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CD8$^+$ T CELL MEDIATED NONCYTOLYTIC INHIBITION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE I

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1. ABSTRACT

The development of cellular immune responses in primary human immunodeficiency virus type-1 (HIV-1) infection is accompanied by a dramatic decrease in plasma viremia and resolution of the acute clinical syndrome. The full nature of the immunological response and its consequences on HIV pathogenesis is still largely a mystery, but significant progress has been achieved in the characterization of some of the players involved. Several studies indicate that noncytolytic HIV suppression by CD8$^+$ T lymphocytes may be inversely associated with viral load

Table 1. Characteristics of CD8$^+$ Antiviral Activity

- Non-cytolytic inhibition of virus replication
- Not HLA restricted, but some enhancement is seen when matched
- Dose dependent
- Effective against acutely or endogenously infected CD4$^+$T-cells
- Declines with progression to AIDS
- Soluble and Contact mediated
- Effective against CCR5 and CXCR4 utilizing viruses
- Present in some exposed uninfected individuals

suggesting that this antiviral activity is important in host control of HIV replication. This review focuses on this antiviral activity by CD8$^+$ T lymphocytes, which is distinct from that activity elicited by some cytolytic CD8$^+$ T lymphocytes (CTLs).

2. INTRODUCTION

CD8$^+$ T lymphocytes act as agents of the cellular immune response in combating a viral infection. These cells are critically important in the host defense against human immunodeficiency virus (HIV) infection, the causative agent of acquired immunodeficiency syndrome (AIDS) (1). HIV was first cultured from the peripheral blood mononuclear cells of individuals with AIDS, AIDS-related conditions (ARC) and those at high risk for infection (2-4). Due to the host virus relationship, the clinical course of the infection can be divided into three stages: acute HIV infection, an asymptomatic period, and finally a symptomatic stage accompanied by opportunistic infections and disease often leading to death. An understanding of the host's immune response during the acute and asymptomatic period, when virus replication is controlled, will help elucidate the correlates of immune protection in HIV infection. Detailed examination of these immune responses will provide practical insights for the development of new antiviral agents as well as immune enhancing vaccines and immunotherapies. Recently, two compelling studies demonstrated the importance of CD8$^+$ T cells in antiviral immunity (5, 6). Both papers addressed this issue by depleting CD8$^+$ T cells in the blood and lymph nodes of the SIVmac/rhesus monkey model of AIDS using anti-CD8$^+$monoclonal antibodies. After antibody treatment there was a pronounced increase in viral replication and a faster progression to disease. From these and other studies, it is clear that CD8$^+$ T cells play a central protective role against viral invasion. CD8$^+$ T lymphocytes are no longer confined to the demarcation of "lytic" cytotoxic T lymphocytes due to the recognition that they possess an armamentarium of cytokines and other unknown nonlytic molecules with which to fight a virally infected cell. Therefore in the course of our studies into the correlates of immune protection, it will be important to not only identify and examine the existing immune defenses, but to enhance and broaden them. This will only be achieved by in depth analysis of the nature and mechanisms of antiviral activity such as the noncytolytic CD8$^+$ T cell mediated responses.

One of the first antiviral immune responses documented in HIV seropositive individuals were neutralizing antibodies to HIV encoded proteins (7). Antibodies detected in HIV infected individuals are also capable of mounting antibody-dependent cellular cytotoxicity (ADCC), (8-12) and complement-dependent lysis (13). Of major importance to the cellular immune response to HIV infection is viral suppression by cytotoxic CD8$^+$ T cells (14, 15) and noncytolytic suppression by CD8$^+$ T cells (16). Noncytolytic suppression of HIV replication can be described overall by some general characteristics as listed in Table 1. Elucidation of the mechanisms employed by this cellular immune response will be advantageous to improving both current HIV therapeutics and new vaccine development.

3. DISCOVERY OF CD8$^+$ T CELL NONCYTOLYTIC ACTIVITY

The ability to isolate HIV directly correlates with the clinical status of the HIV$^+$ individual (17, 18). Virus is easily recovered from the blood of those HIV$^+$ individuals who have progressed to AIDS. The difficulty lies in culturing HIV from the peripheral blood mononuclear cells (PBMCs) of asymptomatic HIV$^+$ individuals. The concept of non-cytolytic CD8$^+$ T cell mediated suppression of HIV-1 unfolded pursuant to the observation that virus recovery from PBMCs of asymptomatic individuals was significantly enhanced by the removal of the CD8$^+$ subset of T lymphocytes. The hypothesis became that CD8$^+$ T lymphocytes were inhibiting viral replication *in vitro* and thus would be representative of a cellular immune response that is functioning *in vivo* to control viral replication in asymptomatic individuals. A seminal study (16) by Levy's group demonstrated that CD8$^+$ T lymphocytes were indeed responsible for inhibiting HIV replication. CD8$^+$ T cells were depleted from the PBMCs of four seropositive asymptomatic individuals by the panning method. Consequently, HIV replication, as measured by a reverse transcriptase assay, occurred 10-20 days later. Additionally, virus replication was inhibited in a dose dependent manner when autologous but not allogeneic CD8$^+$ T cells from a HIV$^+$ individual were added back to the culture (see later discussion in section 9. HLA vs. non-HLA matched). The nature of this activity was determined to be noncytolytic due to the observation that if CD8$^+$ T cells were removed from the culture, viral replication would ensue. This would not be possible if the mechanism of suppression was to lyse the virally infected cells. Since the discovery of this immune response in 1986, many labs have engaged in the search for both the nature of the molecules mediating this activity and the mechanisms by which they act. The identification of the HIV suppressive beta-chemokines (MIP1-alpha, MIP1-beta and RANTES) enhanced our understanding of this noncytolytic antiviral activity (19). These molecules can be released from CD8$^+$ T cells (as well as CD4$^+$ T cells (20-23)) and can inhibit viral replication of viruses that use the coreceptor, CCR5, for entry. However, these molecules are unable to inhibit viral replication of CXCR4 using viruses; whereas, CD8$^+$ T cells with the noncytolytic antiviral phenotype are quite capable of potently inhibiting HIV that gains entry into

cells via CXCR4 (24). Hence, CD8$^+$ T cell mediated activity must be multifactorial. Mechanistic studies performed in our laboratory have elucidated possible targets for suppression within the virus life cycle. To date however, the molecules largely responsible for the antiviral activity and their cellular or viral target(s) are still largely unknown.

4. CD8$^+$ T CELL SUPPRESSION ASSAYS

There are several assay systems employed by various laboratories for the analysis of CD8$^+$ T cell mediated suppressive activity. Consequently, differences in the setup of the assay may account for the contrasting results achieved. For example, Barker *et al*. (25) observed that one can distinguish two sets of CD8$^+$ T cells using a variation of the acute and endogenous assay with an *in vitro* model of HIV replication that resembles the interactions between dendritic cell and T cells *in vivo*. CD8$^+$ T cells from both uninfected individuals and individuals at early stages of the disease course can suppress HIV replication in the endogenous assay system. This activity is insensitive to irradiation and does not require *in vitro* activation of the CD8$^+$ T cells. In contrast, CD8$^+$ T cells from infected individuals at all stages of the disease, but not uninfected individuals will suppress HIV replication in the acute infection assay. This activity is abrogated by irradiation of the CD8$^+$ T cell. Thus, it is likely that these two assay systems are capable of measuring a different set of antiviral factors derived from CD8$^+$ T cells. A possible and highly probable explanation is that CD8$^+$ T cell antiviral activity is multifactorial and this must be kept in mind when analyzing data utilizing a particular assay system. The acute assay format can be combined with either a quantitative, semi-quantitative, or a single cycle infection assay to measure CD8$^+$ T cell mediated antiviral activity. Whereas, the endogenous assay format can only be used in conjunction with the semi-quantitative antiviral assay.

4.1. Endogenous CD8$^+$ T cell suppression assay

An endogenous CD8$^+$ T cell suppression assay measures the ability of an individual's CD8$^+$ T cells to suppress their own virus growing in their CD4$^+$ T cells in vitro. The CD4$^+$ and CD8$^+$ T cells are isolated from peripheral blood mononuclear cells (PBMCs) of HIV-infected individuals. Usually, this is done through the use of magnetic beads conjugated to antibodies to CD4 or CD8. The isolated cells are then activated for two to three days with either PHA or antibodies to CD3 and/or CD28. One study (26) determined that anti-CD3 monoclonal antibody was more effective at inducing endogenous HIV production than PHA stimulation. The CD8$^+$ T cells are added back to the endogenously infected CD4$^+$ T cells at various ratios for 7-14 days. Fresh media is added every 2-3 days and supernatants are analyzed for viral replication using p24 antigen or reverse transcriptase activity as a marker. This assay is useful for analyzing the ability of an individual's CD8$^+$ T cells to suppress their own virus in their own CD4$^+$ T cells. However, it is difficult to compare the potency of the CD8$^+$ T cells between patients. Additionally, the CD4$^+$ T cell count of some HIV infected people is too low to acquire enough CD4$^+$ T cells to perform this assay. Other potentially confounding issues can involve controlling the amount of virus being produced from these CD4$^+$ T cells. Differences in endogenous virus production can lead to quantitative differences in the number of CD8$^+$ T cells that are required to suppress from assay to assay.

4.2. Acute CD8$^+$ T cell suppression assay

An acute CD8$^+$ T cell suppression assay can measure the antiviral activity of CD8$^+$ T cells using a variety of different viruses and different CD4$^+$ target cells. The CD8$^+$ T cells used in this assay are obtained from the PBMCs of HIV$^+$ individuals. These cells are either immediately used for an experiment or they can be transformed with Herpesvirus saimiri or HTLV to produce an IL-2 dependent continuous cell line. The CD4$^+$ target cells are obtained from either a single donor or a pool of seronegative donors. They are activated for 2-3 days with PHA or antibodies to CD3 and/or CD28 and then infected with a virus inoculum at a known $TCID_{50}$ and CD8$^+$ T cells are added at various ratios to the culture. This system overcomes several limitations of the endogenous suppression assay. Here one can control both the titer of the input virus and the type of virus. Thus one can analyze the ability to suppress diverse strains of HIV in addition to the host strain. This assay is also valuable in comparing the activity between different CD8$^+$ T cells while keeping the virus and target CD4$^+$ T cells constant.

4.3. Quantitative CD8$^+$ T cell suppression assay

The quantitative CD8$^+$ T cell suppression assay (quantitative infectivity reduction assay) was adapted from antibody neutralization studies in our laboratory (22, 27). CD4$^+$ target cells are obtained from the PBMCs of either a single donor or a pool of seronegative donors by similar activation and separation methods as described above. They are aliquoted into a 96 well microtiter plate. The assay is set up in two dimensions by titering the infectious virus in one direction and the CD8$^+$ T cells (or supernatant) in the other direction. After 7-14 days, reverse transcription is measured and the virus titer of each condition is determined. The number of CD8$^+$ T cells (or the concentration of supernatant) required to reduce virus infectivity by one log or greater (Vn/Vo = 0.1) is considered to be positive for suppressive activity. This assay has all of the advantages of the acute suppression assay such as controlling the titer and nature of the input virus and CD4$^+$ T cells in order to permit comparisons between different CD8$^+$ T cell effector populations in question. Most importantly, this assay allows quantification of the suppressive activity since multiple doses of virus and CD8$^+$ T cells are used. This provides a significantly enhanced power in distinguishing CD8$^+$ T cells with a strong antiviral activity.

4.4. Semi-quantitative CD8$^+$ T cell suppression assay

A semi-quantitative analysis can be used for both an acute and an endogenous CD8$^+$ T cell suppression assay (28). The claim is that this setup utilizes a low number of fresh or frozen-thawed PBMC and has a wide dynamic range. The method is unique because a stock of HIV_{BRU} infected CD4$^+$ T cells (from a seronegative donor) is made

and the viral load is determined. These can be frozen in aliquots to be used for later assays. For an endogenous CD8$^+$ T cell suppression assay, CD4$^+$ T cells are purified from a seropositive individual, the viral load tested, and CD4$^+$ T cells frozen into aliquots to be used for the assay. CD8$^+$ T cells from a seropositive individual are then added to a culture of infected CD4$^+$ T cells (either exogenously infected or endogenously infected) and p24 antigen or reverse transcriptase is measured after 10 days in culture. The results are semi-quantitative because there are five five-fold dilutions of CD8$^+$ T cells added; however, only a single input of virus-infected CD4$^+$ T cells are utilized.

4.5. Single cycle CD8$^+$ T cell suppression assay

In some instances, it is beneficial to have a more expedient system for determination of CD8$^+$ T cell mediated suppressive activity. For example, the analysis of vaccine recipients for the induction of CD8$^+$ T cell suppressive activity would be less time consuming with an experimental system that has readout of two to five days. We have developed a single cycle assay using HIV_{NL4-3} virus pseudotyped with an HIV envelope for the detection of noncytolytic anti-HIV activity (27). Pseudotyped viruses are produced by transfection of plasmid DNA into the human embryonic kidney cell line, 293T, using a modified calcium phosphate method (29). Both reporter virus DNA (pNL4-3 LUCR$^-$E$^-$) and an expression vector for an HIV-1 envelope are cotransfected. The reporter vector lacks *vpr*, lacks a functional envelope-coding region, and contains the luciferase gene in place of the *nef* gene. An assortment of envelope expressing vectors (CCR5 restricted, CXCR4 restricted, dualtropic, multitropic, etc.) exist that can be utilized to suit the experimental design. We have demonstrated that CD8$^+$ T cells will suppress virus pseudotyped with both HIV containing envelopes and the amphotropic murine leukemia virus envelope suggesting that the suppressive activity is not HIV envelope dependent (27). Thus, this is a versatile, quick and non-radioactive assay system for the screening of CD8$^+$ T cells believed to have anti-viral activity. This assay is easily adapted to the quantitative format after the initial screening for antiviral activity.

4.6. Assay criteria for determination of CD8$^+$ T cells with noncytolytic anti-HIV activity

Some controversy exists over the ability of CD8$^+$ T lymphocytes from uninfected persons to exhibit a suppressive phenotype. Several laboratories, including our own, demonstrated that CD8$^+$ T lymphocytes from seronegative individuals do not have significant anti-HIV activity (27, 30, 31). However, other groups have concluded otherwise (32, 33). Some of this discrepancy lies in the different assay systems used and the criteria for determining that CD8$^+$ T cells have suppressive activity. Brinchman *et al.* (32) demonstrated that CD8$^+$ T cells from seronegative individuals could inhibit viral replication in lymphocytes from naturally infected individuals (endogenous CD8$^+$ T cell suppression assay). Bagasra *et al.* (33) contends that this observation holds true when an acute infection assay is used. In the latter assays, CD4$^+$ and CD8$^+$ T cells from seronegative individuals were isolated, the CD4$^+$ T cells were infected in vitro, and subsequent virus replication was inhibited upon addition of CD8$^+$ T cells back to the culture. These studies are promising in that they suggest that CD8$^+$ T cells can act as a first line endogenous defense against HIV. However, since many of the assay systems vary in the level of quantitation that can be resolved, interpretation of the results can be difficult. In many experiments the level of viral replication, as either p24 production or RT levels, is measured with a single inocula of virus. A more stringent test, utilized by our laboratory, is a quantitative CD8$^+$ T cell suppression assay that uses serial dilutions of the viral inocula, thus preventing responses that may be seen only with a specific virus dose. Additionally, the criterion used in our laboratory is that CD8$^+$ T cells are scored as having anti-HIV activity if at least a 90% or greater reduction in viral titer occurs. In many cases, we have observed a 40-70% inhibition in viral replication as judged by the release of viral RT using CD8$^+$ T cells from seronegative individuals, but the inhibition was well below the cutoff of a log reduction in viral titer. Since the antiviral activity derived from seronegative individuals is inconsistent and considerably less potent than CD8$^+$ T cells acquired from HIV$^+$ individuals, the mechanism of action is likely to be different. Another possible consideration is that the antiviral activity may be an enhancement of a pre-existing immune function due to another infectious disease. However, Bagasra *et al.* (33) portends that the difference may lie in the activation status of the CD8$^+$ T cells from the seronegative individual. They provide no stimulation to the cells before coculture with infected CD4$^+$ T cells and thus attempt to analyze the *in vivo* condition of the cells. Rosok *et al.* (34) demonstrated that CD8$^+$ T cells from seronegative individuals, activated with antibodies against the CD3 molecule or T cell receptor, suppressed HIV-1 and HIV-2 replication in acutely infected CD4$^+$ T cells. Our studies demonstrate that CD8$^+$ T cells from seronegative individuals which are activated with soluble antibodies to CD3 and CD28 do not demonstrate anti-viral activity (27). A conclusive study comparing different activation schemes combined with the most quantitative CD8$^+$ T cell suppression assay is needed in order to clarify the presence or absence of HIV antiviral activity due to CD8$^+$ T cells from HIV unexposed and seronegative individuals.

4.7. Expansion of CD8$^+$ T cells with suppressive activity

4.7.1. Immortalization of CD8$^+$ T cells with antiviral activity

In order to characterize CD8$^+$ T cell suppressive activity, it is useful to immortalize primary CD8$^+$ T cell that exhibit the phenotypes one wishes to study. Then one has access to a continuous cell line to perform experiments. The establishment of this cell line can be done by transformation with a range of different viruses. The oncogenic, lymphotropic $gamma_2$-herpesvirus, Herpesvirus saimiri (HVS), strain 488-77 has been used by several investigators (35-37). These HVS-immortalized CD4$^+$ and CD8$^+$ T cells maintain the phenotype of the parent T cell, including the T cell receptor, and antigen specific responses (38-40). It is important to note that the transformation process itself does not produce the antiviral activity. In other words, many HVS transformed cell lines derived in the same manner do not suppress HIV replication in target

cells. Cocchi *et al.* (19) utilized CD8$^+$ T cell lines from a seronegative individual immortalized *in vitro* with human T cell leukemia/lymphotropic virus (HTLV) type I for the isolation and identification of the soluble CD8$^+$ T cell derived suppressive factors, MIP1-alpha, MIP1-beta, and RANTES. An analogous transformation process can produce continuous cell lines from HIV-1 infected individuals (41). Transformation of CD8$^+$ T cells exhibiting a desired phenotype has been quite useful as a consistent and reliable source to examine the details of CD8$^+$ T cell mediated suppressive activity.

4.7.2. Cloning of CD8$^+$ T cells with antiviral activity

Identification of CD8$^+$ T cell subsets can be achieved by cloning from a bulk population of cells containing the desired phenotype. CD8$^+$ T lymphocytes from HIV$^+$ individuals have been cloned in several laboratories in order to decipher the phenotype of the antiviral CD8$^+$ T cells (42-46). Cloned CD8$^+$ T cells with antiviral activity were obtained at a frequency of 1-20% by Hsueh *et al.* (43). Interestingly, clones from seronegative individuals also demonstrated antiviral activity in their assays. Toso *et al.* (44) demonstrated that the cloned CD8$^+$ T cells from HIV$^+$ individuals with antiviral activity were oligoclonal in nature due to the heterogeneity with respect to phenotypic markers, cytokine production, and T cell receptor VBeta expression. Additionally, for many of the CD8$^+$ T cell clones, CTL activity and noncytolytic suppressive activity were mutually exclusive. However, two clones were identified that demonstrated both activities. This last finding is similar to other studies (45, 46) that reported that HIV-1 specific CTL clones could exert both cytolytic and noncytolytic antiviral activities. Interestingly, Yang *et al.* (46) further described the sequence of events by these CTL clones. First, the CTL makes direct contact with the infected cell resulting in cytolysis. After this antigen-specific, HLA-restricted activation through the T cell receptor, the CTL is able to produce soluble inhibitory factors that can suppress HIV replication. Populations of cloned CD8$^+$ T cells from asymptomatic HIV$^+$ individuals such as these can be valuable in determining the differences between suppressive and non-suppressive clones. These types of insights could be used to discover both the molecules and mechanisms involved in CD8$^+$ T cell mediated anti-HIV activity.

4.8. Superinfection

For some experiments, it may be useful to infect the target CD4$^+$ T cells, derived from an HIV infected individual, with a HIV reporter virus in a single cycle infection assay to analyze CD8$^+$ T cell activity against autologous CD4$^+$ T cells (Tomaras, G.D., Greenberg, M.L. unpublished observations). One group reported that infected cells (PBMCs) were resistant to superinfection unless the CD8$^+$ T cells were removed (47). They demonstrated that peripheral blood lymphocytes (PBL) from asymptomatic HIV-1 individuals will suppress viral replication of exogenously added HIV-1 (IIIB) but not HIV-2 (CEM/LAV-2). HIV-2 superinfects even in the presence of these CD8$^+$ T cells. Further investigation into the idea that CD8$^+$ T cells can prevent superinfection was performed by Barker *et al.* (48). They demonstrated that although the PBMC of asymptomatic HIV$^+$ individuals are resistant to superinfection with HIV-1_{SF33} and HIV-2 (HIV-2_{UC2} and HIV-2_{UC3}), their CD4$^+$ T cells were susceptible. However, during the symptomatic phase of HIV infection when the CD8$^+$ T cell antiviral response is diminished or absent, superinfection occurs readily. As a result, if CD8$^+$ T cells are removed first, superinfection of the CD4$^+$ T cells is possible.

5. CLINICAL RELEVANCE

5.1. CD8$^+$ T cell mediated activity correlates with a healthy clinical status

The length of the disease course varies considerably among individuals due to both the host's immune response and the viral phenotype. The initial asymptomatic period is characterized by the predominance of non-syncytium inducing HIV (CCR5 dependent). Progression to disease is often accompanied by a shift toward CXCR4 viruses, or syncytium-inducing viruses. The asymptomatic period is increasing in length due to the frequent use of antiretroviral therapy in developed countries. This period of time is denoted by a lack of clinical symptoms between the two periods with bursts of antigenemia. The first peak of antigenemia is during the initial HIV infection and the second is upon the onset of opportunistic infections that characterize AIDS. It is believed that the host immune response is responsible for suppressing viral replication between these two outbreaks of enhanced viral replication. One of the cell types believed to play a large role in fighting virus replication during the asymptomatic stage is the noncytolytic CD8$^+$ T lymphocyte. The potency of CD8$^+$ T cells from HIV infected individuals for inhibiting HIV replication *in vitro* correlates with their disease progression. Brinchman *et al.* (32) demonstrated that CD8$^+$ T cells from AIDS patients were either completely unable to inhibit HIV replication in an autologous *in vitro* culture or they inhibited to a significantly less extent than those CD8$^+$ T cells from an asymptomatic individual. Additionally, Mackewicz *et al.* (31) performed a quantitative study analyzing the inhibitory activity of CD8$^+$ T cells from individuals at different stages in the progression to AIDS. They demonstrated that HIV-1 replication was inhibited by 90% or greater at an effector to target ratio between 0.05 to 0.25 for asymptomatic individuals. The effective ratio was increased for symptomatic patients (AIDS Related Complex) to between 0.05 to 1, and for AIDS patients the required ratio was between 0.1 -2.0. They demonstrated that the potency of the activity did not depend on the target CD4$^+$ T cells or the endogenous virus. However, there were noted differences in the acute vs. the endogenous CD8$^+$ T cell suppression assay. More CD8$^+$ T cells were required to efficiently inhibit viral replication in the acute assay. This finding may be due to a difference in the number of infected cells in each assay, with the acute assay having more infected cells and therefore requiring more CD8$^+$ T cells for suppression. Another possibility is that the mechanism for inhibiting virus from a proviral state could be different from that of an acute infection.

Another method of addressing this question is the use of an *in vitro* study to determine how the clinical stage

of HIV affects CD8$^+$ T cell mediated suppression (47). PBMCs from seronegative individuals, an asymptomatic HIV-1 carrier, and a patient with AIDS were exogenously infected with HIV and the ability to support viral replication was measured by reverse transcriptase activity. Although the number of patients was small, they demonstrated that PBMCs from the asymptomatic HIV-1 carrier was able to resist viral infection; whereas, PBMCs from individuals with ARC and AIDS did not resist exogenously added virus. The resistance to exogenous HIV infection by asymptomatic individuals was attributed to the CD8$^+$ T cells, because upon their removal HIV suppression was abrogated.

A longitudinal evaluation of five clinically healthy individuals over a six year period reached similar conclusions in that the CD8$^+$ T cell mediated activity positively correlated with a healthier clinical status (49). CD8$^+$ T cells from two individuals who progressed to AIDS demonstrated a decline in antiviral response accompanying a depletion in their CD4$^+$ T cells. The other three individuals progressed to develop thrush without an AIDS defining condition and maintained a strong CD8$^+$ T cell antiviral activity. They conclude from this that there is a correlation between the stage of the HIV illness and the potency of the CD8$^+$ T cell antiviral response. Interestingly, the diminution of the CD8$^+$ T cell antiviral activity upon progression to AIDS also correlates strongly with a decrease in the cytotoxic CD8$^+$ T lymphocyte subset. In another study, (50) a cohort of 10 asymptomatic HIV$^+$ individuals with a stable CD4$^+$ T cell count for at least 12 years were analyzed. In eight of these individuals, they detected a 90 percent inhibition in peak HIV-1 replication in a CD8$^+$ T cell suppression assay. Interestingly, they also demonstrated a strong neutralizing antibody response in these individuals. In order to further substantiate the claim that CD8$^+$ T cell suppressive activity correlates with a healthy clinical state, Barker *et al.* (51) performed a study with 42 individuals, 50% of which were long-term survivors for greater than 10 years. The other half had progressed to disease. They found no correlation with viral growth kinetics, neutralizing antibody response, or heterozygosity for CCR5 to explain the difference between the two groups. However, it was noted that the progressors had an increased number of CD8$^+$ T cells expressing CD38, as described previously by Giorgi *et al.* (52). Additionally, the ability of the CD8$^+$ T cells to suppress viral replication in an *in vitro* noncytolytic CD8$^+$ T cell suppression assay did positively correlate with the long-term non-progressor status.

5.2. Correlation between CD8$^+$ T cell mediated activity and viral load

Two different studies noted an inverse correlation between CD8$^+$ T cell activity and viral load (53, 54). Mackewicz *et al.* (53) studied seven individuals who were participants in the San Francisco Men's Health study at five time points: 12 and 6 months pre-seroconversion, seroconversion date (estimated as the midpoint between the last seronegative and the first seropositive time point), and 6 and 12 months after the seroconversion visit. Three of these individuals demonstrated an inverse correlation between antiviral activity and plasma viremia. However, in all seven patients examined, CD8$^+$ T cell suppressive activity developed by their seroconversion visit. It is unclear from this study if the suppressive activity occurred by prior exposure to HIV or as a result of their current infection. Further work needs to be done to demonstrate if CD8$^+$ T cell activity is present in the acute stages of HIV infection.

Blackbourn *et al.* analyzed five individuals at different stages of HIV infection for the ability of their lymphoid and peripheral blood CD8$^+$ T cells to suppress HIV replication (54). Higher levels of CD8$^+$ T cell mediated suppressive activity were apparent from those with a healthier clinical state and a lower viral burden. This was determined by the lower ratio of CD8$^+$ T cells: CD4$^+$ T cells needed to effect a 90% or greater reduction in viral replication (measured in an endogenous assay at the peak of viral replication by either a p24 antigen or RT assay). Although the sample number is limited, this study also demonstrates the ability of lymph node derived CD8$^+$ T cells to suppress *in vitro* HIV replication. However, in another study, no correlation between viral load and CD8$^+$ T cell activity was observed. Ferbas *et al.* (55) demonstrated that CD8$^+$ T cell antiviral activities were decreased in nonprogressors with the lowest viral load and the highest CD4$^+$ T cell counts. They studied a group of 35 HIV-infected persons that contained a subgroup of 14 chronically infected individuals. For eight of these individuals, they performed a non-cytolytic CD8$^+$ T cell suppression assay. Two of these individuals were characterized as having a lower viral load and higher CD4$^+$ T cell count than the rest of the individuals. Both of them did not demonstrate a spontaneous non-cytolytic CD8$^+$ T cell anti-viral response. However, one of them could be induced to produce this soluble anti-viral factor. All six of the other individuals produced detectable levels of soluble antiviral activity. In addition, 5 of the 6 were tested for contact mediated suppression and all of these were positive. They suggest that their results were the opposite of what they had originally hypothesized. For they initially believed that those who were controlling the virus the best, those with a low viral load would have the highest levels of CD8$^+$ T cell antiviral activity (both cytolytic and non-cytolytic). It is likely that the occurrence of a low viral load leads to a lack of antigen stimulation for the CD8$^+$ T cells and thus a diminution in their activity. However, one must also take into account the low numbers of individuals analyzed. Most importantly, this study does provide additional evidence for the detection of non-cytolytic CD8$^+$ T cell antiviral activity in HIV-infected persons.

5.3. Correlation of CD8$^+$ T cell mediated activity with CD4$^+$ T cell counts

Conflicting findings have been reported in the literature regarding the relationship between CD8$^+$ T cell mediated antiviral activity and CD4$^+$ T cell counts in infected individuals. Mackewicz *et al.* demonstrated that the potency of this CD8$^+$ T cell activity directly correlated with the individual's CD4$^+$ T cell count (31). Subjects were stratified according to their CD4$^+$ T cell counts and it was observed that those individuals having lower CD4$^+$ T

cell counts required a larger number of CD8$^+$ T cells to mediate the same antiviral effect as those individuals whose CD4$^+$ T cell count were high. In another paper that analyzes the relationship between CD4$^+$ T cell count and CD8$^+$ T cell antiviral activity, two studies are reported (56). One involves 16 HIV$^+$ infected individuals (stage of disease not reported) where 25% had the ability to noncytolytically suppress autologous HIV replication *in vitro* (56). A second study described in this paper states that 13 out of 52 HIV$^+$ individuals displayed antiviral activity, also 25%. In both cases, this activity correlated with a high CD4$^+$ T cell count, (but not CD8$^+$ T cell count) in the peripheral blood. In the second study, the antiviral activity also correlated with a lack of opportunistic infections, but did not correlate with the presence of antiretroviral therapy.

There are also a few studies that did not find a correlation between clinical status and CD8$^+$ T cell suppressive activity. These studies use different methodologies than those studies that found a link. A longitudinal study utilizing both primary HIV-1 isolates and CD8$^+$ T lymphocytes from 7 healthy blood donors, 8 HIV-1 uninfected homosexual men, and 13 HIV-1 infected homosexual men measured the clinical course of disease in relation to CD8$^+$ T cell antiviral activity (57). Interestingly, strong CD8$^+$ T cell antiviral activity was found in all three groups and there was no correlation with CD4$^+$ T cell counts. Thus, it was concluded that CD8$^+$ T cell antiviral activity did not correlate with the clinical status of the individual. In a study using the suppression of HIV-1 LTR mediated transcription as the measurement for CD8$^+$ T cell mediated suppression, there was also no correlation found between the clinical status of the individual and suppression of gene expression (58). HIV$^+$ infected individuals were classified as asymptomatic, symptomatic, or AIDS and analyzed for this study. Of note, some suppressive activity was stable over time, whereas others had a transient activity that fluctuated over time. Although suppression of gene expression in this study did not correlate with the clinical status of the individual, there was a correlation with a higher CD8$^+$ T cell count and higher levels of beta-chemokines in general. In agreement with others (57), this study also demonstrated that there was no correlation between the potency of CD8$^+$ T cell antiviral activity and the *in vivo* CD4$^+$ T cell count.

5.4. CD8$^+$ T cell mediated antiviral activity in exposed uninfected individuals

One can calculate one's risk for acquisition of HIV by the number of possible exposures to the virus. There is a direct correlation between the number of encounters, or risk factors, and a positive HIV result (59). However, there are interesting exceptions to this and they are called exposed uninfected individuals (EU). This group may possess natural and/or acquired anti-HIV immune protective mechanisms. Although genetic variations such as the inherited mutation of a 32-bp deletion in the CCR5 molecule (CCR5delta32) (60, 61), and the CCR2-64I polymorphism (62) can account for the protection seen in some of these individuals, many other individuals do not possess known genetically acquired resistance factors. Consequently, immunological function studies of these individuals are important in deciphering what may represent the correlates of immune protection. It may be possible to specifically enhance these immune responses in the creation of new candidate vaccines. One interesting study demonstrated that a potent CD8$^+$ T cell mediated suppressive activity could be found in individuals uninfected with HIV, but who have had multiple exposures to the virus (63). Four different cohorts of exposed uninfected individuals totaling 60 were used to analyze CD8$^+$ T cell suppressive activity, CTL activity, susceptibility of CD4$^+$ T cells to HIV, and correlation with CCR5delta32 mutations. Significantly, only a strong CD8$^+$ T cell antiviral activity was found in individuals having a possible exposure to HIV within one year of the study.

5.5. Pediatric HIV

Another group of individuals that are likely to provide information about protective anti-HIV immune responses are uninfected infants who are exposed to HIV in utero or parturition. It is known that vertical transmission depends both on the viral load and immunological responses present in the mother (64). However, knowledge about the fetal immune responses that may play a role in protection is scarce. In many cases, a strong antiviral CD8$^+$ T cell mediated response can be detected in infants born to infected mothers within the first year of life (65), (Tomaras, G.D., Greenberg, M.L., unpublished observations). CD8$^+$ T cell mediated suppression was detected sometimes by three weeks of age. There were positive correlations between strong antiviral activity, favorable clinical outcomes and high CD4$^+$ T cell counts. Others have demonstrated that there is an association between a strong CD8$^+$ T cell antiviral response and lack of HIV infection in infants born to HIV infected mothers (66). In 16 of 31 uninfected infants born to HIV-infected mothers, their CD8$^+$ T cells suppressed HIV_{SF33} in an acute infection assay. This activity was not mediated by beta-chemokines, since a beta-chemokine insensitive HIV strain was used. However, a different study demonstrated that protection from vertical transmission correlates with HIV specific TH responses involving the production of the beta-chemokines (67). These studies indicate that a strong CD8$^+$ T cell suppressive activity may play a protective role in inhibiting maternal-fetal transmission of HIV.

6. PHENOTYPIC MARKERS FOR CD8$^+$ T CELL MEDIATED SUPPRESSIVE ACTIVITY

A consistent feature of HIV infection is a rise in the number of CD8$^+$ T lymphocytes (68, 69). However, no clear correlation has yet been made on the absolute number of CD8$^+$ T cells and the potency of antiviral activity suggesting that a specific subset of CD8$^+$ T cells might be involved. Ideally, this subset of CD8$^+$ suppressive T cells would be identified and isolated based on cell surface marker(s) and then used in a detailed analysis of its functional properties. Thus, many investigators have tried to phenotypically characterize CD8$^+$ suppressive T cells. Although some markers have been associated with a suppressive phenotype, it is still not known if one can directly link suppressive activity to a molecule expressed on the cell surface. Plaeger-Marshall *et al.* (70) demonstrated that

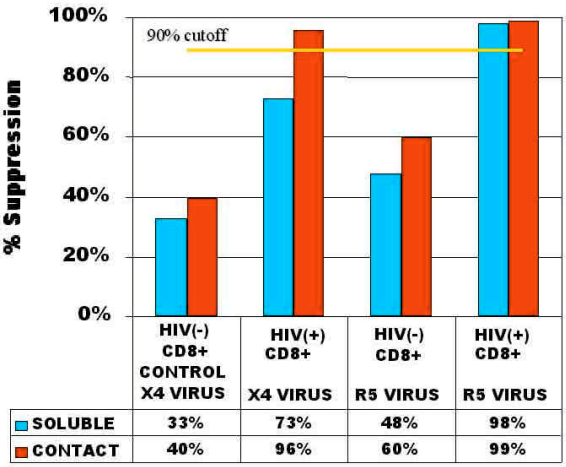

Figure 1. Contact and Soluble Mediators of CD8$^+$ Antiviral Activity. Soluble suppressive activity was assessed by culturing CD4$^+$ T cells in the top well and CD8$^+$ T cells in the bottom well of a transwell tissue culture plate. Contact mediated suppressive activity was assessed by culturing both CD4$^+$ T cells and CD8$^+$ T cells together in a 24 well tissue culture plate. Percent suppression is the measurement of viral reverse transcriptase abolished by the presence of CD8$^+$ cells. The yellow line indicates the one log reduction in viral replication that is the cutoff for this suppression assay. These results are representative of three separate experiments performed at a 2:1 effector to target ratio. Both soluble and contact mediators from CD8$^+$ T cells were responsible for the observed suppression of viral replication.

CD57 depleted cells did not inhibit HIV replication as well as the whole population of CD8$^+$ T cells. They inferred that the adhesion molecule, CD57, was a marker for CD8$^+$ T cells with anti-HIV activity. Other studies have not found a correlation between the expression of CD57 with either disease stage or antiviral activity (49). However, they did find that CD8$^+$ T cells expressing HLA-DR and/or CD28 are significantly associated with suppressive activity against HIV replication. They also demonstrated that activated CD8$^+$ T cells (CD28$^+$) are decreased in AIDS patients compared to asymptomatic individuals. The expression of the activation markers, HLA-DR and CD38 are higher in asymptomatic individuals and AIDS patients compared to uninfected controls (71). The accumulation of DR$^+$CD38$^+$ double positive CD8$^+$ T cells in the peripheral blood of individuals progressing towards AIDS and the selective accumulation of DR$^+$CD38$^-$ CD8$^+$ T cells in asymptomatic individuals has led some investigators to posit that the later subset may represent the subpopulation of CD8$^+$ T cells with the highest viral suppressive activity (71). In another study (44) that analyzed clonal populations, HLA-DR, S6F1, and CD25 were expressed to a higher degree in clones that demonstrated suppressive activity as compared to clones that did not have antiviral activity. Although a few markers on the cell surface have been identified that correlate with CD8$^+$ T cell mediated antiviral activity, the exact nature or category of these cells is still unknown. Identification of the particular subset of CD8$^+$ T cells that is responsible for this activity would not only be beneficial in the isolation of these cells, but also in the characterization of their mechanism of action. However, it remains equally plausible that a defined population may not exist because there may be great heterogeneity within the CD8 positive cells that have acquired this antiviral phenotype.

7. CONTACT & SOLUBLE MEDIATED ACTIVITY

Abrogation of HIV replication in a host cell is mediated by interaction of currently unknown "suppressor" molecule(s) at the infected host cell surface. This molecular interaction leads to either inhibition of virus entry into the cell or triggering of an intracellular signaling pathway that negatively affects the completion of the virus life cycle. Two possible modes by which this suppression can occur is either through "suppressor" molecule(s) which are membrane bound to another cell requiring cell-cell contact for its effects or the "suppressor" molecule(s) can be secreted from cells (soluble) and act independently of cell contact. The ability of peripheral blood CD8$^+$ T lymphocytes from HIV infected individuals to suppress HIV replication was first demonstrated to be mediated by both soluble and contact dependent factors by Levy and colleagues (72, 73). A transwell culture dish, which separates the CD4$^+$ T cells from the CD8$^+$ T cells by a semi-permeable membrane, was used to demonstrate that the diffusible portion of this suppressive activity varied between infected individuals. Those individuals whose CD8$^+$ T cells did not display a soluble component or only had a weak soluble anti-viral activity were able to abrogate HIV replication in a contact dependent manner. Interestingly, when the soluble anti-viral activity was present, it was only transient and declined over time. Others demonstrated the ability of both autologous and allogeneic CD8$^+$ T cells to inhibit viral replication across a semi-permeable membrane (32). This group also demonstrated that a concentrated supernatant from these suppressive CD8$^+$ T cells effected an inhibition in HIV viral replication. In agreement with Walker *et al.*, they demonstrated that this soluble activity was not found in all cultures capable of CD8$^+$ T cell mediated suppression. Much of this work has been confirmed and repeated by others. Some laboratories have focused on identifying the soluble mediators, while many others have pursued studying both mechanisms (contact and soluble) of noncytolytic CD8$^+$ T cell mediated suppression.

7.1. Contact mediators

The cell surface molecules responsible for contact mediated suppression have not been identified. However, several groups have used antibodies to cell surface molecules to try to inhibit this activity (73, 74). Studies performed in our laboratory have demonstrated that the significant proportion of the CD8$^+$ T cell antiviral activity is mediated by cell-cell contact (22, 27, 44). Analysis of the antiviral activity of a CD8$^+$ T cell line that we derived from the PBMCs of an asymptomatic HIV$^+$ long-term nonprogressor (27, 37) demonstrates that the substantial portion of the antiviral activity is initiated from cell-cell contact as presented in figure 1. CD8$^+$ T cell effector cells and CD4$^+$ target cells were separated in a

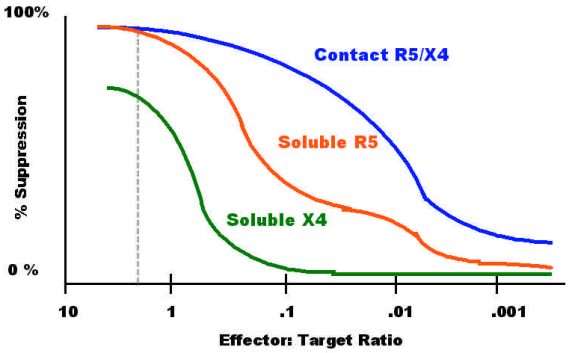

Figure 2. Model of CD8+ T Cell Derived Antiviral Factors. CD8+ T cell mediated suppressive activity is multifactorial. This graph depicts the range of contact and soluble mediated CD8+ T cell mediated suppressive activity seen against CCR5 and CXCR4 tropic viruses.

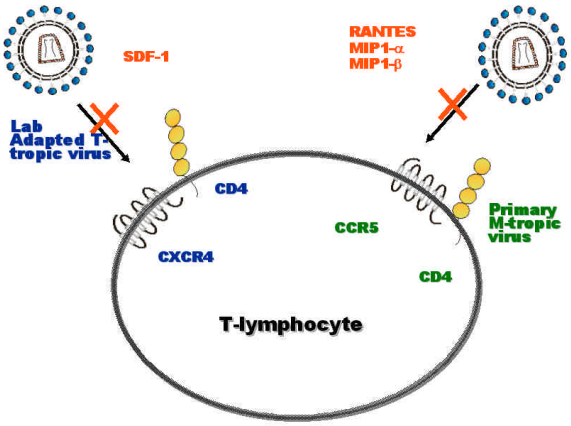

Figure 3. HIV Entry. The most common mode of entry of HIV into the cell is through the use of CD4 and a chemokine coreceptor. Lab adapted T-tropic viruses (SI) utilize the coreceptor, CXCR4, in addition to CD4. The natural ligand for CXCR4 is SDF-1. Although SDF-1 can block HIV infection, it is not responsible for CD8+ T cell mediated inhibition of CXCR4 tropic viruses. Primary M-tropic viruses (NSI) utilize the coreceptor, CCR5, in addition to CD4. The natural ligands for CCR5 are RANTES, MIP-1 alpha, and MIP-1 beta. These molecules can be secreted from CD8+ T cells to mediate inhibition of viral entry.

transwell tissue culture dish by a semipermeable membrane and their ability to suppress HIV replication was compared to the coculture of CD8+ and CD4+ T cells. As demonstrated in the graph, a CCR5 using virus was suppressed by both contact and soluble mediators above the cutoff of a 1 log reduction in viral replication (>90% inhibition). Although there is some viral suppression (73%) by soluble mediators for the CXCR4 using virus, it was significantly suppressed only if the cells were allowed to remain in contact (>90%). In the case of R5 utilizing viruses it appears from the transwell experiment that the soluble and contact mediated activity are of equivalent potency. However, the data in this figure were from an experiment employing a single E: T ratio. From other data generated in our laboratory using a range of different E: T ratios, it is apparent that the contact mediated activity is of greater potency (even against R5 isolates) than the soluble activity for these cells. In figure 2, we describe a model that more accurately depicts the situation for soluble and contact suppressive activities for both CCR5 and CXCR4 HIV-1 isolates. The graph shows a significant distinction between the soluble and contact mediated activity against CXCR4 viruses as also demonstrated in figure 1. However, for CCR5 viruses, as the E: T ratio is decreased, there appears to be a difference in activity between the soluble and contact activities which is not apparent from using a single E: T (2:1) as used in figure 1. We ascribe much of the soluble activity against R5 viruses to beta-chemokines since a large amount of these molecules can be secreted from this cell line. However, the molecule(s) responsible for the soluble activity against X4 viruses and the contact mediated activity against both R5 and X4 viruses remain unknown.

7.2. Soluble mediators

7.2.1. Beta-chemokines

RANTES, MIP1-alpha, MIP1-beta were identified as having anti-HIV activity (19). These chemokines belong to a superfamily of chemotactic factors that are proinflammatory in their ability to recruit and activate leukocytes (75). Utilizing a highly sensitive cell line (PM1) and cloned CD8+ T cells with potent anti-viral activity, Cocchi *et al.* (19) discovered that three beta-chemokines, RANTES, MIP1-alpha, MIP1-beta, could mediate CD8+ T cell noncytolytic antiviral activity. Neutralizing antibodies to all three chemokines blocked the antiviral activity mediated by these CD8+ T cells. Additionally, a combination of these recombinant human proteins could block HIV infection in a dose dependent manner. However, higher doses of the three chemokines were necessary to inhibit HIV in PBMC than in the PM1 cells. Interestingly, these chemokines were not able to block infection by HIV_{IIIB}. The reason for this was clear upon the discovery of the HIV coreceptors and the difference in binding by select viruses. Initially, the biological phenotypes of primary isolates of HIV-1 were divided into two types based on their ability to induce syncytia in the MT-2 cell line. As such, viruses were classified into rapid high syncytium-inducing (SI) or slow/low syncytia inducing (NSI) (76). The molecular differences between these two types of viruses were determined to be due to changes in particular amino acid positions in the third variable region of the gp120 envelope protein (77-84). Identification of fusin (LESTR) (85), a member of the 7-transmembrane G protein-coupled receptor family, as the coreceptor for T cell line adapted HIV-1 isolates (SI), further substantiated the idea that different mechanisms were required for entry of different strains of HIV-1. Eventually, the chemokine receptor, CCR-5, was discovered to be the second receptor for non-syncytia inducing viruses (NSI) (86-90). One of the mechanisms for beta-chemokine inhibition of viral replication is a block in cell fusion subsequent to CD4 binding (86). Others have demonstrated that another mechanism by which the beta-chemokines inhibit viral replication is through the downregulation of the chemokine receptors (91). Figure 3 depicts the coreceptors (CXCR4

and CCR5) that mediate HIV entry and the ligands to these receptors that are capable of blocking infection. Different HIV-1 isolates were shown to have different sensitivities to beta-chemokines (92). Only NSI viruses were inhibited by beta-chemokines, whereas, SI viruses were generally resistant. In support of this, patients whose virus phenotype switched from NSI to SI demonstrated a reduced sensitivity to beta-chemokines.

Chemokines are secreted by a wide range of cell types, including monocytes and various lymphocyte subsets (75). Specifically, MIP-1 alpha, MIP-1 beta, and RANTES are produced by both CD4$^+$ T cells (20, 21, 93, 94) and monocytes (95). It was demonstrated that the secretion of these molecules by CD4$^+$ T cells and not CD8$^+$ T cells correlated with a more favorable clinical outcome (23). Additionally, only particular types of CD8$^+$ T cells mediate antiviral activity. This led many to believe that other molecules/mechanisms must be involved in the CD8$^+$ T cell suppressive activity. Paliard *et al.* (96) tested different CD8$^+$ T cell clones both for the potency of their anti-viral activity and the amount of secreted beta-chemokines. Not only was there no correlation between antiviral activity and secretion of MIP-1 alpha, MIP-1 beta, and RANTES, but anti-chemokine antibodies did not block the CD8$^+$ T cell antiviral effect (37, 96). Additionally, Levy's group argued that the beta-chemokines were not the long sought after "CAF" (24) because chemokine levels in the cell supernatant did not correlate with anti-viral activity and antibodies to the chemokines did not abrogate the anti-viral effect with the viruses HIV$_{SF2}$ and $_{HIV\ SF33}$. In response, it can be argued that there may not be one factor released from CD8$^+$ T cells, but several with different activities against the various viral isolates. Additionally, these factors may be working collectively to promote a potent anti-viral response. It is also likely that they may target different stages of the viral life cycle. Levy's group went on to demonstrate that the *in vivo* levels of beta-chemokines do not prevent progression to disease, although the replication of NSI viruses can be inhibited by beta-chemokines (97). They performed a cross-sectional study from both seronegative donors and HIV-infected patients at different stages of the disease. No significant difference in the levels of beta-chemokines was observed between the asymptomatic and symptomatic stages of HIV infection. A study by Rubbert *et al.* (98) is in agreement with these findings. Beta-chemokine levels were not significantly different between purified CD8$^+$ T cells from seven HIV-uninfected and 14 HIV-infected individuals. In contrast, a cross-sectional analysis (99) of 100 subjects enrolled in the Multicenter AIDS Cohort Study demonstrated that beta-chemokine production is lower in those individuals with AIDS than in those without. The variation between these studies as to whether or not the presence of beta-chemokines are protective in disease progression may be due to the different assay systems and/or activation schemes employed.

In the system of using cocultures of dendritic cells and autologous CD4$^+$ T cells (DC/CD4$^+$), unidentified molecules in addition to beta-chemokines were shown to be responsible for the CD8$^+$ T cell suppressive activity (98). In the acute DC/CD4$^+$ T cell system, the recombinant beta-chemokines did not inhibit viral replication; whereas, the CD8$^+$ T cells potently suppressed both X4 and R5 HIV. However, in an endogenous DC/CD4$^+$ T cell assay, the beta-chemokines did inhibit viral replication, but polyclonal neutralizing antibodies to all three chemokines did not abrogate the suppressive function of the CD8$^+$ T cells. Interestingly, it was demonstrated that another source of the beta-chemokines that are effective against HIV replication is the CD4$^+$ T cell (21). Neutralization of beta-chemokines enhanced viral replication in either CD8$^+$ depleted or CD4$^+$ PBMCs. This idea was further expanded by Saha *et al.* (23). They measured the production of beta-chemokines from both CD4$^+$ and CD8$^+$ T cell clones from nonprogressors and AIDS patients. CD4$^+$ T cell clones from nonprogressors produced high levels of beta-chemokines; whereas, CD4$^+$ T cell clones from AIDS patients produced no RANTES and little MIP-1 alpha and MIP-1 beta. There was an inverse correlation between beta-chemokine production and susceptibility to infection by the R5 virus, HIV$_{ADA}$. Anti-beta-chemokine antibodies made CD4$^+$ cells from the nonprogressors more susceptible to HIV infection. These data indicate that the beta-chemokine production from CD4$^+$ T cells is more important for protection from R5-HIV infection than beta-chemokine production from CD8$^+$ T cells. This work is in agreement with Scala *et al.* (100), who also demonstrated that high levels of beta-chemokines were produced by CD4$^+$ T cells. Additionally it was demonstrated that CD8$^+$ T cell supernatants suppressed HIV$_{Ba-L}$ replication in monocyte/macrophages (MM) and HIV$_{IIIB}$ replication in activated PBMCs, but the beta-chemokines did not (101). Antibodies to the beta-chemokines did not modulate CD8$^+$ T cell mediated suppressive activity of HIV$_{Ba-L}$ infected MDM, but they did diminish the suppressive activity of CD8$^+$ T cells on HIV$_{Ba-L}$ infected PBMCs. Interestingly, supernatant from LPS -stimulated macrophages could inhibit HIV-1 infection in both primary macrophages and T cells (95). It was found that lipopolysaccharide (LPS) could stimulate the release of beta-chemokines from macrophages. Barker *et al.* (102) demonstrated that the beta-chemokines did inhibit infection of R5-HIV (HIV-1$_{SF162}$ and HIV-1$_{SF128A}$) in monocyte derived macrophages. However, neutralizing antibodies to the beta-chemokines did not inhibit suppression of viral replication in macrophages by CD8$^+$ T cells. It was later demonstrated that the form in which chemokines are presented to the cells will affect their inhibitory function (103). RANTES, MIP-1 alpha, MIP-1 beta can be secreted from cytotoxic T cells in a complex with proteoglycans. The association of RANTES with heparin sulfate enhanced its HIV inhibitory activity in monocytes. This study provides some insight on the discrepancy of some of the studies with the M/M system that show a lack of inhibition by the recombinant chemokines. The question is now also raised as to how efficient the beta-chemokine blocking antibodies are at inhibiting a complex of beta-chemokines and proteoglycans.

In other studies which utilized HIV-1 LTR mediated gene expression as the readout for CD8$^+$ T cell mediated suppression, it was observed in both monocytes

(104) and Jurkat T cells (105) that MIP-1 alpha, MIP-1 beta and RANTES were not responsible for the CD8$^+$ T cell derived suppressive effect. Once again, it was concluded that RANTES, MIP-1 alpha and MIP-1 beta could not account for the entire array of CD8$^+$ T cell derived anti-viral activity. Garzino-Demo *et al.* (106) also showed that recombinant RANTES could not inhibit either basal or Tat mediated HIV LTR transcription in T cells. In contrast to these studies, Handen, *et al.* (107) claims that the beta-chemokines do inhibit HIV-LTR mediated gene transcription in Jurkat cells and this is independent of the NFAT regulatory region. This study differs from that of Rosenthal's group in that it does not use cotransfection of the pSV40-*tat* plasmid to enhance the level of HIV-1 transcription. The absence of Tat may not only lower the level of transcription sensitivity that can be measured, but also indicates that these studies were based solely on basal LTR activity. Another difference between the studies is that Handen *et al.* conclusions are based on using a combination of beta-chemokines instead of using the effects of the individual beta-chemokines on transcription like in Leith *et al.* and Copeland *et al.* Leith *et al.* does note that when they tried a combination of the three beta-chemokines, a 37% decrease relative to control was observed (unpublished data). However, they still were unable to block the CD8$^+$ T cell mediated suppressive activity with antibodies to all three beta-chemokines. In light of the studies demonstrating that beta-chemokines act at viral entry and the inability of beta-chemokine antibodies to block transcriptional suppression (104, 105), it is more likely that the beta-chemokines are not responsible for the LTR-mediated transcriptional repression mediated by CD8$^+$ T cells.

7.2.2. Cytokines

Although there are many cytokines, such as IL-6 (IFN-beta2), TNF-alpha, TNF-beta, TGF beta, IFN-alpha, IFN-beta, IL-8 and IL-4 that demonstrate anti-HIV activity, none have thus far been deemed responsible for CD8$^+$ T cell mediated suppression (108, 109). Additionally, the CD8$^+$ T cell derived suppressive factor, termed CAF by Levy's group, did not induce 2' 5'-oligo A synthetase. Progression to AIDS was shown to correlate with a shift in the cytokine pattern from a T helper cell type 1 response (T_H1) to a T helper cell type 2 (T_H2) response. This means that production of certain cytokines, like IL-2, which enhance cell-mediated immunity, decline over time. Cytokines, such as IL-4 and IL-10, which enhance humoral mediated immunity, begin to take over as the disease progresses. Interestingly, several investigators have observed that the anti-viral effect of CD8$^+$ T cells declines with the progression from the asymptomatic stage to AIDS. The role of the modulation of these cytokines in CD8$^+$ T cell mediated antiviral activity was explored by Barker *et al.* (110). They demonstrated that IL-2 enhanced the anti-viral activity of CD8$^+$ T cells from HIV$^+$ individuals. Conversely, culture in IL-4 or IL-10 inhibited their antiviral activity. However, the addition of IL-2 could overcome the inhibitory effects of the T_H2 cytokines. T cells are dependent on IL-2 for proliferation, so it was not entirely surprising that CD8$^+$ T cell suppressive activity is enhanced by IL-2 production. The costimulatory molecule, CD28, is important for both IL-2 and IL-2R (CD25) production (111). Stimulation of CD8$^+$ T cells with both antibodies to CD3 and CD28 enhanced the antiviral capability of these cells. One study suggests that tumor necrosis factor alpha (TNF-alpha) may negatively affect the ability of CD8$^+$ T cells to suppress viral replication (112). Staphylococcal enterotoxin B (SEB) and phorbol 12-myristate 13-acetate (PMA) enabled HIV replication to occur despite the presence of CD8$^+$ T cells. This effect was abrogated by the addition of neutralizing antibody to TNF-alpha. However, since purified recombinant TNF-alpha did not have a viral inductive effect in the presence of CD8$^+$ T cells, a more complex network of signaling involving TNF-alpha must be occurring. In the monocyte/macrophage system, several cytokines are known to suppress HIV replication. In order to determine if these cytokines account for the CD8$^+$ T cell mediated suppressive activity against HIV replication in the M/M system, neutralizing antibodies to IL-10, IL-13, IFN-alpha and IFN-gamma were added simultaneously to the CD8$^+$ T cell supernatant (101). These antibody treatments modestly affected the ability of supernatants from some individuals to suppress HIV replication, indicating that if these cytokines are involved in the M/M system, they are not the predominant mediators.

7.2.3. Macrophage derived chemokine

CD8$^+$ T cells from HIV$^+$ infected individuals were immortalized by HTLV-I and tested for their suppressive function against both CCR5 and CXCR4 utilizing viruses (41). The supernatant from one T cell clone that had activity against both IIIB and a primary SI isolate was subjected to analysis by high-speed centrifugation, fractionation by heparin affinity chromatography, and repeated rounds of reversed-phase high performance liquid chromatography (HPLC). One protein peak that appeared as an 8-kD band by SDS-polyacrylamide gel electrophoresis had suppressive activity against IIIB and amino-terminal sequence analysis revealed the identity of macrophage-derived chemokine (MDC). MDC is a CCR4 ligand that had not previously been identified as an important molecule in HIV infection. The MDC that was purified (described as the native form) lacked the first two NH2-terminal amino acids ((-2) MDC). It was later noted that several forms of MDC exist. However, when recombinant forms of MDC, both truncated and full length, were independently tested no HIV inhibition in T cells was seen (113, 114). Other work confirmed the lack of MDC mediated HIV suppression in activated T cells, but demonstrated that MDC is capable of suppressing HIV replication in monocyte-derived macrophages (115). From this work, it is clear that MDC can not account for the suppressive activity of CD8$^+$ T cells since these cells will inhibit HIV replication in T cells, unlike MDC.

7.2.4. Interleukin 16

The soluble inhibitory factor released by CD8$^+$ T lymphocytes from seropositive African Green Monkeys (Cercopithecus aethiops) was described as, ISL, immunodeficiency-virus-suppressing lymphokine (116). In confirmation of a soluble antiviral activity being reactive against diverse strains, Ennen *et al.* (116) performed cross

coculture experiments using CD8$^+$ T lymphocytes and CD4$^+$ T cells from both humans and the non-human primate African green monkey (AGM). They demonstrated that CD8$^+$ T cells from both seropositive and seronegative AGMs and from a pool of seronegative humans inhibited replication in SIVagm-infected AGM CD4$^+$ T cells. In later work (117), this factor was identified as lymphocyte chemoattractant factor (LCF), or interleukin-16 (IL-16). Both recombinant human and African green monkey IL-16 inhibited HIV replication in PBMCs. It was stated that a fraction of the ISL was likely to be IL-16. IL-16 will bind to CD4 and induce signal transduction. A likely mechanism for HIV inhibition by IL-16 is the production of a negative signal after crosslinking of the cell surface CD4 molecule and not inhibition of viral entry (118). In another study, recombinant IL-16 repressed transcription from the HIV-1 LTR-reporter gene despite PMA and Tat activation (119). These effects are mediated through the CD4 molecule because cell lines that lacked CD4 did not respond to IL-16. Additionally, it appears that IL-16 may affect the binding of transcription factors to the HIV enhancer, possibly by inducing a repressor complex. These studies are supported by evidence that IL-16 downregulates HIV-LTR promoter activity in infected Jurkat T cells (120).

Although IL-16 is an intriguing molecule and has effects that certainly coincide with CD8$^+$ T cell mediated suppressive activity, it is still not possible to make a definitive statement about the importance of its role in CD8$^+$ T cell mediated antiviral activity. It has been reported that many preparations of CD8$^+$ T cell antiviral activity do not contain IL-16 because monoclonal antibodies against IL-16 do not neutralize the antiviral activity of CD8$^+$ T cells (121). Another study measured IL-16 levels from both PBMC and T cell clones from asymptomatic HIV$^+$, long-term nonprogressors (LTNP) and AIDS patients (100). In these studies there was no correlation of soluble suppressive activity attributable to IL-16 and viral suppression. More definitive studies into the possible role of IL-16 in CD8$^+$ T cell mediated virus suppression are needed to clarify this issue.

7.2.5. Stromal cell-derived factor 1

Stromal cell-derived factor 1 (SDF-1) is a CXC chemokine and as the ligand for the HIV coreceptor, CXCR4 (LESTR/fusin), it will block entry of CXCR4 dependent viruses as depicted in figure 3 (122, 123). It was originally described as a bone-marrow stromal cell-derived factor (124) and later it was demonstrated to be a lymphocyte chemoattractant (125). Although it will inhibit X4-dependent HIV strains in vitro, it is not secreted by CD8$^+$ T cells as part of the CD8$^+$ T cell mediated suppressive activity (126). The expression of SDF-1 is constitutive in most cell types, but CD8$^+$ T cells contain low levels of SDF mRNA transcripts (126). It was found that the level of SDF expression does not correlate with CD8$^+$ T cell mediated suppressive activity. In agreement with this, Ohashi et al (127) also demonstrated that the levels of SDF-1 mRNA do not correlate with the level of CD8$^+$ T cell mediated antiviral activity. Consequently, the full spectrum of molecules responsible for the CD8$^+$ T cell mediated suppression of X4 dependent HIV infection is still unknown.

8. CYTOLYTIC INITIATED SUPPRESSION

Cytotoxic CD8$^+$ T cells (CTL) specific for HIV antigens have been described in HIV infected individuals (128, 129). CTL function is associated with HLA class I restriction and the ability to kill HIV-infected targets, such as autologous macrophages, lymphoblasts and EBV-transformed cell lines that present HIV antigens. There has been some question of differentiating CD8$^+$ T cell mediated suppressive activity from CTL function. The most apparent difference is the lack of targeted cell killing by the CD8$^+$ T cells with the suppressive phenotype. This was first addressed by recovering CD4$^+$ T cells from the coculture of CD8$^+$ and CD4$^+$ T cells that could support viral replication. A quantitative analysis, utilizing an immunofluorescence assay for HIV-1 antigens (IFA), provided further evidence in support of a noncytolytic CD8$^+$ T lymphocyte mediated suppression of HIV replication (130). This work demonstrated that if infected CD4$^+$ T cells were cultured with CD8$^+$ T cells and then removed, the number of HIV positive cells after a subsequent 13 days in culture without CD8$^+$ T cells was greater than or equivalent to the number of HIV positive cells in CD4$^+$ T cells that were never exposed to CD8$^+$ T cells. Further work went on to demonstrate the absence of cytotoxicity in the CD8$^+$ T cell mediated antiviral mechanism (73).

Although much work has demonstrated the absence of cytolysis in CD8$^+$ T cell mediated suppression of HIV, there are a multitude of other CD8$^+$ T cell clones generated from HIV infected individuals that are diverse in phenotype and function. Some will demonstrate either HLA-restricted CTL activity or non-HLA restricted inhibition of virus replication (44). Many clones also demonstrate both cytolytic and non-cytolytic activities (44-46). Further evidence in support of the dual function of HIV-1-specific cytotoxic T lymphocytes came with the discovery that MIP-1 alpha and granzyme A colocalize in the cytolytic granules (103). Hence, both non-cytolytic and cytolytic molecules can be released jointly upon activation of CTLs.

9. HLA VS. NON-HLA MATCHED

Tsubota *et al.* concluded that autologous, but not allogeneic CD8$^+$ T cells inhibited HIV replication in CD4$^+$ T cell cultures (74). However others have demonstrated that both autologous and allogeneic CD8$^+$ T cells suppressed HIV replication of endogenously infected CD4$^+$ T cells (32). Additionally, CD8$^+$ T cells from seronegative individuals also suppressed HIV replication in a non-MHC specific manner, whereas CD4$^+$ T cells had no effect and B cells had a minor effect. However, Brinchman *et al.* also demonstrated that allogeneic CD8$^+$ cells required a higher concentration than that of autologous CD8$^+$ T cells for efficient suppression (32). Other studies came to the conclusion that suppressive activity was more efficient when MHC alleles were shared (45, 131). In a syngeneic setting, more than 20-fold fewer CD8$^+$ T cells were required to inhibit HIV replication compared to the heterologous system (131). Thus, HLA matching was

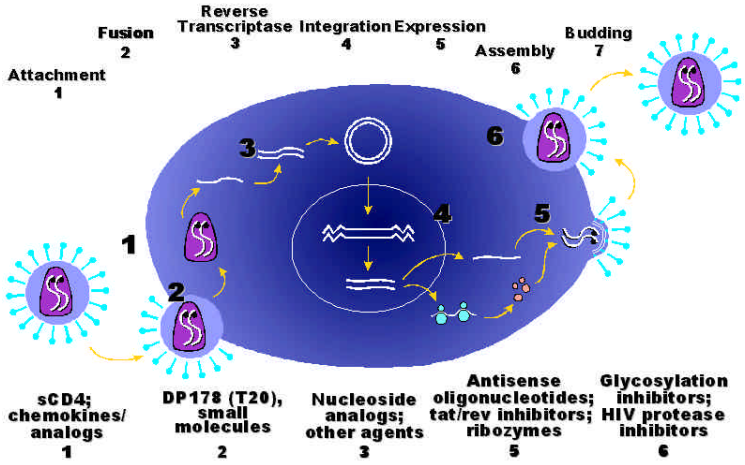

Figure 4. Possible Points of Intervention. The replication of HIV inside a cell can be described by the various stages it must go through to complete its replication cycle. Analysis of the various steps in the life cycle has been useful in determining the mechanism of action of a specific inhibitor (such as $CD8^+$ T cell derived inhibitors). Many different inhibitors have been identified for each of the stages of the life cycle. In some cases, they are used only in the laboratory; others are approved clinical therapies for the treatment of HIV disease.

unnecessary, but it raised the potency of the $CD8^+$ T cell mediated antiviral response. Interestingly, the higher sensitivity was not linked to either HLA class I or class II locus. Another study (132) also demonstrated that there was both HLA class I restricted and unrestricted $CD8^+$ T cell mediated suppressive activity. In agreement with others, MHC class I matching resulted in a more efficient suppression of viral replication. Over the disease course, they demonstrated that the MHC class I unrestricted suppression disappeared before the MHC class I restricted suppression. Additionally, the maintenance of just the MHC class I restricted suppression correlated with a decline in the $CD4^+$ T cell count suggesting that MHC class I unrestricted suppression may be required for a healthier clinical status.

The controversy over the requirement of HLA restriction for $CD8^+$ T cell suppressive function is tightly linked to the cell type that is being analyzed. Yang et al (46) maintains that HIV-1 -specific cytotoxic T lymphocytes (CTL) suppress viral replication by both a cytolytic and noncytolytic mechanisms. The initial interaction of $CD8^+$ T cells with infected $CD4^+$ T cells is by direct contact and is HLA class I restricted. Subsequently, these cells can inhibit viral replication in a noncytolytic fashion and without HLA restriction. They also demonstrated that the noncytolytic activity is in part mediated by the soluble and proteoglycan complexed beta-chemokines (MIP-1 alpha, MIP-1 beta and RANTES) but there are also unidentified factors involved (103).

10. MECHANISMS OF INHIBITION

10.1. Entry

Suppression of HIV replication is potentially mitigated at several different stages in the viral life cycle including entry (attachment and fusion), provirus synthesis (reverse transcriptase), integration, gene expression, assembly of viral proteins, and budding from the cellular membrane as depicted in figure 4. The currently approved HIV therapeutics either inhibit reverse transcription or the HIV protease. However, new studies have demonstrated that HIV entry can be inhibited and may be an important addition to the current supply of HIV treatment options (133). It has been established that HIV inhibition by $CD8^+$ T cells can occur at viral entry. This discovery began with work (19) that identified the beta-chemokines as the $CD8^+$ T cell derived suppressive factors. The mechanism of inhibition is the result of competitive inhibition of virus-coreceptor binding and downregulation of coreceptor cell surface expression (134). Studies have shown that the beta-chemokine, RANTES, did not inhibit HIV-1 gene expression (106). Although, the beta-chemokines are clearly important inhibitors of HIV replication, our lab and others have demonstrated that these molecules do not account for all of the suppressive activity demonstrated by $CD8^+$ T cells (22, 24, 27, 98, 101). However, the study of these molecules and their role in suppressing HIV replication has led to numerous important findings, and provided significant insight into the multifactorial nature of $CD8^+$ T cells.

10.2. Transcription & gene expression

The question of $CD8^+$ T cell mediated effects on HIV transcription has been approached in a multitude of ways. The first study to directly address this issue demonstrated that in primary $CD4^+$ T lymphocytes both basal and Tat mediated transcription of the HIV-1 LTR were inhibited by $CD8^+$ T cells from asymptomatic HIV-1 infected individuals (135). This activity was not MHC class I restricted because Tat mediated transcription was suppressed in heterologous $CD4^+$ target cells. Additionally, supernatants from $CD8^+$ T lymphocyte cultures of asymptomatic HIV^+ individuals suppressed HIV-LTR transcription demonstrating that soluble factors

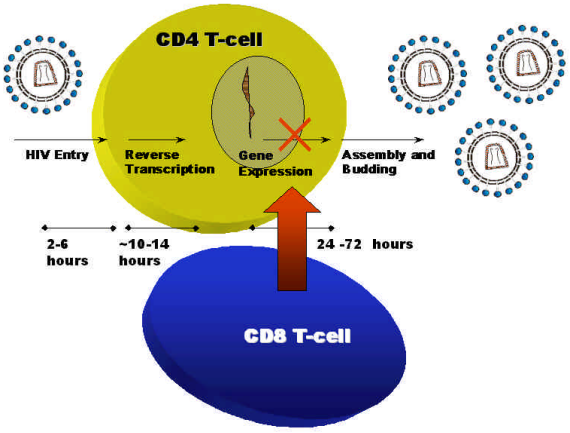

Figure 5. Kinetics of HIV Infection and CD8$^+$ Suppressive Activity. The time course of the different stages of a single cycle of HIV replication in primary CD4$^+$ T lymphocytes is depicted in this model. In studies that specifically remove beta-chemokine mediated effects on entry, CD8$^+$ T cell mediated activity targeted HIV gene expression.

are involved in mediating the inhibition. The concepts developed in these studies were then confirmed by others (136, 137). Copeland *et al.* (137) utilized an HIV-1 LTRCAT plasmid that was either transfected alone or in combination with pSVtat in Jurkat T cells. Supernatants from T cells displaying suppressive activity from either a cloned CD8$^+$ T cell line or activated CD8$^+$ T cells from asymptomatic HIV infected individuals were able to reduce the levels of CAT production under the control of the HIV-1 LTR in activated Jurkat cells. So, in agreement with Chen *et al.* (135), Tat mediated transactivation of the HIV LTR could be inhibited by CD8$^+$ T cells. CD8$^+$ T cell mediated suppression on CAT production was also effective if just the HIV dimer of NF-kappa B sites was placed upstream of the LTR. Additionally, because they demonstrated that CD8$^+$ T cells also inhibited transcription from the HTLV-1 LTR and the RSV LTR, they concluded that CD8$^+$ T cell mediated suppression is not lentivirus specific. Others demonstrated that the number of cells expressing HIV antigens and RNA was reduced when exposed to CD8$^+$ T cell antiviral factor (CAF) and that supernatant containing CAF inhibited LTR driven transcription in HIV-1$_{SF33}$ infected 1G5 cells (Jurkat cells transfected with a LTR-luciferase gene construct) (136). Basal levels of LTR driven transcription were not affected by CAF in these studies. However, from these experiments one can not discern whether entry, reverse transcriptase, nuclear translocation, or integration is the real target. A block at any one of these steps will also lead to an inhibition of viral RNA and HIV antigens. In contrast, other studies performed in a B-cell line demonstrated that CD8$^+$ T cells suppress basal transcription but not Tat mediated transcription (138).

Knuchel *et al.* (139) described a soluble factor from CD8$^+$ T cells from naturally infected disease-resistant sooty mangabeys (Cercocebus atys) that inhibited production of CAT mRNA in target cells stably transfected with a plasmid containing the SIV LTR upstream of the CAT gene. They concluded that CD8$^+$ T cells enhance early phases of transcription and inhibit the later stages. It is not clear from these studies what the mechanism is, but their results suggest that the CD8$^+$ T cells are secreting a soluble factor that can act on expression of an integrated gene controlled by an SIV LTR promoter. The quantitative amount of inhibition was rather limited in these assays, so it is difficult to extrapolate what is occurring in the naturally infected state. This same group (138) had previously demonstrated that Epstein-Barr virus (EBV)-transformed cell line (FEc1) from a simian immunodeficiency virus (SIV) seropositive sooty mangabey monkey transfected with a human CD4 gene was sensitive to infection with HIV-1, HIV-2, and SIV. CD8$^+$ T cells were able to inhibit CAT activity when these transformed B cells were transiently transfected with an LTR-driven CAT reporter gene. Interestingly, when these cells were cotransfected with an LTR-*tat* plasmid the suppression by whole lymphocytes was diminished. In contrast to the conclusions drawn from our studies (135), Powell *et al.* (138) concluded that CD8$^+$ T cells inhibited basal LTR transcription but not Tat driven HIV-LTR promoter activity.

The role of CD8$^+$ T cell supernatants from HIV-1 infected individuals on HIV-LTR mediated transcription in monocytes has been examined (104). These supernatants significantly enhanced Tat mediated transcription of the integrated HIV-1 LTR in a dose dependent manner. They did so both in the presence and absence of mitogenic stimulation. This enhancement is in direct contrast to that observed in Jurkat T cells. In Jurkat T cells, the same CD8$^+$ T cell supernatants demonstrated a marked reduction in Tat mediated transcription of an integrated HIV-1 LTR, in agreement with the findings of Chen *et al.* (135). Additionally, the beta-chemokines did not influence the HIV-1 LTR-mediated transcription in monocytes (U38 cells), indicating that these are not the CD8$^+$ T cell derived factors that are acting upon transcriptional regulation. These studies suggest that the effects of CD8$^+$ T cells may be different depending on the target cell and the local cytokine milieu. In a promonocytic cell line, (U1 cells) CD8$^+$ T -cell supernatants were able to suppress HIV-1 expression of an integrated provirus (101). Not only are these findings in contrast to the results obtained in monocytes, but they also demonstrate that in promonocytes suppression can act after virus integration into the host chromosome.

Studies performed in our laboratory demonstrate that the mechanism of action for CD8$^+$ T cell mediated suppression is postentry for X4 HIV strains (27) and R5 strains (Tomaras, G.D., Greenberg, M.L., unpublished observations). The kinetics of HIV pseudotyped virus infection in primary CD4$^+$ T cells was determined in order to decipher the stage in the virus life cycle targeted by CD8$^+$ T cells (27). Utilizing this single cycle infection assay, we demonstrated that CD8$^+$ T cell mediated suppressive activity acted after entry of virus into the cells unlike the entry inhibition mediated by the beta-chemokines. CD8$^+$ T cell mediated antiviral activity also acted after reverse transcription. The kinetics of inhibition implicated that CD8$^+$ T cells acted at the level of gene expression as depicted in figure 5. This assay system is

different from prior studies of the effects of CD8$^+$ T cells on HIV transcription, because here one is looking at inhibition in an ongoing infection. The prior studies demonstrated that it is possible to inhibit transcription of a transfected HIV LTR construct, but this new study shows that HIV gene expression is actually inhibited in an ongoing cycle of viral replication. Studies are currently underway to determine precisely the molecules involved in the alteration of viral gene expression.

11. ANIMAL MODELS

Animal models for acquired immunodeficiency syndrome are important for understanding the progression of the disease, for developing therapeutic interventions, and preventative strategies. There are several primate species that are infected in the wild with lentiviruses similar to HIV, but they do not succumb to the disease. Thus, many investigators have pursued the study of these primate virus infections to compare them with HIV infection in humans. Numerous studies have shed light on the virus/host interactions and the immune responses to infection that may play an important protective role in these animals. Several primate models are based on infection by the CD4$^+$ T-tropic simian immunodeficiency virus (SIV) to study the course of disease in animals.

11.1. Primate

One of the natural hosts for SIV is the African green monkey (AGM). About 50% of wild African green monkeys and 80% of captive AGM are apathogenically infected with SIVagm (140). Interestingly, AGMs have CD4$^+$/CD8$^+$ ratio of 0.13 compared to humans of 2.2. The increased number of circulating CD8$^+$ T cells in AGM may be important for controlling virema, for it has been demonstrated that there is a soluble anti-viral factor secreted from these CD8$^+$ T cells (116). This factor has interspecies activity, for it also suppressed replication of HIV-1 in human lymphocytes. In contrast to SIV infection, it does not appear that CD8$^+$ T cell mediated inhibition acquired by infection with HIV-1 will protect against a superinfection with HIV-2. CD8$^+$ T cells from asymptomatic HIV-1 individuals will suppress viral replication of exogenously added HIV-1 (IIIB) but not HIV-2 (CEM/LAV-2) (47). Although there has been conflicting data regarding the protective effects of HIV-2 infection for preventing HIV-1 infection (141, 142), it has been shown that individuals can be coinfected with both HIV-1 and HIV-2 (143).

Another natural host for SIV is sooty mangabeys (Cercocebus atys). Since they remain clinically asymptomatic, many investigators utilize infected sooty mangabeys to study which protective immune responses are employed. Interestingly, CD8$^+$ T cells from infected sooty mangabeys inhibited both SIV and HIV-2 replication in EBV transformed B cells expressing the CD4 gene (144). In contrast, in a study of SIV infection of sooty mangabeys, it was demonstrated that the isolation of SIV smFr74 was not facilitated by the removal of the CD8$^+$ T cells from the PBMC culture (145). This suggests that the CD8$^+$ T cell mediated antiviral response was not strong in these animals. Further studies utilizing CD8$^+$ T cell suppression assays with these animals are needed to confirm or deny this supposition.

Simian immunodeficiency virus (SIV) does induce an AIDS-like disease in rhesus macaques (Macaca mulatta). These animals die of diseases similar to that seen in humans (146). Macaques infected with SIV have a lymphadenopathy characterized by an increased number of activated CD8$^+$ T lymphocytes in the paracortex of the lymph nodes (147). This group also confirmed the original observation (16) that virus isolation was enhanced upon the removal of CD8$^+$ T cells. They also concurred that CD8$^+$ T cell derived antiviral activity was present from SIV-infected, but not uninfected animals.

HIV-1 infected chimpanzees do not exhibit clinical signs of disease (148). It appears that there is an innate ability of a chimpanzee's CD8$^+$ T cells to control HIV replication. This study by Castro *et al*. demonstrated that CD8$^+$ T cells from 6 HIV-1 infected and 5/10 uninfected animals caused a 75-100% suppression of HIV replication. CD8$^+$ T cells from an additional 3 uninfected animals demonstrated the ability to slow down HIV replication and cause a moderate suppression of peak virus production. However, the conclusion by these authors that uninfected chimps have the ability to innately suppress HIV replication is confounded by the fact that some of the animals used in this study were previously used for hepatitis B vaccine research.

Another non-human primate model of AIDS is the infection of baboons with HIV-2 (149). These animals are viremic and although some do succumb to an AIDS like clinical state, many remain relatively healthy. As a result, this animal model was used to study the host immune response to viral replication. It was demonstrated that CD8$^+$ T cells from HIV-2 infected baboons had anti-HIV activity against acutely infected autologous CD4$^+$ T cells. Although CD8$^+$ T cell supernatant demonstrated antiviral activity, there was also a lytic component to the observed suppression. Baboons (*Papio cynocephalus*) are also used to investigate protection against superinfection with HIV-2 (149). Antiviral activity by CD8$^+$ T cells was demonstrated to be an important correlate of protection; whereas neutralizing antibodies were not. Part of the antiviral activity was a soluble component from filtered cell supernatants. This soluble activity could not be attributed to beta-chemokines, was heat stable (100ºC, 10 min.), and could inhibit HIV-LTR transcription all of which is similar to the CD8$^+$ T cell derived antiviral factor from human HIV infected individuals (150).

11.2. Feline

Feline immunodeficiency virus (FIV) is a lentivirus, similar to HIV, that preferentially infects CD4$^+$ T lymphocytes in cats and produces feline AIDS with characteristics similar to human AIDS (151, 152). Not only is the clinical course of infection in cats similar to HIV, but anti-FIV CD8$^+$ T cells are capable of suppressing FIV replication in a noncytolytic manner (153, 154). Interestingly, this activity has been demonstrated in acute

FIV infection in association with reduced or absent PBMC-associated virus (155). This study is similar to that of Mackewicz *et al.* (53) in that it shows the presence of this activity during acute infection. However, it is still unclear if there is a direct casual relationship between the presence of noncytolytic antiviral CD8$^+$ T cells and reduction in viremia in the acute stage of infection.

12. VACCINE APPROACHES

The SIV and HIV-2 macaque models are useful to study the immunological effects of various vaccine strategies before they are tested in humans. There are many types of vaccines that have been employed in animal models, such as live attenuated virus, live recombinant vectors with and without a recombinant subunit boost, recombinant subunit vaccines, and inactivated virus vaccines. Since it is still uncertain as to what the correlates of immune protection are, there are many different beliefs as to what types of immune responses a candidate vaccine should elicit. Several studies discussed in this review indicate that CD8$^+$ T cell mediated antiviral activity may correlate with protection and thus induction or enhancement of this cell-mediated immune response should be considered when developing new vaccines. Some laboratories have investigated whether or not this CD8$^+$ T cell mediated antiviral activity is induced in vaccine trials using animal models.

Effective immunization of macaques with SIV grown in human T cells (156-159) was previously thought to be due to human T cell surface components since the observed protection could be mimicked by the T cells alone in the absence of virus (160). Later it was demonstrated that immunization with recombinant SIVgp120 and p27 induced protection in macaques (161). This protection correlated with CD8$^+$ T cell suppressive activity and beta-chemokine production. Therefore, this same group performed experiments to determine if CD8$^+$ T cell suppressive activity and beta-chemokine production were the cause of the protective effect of immunization with SIV grown in human T cells (162). They demonstrated that beta-chemokines were produced both from simian T cell cocultured with human CD4$^+$ T cells and from immunization of macaques with SIV grown in human T cells (or just human T cells alone). Production of chemokines correlated with CD8$^+$ T cell mediated suppressive activity and protection in the immunized macaques. Another study demonstrated an enhancement of CD8$^+$ T cell mediated antiviral activity in rhesus macaques after vaccination with a highly attenuated vaccinia (NYVAC)-SIV recombinant (163). However, interpretation of these results is difficult due to the high levels of pre-existing CD8$^+$ T cell mediated antiviral activity in naive macaques. When rhesus macaques were immunized with live attenuated SIV (SIVmac239?nef or SIVmac239?3), their CD8$^+$ T lymphocytes were able to suppress SIV replication in autologous SIV-infected CD4$^+$ T cells (164). Without stimulation, the CD8$^+$ T cell suppressive activity was MHC restricted and required cell contact. After CD3-stimulation, these CD8$^+$ T cells produced MHC unrestricted antiviral soluble factors in addition to the beta-chemokines. This conclusion was based on the fact that beta-chemokine production was high, but neutralizing antibodies to the beta-chemokines did not completely abrogate the CD8$^+$ T cell mediated suppressive activity. However, others have suggested that CD8$^+$ T cell mediated antiviral activity may not play a large role in the controlling SIV infection. It remains to be seen if the results of the vaccine trials in animal models will correlate with human trials. Although strengthening the argument for control of SIV infection by CD8$^+$ T cells are two independent studies (5, 6) that employed *in vivo* depletion of CD8$^+$ T cells in SIV infection. After removal of CD8$^+$ T cells there was a dramatic rise in virus replication indicating the potent suppressive effect CD8$^+$ T cells had on viral replication. Additionally after analysis, Jin *et al.* (6) determined that the effects of increased virus replication after CD8$^+$ T cell removal could be best explained by the absence of noncytolytic CD8$^+$ T cell mediated suppression of viral gene expression.

Currently, some analysis of CD8$^+$ T cell mediated suppressive activity is being evaluated in ongoing human vaccine trials through the Vaccine Trial Network (VTN). However, the use of a reliable and simplistic assay system to screen for CD8$^+$ T cell mediated suppressive activity would greatly facilitate the analysis of vaccinee samples. We have developed a CD8$^+$ T cell suppression assay involving a single cycle of replication using a reporter virus (27) that would lend itself to these types of studies in vaccinee samples. Other studies in our laboratory are focusing on longitudinal cohorts of HIV infected individuals to examine if CD8$^+$ T cells exert selective pressure on the virus *in vivo* over time. If so, this would indicate that the noncytolytic CD8$^+$ T cell antiviral activity is important, *in vivo*, at restricting viral replication and provide a greater impetus for designing future vaccines that would elicit this activity.

13. CD8$^+$ T CELL MEDIATED SUPPRESSIVE ACTIVITY AND ANTIRETROVIRAL THERAPY

In studies of chronically infected HIV individuals undergoing combination antiretroviral therapy, noncytolytic CD8$^+$ T cell responses were examined and shown to correlate with ongoing viral replication (165). Suppression of the viral load below 400 copies/ml correlated with a loss of noncytolytic activity. This suggests that maintenance of this antiviral activity is highly dependent on continued viral replication in chronically infected HIV individuals. In a different approach, another study analyzed baseline CD8$^+$ T cell antiviral responses before the initiation of antiretroviral therapy (166). The presence of a strong CD8$^+$ T cell mediated antiviral response at the onset of therapy correlated with a favorable response to antiretroviral therapy after 1 year. This study suggests that the immune function of individuals before initiating therapy will greatly affect their ability to suppress viral replication. It remains to be seen however, what the immunological response will be for those individuals that are treated during the acute stages of HIV infection. One study suggests that early antiretroviral treatment allows significant preservation of the immune response (167).

Whether or not CD8$^+$ T cell mediated suppression is one of those activities that is preserved if therapy is instituted shortly after infection is not known, but should be the basis for further investigation in acute HIV infection cohorts.

14. PERSPECTIVES

14.1. Therapeutic implications

With the advent of technology for cloning and expansion of CD8$^+$ T cells with a suppressive phenotype one could likely imagine adoptively transferring these cells back to the patient. However, these studies would need to be carefully monitored to prevent any adverse effects. Additionally, measurement of CD8$^+$ T cell suppressive activity may have a useful prognostic value. Understanding the mechanism of suppression could identify new targets and lead to novel therapeutic agents that specifically target HIV replication. Alternatively, identification of the soluble factor(s) responsible for suppression could lead to synthesis and purification of this factor for clinical use. Discerning the events involved in induction of this antiviral response by CD8$^+$ T cells could also enable development of biological response modifiers for treatment regimens.

There is some indication that cytokines affect the potency of CD8$^+$ T cell mediated antiviral activity. IL-2 augments this response and IL-10 diminishes this response (168). This correlates with other findings that demonstrates a switch from a T helper cell type I cytokine response during the asymptomatic stages to a T helper cell type II response during the symptomatic stages of HIV infection (169, 170). Since the CD8$^+$ T cell mediated antiviral activity positively correlates with the asymptomatic period, it may be beneficial to try to maintain the T_H1 response in patients to prevent a switch to a T_H2 response and therefore loss of CD8$^+$ T cell mediated suppressive activity.

Interestingly, noncytolytic CD8$^+$ T cell mediated suppressive activity may be produced from CD8$^+$ T cells that have been stimulated with alloantigens (171, 172). These studies may potentially be applicable for an HIV vaccine design to elicit a CD8$^+$ T cell noncytolytic suppressive activity. However, we must be careful in the development of potential therapies that would aim to enhance CD8$^+$ T cell antiviral activity to ensure that untoward effects are not manifested. It is essential to determine the full range of suppressive activities that are produced by CD8$^+$ T cells, the mechanism of each, and the range of target cells that can be affected.

14.2. Multifactorial nature of CD8$^+$ T cell mediated suppression

Cellular immunity is known to be an important component of the host's arsenal for defense against viral infections. This arm of the immune system utilizes CD8$^+$ T cell clones, natural killer cells (NK), macrophages and lymphokine activated killer cells (LAK). The importance of the cellular immune response for control of HIV infection has been documented. Several groups, including our own, have observed an inverse correlation between CD8$^+$ T cell mediated suppressive activity and progression to AIDS. The precise mechanism of CD8$^+$ T cell mediated suppression, as well as the means by which a cell acquires this phenotype is largely unknown and is a question pursued vigorously both by our laboratory and others. Whether or not this suppressive activity is restricted to HIV infection has not yet been proven, neither is it known what the requirements for induction of this response are. It is possible that other types of viral infections also provoke this type of immune response. Of further interest will be to study these antiviral CD8$^+$ T cells and their ability to inhibit other viral infections. The idea that the nature of the CD8$^+$ T cell suppressive activity is multifactorial easily lends itself to the notion that other types of (viral) infections may induce this response and/or become affected by it. Most investigators studying the phenomena of CD8$^+$ T cell mediated suppression of HIV are in agreement that not one, but several molecules are responsible.

14. ACKNOWLEDGEMENTS

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16. REFERENCES

1. Coffin, J., A. Haase, J.A. Levy, L. Montagnier, S. Oroszlan, N. Teich, H. Temin, K. Toyoshima, H. Varmus, P. Vogt and *et al.*: Human immunodeficiency viruses. *Science* 232, 697 (1986)

2. Barre-Sinoussi, F., J.C. Chermann, F. Rey, M.T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum and L. Montagnier: Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 220, 868-871 (1983)

3. Gallo, R.C., S.Z. Salahuddin, M. Popovic, G.M. Shearer, M. Kaplan, B.F. Haynes, T.J. Palker, R. Redfield, J. Oleske, B. Safai and *et al.*: Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 224, 500-503 (1984)

4. Levy, J.A., A.D. Hoffman, S.M. Kramer, J.A. Landis, J.M. Shimabukuro and L.S. Oshiro: Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. *Science* 225, 840-842 (1984)

5. Schmitz, J.E., M.J. Kuroda, S. Santra, V.G. Sasseville, M.A. Simon, M.A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B.J. Scallon, J. Ghrayeb, M.A. Forman, D.C. Montefiori, E.P. Rieber, N.L. Letvin and K.A. Reimann: Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* 283, 857-860 (1999)

6. Jin, X., D.E. Bauer, S.E. Tuttleton, S. Lewin, A. Gettie, J. Blanchard, C.E. Irwin, J.T. Safrit, J. Mittler, L. Weinberger, L.G. Kostrikis, L. Zhang, A.S. Perelson and D.D. Ho: Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med* 189, 991-998 (1999)

7. Weiss, R.A., P.R. Clapham, R. Cheingsong-Popov, A.G. Dalgleish, C.A. Carne, I.V. Weller and R.S. Tedder:

Neutralization of human T-lymphotropic virus type III by sera of AIDS and AIDS-risk patients. *Nature* 316, 69-72 (1985)

8. Lyerly, H.K., T.J. Matthews, A.J. Langlois, D.P. Bolognesi and K.J. Weinhold: Human T-cell lymphotropic virus IIIB glycoprotein (gp120) bound to CD4 determinants on normal lymphocytes and expressed by infected cells serves as target for immune attack. *Proc Natl Acad Sci U S A* 84, 4601-4605 (1987)

9. Rook, A.H., H.C. Lane, T. Folks, S. McCoy, H. Alter and A.S. Fauci: Sera from HTLV-III/LAV antibody-positive individuals mediate antibody- dependent cellular cytotoxicity against HTLV-III/LAV-infected T cells. *J Immunol* 138, 1064-1067 (1987)

10. Ojo-Amaize, E.A., P. Nishanian, D.E. Keith, Jr., R.L. Houghton, D.F. Heitjan, J.L. Fahey and J.V. Giorgi: Antibodies to human immunodeficiency virus in human sera induce cell- mediated lysis of human immunodeficiency virus-infected cells. *J Immunol* 139, 2458-2463 (1987)

11. Ljunggren, K., B. Bottiger, G. Biberfeld, A. Karlson, E.M. Fenyo and M. Jondal: Antibody-dependent cellular cytotoxicity-inducing antibodies against human immunodeficiency virus. Presence at different clinical stages. *J Immunol* 139, 2263-2267 (1987)

12. Shepp, D.H., S. Chakrabarti, B. Moss and G.V. Quinnan, Jr.: Antibody-dependent cellular cytotoxicity specific for the envelope antigens of human immunodeficiency virus. *J Infect Dis* 157, 1260-1264 (1988)

13. Spear, G.T., A.L. Landay, B.L. Sullivan, B. Dittel and T.F. Lint: Activation of complement on the surface of cells infected by human immunodeficiency virus. *J Immunol* 144, 1490-1496 (1990)

14. Walker, B.D. and F. Plata: Cytotoxic T lymphocytes against HIV. *Aids* 4, 177-184 (1990)

15. Nixon, D.F., A.R. Townsend, J.G. Elvin, C.R. Rizza, J. Gallwey and A.J. McMichael: HIV-1 gag-specific cytotoxic T lymphocytes defined with recombinant vaccinia virus and synthetic peptides. *Nature* 336, 484-487 (1988)

16. Walker, C.M., D.J. Moody, D.P. Stites and J.A. Levy: CD8+ lymphocytes can control HIV infection *in vitro* by suppressing virus replication. *Science* 234, 1563-1566 (1986)

17. von Briesen, H., W.B. Becker, K. Henco, E.B. Helm, H.R. Gelderblom, H.D. Brede and H. Rubsamen-Waigmann: Isolation frequency and growth properties of HIV-variants: multiple simultaneous variants in a patient demonstrated by molecular cloning. *J Med Virol* 23, 51-66 (1987)

18. Chiodi, F., J. Albert, E. Olausson, G. Norkrans, L. Hagberg, A. Sonnerborg, B. Asjo and E.M. Fenyo: Isolation frequency of human immunodeficiency virus from cerebrospinal fluid and blood of patients with varying severity of HIV infection. *AIDS Res Hum Retroviruses* 4, 351-358 (1988)

19. Cocchi, F., A.L. DeVico, A. Garzino-Demo, S.K. Arya, R.C. Gallo and P. Lusso: Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV- suppressive factors produced by CD8+ T cells. *Science* 270, 1811-1815 (1995)

20. Paxton, W.A., S.R. Martin, D. Tse, T.R. O'Brien, J. Skurnick, N.L. VanDevanter, N. Padian, J.F. Braun, D.P. Kotler, S.M. Wolinsky and R.A. Koup: Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposure. *Nat Med* 2, 412-417 (1996)

21. Kinter, A.L., M. Ostrowski, D. Goletti, A. Oliva, D. Weissman, K. Gantt, E. Hardy, R. Jackson, L. Ehler and A.S. Fauci: HIV replication in CD4+ T cells of HIV-infected individuals is regulated by a balance between the viral suppressive effects of endogenous beta-chemokines and the viral inductive effects of other endogenous cytokines. *Proc Natl Acad Sci U S A* 93, 14076-14081 (1996)

22. Greenberg, M.L., S.F. Lacey, C.H. Chen, D.P. Bolognesi and K.J. Weinhold: Noncytolytic CD8 T cell-mediated suppression of HIV replication. *Springer Semin Immunopathol* 18, 355-369 (1997)

23. Saha, K., G. Bentsman, L. Chess and D.J. Volsky: Endogenous production of beta-chemokines by CD4+, but not CD8+, T-cell clones correlates with the clinical state of human immunodeficiency virus type 1 (HIV-1)-infected individuals and may be responsible for blocking infection with non-syncytium-inducing HIV-1 in vitro. *J Virol* 72, 876-881 (1998)

24. Mackewicz, C.E., E. Barker and J.A. Levy: Role of beta-chemokines in suppressing HIV replication [letter; comment]. *Science* 274, 1393-1395 (1996)

25. Barker, T.D., D. Weissman, J.A. Daucher, K.M. Roche and A.S. Fauci: Identification of multiple and distinct CD8+ T cell suppressor activities: dichotomy between infected and uninfected individuals, evolution with progression of disease, and sensitivity to gamma irradiation. *J Immunol* 156, 4476-4483 (1996)

26. Hausner, M.A., J.V. Giorgi and S. Plaeger-Marshall: A reproducible method to detect CD8 T cell mediated inhibition of HIV production from naturally infected CD4 cells. *J Immunol Methods* 157, 181-187 (1993)

27. Tomaras, G.D., S.F. Lacey, C.B. McDanal, G. Ferrari, K.J. Weinhold and M.L. Greenberg: CD8+ T cell-mediated suppressive activity inhibits HIV-1 after virus entry with kinetics indicating effects on virus gene expression. *Proc Natl Acad Sci U S A* 97, 3503-3508 (2000)

28. Chen, Y., C. Rinaldo and P. Gupta: A semiquantitative assay for CD8+ T-cell-mediated suppression of human immunodeficiency virus type 1 infection. *Clin Diagn Lab Immunol* 4, 4-10 (1997)

29. Connor, R.I., B.K. Chen, S. Choe and N.R. Landau: Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. *Virology* 206, 935-944 (1995)

30. Walker, C.M., G.A. Thomson-Honnebier, F.C. Hsueh, A.L. Erickson, L.Z. Pan and J.A. Levy: CD8+ T cells from HIV-1-infected individuals inhibit acute infection by human and primate immunodeficiency viruses. *Cellular Immunology* 137, 420-428 (1991)

31. Mackewicz, C.E., H.W. Ortega and J.A. Levy: CD8+ cell anti-HIV activity correlates with the clinical state of the infected individual. *J Clin Invest* 87, 1462-1466 (1991)

32. Brinchmann, J.E., G. Gaudernack and F. Vartdal: CD8+ T cells inhibit HIV replication in naturally infected

CD4+ T cells. Evidence for a soluble inhibitor. *J Immunol* 144, 2961-2966 (1990)
33. Bagasra, O. and R.J. Pomerantz: The role of CD8-positive lymphocytes in the control of HIV-1 infection of peripheral blood mononuclear cells. *Immunol Lett* 35, 83-92 (1993)
34. Rosok, B., P. Voltersvik, B.M. Larsson, J. Albert, J.E. Brinchmann and B. Asjo: CD8+ T cells from HIV type 1-seronegative individuals suppress virus replication in acutely infected cells. *AIDS Res Hum Retroviruses* 13, 79-85 (1997)
35. Saha, K., P. Sova, W. Chao, L. Chess and D.J. Volsky: Generation of CD4+ and CD8+ T-cell clones from PBLs of HIV-1 infected subjects using herpesvirus saimiri. *Nat Med* 2, 1272-1275 (1996)
36. Mackewicz, C.E., R. Orque, J. Jung and J.A. Levy: Derivation of herpesvirus saimiri-transformed CD8+ T cell lines with noncytotoxic anti-HIV activity. *Clin Immunol Immunopathol* 82, 274-281 (1997)
37. Lacey, S.F., K.J. Weinhold, C.H. Chen, C. McDanal, C. Oei and M.L. Greenberg: Herpesvirus saimiri transformation of HIV type 1 suppressive CD8+ lymphocytes from an HIV type 1-infected asymptomatic individual. *AIDS Res Hum Retroviruses* 14, 521-531 (1998)
38. Weber, F., E. Meinl, K. Drexler, A. Czlonkowska, S. Huber, H. Fickenscher, I. Muller-Fleckenstein, B. Fleckenstein, H. Wekerle and R. Hohlfeld: Transformation of human T-cell clones by Herpesvirus saimiri: intact antigen recognition by autonomously growing myelin basic protein-specific T cells. *Proc Natl Acad Sci U S A* 90, 11049-11053 (1993)
39. Broker, B.M., A.Y. Tsygankov, I. Muller-Fleckenstein, A.H. Guse, N.A. Chitaev, B. Biesinger, B. Fleckenstein and F. Emmrich: Immortalization of human T cell clones by Herpesvirus saimiri. Signal transduction analysis reveals functional CD3, CD4, and IL-2 receptors. *J Immunol* 151, 1184-1192 (1993)
40. Mittrucker, H.W., I. Muller-Fleckenstein, B. Fleckenstein and B. Fleischer: Herpes virus saimiri-transformed human T lymphocytes: normal functional phenotype and preserved T cell receptor signalling. *Int Immunol* 5, 985-990 (1993)
41. Pal, R., A. Garzino-Demo, P.D. Markham, J. Burns, M. Brown, R.C. Gallo and A.L. DeVico: Inhibition of HIV-1 infection by the beta-chemokine MDC. *Science* 278, 695-698 (1997)
42. Bollinger, R.C., T.C. Quinn, A.Y. Liu, P.E. Stanhope, S.A. Hammond, R. Viveen, M.L. Clements and R.F. Siliciano: Cytokines from vaccine-induced HIV-1 specific cytotoxic T lymphocytes: effects on viral replication. *AIDS Res Hum Retroviruses* 9, 1067-1077 (1993)
43. Hsueh, F.W., C.M. Walker, D.J. Blackbourn and J.A. Levy: Suppression of HIV replication by CD8+ cell clones derived from HIV-infected and uninfected individuals. *Cellular Immunology* 159, 271-279 (1994)
44. Toso, J.F., C.H. Chen, J.R. Mohr, L. Piglia, C. Oei, G. Ferrari, M.L. Greenberg and K.J. Weinhold: Oligoclonal CD8 lymphocytes from persons with asymptomatic human immunodeficiency virus (HIV) type 1 infection inhibit HIV-1 replication. *J Infect Dis* 172, 964-973 (1995)
45. Buseyne, F., M. Fevrier, S. Garcia, M.L. Gougeon and Y. Riviere: Dual function of a human immunodeficiency virus (HIV)-specific cytotoxic T-lymphocyte clone: inhibition of HIV replication by noncytolytic mechanisms and lysis of HIV-infected CD4+ cells. *Virology* 225, 248-253 (1996)
46. Yang, O.O., S.A. Kalams, A. Trocha, H. Cao, A. Luster, R.P. Johnson and B.D. Walker: Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms. *J Virol* 71, 3120-3128 (1997)
47. Kannagi, M., T. Masuda, T. Hattori, T. Kanoh, K. Nasu, N. Yamamoto and S. Harada: Interference with human immunodeficiency virus (HIV) replication by CD8+ T cells in peripheral blood leukocytes of asymptomatic HIV carriers in vitro. *J Virol* 64, 3399-3406 (1990)
48. Barker, E., K.N. Bossart, C.P. Locher, B.K. Patterson and J.A. Levy: CD8+ cells from asymptomatic human immunodeficiency virus-infected individuals suppress superinfection of their peripheral blood mononuclear cells. *J Gen Virol* 77, 2953-2962 (1996)
49. Landay, A.L., C.E. Mackewicz and J.A. Levy: An activated CD8+ T cell phenotype correlates with anti-HIV activity and asymptomatic clinical status. *Clin Immunol Immunopathol* 69, 106-116 (1993)
50. Cao, Y., L. Qin, L. Zhang, J. Safrit and D.D. Ho: Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. *N Engl J Med* 332, 201-208 (1995)
51. Barker, E., C.E. Mackewicz, G. Reyes-Teran, A. Sato, S.A. Stranford, S.H. Fujimura, C. Christopherson, S.Y. Chang and J.A. Levy: Virological and immunological features of long-term human immunodeficiency virus-infected individuals who have remained asymptomatic compared with those who have progressed to acquired immunodeficiency syndrome. *Blood* 92, 3105-3114 (1998)
52. Giorgi, J.V. and R. Detels: T-cell subset alterations in HIV-infected homosexual men: NIAID Multicenter AIDS cohort study. *Clin Immunol Immunopathol* 52, 10-18 (1989)
53. Mackewicz, C.E., L.C. Yang, J.D. Lifson and J.A. Levy: Non-cytolytic CD8 T-cell anti-HIV responses in primary HIV-1 infection. *Lancet* 344, 1671-1673 (1994)
54. Blackbourn, D.J., C.E. Mackewicz, E. Barker, T.K. Hunt, B. Herndier, A.T. Haase and J.A. Levy: Suppression of HIV replication by lymphoid tissue CD8+ cells correlates with the clinical state of HIV-infected individuals. *Proc Natl Acad Sci U S A* 93, 13125-13130 (1996)
55. Ferbas, J., A.H. Kaplan, M.A. Hausner, L.E. Hultin, J.L. Matud, Z. Liu, D.L. Panicali, H. Nerng-Ho, R. Detels and J.V. Giorgi: Virus burden in long-term survivors of human immunodeficiency virus (HIV) infection is a determinant of anti-HIV CD8+ lymphocyte activity. *J Infect Dis* 172, 329-339 (1995)
56. Gomez, A.M., F.M. Smaill and K.L. Rosenthal: Inhibition of HIV replication by CD8+ T cells correlates with CD4 counts and clinical stage of disease. *Clin Exp Immunol* 97, 68-75 (1994)
57. Kootstra, N.A., F. Miedema and H. Schuitemaker: Analysis of CD8+ T lymphocyte-mediated nonlytic suppression of autologous and heterologous primary human immunodeficiency virus type 1 isolates. *AIDS Res Hum Retroviruses* 13, 685-693 (1997)

58. Copeland, K.F., J.G. Leith, P.J. McKay, L. Kelleher, F.M. Smaill and K.L. Rosenthal: CD8+ T cell-mediated suppression of HIV long terminal repeat-driven gene expression is not associated with improved clinical status. *Aids* 11, 581-586 (1997)
59. Fowke, K.R., N.J. Nagelkerke, J. Kimani, J.N. Simonsen, A.O. Anzala, J.J. Bwayo, K.S. MacDonald, E.N. Ngugi and F.A. Plummer: Resistance to HIV-1 infection among persistently seronegative prostitutes in Nairobi, Kenya. *Lancet* 348, 1347-1351 (1996)
60. Liu, R., W.A. Paxton, S. Choe, D. Ceradini, S.R. Martin, R. Horuk, M.E. MacDonald, H. Stuhlmann, R.A. Koup and N.R. Landau: Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 86, 367-377 (1996)
61. Samson, M., F. Libert, B.J. Doranz, J. Rucker, C. Liesnard, C.M. Farber, S. Saragosti, C. Lapoumeroulie, J. Cognaux, C. Forceille, G. Muyldermans, C. Verhofstede, G. Burtonboy, M. Georges, T. Imai, S. Rana, Y. Yi, R.J. Smyth, R.G. Collman, R.W. Doms, G. Vassart and M. Parmentier: Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene [see comments]. *Nature* 382, 722-725 (1996)
62. Smith, M.W., M. Dean, M. Carrington, C. Winkler, G.A. Huttley, D.A. Lomb, J.J. Goedert, T.R. O'Brien, L.P. Jacobson, R. Kaslow, S. Buchbinder, E. Vittinghoff, D. Vlahov, K. Hoots, M.W. Hilgartner and S.J. O'Brien: Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. *Science* 277, 959-965 (1997)
63. Stranford, S.A., J. Skurnick, D. Louria, D. Osmond, S.Y. Chang, J. Sninsky, G. Ferrari, K. Weinhold, C. Lindquist and J.A. Levy: Lack of infection in HIV-exposed individuals is associated with a strong CD8(+) cell noncytotoxic anti-HIV response. *Proc Natl Acad Sci U S A* 96, 1030-1035 (1999)
64. Weiser, B., S. Nachman, P. Tropper, K.H. Viscosi, R. Grimson, G. Baxter, G. Fang, C. Reyelt, N. Hutcheon and H. Burger: Quantitation of human immunodeficiency virus type 1 during pregnancy: relationship of viral titer to mother-to-child transmission and stability of viral load. *Proc Natl Acad Sci U S A* 91, 8037-8041 (1994)
65. Pollack, H., M.X. Zhan, J.T. Safrit, S.H. Chen, G. Rochford, P.Z. Tao, R. Koup, K. Krasinski and W. Borkowsky: CD8+ T-cell-mediated suppression of HIV replication in the first year of life: association with lower viral load and favorable early survival. *Aids* 11, F9-13 (1997)
66. Levy, J.A., F. Hsueh, D.J. Blackbourn, D. Wara and P.S. Weintrub: CD8 cell noncytotoxic antiviral activity in human immunodeficiency virus-infected and -uninfected children. *J Infect Dis* 177, 470-472 (1998)
67. Wasik, T.J., J. Bratosiewicz, A. Wierzbicki, V.E. Whiteman, R.R. Rutstein, S.E. Starr, S.D. Douglas, D. Kaufman, A.V. Sison, M. Polansky, H.W. Lischner and D. Kozbor: Protective role of beta-chemokines associated with HIV-specific Th responses against perinatal HIV transmission. *J Immunol* 162, 4355-4364 (1999)
68. Cooper, D.A., B. Tindall, E.J. Wilson, A.A. Imrie and R. Penny: Characterization of T lymphocyte responses during primary infection with human immunodeficiency virus. *J Infect Dis* 157, 889-896 (1988)
69. Yagi, M.J., M.E. Joesten, J. Wallace, J.P. Roboz and J.G. Bekesi: Human immunodeficiency virus type 1 (HIV-1) genomic sequences and distinct changes in CD8+ lymphocytes precede detectable levels of HIV-1 antibodies in high-risk homosexuals. *J Infect Dis* 164, 183-188 (1991)
70. Plaeger-Marshall, S., M.A. Hausner, V. Isacescu and J.V. Giorgi: CD8 T-cell-mediated inhibition of HIV replication in HIV infected adults and children. *AIDS Res Hum Retroviruses* 8, 1375-1376 (1992)
71. Ho, H.N., L.E. Hultin, R.T. Mitsuyasu, J.L. Matud, M.A. Hausner, D. Bockstoce, C.C. Chou, S. O'Rourke, J.M. Taylor and J.V. Giorgi: Circulating HIV-specific CD8+ cytotoxic T cells express CD38 and HLA-DR antigens. *J Immunol* 150, 3070-3079 (1993)
72. Walker, C.M. and J.A. Levy: A diffusible lymphokine produced by CD8+ T lymphocytes suppresses HIV replication. *Immunology* 66, 628-630 (1989)
73. Walker, C.M., A.L. Erickson, F.C. Hsueh and J.A. Levy: Inhibition of human immunodeficiency virus replication in acutely infected CD4+ cells by CD8+ cells involves a noncytotoxic mechanism. *Journal of Virology* 65, 5921-5927 (1991)
74. Tsubota, H., C.I. Lord, D.I. Watkins, C. Morimoto and N.L. Letvin: A cytotoxic T lymphocyte inhibits acquired immunodeficiency syndrome virus replication in peripheral blood lymphocytes. *J Exp Med* 169, 1421-1434 (1989)
75. Taub, D.D. and J.J. Oppenheim: Chemokines, inflammation and the immune system. *Ther Immunol* 1, 229-246 (1994)
76. Fenyo, E.M., L. Morfeldt-Manson, F. Chiodi, B. Lind, A. von Gegerfelt, J. Albert, E. Olausson and B. Asjo: Distinct replicative and cytopathic characteristics of human immunodeficiency virus isolates. *J Virol* 62, 4414-4419 (1988)
77. Westervelt, P., H.E. Gendelman and L. Ratner: Identification of a determinant within the human immunodeficiency virus 1 surface envelope glycoprotein critical for productive infection of primary monocytes. *Proc Natl Acad Sci U S A* 88, 3097-3101 (1991)
78. Hwang, S.S., T.J. Boyle, H.K. Lyerly and B.R. Cullen: Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. *Science* 253, 71-74 (1991)
79. Cann, A.J., M.J. Churcher, M. Boyd, W. O'Brien, J.Q. Zhao, J. Zack and I.S. Chen: The region of the envelope gene of human immunodeficiency virus type 1 responsible for determination of cell tropism. *J Virol* 66, 305-309 (1992)
80. Chesebro, B., K. Wehrly, J. Nishio and S. Perryman: Macrophage-tropic human immunodeficiency virus isolates from different patients exhibit unusual V3 envelope sequence homogeneity in comparison with T-cell-tropic isolates: definition of critical amino acids involved in cell tropism. *J Virol* 66, 6547-6554 (1992)
81. De Jong, J.J., A. De Ronde, W. Keulen, M. Tersmette and J. Goudsmit: Minimal requirements for the human immunodeficiency virus type 1 V3 domain to support the syncytium-inducing phenotype: analysis by single amino acid substitution. *J Virol* 66, 6777-6780 (1992)

82. Fouchier, R.A., M. Groenink, N.A. Kootstra, M. Tersmette, H.G. Huisman, F. Miedema and H. Schuitemaker: Phenotype-associated sequence variation in the third variable domain of the human immunodeficiency virus type 1 gp120 molecule. *J Virol* 66, 3183-3187 (1992)
83. Shioda, T., J.A. Levy and C. Cheng-Mayer: Small amino acid changes in the V3 hypervariable region of gp120 can affect the T-cell-line and macrophage tropism of human immunodeficiency virus type 1. *Proc Natl Acad Sci U S A* 89, 9434-9438 (1992)
84. Westervelt, P., D.B. Trowbridge, L.G. Epstein, B.M. Blumberg, Y. Li, B.H. Hahn, G.M. Shaw, R.W. Price and L. Ratner: Macrophage tropism determinants of human immunodeficiency virus type 1 *in vivo*. *J Virol* 66, 2577-2582 (1992)
85. Feng, Y., C.C. Broder, P.E. Kennedy and E.A. Berger: HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 272, 872-877 (1996)
86. Dragic, T., V. Litwin, G.P. Allaway, S.R. Martin, Y. Huang, K.A. Nagashima, C. Cayanan, P.J. Maddon, R.A. Koup, J.P. Moore and W.A. Paxton: HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC- CKR-5. *Nature* 381, 667-673 (1996)
87. Deng, H., R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R.E. Sutton, C.M. Hill, C.B. Davis, S.C. Peiper, T.J. Schall, D.R. Littman and N.R. Landau: Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 381, 661-666 (1996)
88. Alkhatib, G., C. Combadiere, C.C. Broder, Y. Feng, P.E. Kennedy, P.M. Murphy and E.A. Berger: CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 272, 1955-1958 (1996)
89. Doranz, B.J., J. Rucker, Y. Yi, R.J. Smyth, M. Samson, S.C. Peiper, M. Parmentier, R.G. Collman and R.W. Doms: A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* 85, 1149-1158 (1996)
90. Choe, H., M. Farzan, Y. Sun, N. Sullivan, B. Rollins, P.D. Ponath, L. Wu, C.R. Mackay, G. LaRosa, W. Newman, N. Gerard, C. Gerard and J. Sodroski: The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* 85, 1135-1148 (1996)
91. Amara, A., S.L. Gall, O. Schwartz, J. Salamero, M. Montes, P. Loetscher, M. Baggiolini, J.L. Virelizier and F. Arenzana-Seisdedos: HIV coreceptor downregulation as antiviral principle: SDF-1alpha- dependent internalization of the chemokine receptor CXCR4 contributes to inhibition of HIV replication. *J Exp Med* 186, 139-146 (1997)
92. Jansson, M., M. Popovic, A. Karlsson, F. Cocchi, P. Rossi, J. Albert and H. Wigzell: Sensitivity to inhibition by beta-chemokines correlates with biological phenotypes of primary HIV-1 isolates. *Proc Natl Acad Sci U S A* 93, 15382-15387 (1996)
93. Conlon, K., A. Lloyd, U. Chattopadhyay, N. Lukacs, S. Kunkel, T. Schall, D. Taub, C. Morimoto, J. Osborne, J. Oppenheim and *et al.*: CD8+ and CD45RA+ human peripheral blood lymphocytes are potent sources of macrophage inflammatory protein 1 alpha, interleukin-8 and RANTES. *Eur J Immunol* 25, 751-756 (1995)
94. Furci, L., G. Scarlatti, S. Burastero, G. Tambussi, C. Colognesi, C. Quillent, R. Longhi, P. Loverro, B. Borgonovo, D. Gaffi, E. Carrow, M. Malnati, P. Lusso, A.G. Siccardi, A. Lazzarin and A. Beretta: Antigen-driven C-C chemokine-mediated HIV-1 suppression by CD4(+) T cells from exposed uninfected individuals expressing the wild-type CCR- 5 allele. *J Exp Med* 186, 455-460 (1997)
95. Verani, A., G. Scarlatti, M. Comar, E. Tresoldi, S. Polo, M. Giacca, P. Lusso, A.G. Siccardi and D. Vercelli: C-C chemokines released by lipopolysaccharide (LPS)-stimulated human macrophages suppress HIV-1 infection in both macrophages and T cells. *J Exp Med* 185, 805-816 (1997)
96. Paliard, X., A.Y. Lee and C.M. Walker: RANTES, MIP-1 alpha and MIP-1 beta are not involved in the inhibition of HIV-1SF33 replication mediated by CD8+ T-cell clones. *Aids* 10, 1317-1321 (1996)
97. Mackewicz, C.E., E. Barker, G. Greco, G. Reyes-Teran and J.A. Levy: Do beta-chemokines have clinical relevance in HIV infection? *J Clin Invest* 100, 921-930 (1997)
98. Rubbert, A., D. Weissman, C. Combadiere, K.A. Pettrone, J.A. Daucher, P.M. Murphy and A.S. Fauci: Multifactorial nature of noncytolytic CD8+ T cell-mediated suppression of HIV replication: beta-chemokine-dependent and -independent effects. *AIDS Res Hum Retroviruses* 13, 63-69 (1997)
99. Garzino-Demo, A., R.B. Moss, J.B. Margolick, F. Cleghorn, A. Sill, W.A. Blattner, F. Cocchi, D.J. Carlo, A.L. DeVico and R.C. Gallo: Spontaneous and antigen-induced production of HIV-inhibitory beta- chemokines are associated with AIDS-free status. *Proc Natl Acad Sci U S A* 96, 11986-11991 (1999)
100. Scala, E., G. D'Offizi, R. Rosso, O. Turriziani, R. Ferrara, A.M. Mazzone, G. Antonelli, F. Aiuti and R. Paganelli: C-C chemokines, IL-16, and soluble antiviral factor activity are increased in cloned T cells from subjects with long-term nonprogressive HIV infection. *J Immunol* 158, 4485-4492 (1997)
101. Moriuchi, H., M. Moriuchi, C. Combadiere, P.M. Murphy and A.S. Fauci: CD8+ T-cell-derived soluble factor(s), but not beta-chemokines RANTES, MIP-1 alpha, and MIP-1 beta, suppress HIV-1 replication in monocyte/macrophages. *Proc Natl Acad Sci U S A* 93, 15341-15345 (1996)
102. Barker, E., K.N. Bossart and J.A. Levy: Primary CD8+ cells from HIV-infected individuals can suppress productive infection of macrophages independent of beta-chemokines. *Proc Natl Acad Sci U S A* 95, 1725-1729 (1998)
103. Wagner, L., O.O. Yang, E.A. Garcia-Zepeda, Y. Ge, S.A. Kalams, B.D. Walker, M.S. Pasternack and A.D. Luster: Beta-chemokines are released from HIV-1-specific cytolytic T-cell granules complexed to proteoglycans. *Nature* 391, 908-911 (1998)
104. Copeland, K.F., J.G. Leith, P.J. McKay and K.L. Rosenthal: CD8+ T cell supernatants of HIV type 1-infected individuals have opposite effects on long terminal repeat-mediated transcription in T cells and monocytes. *AIDS Res Hum Retroviruses* 13, 71-77 (1997)
105. Leith, J.G., K.F. Copeland, P.J. McKay, C.D. Richards and K.L. Rosenthal: CD8+ T-cell-mediated suppression of HIV-1 long terminal repeat-driven gene expression is not modulated by the CC chemokines RANTES, macrophage inflammatory protein (MIP)-1 alpha and MIP-1 beta. *Aids* 11, 575-580 (1997)
106. Garzino-Demo, A., S.K. Arya, A.L. Devico, F. Cocchi, P. Lusso and R.C. Gallo: C-C chemokine RANTES and HIV long terminal repeat-driven gene expression. *AIDS Res Hum Retroviruses* 13, 1367-1371 (1997)

107. Handen, J.S. and H.F. Rosenberg: Suppression of HIV-1 transcription by beta-chemokines RANTES, MIP1-alpha, and MIP-1beta is not mediated by the NFAT-1 enhancer element. *FEBS Lett* 410, 301-302 (1997)
108. Mackewicz, C.E., H. Ortega and J.A. Levy: Effect of cytokines on HIV replication in CD4+ lymphocytes: lack of identity with the CD8+ cell antiviral factor. *Cell Immunol* 153, 329-343 (1994)
109. Brinchmann, J.E., G. Gaudernack and F. Vartdal: *In vitro* replication of HIV-1 in naturally infected CD4+ T cells is inhibited by rIFN alpha 2 and by a soluble factor secreted by activated CD8+ T cells, but not by rIFN beta, rIFN gamma, or recombinant tumor necrosis factor-alpha. *J Acquir Immune Defic Syndr* 4, 480-488 (1991)
110. Barker, E., C.E. Mackewicz and J.A. Levy: Effects of TH1 and TH2 cytokines on CD8+ cell response against human immunodeficiency virus: implications for long-term survival. *Proc Natl Acad Sci U S A* 92, 11135-11139 (1995)
111. Barker, E., K.N. Bossart, S.H. Fujimura and J.A. Levy: CD28 costimulation increases CD8+ cell suppression of HIV replication. *J Immunol* 159, 5123-5131 (1997)
112. Kubo, M., T. Ohashi, M. Fujii, S. Oka, A. Iwamoto, S. Harada and M. Kannagi: Abrogation of *in vitro* suppression of human immunodeficiency virus type 1 (HIV-1) replication mediated by CD8+ T lymphocytes of asymptomatic HIV-1 carriers by staphylococcal enterotoxin B and phorbol esters through induction of tumor necrosis factor alpha. *J Virol* 71, 7560-7566 (1997)
113. Arenzana-Seisdedos, F., A. Amara, D. Thomas and J.L. Virelizier: B-Chemokine MDC and HIV-1 Infection. *Science* 281, 487-487a (1998)
114. Lee, B., J. Rucker, R.W. Doms, M. Tsang, X. Hu, M. Dietz, R. Bailer, L.J. Montaner, C. Gerard and N. Sullivan: B-Chemokine MDC and HIV-1 Infection. *Science* 281, 487-487a (1998)
115. Cota, M., M. Mengozzi, E. Vicenzi, P. Panina-Bordignon, F. Sinigaglia, P. Transidico, S. Sozzani, A. Mantovani and G. Poli: Selective inhibition of HIV replication in primary macrophages but not T lymphocytes by macrophage-derived chemokine. *Proc Natl Acad Sci U S A* 97, 9162-9167 (2000)
116. Ennen, J., S. Norley and R. Kurth: The activated CD8 T-lymphocyte-derived immunodeficiency-virus-suppressing lymphokine in African green monkeys: evidence for a role in control of infection? *Res Immunol* 145, 647-652 (1994)
117. Baier, M., A. Werner, N. Bannert, K. Metzner and R. Kurth: HIV suppression by interleukin-16. *Nature* 378, 563 (1995)
118. Center, D.M., H. Kornfeld and W.W. Cruikshank: Interleukin 16 and its function as a CD4 ligand. *Immunol Today* 17, 476-481 (1996)
119. Maciaszek, J.W., N.A. Parada, W.W. Cruikshank, D.M. Center, H. Kornfeld and G.A. Viglianti: IL-16 represses HIV-1 promoter activity. *J Immunol* 158, 5-8 (1997)
120. Zhou, P., S. Goldstein, K. Devadas, D. Tewari and A.L. Notkins: Human CD4+ cells transfected with IL-16 cDNA are resistant to HIV-1 infection: inhibition of mRNA expression. *Nat Med* 3, 659-664 (1997)
121. Mackewicz, C.E., J.A. Levy, W.W. Cruikshank, H. Kornfeld and D.M. Center: Role of IL-16 in HIV replication. *Nature* 383, 488-489 (1996)
122. Bleul, C.C., M. Farzan, H. Choe, C. Parolin, I. Clark-Lewis, J. Sodroski and T.A. Springer: The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* 382, 829-833 (1996)
123. Oberlin, E., A. Amara, F. Bachelerie, C. Bessia, J.L. Virelizier, F. Arenzana-Seisdedos, O. Schwartz, J.M. Heard, I. Clark-Lewis, D.F. Legler, M. Loetscher, M. Baggiolini and B. Moser: The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature* 382, 833-835 (1996)
124. Tashiro, K., H. Tada, R. Heilker, M. Shirozu, T. Nakano and T. Honjo: Signal sequence trap: a cloning strategy for secreted proteins and type I membrane proteins. *Science* 261, 600-603 (1993)
125. Bleul, C.C., R.C. Fuhlbrigge, J.M. Casasnovas, A. Aiuti and T.A. Springer: A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). *J Exp Med* 184, 1101-1109 (1996)
126. Lacey, S.F., C.B. McDanal, R. Horuk and M.L. Greenberg: The CXC chemokine stromal cell-derived factor 1 is not responsible for CD8+ T cell suppression of syncytia-inducing strains of HIV-1. *Proc Natl Acad Sci U S A* 94, 9842-9847 (1997)
127. Ohashi, T., M. Arai, H. Kato, M. Kubo, M. Fujii, N. Yamamoto, A. Iwamoto and M. Kannagi: High SDF-1 expression in HIV-1 carriers does not correlate with CD8+ T- cell-mediated suppression of viral replication. *Virology* 244, 467-472 (1998)
128. Walker, B.D., S. Chakrabarti, B. Moss, T.J. Paradis, T. Flynn, A.G. Durno, R.S. Blumberg, J.C. Kaplan, M.S. Hirsch and R.T. Schooley: HIV-specific cytotoxic T lymphocytes in seropositive individuals. *Nature* 328, 345-348 (1987)
129. Plata, F., B. Autran, L.P. Martins, S. Wain-Hobson, M. Raphael, C. Mayaud, M. Denis, J.M. Guillon and P. Debre: AIDS virus-specific cytotoxic T lymphocytes in lung disorders. *Nature* 328, 348-351 (1987)
130. Wiviott, L.D., C.M. Walker and J.A. Levy: CD8+ lymphocytes suppress HIV production by autologous CD4+ cells without eliminating the infected cells from culture. *Cellular Immunology* 128, 628-634 (1990)
131. Mackewicz, C.E., M.R. Garovoy and J.A. Levy: HLA compatibility requirements for CD8(+)-T-cell-mediated suppression of human immunodeficiency virus replication. *J Virol* 72, 10165-10170 (1998)
132. Ohashi, T., M. Kubo, H. Kato, A. Iwamoto, H. Takahashi, M. Fujii and M. Kannagi: Role of class I major histocompatibility complex-restricted and - unrestricted suppression of human immunodeficiency virus type 1 replication by CD8+ T lymphocytes. *J Gen Virol* 80, 209-216 (1999)
133. Tomaras, G.D. and M.L. Greenberg: Mechanisms for HIV-1 Entry: Current Strategies to Interfere with This Early Step. *Current Science Review* in press, (2000)
134. Trkola, A., W.A. Paxton, S.P. Monard, J.A. Hoxie, M.A. Siani, D.A. Thompson, L. Wu, C.R. Mackay, R. Horuk and J.P. Moore: Genetic subtype-independent inhibition of human immunodeficiency virus type 1

replication by CC and CXC chemokines. *J Virol* 72, 396-404 (1998)
135. Chen, C.H., K.J. Weinhold, J.A. Bartlett, D.P. Bolognesi and M.L. Greenberg: CD8+ T lymphocyte-mediated inhibition of HIV-1 long terminal repeat transcription: a novel antiviral mechanism. *AIDS Res Hum Retroviruses* 9, 1079-1086 (1993)
136. Mackewicz, C.E., D.J. Blackbourn and J.A. Levy: CD8+ T cells suppress human immunodeficiency virus replication by inhibiting viral transcription. *Proc Natl Acad Sci U S A* 92, 2308-2312 (1995)
137. Copeland, K.F., P.J. McKay and K.L. Rosenthal: Suppression of activation of the human immunodeficiency virus long terminal repeat by CD8+ T cells is not lentivirus specific. *AIDS Res Hum Retroviruses* 11, 1321-1326 (1995)
138. Powell, J.D., D.P. Bednarik, T.M. Folks, T. Jehuda-Cohen, F. Villinger, K.W. Sell and A.A. Ansari: Inhibition of cellular activation of retroviral replication by CD8+ T cells derived from non-human primates. *Clin Exp Immunol* 91, 473-481 (1993)
139. Knuchel, M., D.P. Bednarik, N. Chikkala and A.A. Ansari: Biphasic *in vitro* regulation of retroviral replication by CD8+ cells from nonhuman primates. *J Acquir Immune Defic Syndr* 7, 438-446 (1994)
140. Norley, S.G.: SIVagm infection of its natural African green monkey host. *Immunol Lett* 51, 53-58 (1996)
141. Travers, K., S. Mboup, R. Marlink, A. Gueye-Nidaye, T. Siby, I. Thior, I. Traore, A. Dieng-Sarr, J.L. Sankale, C. Mullins and *et al.*: Natural protection against HIV-1 infection provided by HIV-2 [see comments] [published erratum appears in Science 1995 Jun 30;268(5219):1833]. *Science* 268, 1612-1615 (1995)
142. Norrgren, H., S. Andersson, A.J. Biague, Z.J. da Silva, F. Dias, A. Naucler and G. Biberfeld: Trends and interaction of HIV-1 and HIV-2 in Guinea-Bissau, west Africa: no protection of HIV-2 against HIV-1 infection. *Aids* 13, 701-707 (1999)
143. Kanki, P.J., S. M'Boup, D. Ricard, F. Barin, F. Denis, C. Boye, L. Sangare, K. Travers, M. Albaum, R. Marlink and *et al.*: Human T-lymphotropic virus type 4 and the human immunodeficiency virus in West Africa. *Science* 236, 827-831 (1987)
144. Powell, J.D., T. Yehuda-Cohen, F. Villinger, H.M. McClure, K.W. Sell and A. Ahmed-Ansari: Inhibition of SIV/SMM replication *in vitro* by CD8+ cells from SIV/SMM infected seropositive clinically asymptomatic sooty mangabeys. *J Med Primatol* 19, 239-249 (1990)
145. Rey-Cuille, M.A., J.L. Berthier, M.C. Bomsel-Demontoy, Y. Chaduc, L. Montagnier, A.G. Hovanessian and L.A. Chakrabarti: Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease. *J Virol* 72, 3872-3886 (1998)
146. McClure, H.M., D.C. Anderson, P.N. Fultz, A.A. Ansari, E. Lockwood and A. Brodie: Spectrum of disease in macaque monkeys chronically infected with SIV/SMM. *Vet Immunol Immunopathol* 21, 13-24 (1989)
147. Kannagi, M., L.V. Chalifoux, C.I. Lord and N.L. Letvin: Suppression of simian immunodeficiency virus replication *in vitro* by CD8+ lymphocytes. *J Immunol* 140, 2237-2242 (1988)
148. Castro, B.A., C.M. Walker, J.W. Eichberg and J.A. Levy: Suppression of human immunodeficiency virus replication by CD8+ cells from infected and uninfected chimpanzees. *Cellular Immunology* 132, 246-255 (1991)
149. Blackbourn, D.J., C.P. Locher, B. Ramachandran, S.W. Barnett, K.K. Murthy, K.D. Carey, K.M. Brasky and J.A. Levy: CD8+ cells from HIV-2-infected baboons control HIV replication. *Aids* 11, 737-746 (1997)
150. Locher, C.P., D.J. Blackbourn and J.A. Levy: Suppression of human immunodeficiency virus type 1 replication by a soluble factor produced by CD8+ lymphocytes from HIV-2-infected baboons. *Immunol Lett* 66, 151-157 (1999)
151. English, R.V., C.M. Johnson, D.H. Gebhard and M.B. Tompkins: *In vivo* lymphocyte tropism of feline immunodeficiency virus. *J Virol* 67, 5175-5186 (1993)
152. Pedersen, N.C., E.W. Ho, M.L. Brown and J.K. Yamamoto: Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* 235, 790-793 (1987)
153. Jeng, C.R., R.V. English, T. Childers, M.B. Tompkins and W.A. Tompkins: Evidence for CD8+ antiviral activity in cats infected with feline immunodeficiency virus. *J Virol* 70, 2474-2480 (1996)
154. Choi, I.S., R. Hokanson and E.W. Collisson: Anti-feline immunodeficiency virus (FIV) soluble factor(s) produced from antigen-stimulated feline CD8(+) T lymphocytes suppresses FIV replication. *J Virol* 74, 676-683 (2000)
155. Bucci, J.G., R.V. English, H.L. Jordan, T.A. Childers, M.B. Tompkins and W.A. Tompkins: Mucosally transmitted feline immunodeficiency virus induces a CD8+ antiviral response that correlates with reduction of cell-associated virus. *J Infect Dis* 177, 18-25 (1998)
156. Desrosiers, R.C., M.S. Wyand, T. Kodama, D.J. Ringler, L.O. Arthur, P.K. Sehgal, N.L. Letvin, N.W. King and M.D. Daniel: Vaccine protection against simian immunodeficiency virus infection. *Proc Natl Acad Sci U S A* 86, 6353-6357 (1989)
157. Murphey-Corb, M., L.N. Martin, B. Davison-Fairburn, R.C. Montelaro, M. Miller, M. West, S. Ohkawa, G.B. Baskin, J.Y. Zhang, S.D. Putney and *et al.*: A formalin-inactivated whole SIV vaccine confers protection in macaques. *Science* 246, 1293-1297 (1989)
158. Carlson, J.R., T.P. McGraw, E. Keddie, J.L. Yee, A. Rosenthal, A.J. Langlois, R. Dickover, R. Donovan, P.A. Luciw, M.B. Jennings and *et al.*: Vaccine protection of rhesus macaques against simian immunodeficiency virus infection. *AIDS Res Hum Retroviruses* 6, 1239-1246 (1990)
159. Stott, E.J., W.L. Chan, K.H. Mills, M. Page, F. Taffs, M. Cranage, P. Greenaway and P. Kitchin: Preliminary report: protection of cynomolgus macaques against simian immunodeficiency virus by fixed infected-cell vaccine. *Lancet* 336, 1538-1541 (1990)
160. Stott, E.J.: Anti-cell antibody in macaques. *Nature* 353, 393 (1991)
161. Lehner, T., Y. Wang, M. Cranage, L.A. Bergmeier, E. Mitchell, L. Tao, G. Hall, M. Dennis, N. Cook, R. Brookes, L. Klavinskis, I. Jones, C. Doyle and R. Ward: Protective mucosal immunity elicited by targeted iliac lymph node

immunization with a subunit SIV envelope and core vaccine in macaques. *Nat Med* 2, 767-775 (1996)
162. Wang, Y., L. Tao, E. Mitchell, W.M. Bogers, C. Doyle, C.A. Bravery, L.A. Bergmeier, C.G. Kelly, J.L. Heeney and T. Lehner: Generation of CD8 suppressor factor and beta chemokines, induced by xenogeneic immunization, in the prevention of simian immunodeficiency virus infection in macaques. *Proc Natl Acad Sci U S A* 95, 5223-5228 (1998)
163. Leno, M., L. Carter, D.J. Venzon, J. Romano, P.D. Markham, K. Limbach, J. Tartaglia, E. Paoletti, J. Benson, G. Franchini and M. Robert-Guroff: CD8+ lymphocyte antiviral activity in monkeys immunized with SIV recombinant poxvirus vaccines: potential role in vaccine efficacy. *AIDS Res Hum Retroviruses* 15, 461-470 (1999)
164. Gauduin, M.C., R.L. Glickman, R. Means and R.P. Johnson: Inhibition of simian immunodeficiency virus (SIV) replication by CD8(+) T lymphocytes from macaques immunized with live attenuated SIV. *J Virol* 72, 6315-6324 (1998)
165. Wilkinson, J., J.J. Zaunders, A. Carr and D.A. Cooper: CD8+ anti-human immunodeficiency virus suppressor activity (CASA) in response to antiretroviral therapy: loss of CASA is associated with loss of viremia. *J Infect Dis* 180, 68-75 (1999)
166. Fransen, S., K.F. Copeland, M. Smieja, F. Smaill and K.L. Rosenthal: RANTES production by T cells and CD8-mediated inhibition of human immunodeficiency virus gene expression before initiation of potent antiretroviral therapy predict sustained suppression of viral replication. *J Infect Dis* 181, 505-512 (2000)
167. Rosenberg, E.S., M. Altfeld, S.H. Poon, M.N. Phillips, B.M. Wilkes, R.L. Eldridge, G.K. Robbins, R.T. D'Aquila, P.J. Goulder and B.D. Walker: Immune control of HIV-1 after early treatment of acute infection. *Nature* 407, 523-526 (2000)
168. Blackbourn, D.J., C. Mackewicz, E. Barker and J.A. Levy: Human CD8+ cell non-cytolytic anti-HIV activity mediated by a novel cytokine. *Res Immunol* 145, 653-659; (1994)
169. Clerici, M., F.T. Hakim, D.J. Venzon, S. Blatt, C.W. Hendrix, T.A. Wynn and G.M. Shearer: Changes in interleukin-2 and interleukin-4 production in asymptomatic, human immunodeficiency virus-seropositive individuals. *J Clin Invest* 91, 759-765 (1993)
170. Clerici, M., C. Balotta, L. Meroni, E. Ferrario, C. Riva, D. Trabattoni, A. Ridolfo, M. Villa, G.M. Shearer, M. Moroni and M. Galli: Type 1 cytokine production and low prevalence of viral isolation correlate with long-term nonprogression in HIV infection. *AIDS Res Hum Retroviruses* 12, 1053-1061 (1996)
171. Bruhl, P., A. Kerschbaum, K. Zimmermann, M.M. Eibl and J.W. Mannhalter: Allostimulated lymphocytes inhibit replication of HIV type 1. *AIDS Res Hum Retroviruses* 12, 31-37 (1996)
172. Wang, Y., L. Tao, E. Mitchell, C. Bravery, P. Berlingieri, P. Armstrong, R. Vaughan, J. Underwood and T. Lehner: Allo-immunization elicits CD8+ T cell-derived chemokines, HIV suppressor factors and resistance to HIV infection in women. *Nat Med* 5, 1004-1009 (1999)

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