.

There is substantial heterogeneity in the clinical response to HIV-1 infection, with some individuals progressing rapidly to AIDS, while others remain in the asymptomatic phase for several years. While this may simply represent a stochastic phenomenon, the recent finding of rare individuals that appear to be actually resistant to infection with HIV-1 strongly suggests that this clinical heterogeneity is based on varying levels of protective immunity to infection with HIV-1 [34]. This review will focus on the recent progress in understanding immunologic and viral characteristics of the host response to HIV-1.

Viral Factors in HIV-1 Pathogenesis

The Viral Envelope V3 Loop - Primary Determinant of Cell Tropism in HIV-1

The HIV-1 isolates can be divided into two major subgroups on the basis of their cellular host range *in vitro* [25,36]. Macrophage tropic (M-tropic) isolates efficiently infect both macrophages and CD4 peripheral blood lymphocytes (PBL's) but are unable to replicate efficiently in many transformed cell lines. A second class of viruses termed T lymphocyte tropic (T-tropic), replicate efficiently in both PBL's and transformed T cell lines but poorly in macrophages. M-tropic strains generally do not induce syncytium formation and thus the term non-syncytia-inducing (NSI) has been used synonymously with M-tropism. T-tropic strains, in contrast, typically induce syncytia formation and have been referred to as syncytia-inducing (SI) strains. This may not be entirely correct, as the transition from M-tropic/NSI strains to T-tropic/SI strain may produce dual tropic viruses that are M-tropic despite SI characteristics

Table 6

	M-Tropic	T-Tropic
Infection of $CD4^+$ PBLs	✔	✔
Infection of Macrophages	✔	
Replication in T Cell Lines		✔
Replication in Macrophages	✔	
Syncytia Formation		✔

The HIV-1 envelope gene (*env*) codes for gp120 and gp41. Sequences encoding CD4 binding and membrane fusion are conserved while the sequence determining tropism is variable. The primary determinant responsible for HIV-1 specific cell tropism has been

mapped to the third variable domain (V3 loop) of gp120, the surface glycoprotein of HIV-1 [24,36]. The V3 loop is a disulfide-linked loop of 34-37 amino acids with a conserved motif at its tip. It is the principal neutralizing domain of gp120 [23]. Very recently, the binding site on gp120 for the chemokine receptor crucial to M-tropic strains, CCR-5 (*vide infra*) has been narrowed down to a fragment retaining the CD4-binding site and overlapping epitopes within the V3 loop [37].

Figure 1

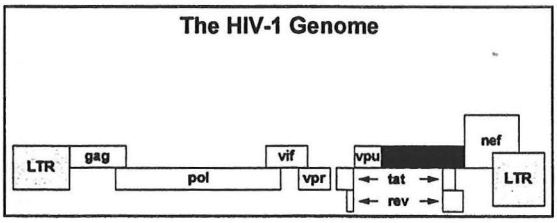
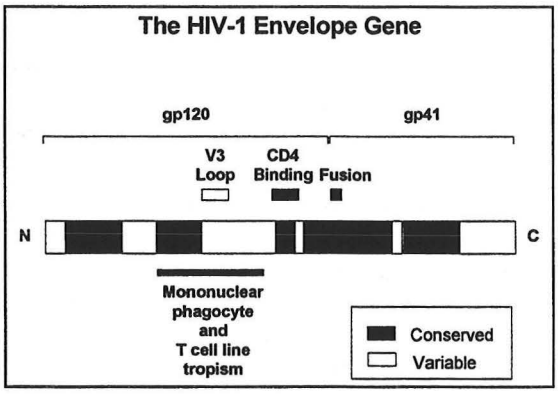


Figure 2



M-tropic Strains Initiate HIV-1 Infection

Roos *et al.* studied virologic and immunologic events in primary HIV-1 infection and could detect only M-tropic strains in 16/19 individuals [28]. Studies of transmitter-recipient pairs of individuals showed that both T-tropic and M-tropic strains were transmitted, implying that T-tropic strains were suppressed while M-tropic strains established infection. CD8 numbers were highest in those individuals who had the most symptoms, likely representing an antiviral immune response. A similar study of transmitter-recipient pairs by van't Wout *et al* [27] used actual sequence analysis of the V3 loop. Sexual, parenteral and vertical transmission routes were studied, showing that while both M-tropic and T-tropic strains were transmitted, a selective expansion of M-tropic variants seemed to occur in a newly infected individual

Mononuclear Phagocytes are Important Reservoirs of HIV-1

The CD4 T-lymphocyte was the first cell identified as a target for HIV-1 infection *in vivo*. However, cells of the mononuclear phagocyte lineage are the predominant cell type producing HIV-1 in extravascular tissues. The relative importance of this was underestimated because isolation of HIV-1 from peripheral blood mononuclear cells (PBMCs) revealed an infection frequency of 1/10,000 or less [31]. In a study of subjects with long-term nonprogressive HIV-1 infection [35], the virus was consistently cultured from mononuclear cells in lymph nodes while plasma levels of viral burden in PBMCs were consistently low. Macrophages are likely the reservoirs of HIV-1 outside the blood and are probably important in carrying infection to different organs (the Trojan horse metaphor). Several manifestations of HIV-1, distinct from the severe immunodeficiency caused by T helper cell depletion, have been associated with mononuclear phagocyte infection: for example, AIDS dementia, neuropathy, enteropathic and wasting syndromes are all thought to be direct consequences of infection of mononuclear phagocytes.

Progression of Disease is Associated with a Shift from Monocytotropic to T cell Tropic Virus Populations

Shuitemaker and his colleagues [30] from the University of Amsterdam postulated that since M-tropic strains preferentially establish early HIV-1 infection, peripheral blood T cells are infected by progeny from HIV-1-infected tissue macrophages. To determine whether M-tropic variants are indeed most predominant in the asymptomatic phase of infection, dynamics of virus populations were studied at the clonal level

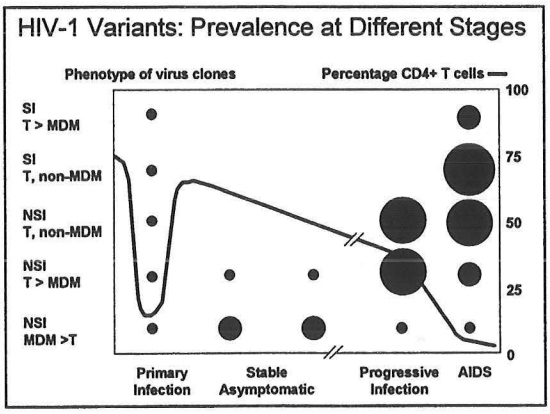
in two asymptomatic and two symptomatic individuals. In addition an accidental donor-recipient pair was investigated.

In the asymptomatic individuals the majority of clones isolated were M-tropic, NSI strains, while the reverse phenomenon was observed in the symptomatic individuals with a high-yield recovery of predominantly T-tropic, SI strains. Interestingly, while the T-tropic clones greatly outnumbered the M-tropic clones, the yield of the latter was comparable to the yield obtained from the asymptomatic individuals indicating that the increase in frequency of HIV-1 infected cells was a result of the selective expansion of T-tropic clones.

Studies on the accidental donor-recipient pair, in which the recipient was accidentally infected by a minute amount of blood from his donor, a patient with advanced AIDS, revealed a shift from T-tropic to M-tropic populations. Eventually a shift back to a T-tropic virus population was observed.

Finally, the hypothesis that M-tropic variants persist in tissue during all stages of infection, was tested by analyzing parallel virus isolation from cells in bronchioalveolar lavage and peripheral blood in one AIDS patient. While 85% of the clones isolated from bronchioalveolar lavage were M-tropic, the isolation rate from peripheral blood was only 34%.

Figure 3



Shuitemaker came to many interesting conclusions based on these data. An inverse correlation was noted between the proportion of M-tropic clones and progression of disease. Persons with AIDS had a lower *proportion* of M-tropic clones while the *frequency* of M-tropic clones remained relatively constant. This change in proportion came about as the result of the selective expansion of T-tropic clones. Shuitemaker postulated that while both M-tropic and T-tropic strains are transmissible, the T-tropic strains are likely eliminated by a competent immune response while M-tropic strains, by virtue of their capacity to replicate in macrophages, are adapted to survival in early HIV-1 infection. The length of the asymptomatic period may be critically dependent on the effectiveness of the immune system to eliminate T-tropic variants. He also suggested that rapid progression may result from intermediate M-tropic strains with SI characteristics. The results in this study also suggested that initial macrophage infection results in widespread dissemination of the virus into tissue compartments from where macrophages act as reservoirs of HIV-1, generating the emergence of new, more virulent T-tropic, SI strains.

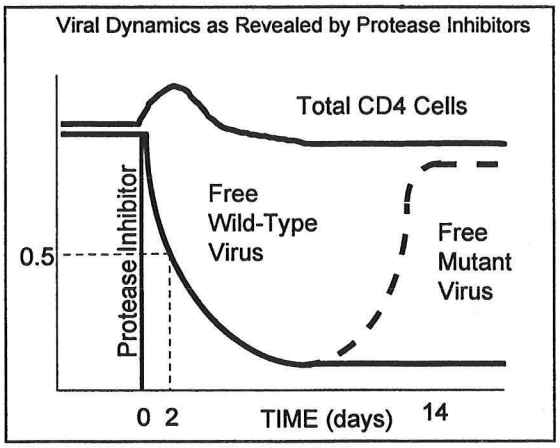
Complementing the above study, McNearney *et al.* [29], evaluated HIV-1 infected patients longitudinally. This study sought to determine whether sequence heterogeneity of the V3 loop correlated directly with HIV-1 stage. It was based on the knowledge that the sequence of the V3 loop (which is involved in fusion of the virus) determines tropism. McNearny predicted that sequence heterogeneity should correlate directly with stage of HIV-1 infection. While the tip of the V3 loop is highly conserved, flanking regions demonstrate considerable variability. This is the result of the error prone HIV-1 reverse transcriptase and selective pressures exerted by the host immune system. Interestingly M-tropic strains demonstrate low sequence variability, while the opposite is true for T-tropic strains. The results showed that low V3 sequence heterogeneity is found in the early stages of infection with HIV-1, and when the *same individuals* were tested at later dates, disease progression was associated with increasing V3 sequence heterogeneity. At later stages of disease, 4 to 14-fold more sequence diversity was noted than at earlier stages of infection. In several studies

of V3 sequences no inactivating mutations were detected, suggesting that variants arose as a result of phenotypic selection. Despite the fact that V3 sequences have considerable *inter*patient variability, V3 sequences obtained from different subjects during the early stages of infection were remarkably similar, again implying that a conserved M-tropic strain initiates infection and the V3 sequence is likely critical in establishing initial infection

Viral Dynamics in HIV-1 Infection

The mean duration to AIDS from primary infection with HIV-1 is typically ten years. Most individuals, following primary infection, enter what has been called the "asymptomatic" or "latent" phase of infection that eventually culminates in a precipitous decline in CD4 counts heralding the onset of AIDS. The terms "asymptomatic" and "latent" implied that viral replication was also "latent". Two studies published in *Nature* in January 1995 [38,39] effectively dispelled this misconception. Using anti-HIV-1 drugs as probes, and mathematical kinetic models, the studies showed that billions of virus particles are continuously produced by newly infected cells and then rapidly cleared by the immune system. In Ho's study [39], the researchers gave a protease inhibitor to 20 HIV-1 infected people who initially had CD4 counts between 36 and 490. Shaw's group [38] studied 22 HIV-1 infected persons with CD4 counts ranging from 18 to 251. These persons received either a protease inhibitor or nevaparine (a reverse transcriptase inhibitor). Both groups analyzed plasma levels of HIV-1-RNA which dropped exponentially when drug treatment began. Every 2 days the plasma HIV-1-RNA was reduced by half, indicating that the composite lifespan of plasma virus and virus-producing cells is remarkably short (half-life ~ 2 days). Viral nucleotide sequencing studies indicated that there was almost complete replacement of wild-type virus in plasma by drug-resistant variants in fourteen days. Both researchers concluded that HIV-1 viremia is sustained primarily by a dynamic process involving continuous rounds of *de novo* virus infection and replication. The sharp drop in HIV-1-RNA was accompanied by dramatic rises in CD4 counts. These studies introduced several new concepts: HIV-1 has enormous potential to evolve in response to selection pressures exerted by the immune system; the viral load is largely a function of virus production and is not dependent on the stage of infection; CD4 depletion seen in AIDS is primarily a consequence of the destruction of these cells induced by HIV-1, not a lack of their production. David Ho invoked an interesting analogy: the CD4 lymphocyte depletion seen in advanced HIV-1 infection may be likened to a sink containing a low water level, with the tap (CD4 cell repletion) and drain (CD4 cell destruction) both equally wide open. Since the capacity of the immune system to regenerate is not infinite, it is not difficult to see why the sink eventually empties.

Figure 4

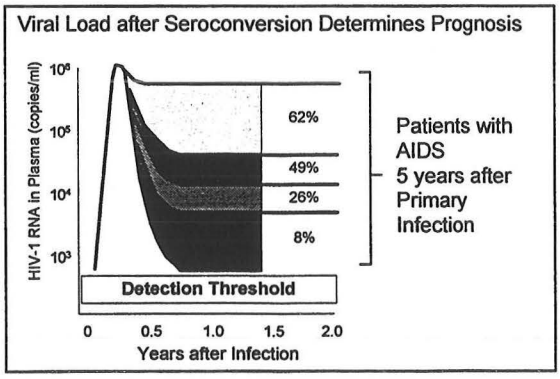


Prognosis in HIV-1 Infection is Predicted by the Quantity of Virus in Plasma

Given the variability in progression to AIDS, many clinical and laboratory markers have been used to estimate prognosis in HIV-1 infection. Although the single best predictor of AIDS onset is the percentage or absolute numbers of circulating CD4 lymphocytes, a marker that could be used to predict prognosis before there has been substantial destruction of the immune system would be useful. Studying 209 HIV-1 infected gay men enrolled in the Pittsburgh portion of the Multicenter AIDS cohort study (MACS),

Mellors and his colleagues [40] showed that baseline viral load provided excellent discrimination of both time to AIDS and time to death. When HIV-1 enters a new host, there is typically a burst of viremia, which is then inhibited by the onset of immune responses. The subsequent level of plasma virus is a reflection of the equilibrium reached between the virus and the host after the initial battle and is generally maintained for years. A single measurement of the plasma viral load after seroconversion predicted the subsequent risk of AIDS or death. The findings were truly striking. For example, 5 years after entering the study only 8% of patients with a viral load of less than 4350 copies of viral RNA per mm^3 of plasma progressed to AIDS, whereas 62% of those with viral loads exceeding 36,270 copies had done so. In contrast to the close relationship between virus load and outcome, baseline CD4 counts failed to show a relationship with the risk of AIDS or death. The risk gradient between viral load and outcome was still evident 10 years after the baseline determinations. The investigators thus concluded that the extent of viremia, measured as HIV-1 RNA, is the best available surrogate marker of HIV-1 disease progression. Along with CD4 counts viral load measurements have become very useful tools to assign risk and assess the benefits of interventions. John Coffin has provided a crude but useful analogy: The development of AIDS can be likened to an impending train wreck, where the viral load indicates the speed with which the train is headed for catastrophe and the CD4 cell count marks the distance from the site of doom.

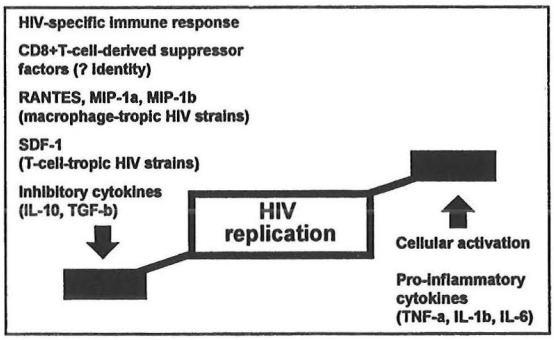
Figure 5



Host Factors in HIV-1 Pathogenesis

The level of HIV-1 replication in patients reflects a balance between stimulatory and inhibitory host factors. Stimulatory host factors include cellular activation and the action of proinflammatory cytokines (TNF-α, IL-1β, IL-6). Inhibitory host factors include CD8 T cell-derived suppressor factors (including the action of inhibitory chemokines), and certain inhibitory cytokines (IL-10, TGF-β). Cellular activation and the role of cytokines has been recently reviewed elsewhere [33]. This review will focus on the role of the host immune response to infection with HIV-1, specifically with regard to CD8 T cells, HLA polymorphisms and the emerging field of chemokines and their receptors.

Figure 6



Variations in the Rate of Disease Progression after HIV-1 Infection

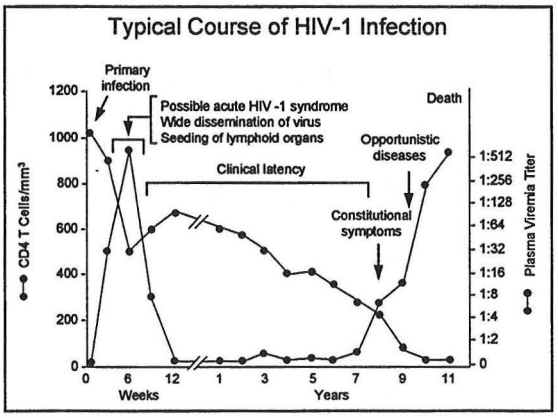
A spectrum of clinical courses can occur after HIV-1 infection. Approximately 10% of HIV-1 infected subjects progress to AIDS in the first 2 to 3 years of HIV-1 infection and are known as rapid progressors [41]. Five to 10% of HIV-1 infected individuals are clinically asymptomatic after 7 to 10 years and have stable peripheral blood CD4 counts.[35,42]. These subjects have been referred to as long-term non-progressors, or healthy long-term positives. Data form the Multicenter AIDS Cohort Study (MACS) suggest that 10 to 17% of HIV-1 infected patients will be AIDS free 20 years after infection [41].The remaining 80 to 85% are projected to develop AIDS within a median

time of 10 years. Such persons are typical progressors.

Typical Progressors

Most patients will develop an infectious mononucleosis-like syndrome three to six weeks after initial infection. This period is associated with high levels of viremia and within one week to three months there is an immune response to HIV-1. During this phase there is widespread dissemination of HIV-1. Detectable viremia declines markedly or disappears within weeks to months after the acute syndrome subsides. The fall in viremia correlates best with the appearance in peripheral blood of anti-HIV-1 major histocompatiblity (MHC) class I-restricted CD8 cytotoxic T cells (CTLs). During acute HIV-1 infection there is an oligoclonal expansion of HIV-1-specific CTLs that are thought to be important in the immune response to HIV-1 during the so-called latent phase of the infection. Serum antibodies capable of neutralizing selected primary HIV-1 isolates *in vitro* are detectable in most seropositive individuals, but as yet there is no direct unequivocal evidence supporting a role for neutralizing antibodies *in vivo*.

Figure 7



As discussed above, while the patient may be clinically latent, there is massive ongoing viral replication, matched by an equally brisk immune clearance of the virus. Thus there is probably never a true state of microbiologic latency. There continues a progressive erosion of the immune system, the most obvious manifestation of which is a gradual decline in CD4 counts peripherally. The inevitable outcome of this decline is clinically apparent disease or an AIDS-defining illness. Progression to AIDS is associated with generalized activation of the immune system. While several components of this generalized activation are reflections of the immune system's efforts to control viral replication, both humoral and cell-mediated arms become severely impaired and immune activation may ultimately have inappropriate and detrimental effects [43].

Rapid Progressors

Such individuals are characterized by a rapid decline in CD4 peripheral T cells, usually within 2 to 3 years following primary infection [41]. In general, rapid progressors have lower levels of antibodies to HIV-1 proteins, and CTL levels are generally lower [42,44]. With the discovery of non-cytolytic CD8 T cell-secreted inhibitors of HIV-1 replication, [45], it will be interesting to see if the production of such inhibitors is defective in rapid progressors. Other characteristics of rapid progressors include elevated numbers of T cells expressing $CD8^+$, $CD38^+$, and DR [46], and an association with certain MHC alleles (*vide infra*). A uniform finding in rapid progressors has been a high viral load, that fails to fall after primary infection [40].

Nonprogressors

Despite all the evidence that all stages of infection with HIV-1 infection are characterized by extensive viral replication [38,39], this group of individuals remains clinically healthy and does not have the declining CD4 lymphocyte counts that are evident in typical progressors. The eventual fate of this group remains unknown. This is likely quite a heterogeneous group; perhaps some of these individuals will not progress and will have normal life expectancy, while others may simply represent the tail of a normally distributed response to HIV-1 infection. Regardless, this group has attracted a lot of attention.

Much of what we know regarding the natural history of HIV-1 infection comes from a cohort of 6,705 homosexual and bisexual

men recruited at the San Francisco City Clinic between 1978 and 1980 for studies of hepatitis B transmission. [42]. A subset of hepatitis B virus (HBV) seronegative men were recruited into a clinical trial of HBV vaccine from 1980 to 1983. Unused sera from these studies was frozen and subjects were recruited into a prospective study of HIV-1 infection. Stored sera was tested for HIV-1 antibodies and participants were evaluated prospectively every 6 to 12 months. The researchers identified 588 men with well-documented dates of seroconversion. Sixty-nine percent had developed AIDS after 14 years of follow-up. Forty-two men (8%) were healthy long-term positives, defined as HIV-1 infection >10 years and CD4 counts >500 $\times$ 10^6/l. When compared to progressors, epidemiologically, there were no differences with regard to race, socioeconomic status, number of sexual partners or lifestyle factors such as diet, sleep habits, vitamin intake or exercise. The healthy long-term positives did have higher CD8 lymphocyte counts (mean CD8 1077 $\times$ 10^6/l vs. 689 $\times$ 10^6/l; P <0.001).

Studies of other cohorts (transfusion recipients, hemophiliacs and vertically transmitted infants) have found similar patterns of immune competence in a small percentage of infected patients [41,47].

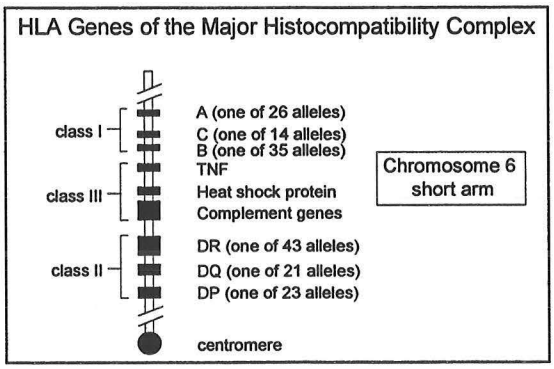
Nonprogressors have high levels of $CD8^+$ $CD38^-$ CTLs [48], high peripheral blood $CD8^+$ MHC class I-restricted anti-HIV-1 CTL levels that do not fall over time [35], strong non-MHC-restricted HIV-1 suppressor activity [49-51] and high levels of antibodies to HIV-1 [35]. Viral loads are typically low [40,49]. The structure and function of lymph node germinal centers are maintained and the follicular dendritic cells are preserved [35]. There is also evidence that some nonprogressors are infected with constitutively less pathogenic HIV-1 strains [52]. In this report a nonprogressor is infected with an HIV-1 strain that has a deletion of portions of the critical *nef* gene of HIV-1. Monkeys infected with simian immunodeficiency virus (SIV) harboring *nef* deletions also have nonprogressive infection. The finding of *nef* mutants in humans is likely a rare finding. It is interesting to speculate that the *nef* mutant may prevent a second infection. That this scenario is possible is suggested by well documented reports that people infected with HIV-1 virtually never show signs of having more than one circulating strain of virus, although sequences within any one individual may vary continually, but are nonetheless closely related. Thus, the virus with *nef* deletions may act as a type of attenuated-virus vaccine.

Hence, nonprogessors likely represent a heterogeneous group in whom host responses and the level of pathogenecity of the virus variably contribute to the state of nonprogression of HIV-1 infection.

MHC Class I and Class II Alleles Modulate the Host Immune Responses to HIV-1 Infection.

There is increasing evidence that the remarkable polymorphisms of MHC genes are maintained through natural selection by infectious pathogens. HLA associations may be more readily identified with a new infectious agent such as HIV-1, because the host repertoire of MHC types has not had time to adjust through elimination of susceptible variants [53].

Figure 8

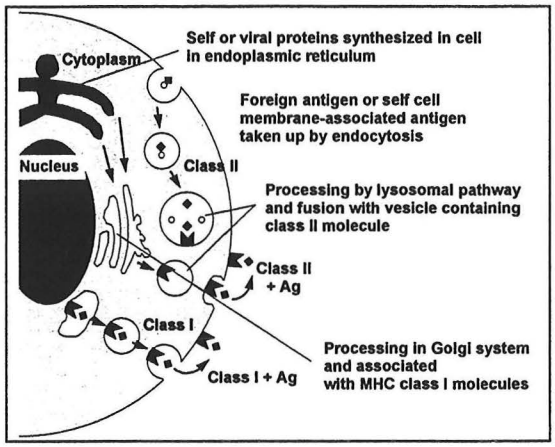


The MHC class I and class II genes play a major role in determining the specificity of T and B cell antiviral immune responses. A number of MHC alleles have been described that may influence predisposition or protection against HIV-1 infection or disease. [11,13,53-57].

There are several mechanisms whereby MHC-encoded molecules might predispose an individual to rapid or nonprogression to AIDS. Having certain MHC class I or class II alleles could protect against HIV-1 progression by serving as a restricting element for one or several immunodominant HIV-1 T-helper or CTL epitopes, thus promoting a salutary immune response to HIV-1 and protection from progression to AIDS. Similarly the lack of a protective MHC allele could predispose to developing AIDS because of the lack of salutary responses to HIV-1 [53]. Having a certain MHC class I or class II allele could predispose an individual to pathogenic immune responses against a viral epitope. The HLA-HIV-1 disease associations may not be absolute and might reflect the associations of genes linked to or within the MHC. For example, genetic markers linked to the HLA A-1, Cw7, B8, DR3 haplotype such as C4 null alleles, and polymorphisms in TNF α promoter may be the more important determinants affecting outcome in HIV-1 infection. Finally, roles for transporter-associated with antigen-processing (TAP) genes in combination with certain haplotypes have been shown to influence the course of HIV-1 infection [53].

However, many of these studies are relatively small-scale and often provide conflicting data. Two concerns are raised by such heterogeneity: did the associations arise by chance and might they result from unrecognized differences in gene frequencies between population subgroups that are unequally represented in the samples compared? An alternative approach would be to perform genetic linkage studies in families. Sibling pairs in HIV-1 infection are rare but Kroner *et al.* [58] identified 95 pairs of hemophiliac brothers with HIV-1 infection and found that sibling pairs sharing one or two haplotypes were significantly concordant in CD4 decline and progression to AIDS.

Figure 9. Antigen Presentation



HIV-1-Specific Cytotoxic T Lymphocytes (CTLs)

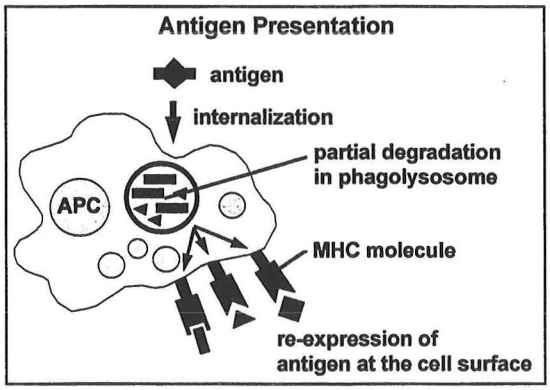
Most viral infections are controlled and may be eliminated by the host immune system. While neutralizing antibodies may prevent viral infections, it is the cellular arm of the immune system that is largely responsible for battling the virus after infection is established. CTLs are thought to be critical players in this endeavor [59,60]. In order to hold HIV-1 infection in check for several years, the immune system of the infected host must mount an effective CTL response. HIV-1, however, elaborates sophisticated strategies to persist and expand over this period. Considering that HIV-1 replicates at an extraordinary rate, continually producing new "escape" variants, it is a tribute to the immune system that most individuals survive for ten years or longer.

Follicular Dendritic Cells in Lymph Nodes Present HIV-1 Antigens to Immune Cells

After HIV-1 enters through the circulation or mucosa, it is carried, like other invading microbes to regional lymph nodes, where follicular dendritic cells trap antigens in the environment of the germinal center and present processed HIV-1 antigens to competent immune cells. Before an antigen can be recognized by a T cell, it must be processed and then expressed on the surface of the antigen-presenting cell in association with either an MHC class I or class II molecule. $CD4^+$ cells are MHC class II restricted. This means that

they will only recognize antigens presented in association with HLA class II molecules. Similarly $CD8^+$ cells are MHC class I restricted and will only respond to antigens presented on the surface of HLA class I molecules. Most CTLs are $CD8^+$ and are therefore MHC class I restricted. Unlike MHC class II molecules, which have a restricted distribution, MHC class I molecules are expressed on all nucleated cells. Since HIV-1 infects a variety of cells, it makes sense that MHC class I restricted cells like CTLs are the prime defense mechanism against HIV-1. CTL T cell receptors are then able to recognize viral epitopes presented on the surface of virally infected cell and destroy that cell. In this manner CTLs diminish viral replication. A corollary of the MHC class I restriction of CTLs is that one would predict that the host's MHC repertoire would influence this mechanism of inhibition of viral replication since the absence of a particular class I allele may cause an important viral epitope to not be recognized (*vide supra*).

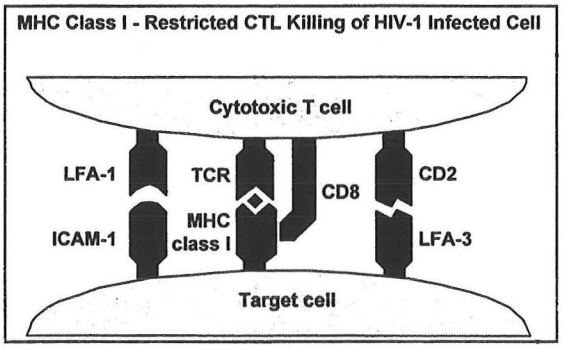
Figure 10



Do HIV-1-Specific CTLs Control Primary Infection and Disease Outcome?

As discussed above, HIV-1-specific CTLs are clearly involved in suppressing the initial burst of viremia in primary HIV-1 infection [44,48]. The viral load that remains after this initial battle is an excellent predictor of the subsequent course of HIV-1 infection [40]. Thus, it stands to reason that persons with an effective anti-HIV-1 CTL response will be more likely to have lower viral loads after seroconversion and will have a better prognosis. Nonprogressors have been shown to generally have higher CD8 T lymphocyte counts in a number of studies [35,42,48,49]. Additionally, studies in subjects with primary infection [28] have shown that those with the most symptoms (hence, vigorous immune response) were more likely to have higher CD8 counts. Yet, the high turnover of HIV-1 [38,39] at all stages of infection indicates that CTLs are not efficient at eradicating the virus [59,60].

Figure 11

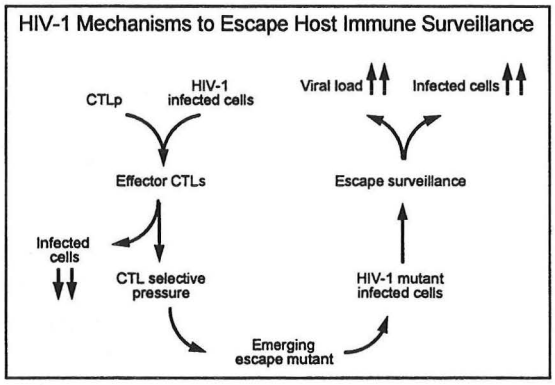


HIV-1 Stays One Step Ahead of the Immune System

HIV-1 replicates at an enormous rate, generating 10^9 - 10^{10} virions per day [38,39]. As a consequence, with a genome of 10^4 bases, even a fairly modest mutation rate of 10^{-5} per base per generation would yield 10^8 - 10^9 mutants per day. Thus, there are endless possibilities for rapid adaptation of the virus to the host immune response, with the formation of escape mutants. Furthermore, HIV-1 infects the heart of the immune system, the CD4 T lymphocyte, further undermining the effectiveness of the immune response against it. Nowak [61] has proposed a mathematical model based on this dynamic evolution of HIV-1. This model confers a survival advantage to a viral variant which persists until discovered and responded to by the immune system. This response would reduce the viral load for a time, but meanwhile other escape mutants would begin to break out, and the cycle would continue, preventing full elimination of the infection. The model additionally distinguished between two kinds of immune responses: those recognizing epitopes that

undergo mutation readily and those recognizing epitopes that are conserved (because the virus cannot tolerate their loss or mutation). A computer simulation managed to reproduce the typical long delay between infection with HIV-1 and the eventual sharp rise in viral levels. The model also provided an explanation for why the cycle of escape and repression does not go on indefinitely but culminates in uncontrolled viral replication, the almost complete loss of the helper T cell population and the onset of AIDS. Such a model predicts that if the initial CTL response to conserved epitopes is strong, the efficiency of the defensive attack on HIV-1 will not be undermined much by mutation in other epitopes. In such individuals progression to AIDS is likely very slow.

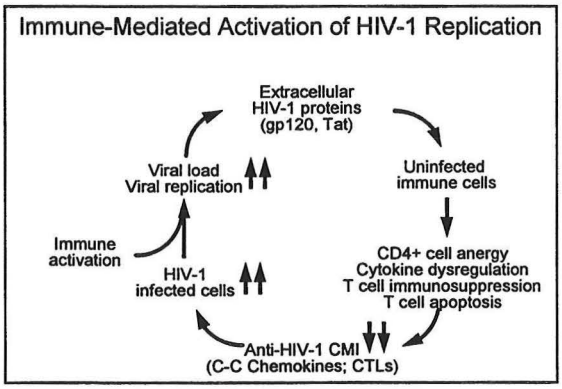
Figure 12



Can HIV-1-Specific CTL Responses be Harmful?

It is generally assumed that HIV-1 infects CD4 cells and destroys them. However, many other cell types are also infected by HIV-1, including epithelial cells, monocytes and neurons. From various examples of persistent infections by viruses that infect many cell types, the prediction should be that HIV-1 is not a cytopathogenic virus. If HIV-1 is not cytopathogenic, the question of why CD4 cells die arises. One possible answer is that both the disappearance of T cells and AIDS is a consequence of cell damage caused by anti-HIV-1 CTLs. The subtle perversity of HIV-1 infection in humans may thus be summarized as follows: this noncytopathogenic virus infects macrophages, dendritic cell , T helper cells and other host cells, which are then destroyed by the CTL response. In this model, HIV-1 infects some of the key cells that are involved in the immune response and then allows the immune system to destroy itself, thereby enabling the virus to persist. A good example is HBV infection, where liver damage is caused by anti-HBV CTLs. There is no evidence for the direct cytopathogenecity of HIV-1 *in vivo* but there is no evidence for the absence of it either. Therefore, an imaginary patient with severe combined immunodeficiency (SCID) who is infected with HIV-1 may never develop AIDS if HIV-1 is truly non-cytopathogenic. The model also predicts that if there are only a few HIV-1 infected cells, and the CTL response develops quickly, HIV-1 is eliminated or well controlled. If the initial response is weak, then extensive viral replication may lead to extensive CTL-mediated killing of CD4 cells. Thus the query, are HIV-1-specific CTLs helpful or harmful can both be answered with yes. But, the more important question regarding whether HIV-1 is a cytopathogenic virus *in vivo* cannot be answered yet and the proposal that AIDS is a consequence of CTL-mediated immunopathology remains hypothetical.

Figure 13



HIV-1 Exposed Seronegatives

In the quest for a successful vaccine against HIV-1 infection, a fundamental question posed is whether even a handful of people can mount a successful immune response that leads to viral clearance after exposure to HIV-1. Inevitably, attention has focused on those individuals who have been exposed to

HIV-1 yet remain seronegative and apparently uninfected. However, given the relatively low transmission efficiency of HIV-1, it remains difficult to determine whether these individuals were simply fortunate to have encountered insufficient virus to establish infection, or whether indeed, they have successfully cleared an infectious dose of HIV-1. Studies have focused on especially high-risk individuals, like prostitutes and sexual partners of HIV-1 infected persons [62,63] and have led to some exciting new discoveries.

Transmission Efficiency of HIV-1

There are considerable differences in the likelihood of seroconverting following an encounter with HIV-1. With the transfusion of blood infected with HIV-1, the risk is 90%. Seroconversion following needlestick injury is exceedingly uncommon and is estimated at 0.36 - 0.42%. The risks of infection following sexual exposure are hard to estimate, but with repeated exposure 10 - 16% of the wives of HIV-1 infected hemophiliacs become seropositive. This relatively low number contrasts with that found in a cohort of prostitutes in Nairobi observed since 1985, of whom more than 90% have become infected. Perinatal exposure leads to infection rates of 15-25%.

Virologic Studies in Exposed Seronegatives

Most studies have shown that silent infection with HIV-1 is extremely rare. Imagawa *et al.* [64] originally described 27 PCR-positive seronegative men at continuing risk for HIV-1 infection, but only one of these turned seropositive for the virus in follow-up studies leading to the speculation that some persons may harbor the virus but never seroconvert. There have been similar reports in wives of hemophiliacs and vertically infected children [47].

CTL Studies in Exposed Seronegatives

The induction of MHC class I-restricted CTLs may be a more reliable indication of exposure to replicating virus. The finding of CTLs in apparently uninfected children born to HIV-1 infected mothers [65] is believed to indicate exposure to live virus. Rowland-Jones *et al.* [62] studied a group of repeatedly exposed but persistently seronegative female prostitutes in The Gambia, West Africa, and found specific CTL activity against one or more HIV-1 peptides in three of six exposed but apparently uninfected women, but none in a panel of controls with no history of HIV-1 exposure. Occult infection was excluded by testing for HIV-1 and HIV-2 by means of the polymerase chain reaction. The most probable explanation of finding HIV-1-specific CTLs in such repeatedly exposed but persistently uninfected individuals is that they have been immunized by exposure to HIV-1.

Non-MHC Restricted CD8 Cell Suppression of HIV-1 Replication by Soluble Factors

Beginning in 1986, Jay Levy [48] reported that CD8 cells mediated noncytolytic suppression of HIV-1 replication. This activity was attributed to a soluble factor [51,66]. Additional evidence for such suppressor activity appeared in clinical reports that correlated nonprogression with high levels of CD8 suppressor activity [49]. It was difficult, however, to reproduce this activity with CD8-derived soluble materials. Studies by Levy and Brinchmann [66,67] suggested that these soluble factors were distinct from cytokines.

Chemokines and their Receptors

The trafficking of immune cells around the body is a complex process achieved by the interactions of cellular adhesion molecules such as selectins and integrins and a newly-discovered superfamily of chemoattractant cytokines (chemokines) and their receptors. The first chemokine, interleukin-8 (IL-8), was not identified until 1987. In 1992, at the Third International Symposium of Chemotactic Cytokines at Baden, the name "chemokines" was proposed to convey that these factors combine chemoattractant and cytokine properties. The recent appreciation of the intersection of chemokines and infec-

tious disease research has brought the field of chemokine biology from obscurity to present-day eminence. Not only is chemokine biology relevant to HIV-1 pathogenesis, it also is relevant to malaria and the herpesviruses.

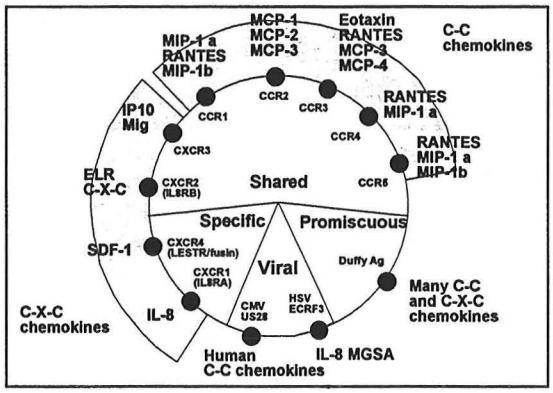
There are Three Structural Branches of Chemokines

Chemokines are a superfamily of proteins 68-120 amino acids in length. They can be divided into three structural branches, based on variations in a shared cysteine motif: the C chemokines have a single cysteine residue and have only one member which appears to be lymphocyte specific. The C-C chemokines (β Chemokines) have adjacent cysteine residues and generally attract monocytes and lymphocytes. The C-X-C chemokines (α Chemokines) have a single amino acid between the first and second cysteine residues, and attract neutrophils, but not monocytes.

There are Four Classes of Chemokine Receptors

Based on their chemokine binding specificity (ligands), the chemokine receptors can be categorized into four separate classes: "specific", "shared", "promiscuous", and "viral". The *specific* chemokine receptors bind only one chemokine. This group contains only two members: CXCR4 (fusin) which binds exclusively to SDF-1, and CXCR1 (IL8RA) which binds exclusively to interleukin-8. *Shared* receptors will bind with more than one chemokine. This group includes all the C-C chemokines (CCR1 through CCR5) and two of the CXC chemokines, CXCR2 and.CXCR3. Ligands for this group include MIP-1α, MIP-1β, RANTES, MCP-1, MCP-2, MCP-3, MCP-4, and eotaxin. The *promiscuous* receptors will bind to many chemokine ligands. This group has only one member, the Duffy blood group antigen (Duffy Ag). The *viral* chemokine receptors represent shared chemokine receptors that have been transduced into viral genomes during evolutionary history. There are two examples, one encoded by cytomegalovirus (CMV US28) and one by the herpes siamiri virus (HSV ECRF3).

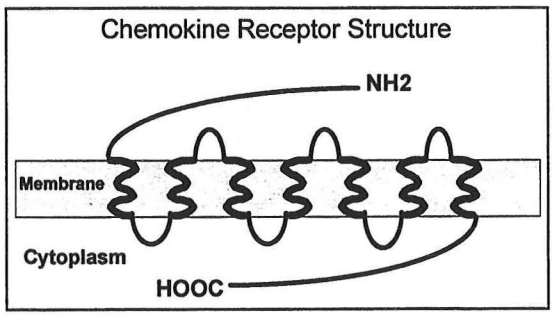
Figure 14. Chemokines and Receptors



Chemokine Receptors are all Seven-Transmembrane (7TM)Glycoproteins

Chemokine receptors are all structurally related, containing a transmembrane segment of seven loops. The shared C-C receptors are all remarkably homologous and localized to human chromosome 3, whereas CXCR 1, 2 and 4 are clustered on human chromosome 2. Chemokine receptor signaling involves a functional link via phospholipases and G proteins, with downstream generation of inositol triphosphate, resulting in intracellular Ca^{2+} release, Ca^{2+} channel opening and protein kinase C activation. Chemotaxis is achieved by actin polymerization, changes in adhesion properties and cytoskeletal rearrangement. Additionally, differentiation and proliferation signals are generated.

Figure 15



Chemokines in Infectious Disease

Other than HIV-1, there are two known situations where infectious agents use chemokine receptors. It has long been known that cytomegalovirus (CMV) possesses three open reading frames (ORF) encoding putative 7TM proteins. One of these, CMV US28, is now known to be a chemokine receptor and is homologous to CCR1. The biological significance of this receptor is currently unknown. The malarial parasite *Plasmodium vivax* takes advantage of the Duffy antigen on the surface of red blood cells to gain entry. As Duffy antigen is also expressed on extracorpuscular tissues, it may determine the tissue tropism of this parasite.

Soluble CD8 Cell HIV-1-Suppressor Factors are β Chemokines

In December 1995, the soluble factors that suppressed entry of primary strains of HIV-1 were identified as three chemokines: RANTES (regulated-upon-activation, normal T expressed and secreted), macrophage inflammatory protein-1 α (MIP-1α), and macrophage inflammatory protein-1 β (MIP-1β) [45]. These chemokines were purified from the culture supernatant of both immortalized and primary CD8 T cell clones and were found to inhibit the entry of HIV-1 in an immortalized CD4 T cell clone (PM1) that has broad susceptibility to infection with primary HIV-1 isolates that infect both macrophages and CD4 T cells.

In April 1996, Paxton *et al.* [63], reported a cohort of 25 HIV-1 seronegative subjects with multiple sexual exposures to HIV-1. He found that their CD8 T cells had greater anti-HIV-1 activity than did CD8 T cells from unexposed controls. Additionally, their CD4 cells were less susceptible to infection with primary HIV-1 isolates, but not to T cell line-adapted strains suggesting that they are resistant to infection by M-tropic isolates This resistance was shown to be mediated by the activity of the β chemokines, RANTES, MIP-1α and MIP-1β. These results suggested that the relative resistance of CD4 lymphocytes may contribute to protection from HIV-1 in multiply exposed persons.

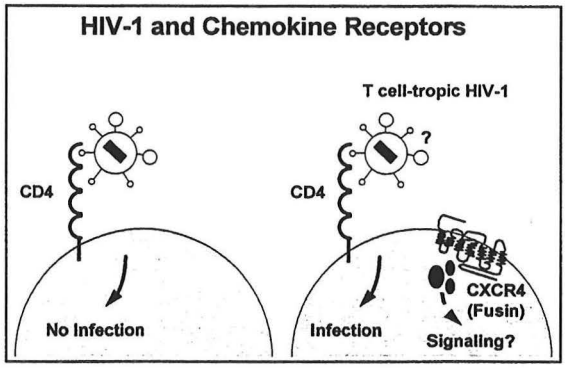
Chemokine Receptors are Essential for HIV-1 Entry

The above experiments suggested that perhaps HIV-1 utilizes a chemokine receptor for entry. The selective inhibition by the chemokines of HIV-1 isolates with differing cellular tropisms suggested that the difference in cellular tropisms could be explained by selective chemokine receptors. As early as 1986, researchers had postulated the presence of a second receptor in addition to the CD4 molecule. Maddon *et al.* [68] showed that HIV-1 was unable to infect mouse cells engineered with human CD4. Additionally, it had been previously shown that the V3 loop is the primary determinant of cell tropism of HIV-1 [36] and that this area is distinct from the CD4 binding domain of HIV-1 [69]. Thus, it was entirely plausible that M-tropic and T-tropic HIV-1 strains could utilize distinct HIV-1 co-receptors to gain entry into cells.

Fusin (CXCR-4) is the Second Receptor for T-tropic HIV-1

In April 1996, Berger and colleagues [70] identified the second receptor for T-tropic HIV-1 entry into transformed T cell lines. The receptor is a seven-transmembrane domain glycoprotein of the chemokine superfamily of receptors. The Berger group named the protein *fusin* because it facilitated HIV-1 fusion. When fusin is coexpressed with CD4 on mouse cells it permits fusion with T-tropic, but not M-tropic, HIV-1 strains. Fusin has been subsequently renamed CXC-chemokine receptor 4 (CXCR-4). Interestingly HIV-2 can infect CD4 negative cells expressing fusin alone [71]. Recently the ligand for CXCR-4 was identified as stromal derived cofactor (SDF-1), an α chemokine. SDF is a chemoattractant for T cells and plays a prominent role in B cell development [72,73]. Mice that are genetically deficient in SDF-1 die perinatally [74].

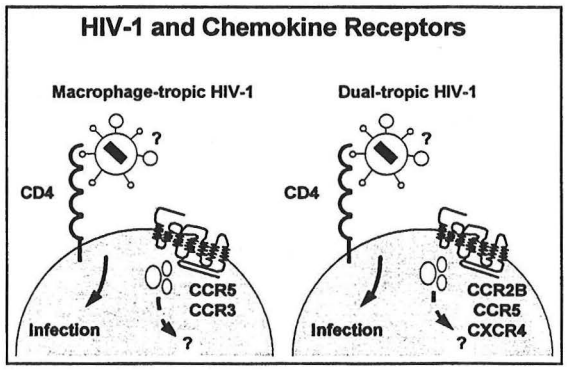
Figure 16



CCR-5 is the Second Receptor for M-tropic HIV-1

The observations that the β chemokines RANTES, MIP-1α and MIP-1β inhibited HIV-1 replication in M-tropic isolates [45] strongly suggested that the second receptor for M-tropic strains of HIV-1 may be a β chemokine receptor. Coincidentally, in March 1996, Samson *et al.* in Brussels, reported the cloning, sequencing and functional characterization of a chemokine receptor that responded to RANTES, MIP-1α and MIP-1β and named it CCR-5 [75]. Subsequently in June 1996, five groups simultaneously reported that CCR-5 is the required second receptor for the entry of M-tropic HIV-1 [33,76-79].

Figure 17



Each group employed different strategies, making the cumulative evidence very convincing. Richard Koup's group at the Aaron Diamond AIDS Research Center [77] showed that CD4 cells from HIV-1 exposed but uninfected individuals cannot fuse with M-tropic HIV-1 and secrete high levels of β chemokines. They expressed several chemokine receptors on human simian and murine cells and found that CCR-5 expression rendered these cells susceptible to M-tropic HIV-1 entry.

A collaborative group from New York, Stanford, Louisville and Palo Alto [76] expressed all known chemokine receptors on several human and murine cell lines and tested for their ability to facilitate fusion with M-tropic HIV-1. Their results showed that CCR-5 co-expression with CD4 was required for efficient entry of M-tropic HIV-1 strains.

Edward Berger's team from the NIH [78] showed that recombinant CCR-5 rendered nonhuman CD4 cells able to fuse preferentially with M-tropic HIV-1 strains. They also found that CCR-5 messenger RNA was detected selectively in cell types susceptible to M-tropic HIV-1 isolates.

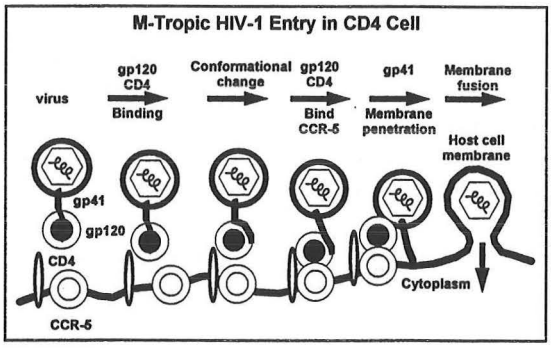
Robert Dom's group from the University of Pennsylvania [33] reproduced the Berger-group findings and additionally showed that although CCR-5 served as the primary cofactor for M-tropic isolates of HIV-1, a dual-tropic primary HIV-1 isolate could utilize fusin (CXCR-4), CCR-5, CCR-3 and CCR-2b as entry cofactors. These results suggested that T-tropic viruses characteristic of disease progression may evolve from purely M-tropic strains prevalent early in viral infection, by mutations in the env gene (codes for gp120 and gp41), thereby enabling it to use multiple coentry factors.

Finally, Sodroski's investigative team from Boston, showed that M-tropic isolates could utilize either CCR-5 or CCR-3 as entry cofactors and that the V3 loop sequences in gp120 play an important role in the interaction with the chemokine receptor.

Recent studies [37,80,81] have demonstrated the binding site on gp120 for CCR-5 likely involves epitopes in the V3 loop. Notably the affinity of gp120 for CCR-5 is greatly increased following the binding of gp120 to CD4. This suggests that CD4 induces a conformational change in gp120 that promotes CCR-5 binding by the V3 loop. This is further substantiated by recent data from Cocchi *et al.* that chemokine-mediated blocking

of HIV-1 entry occurs on the V3 domain [80].

Figure 18



Mutations in CCR-5 Confer Resistance to Infection with HIV-1

Armed with the information that CCR-5 is the co-receptor for infection with M-tropic HIV-1 strains, and recalling that M-tropic strains initiate HIV-1 infection by all routes of transmission [27,28], the next logical step was to examine persons who remain uninfected with HIV-1 despite multiple high-risk exposures. Paxton and Koup studied 25 such subjects and found that their CD8 cells had greater anti-viral activity when compared with unexposed controls [63]. In addition their CD4 cells were less susceptible to infection with M-tropic strains, likely related to the activity of the β chemokines, MIP-1α, MIP-1β, and RANTES.

It was logical to postulate that a genetic variation in CCR-5 may confer resistance to HIV-1. In August 1996, Landau and Koup collaborated to show that two exposed but uninfected individuals had homozygous defects in CCR-5 [82]. The described defect was a 32 base pair deletion, resulting in a nonfunctional, severely truncated protein that could not be detected at the cell surface. The investigators next estimated the allelic frequency of the mutant CCR-5 allele (Δ32) in a European sample of random blood donors and found a heterozygosity rate of 22.7%. Surprisingly this mutation had no obvious phenotype in affected individuals. Also in August 1996, Parmentier and his colleagues, from Brussels, collaborating with Dom's group, in Philadelphia, showed that Δ32 was present at a high frequency in Caucasian populations (0.092), but absent from Western and Central African and Japanese populations [83]. In a cohort of HIV-1 infected Caucasian subjects, no individuals homozygous for Δ32 were found and the frequency of heterozygotes was 35% lower than in a control European population. In September 1996, Stephen O'Brien's team reported a study of 1955 individuals from six well-characterized AIDS cohorts and confirmed that the homozygous Δ32 genotype offers protection against HIV-1 regardless of the route of viral transmission. The latter study did not find that heterozygosity offered an protection from HIV-1.

In November 1996, David Ho's group analyzed the CCR-5 genotype in 1252 homosexual men enrolled in the Chicago component of the Multicenter AIDS Study Cohort (MACS) [84]. No infected participant was found to be homozygous for the Δ32 allele whereas 3.6% of at-risk but uninfected Caucasian participants were homozygous, which is slightly greater than the frequency in a low risk Caucasian population (1.4% in platelet donors to the New York Blood Center), showing the highly protective role of this genotype against sexual acquisition of HIV-1. No evidence was found to suggest that heterozygotes were protected against HIV-1 infection, but a limited protective role against disease progression was noted. Further evidence for the protective role of the homozygous Δ32 genotype came from an examination of its frequency in relation to risk behavior. When uninfected participants who had anal receptive intercourse with the greatest number of men in the six months preceding enrollment were assessed, the frequency of the homozygous Δ32 genotype was 20%. Further examination of those uninfected subjects who had been followed for greater than eight years, and remained seronegative while having anal receptive intercourse with greater than six partners the frequency of the homozygous Δ32 genotype was 33.3%.

All these data provide powerful epidemiological evidence for the protective role of the homozygous Δ32 genotype against infection

with HIV-1. Additionally, the critical role of M-tropic strains in HIV-1 transmission by all routes is certainly more convincing. An unsolved problem is why these resistant individuals who have functional CXCR-4 receptors [82] evade infection by T-tropic isolates. The absence of Δ32 homozygous genotypes in HIV-1-resistant individuals in African populations implies that other factors must operate in conferring HIV-1 resistance in these populations. It is perplexing that the homozygous Δ32 genotype is either absent, or present in extremely low frequencies in non-Caucasian populations, yet HIV-1 is endemic in Africa and Asia. Perhaps this polymorphism arose recently in Caucasian populations as a protective response to other pathogens. An historical precedent exists for such an observation: the Duffy antigen, an erythrocyte marker protein is a chemokine receptor and functions as the receptor for the human malarial parasite *Plasmodium Vivax*.Certain individuals in sub-Saharan Africa lack the Duffy antigen and resist invasion by the malaria parasite [85].

Future Implications

The fields of rheumatology, immunology and infectious disease are now closer than ever, fueled by the intense research in HIV-1 pathogenesis. The study of the phenotypic mimics of Sjögren's syndrome (DILS) in patients infected with HIV-1, has revealed many similarities between such patients and long-term nonprogressors. It will be very interesting to study the role of chemokines and their receptors in patients with DILS. Furthermore, many insights may be gained into the long-held premise that Sjögren's syndrome is caused by a retrovirus [86]. The exploding field of chemokines and their receptors has opened new vistas in pathogenesis research, vaccine discovery and therapeutic targets. Additionally, the study of chemokine biology will likely provide insights in the pathogenesis of the vasculitides, diseases that clearly involve immune cell trafficking. Not all of the resistance to HIV-1 infection is explained by mutations in chemokine receptors. This is especially true for non-Caucasian populations. We still need explanations for persons who are infected with HIV-1 and then go on to eradicate the infection completely. The answer may certainly lie in the genetics of the MHC. While, the literature concerning HLA associations and HIV-1 is somewhat hampered by the small scale of individual studies, the importance of MHC-restricted CTLs in determining the outcome of infection with HIV-1 should be vigorously pursued by designing large-scale studies.

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