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The Effect of IL-7 on Memory $CD8^+$ T Cells Survival and Function During Health and HIV Disease

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THE EFFECT OF IL-7 ON MEMORY $CD8^+$ T CELL SURVIVAL AND FUNCTION DURING HEALTH AND HIV DISEASE

Alison O'Connor

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requirements for the degree of Masters in Science (M.Sc.),
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ABSTRACT

Memory $CD8^+$ T cells proliferate and gain effector function in response to antigen stimulus. It is possible that IL-7 may enhance antigen-mediated $CD8^+$ T cell recall response, since IL-7 is required during a primary immune response. During HIV infection, $CD8^+$ T cell function is impaired, and this could be due to reduced IL-7 activity on $CD8^+$ T cells. In the present study, the effect of IL-7 on antigen-mediated proliferation and function was evaluated in HIV^- and HIV^+ individuals. IL-7 enhanced antigen-mediated proliferation of the $CD8^+CD45RA^-CD127^+$ T cells, but it did not significantly alter IFN-γ production or CD107a/b expression. In HIV^+ individuals, $CD8^+CD45RA^-CD127^+$ T cells did not significantly proliferate in response to IL-7. These data indicate that IL-7 can enhance certain aspects of memory $CD8^+$ T cell function, and IL-7 activity is impaired in HIV infection. These findings are relevant to the ongoing studies of HIV pathogenesis and of IL-7 as a therapeutic in HIV infection and other diseases.

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AIDS – Acquired Immunodeficiency Syndrome
AKT – Protein kinase B family proteins
APC – Antigen presenting cell
ART – Anti-retroviral therapy
BCL-2 – B-cell lymphoma 2
BSA – Bovine serum albumin
CD – Cluster of differentiation
CFSE – Carboxyfluorescein diacetate succinimidyl ester
CMV – Cytomegalovirus
CNS – Central nervous system
CTL – Cytotoxic T lymphocyte
DC – Dendritic cell
DMSO – Dimethylsulfoxide
EBV – Epstein Barr virus
ECD – Phycoerythrin-Texas red
EDTA – ethylenediaminetetraacetic acid
ERK – Extracellular Signal-Regulated Kinase
FCS – Fetal calf serum
FITC – Fluorescein isothiocyanate
FLU – Influenza virus
HAART – Highly-active antiretroviral therapy
HIV – Human Immunodeficiency Virus
IFN-γ – Interferon-γ
IL – Interleukin
Jak – Janus Kinase
LAMP – Lysosomal associated membrane glycoproteins
LTNPs – Long-term non-progressors
MAPK – Mitogen-Activated Protein Kinase
MHC – Major histocompatibility class
MPECs – Memory precursor effector cells
PC5 – Phycoerythrin-Cy5
PC7 – Phycoerythrin-Cy7
PBMC – Peripheral blood mononuclear cells
PBS – Phosphate-buffered saline
PEN/STREP – Penicillin/Streptomycin
PI3K – Phosphoionositide 3-kinase
PHA – Phytohemagglutinin
PMA – Phorbol 12-myristate 13-acetate
RTEs – Recent thymic emigrants
SLECs – Short-lived effector cells
STAT – Signal Transducer and Activator of Transcription
TCR – T-cell receptor
T_N – Naïve $CD8^+$ T cell
T_{CM} – Central memory $CD8^+$ T cell
T_{EM} – Effector memory $CD8^+$ T cell
T_{EMRA} – Terminally-differenitiated effector memory $CD8^+$ T cell

CHAPTER 1: INTRODUCTION

1.1 HUMAN IMMUNODEFICIENCY VIRUS

The human immunodeficiency virus (HIV) is a retrovirus that infects $CD4^+$ T cells, monocytes, macrophages and dendritic cells and leads to destruction of the immune system (Stevenson, 2003). HIV infects cells through interactions between the gp120 molecule on the envelope of the viral particle and the CD4 receptor along with the CCR5 or CXCR4 co-receptors on the host cell (Barre-Sinoussi et al., 1983; Dalgeish AG, 1984; Choe H, 1996; Deng H, 1996). Once inside the cell, the viral genome integrates into the host cell genome and replicates to produce more viral particles. Once the virus has integrated into the cellular genome, it can remain hidden in latently infected cells and establish a chronic infection (Pierson et al., 2000). The newly synthesized HIV particles bud from the infected cells, and can then infect or kill other cells through viral interactions and bystander apoptosis (Stevenson, 2003). During the acute stages of infection, the significant loss of $CD4^+$ T cells is coupled with a high viral load (Douek et al., 2003). The immune system is initially able to control viral replication, and viral loads decrease with $CD4^+$ T cell numbers remaining stable. Clinical latency with chronic infection becomes established, and can persist for a variable number of years depending on the interaction between the individual's immune response and the virus (Letvin and Walker, 2003). Eventually, the immune system becomes unable to control viral replication, and viral loads rapidly increase as $CD4^+$ T cells decrease. Infection with HIV leads to the eventual depletion of the $CD4^+$ T cell pool, dysregulation of the cytokine environment, and the loss of function of $CD8^+$ T cells. Progressive immune dysfunction leads to an increased risk of opportunistic infections and acquired immunodeficiency syndrome (AIDS).

1.2 The acquired immune response by $CD8^+$ T cells

To mount an acquired immune response, $CD4^+$ and $CD8^+$ T cells are activated by antigen presenting cells (APC), such as dendritic cells (DCs), macrophages, and B cells. The APC processes antigen from a pathogen and presents the antigen in the major histocompatibility class I (MHC I) and MHC II receptors on the APC to the T-cell receptor (TCR) on naïve $CD8^+$ or $CD4^+$ T cells, respectively, in the lymph nodes. $CD8^+$ T cells are cytotoxic T lymphocytes (CTL) and $CD4^+$ T cells provide help in activating these cells. $CD8^+$ T cells recognize and kill infected cells in order to control or eliminate infection. Once activated by antigen stimulation, naïve $CD8^+$ T cells go through three phases: expansion and effector $CD8^+$ T cell differentiation, contraction of the effector $CD8^+$ T cell population, and formation of a stable memory $CD8^+$ T cell pool (Williams and Bevan, 2007). During the expansion phase, naïve $CD8^+$ T cells proliferate and differentiate into antigen-specific effector cells which leave the lymphoid tissue and migrate to sites of infection and exhibit cytotoxic function to kill infected cells (Wherry and Ahmed, 2004). Effector $CD8^+$ T cells release cytotoxic granules containing perforin, granzymes, as well as effector molecules such as interferon-γ (IFN-γ), which kill the infected cell (Callan et al., 2000). Effector $CD8^+$ T cells can also kill infected cells through FAS ligand-FAS receptor interactions which induce a signaling cascade that leads to apoptosis of the target cell (Callan et al., 2000). $CD4^+$ T cells are also important for effector $CD8^+$ T cell function. $CD4^+$ T cells help prime $CD8^+$ T cells when they interact with the same dendritic cell (Bennett, SR. 1997). $CD4^+$ T cell help may also be important for activation of $CD8^+$ T cells during a secondary immune response to antigen (Bevan 2004). Naïve and memory $CD8^+$ T cells require co-stimulation, specifically through CD28 and CD137 molecules, in addition to TCR engagement for cell expansion and

survival (Williams and Bevan, 2007; Borowski et al., 2007). The cytokines present in the cellular microenvironment, specifically interleukin (IL)-2, IL-7, and IL-15, are also important for activation of $CD8^+$ T cells (Schluns and Lefrancois, 2003; Ma et al., 2006). IL-2 can control clonal expansion of cells by promoting proliferation at early activation and inducing cell death during late activation (Schluns and Lefrancois, 2003). IL-15 can activate DCs, promote proliferation of antigen specific $CD8^+$ T cells, and enhance memory $CD8^+$ T cell development and homeostasis (Schluns and Lefrancois, 2003). IL-7 is important for generation and homeostasis of memory $CD8^+$ T cells (Schluns et al., 2000). After the pathogen has been cleared, the contraction phase begins, and a majority of effector $CD8^+$ T cells become apoptotic and die. From the effector pool, a small fraction of the effector $CD8^+$ T cells survive and differentiate into long-lived memory $CD8^+$ T cells (Kaech and Wherry, 2007). $CD8^+$ T cells are critical for an effective immune response against many pathogens.

1.3 $CD8^+$ T cell responses during HIV infection

Upon infection with HIV, the acquired immune system is activated to eliminate viral replication in order to contain the infection. To control HIV replication, HIV-specific $CD8^+$ T cells expand and kill infected cells during the initial stages of infection. However, HIV cannot be completely cleared from the infected individual, and the virus establishes itself in host reservoir sites. HIV reservoirs include latently-infected resting naïve and memory $CD4^+$ T cells, macrophages, monocytes, and cells within the central nervous system (CNS), as well as follicular dendritic cells and B cells, which are uninfected but act as reservoirs (Pierson et al., 2000; Blankson et al., 2002). These HIV reservoirs cannot be eradicated with antiretroviral therapy, and are a major reason for persistence of viral infection. As HIV infection persists, $CD8^+$ T cells progressively lose function and are unable to control the

infection. It has yet to be determined why $CD8^+$ T cell function is lost during HIV infection however there are several hypotheses to explain the loss of function. Constant antigenic stimulation may cause $CD8^+$ T cells to become exhausted, causing the cells to lose function (Letvin and Walker, 2003). Exhaustion is characterized by replicative senescence, and may occur within certain HIV-specific $CD8^+$ T cell clones, resulting in the loss of these specific clones (Appay). Impaired $CD8^+$ T cell function may also be due to the lack of $CD4^+$ T cell help, as these cells are significantly depleted during HIV infection. $CD8^+$ T cells become less sensitive to cytokine stimulation and exhibit down-regulated cytokine receptor expression (Vingerhoets et al., 1998), and the loss of $CD8^+$ T cell function may occur because cells do not receive the required activation signals. A critical signaling component of the TCR, CD3ξ, and the co-stimulatory molecule CD28 are down-regulated on HIV-specific $CD8^+$ T cells, indicating the cells may not be appropriately activated (Trimble et al., 2000). It is possible that the loss of function of $CD8^+$ T cells during HIV infection is due to a combination of these factors.

Infection with HIV can affect many aspects of function in HIV-specific $CD8^+$ T cells. Perforin production and lytic capabilities are progressively impaired in HIV-specific $CD8^+$ T cells, but they can produce other antiviral molecules, including IFN-γ and MIP-1β (Appay et al., 2000). In HIV^+ individuals, HIV-specific $CD8^+$ T cells do not have the same polyfunctional capabilities as non-HIV-specific $CD8^+$ T cells, and a single $CD8^+$ T cell cannot produce the proper combination of antiviral molecules (Almeida et al., 2007). In contrast, HIV-specific $CD8^+$ T cells from HIV^+ long-term non-progressors (LTNPs) have greater polyfunctional capabilities compared to HIV-specific $CD8^+$ T cells from HIV^+ progressors, suggesting $CD8^+$ T cell function is an important aspect for controlling HIV

infection (Betts et al., 2006). The loss of function of $CD8^+$ T cells during HIV infection is a major reason why viral replication and disease progression cannot be controlled.

HIV-specific $CD8^+$ T cells are also found in different maturation states compared to $CD8^+$ T cells from other chronic infections, and differentiation of these cells may be impaired during HIV infection (Appay et al., 2002). The HIV-specific $CD8^+$ T cell population has an increased number of effector memory $CD8^+$ T cells and a decreased number of terminally-differentiated effector $CD8^+$ T cells during HIV infection compared to the subset distribution of a CMV-specific $CD8^+$ T cell pool (Champagne et al., 2001). The decreased number of effector cells could be a reason for impaired killing of infected cells as HIV infection progresses. Total central memory $CD8^+$ T cells from HIV^+ individuals also have a shorter half-life compared to those from HIV^- individuals, and the number of central memory $CD8^+$ T cells is positively correlated with CD4 count (Ladell et al., 2008). Since these cells have an increased turnover, it is possible that memory $CD8^+$ T cells do not survive long enough to form an adequate memory cell pool or to mount an effective secondary immune response. The response of HIV-specific $CD8^+$ T cells depends on the antigen, and the cells do not proliferate and produce IFN-γ in response to all HIV antigens (McKinnon et al., 2008). This suggests that memory $CD8^+$ T cells can respond in some ways to antigen stimulation, but are impaired in other functions. While impaired function of HIV-specific $CD8^+$ T cells has been thoroughly investigated, the effect of HIV infection on non-HIV specific $CD8^+$ T cell responses has yet to be well defined. It was found that patients on antiretroviral therapy (ART) have functional memory $CD8^+$ T cell responses to non-HIV viral antigens, suggesting the loss of function can be reversed to some extent with therapy

(Arrode et al., 2005). If memory $CD8^+$ T cell responses are impaired during HIV infection, there is a higher risk of opportunistic infection.

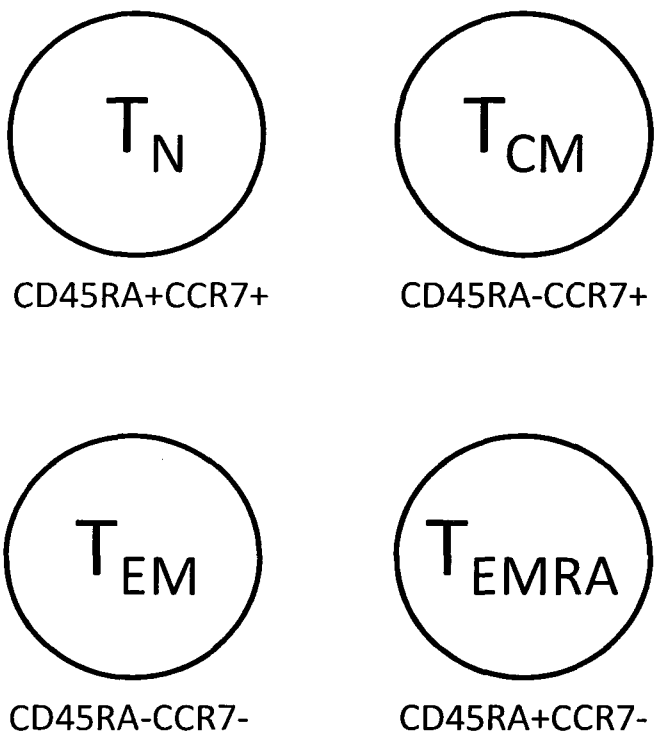
1.4 $CD8^+$ T cell development and function

1.4.1 $CD8^+$ T cell subsets

$CD8^+$ T cell responses are critical for an immune response, and they exist as distinct subsets that can be distinguished by surface receptor expression. Expression of several surface molecules are used to define $CD8^+$ T cell subsets, including CD27, CD28, CD62L, CD127, CD45RO, CD45RA, and CCR7 (Sallusto et al., 2004). Surface expression of CD45RA was initially used to distinguish between cells naïve and memory $CD8^+$ T cells, as $CD45RA^-$ cells had a greater proliferation response to rechallenge with antigen, and CD45RA expression was lost after PHA stimulation (Akbar et al., 1988). The chemokine receptor CD62L expression was then found to be low on effectors and higher on memory CD8+ T cells in mice (Zimmerman et al., 1996). Expression of activation markers, specifically CD28 and CD27, was also used to discriminate between naïve, memory, and effector cells (Zimmerman et al., 1996; Hamman et al., 1997). CCR7, a chemokine homing receptor, was found to be expressed on resting $CD4^+$ T cells but not activated $CD4^+$ T cells (Campbell et al., 2001). The CD45RA and CCR7 receptors are commonly used to identify $CD8^+$ T cell subsets, as cells that are either positive or negative for their expression are easily distinguished experimentally, whereas expression of other markers can occur at lower intensities. Naïve $CD8^+$ T cells (T_N) are $CD45RA^+CCR7^+$, central memory $CD8^+$ T cells (T_{CM}) are $CD45RA^-CCR7^+$, effector memory $CD8^+$ T cells (T_{EM}) are $CD45RA^-CCR7^-$, and terminally-differentiated effector memory $CD8^+$ T cells (T_{EMRA}) are $CD45RA^+CCR7^-$ (Figure 1-1) (Sallusto et al., 2004). The expression of CD127 also changes as $CD8^+$ T cells mature

and differentiate upon antigen exposure, and expression is high on T_N and T_{CM} $CD8^+$ T cells and decreases as they become T_{EM} and T_{EMRA} $CD8^+$ T cells (Kaech et al., 2003). Memory $CD8^+$ T cells include T_{CM}, T_{EM}, and T_{EMRA} cells, and are functionally heterogenic.

Figure 1-1. $CD8^+$ T cell subsets. $CD8^+$ T cells can be phenotypically categorized into subsets based on surface expression of the CD45RA and CCR7 molecules. T_N: $CD45RA^+CCR7^+$, T_{CM}: $CD45RA^-CCR7^+$, T_{EM}: $CD45RA^-CCR7^-$, and T_{EMRA}: $CD45RA^+CCR7^-$.


T_N
CD45RA+CCR7+
T_{CM}
CD45RA-CCR7+
T_{EM}
CD45RA-CCR7-
T_{EMRA}
CD45RA+CCR7-

Memory $CD8^+$ T cell subsets differ not only in their receptor expression, but also in their cytokine profiles and their effector functions. Expansion potential of cells is highest in T_{CM} cells compared to the more differentiated subsets, while the T_{EM} and T_{EMRA} populations exhibit rapid effector function, including perforin expression and IFN-γ production, compared to T_{CM} cells (Sallusto et al., 2004). The $CD8^+$ T cell subsets also differ in their tissue distribution. T_{CM} cells are located predominantly in the lymph nodes, while T_{EM} cells are found in the peripheral blood (Sallusto et al., 2004). Memory $CD8^+$ T cells differentiate into effectors upon stimulation with antigen, however the pathway and required signals have not been fully elucidated. The T_{CM} cells can differentiate into T_{EM} cells, as seen by the decrease of CCR7, CD27, and CD28 expression on $CD45RA^-CD8^+$ T cells after antigenic stimulation (Tomiyama et al., 2002). As T_{CM} cells differentiate and gain effector phenotype and function, the cytokine profiles change, and IFN-γ and TNF-α are produced to greater extents as cells differentiate (Hamann et al., 1997; Tomiyama et al., 2002; Geginat et al., 2003; Romero et al., 2007). In addition, the production of cytotoxic molecules differs between T_{CM} and T_{EM} cells. Early-stage memory $CD8^+$ T cells express granzyme K and granzyme A, whereas more differentiated memory $CD8^+$ T cells express high levels of perforin, granzyme A and granzyme B (Harari et al., 2009). T_{EM} cells can be even further subdivided into functionally distinct populations, and produce different combinations of perforin and granzymes (Romero et al., 2007). T_{CM} cells may also proliferate without gaining an effector phenotype to replenish the memory $CD8^+$ T cell pool, as this subset has a high proliferative capacity (Sallusto et al., 2004). Memory $CD8^+$ T cell responses are coupled with differentiation of the cells, and each subset has specific functions during an immune response.

1.4.2 Differentiation of $CD8^+$ T cells

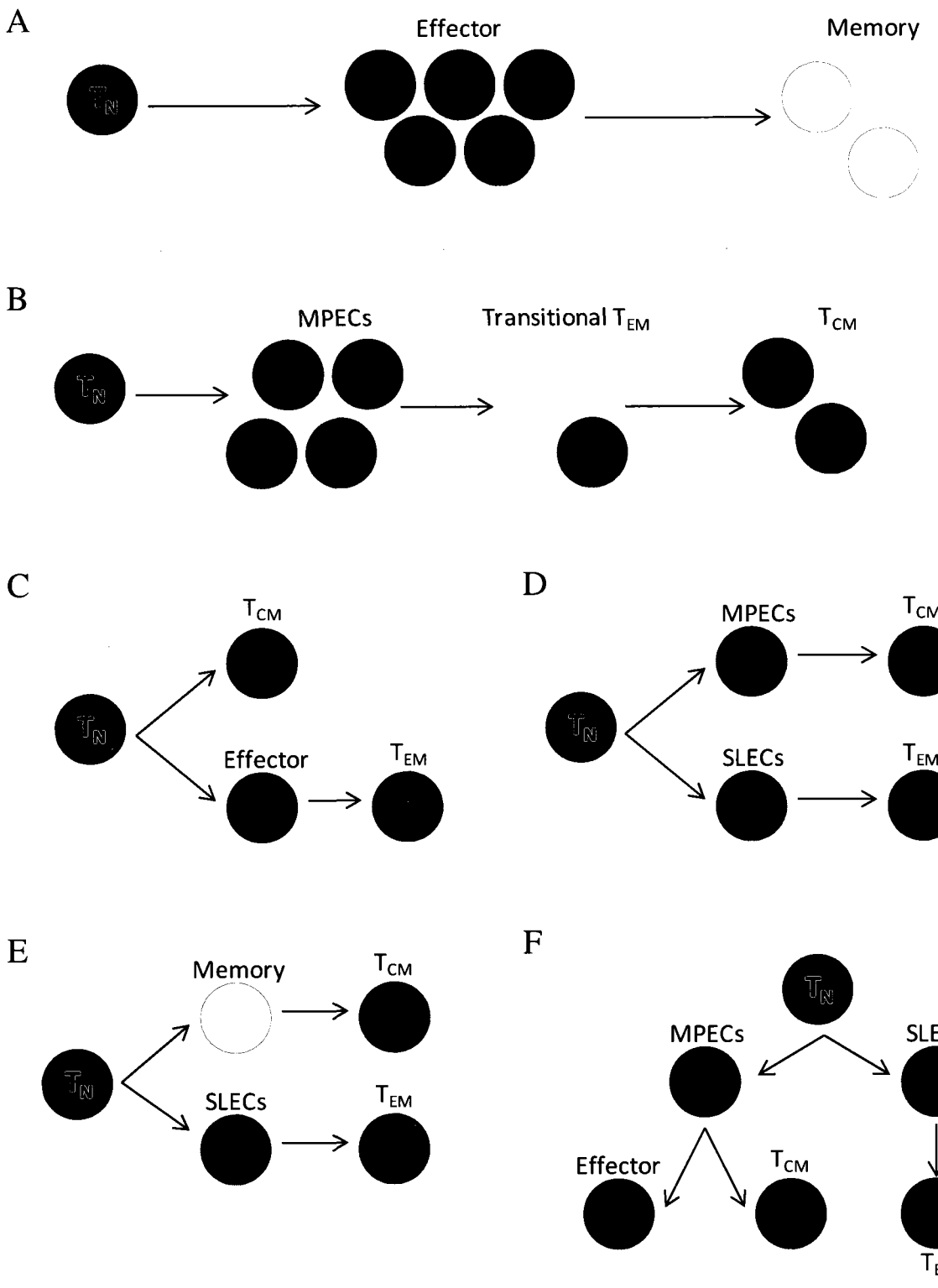
$CD8^+$ T cell activation and differentiation is a complex process, and there are several models of $CD8^+$ T cell development (reviewed in Sallusto and Lanzavecchia, 2001; Sallusto et al., 2004; and Kaech and Wherry, 2007). Differentiation of naïve $CD8^+$ T cells into effector and memory $CD8^+$ T cells upon antigenic stimulation has still not been definitively described, however several models of differentiation have been proposed. A 'linear model' of differentiation (Figure 1-2A) suggests that naïve $CD8^+$ T cells differentiate into effector $CD8^+$ T cells and a small proportion of these become memory $CD8^+$ T cells after a primary immune response (Sallusto and Lanzavecchia, 2001). This model may be too simplistic, as naïve $CD8^+$ T cells have been shown to differentiate into effector or memory cells, depending on the stimuli (Stemberger et al., 2007). A similar model is a 'uniform potential model' of differentiation (Figure 1-2B), in which memory precursor effector cells (MPECs) generated after an immune response can differentiate into transitional effector memory $CD8^+$ T cells and then into central memory $CD8^+$ T cells (Kaech and Wherry, 2007).

A 'signal strength model' of differentiation (Figure 1-2C) states that naïve $CD8^+$ T cells become activated and differentiate depending on the strength of antigen stimulus and the cytokine microenvironment (Lanzavecchia and Sallusto, 2000). Signal strength is determined by the concentration of antigen and co-stimulatory molecules, length of interaction between the TCR and MHC I molecule, and cytokine microenvironment (Sallusto et al., 2004). This model is based on the premise that the $CD8^+$ T cells that receive strong antigenic stimulation die by activation-induced cell death and the cells that receive weak antigenic stimulation die by neglect (Sallusto et al., 2004). $CD8^+$ T cells that receive adequate stimulation become T_{CM} cells, while those that receive slightly stronger antigenic stimulation become effector cells

(Sallusto et al., 2004). Once the primary immune response has cleared the infection, the effector $CD8^+$ T cells become apoptotic or, if the correct signals are present, further differentiate into T_{EM} cells, which can also contain the T_{EMRA} subset (Sallusto et al., 2004). A 'decreasing potential model' of differentiation (Figure 1-2D) suggests a similar response, however brief antigen stimulation leads to the development of MPECs that differentiate into T_{CM} cells, and longer stimulation leads to the development of short-lived effector cells (SLECs) that can become effector memory T cells (Kaech and Wherry, 2007).

Another model of differentiation is a 'fixed lineage model', or non-linear model, of differentiation (Figure 1-2E) which states that after activation, naïve $CD8^+$ T cells differentiate into SLECs or mature memory $CD8^+$ T cells (Farber, 1998; Sallusto and Lanzavecchia, 2001; Kaech and Wherry, 2007). Mature memory $CD8^+$ T cells comprise the self-renewing T_{CM} cell population, and SLECs can differentiate into T_{EM} cells that will die over time. A combined and more comprehensive model is the 'fate commitment with progressive differentiation' model (Figure 1-2F). A naïve $CD8^+$ T cell can either differentiate into a MPEC with weak antigen stimulation, and can then either gain effector function or develop into self-renewing T_{CM} cells. Strong antigen stimulation promotes differentiation of naïve $CD8^+$ T cells into SLECs, most of which die, however cells that persist become T_{EM} cells (Kaech and Wherry, 2007). The mechanism and signals required for $CD8^+$ T cell differentiation into memory $CD8^+$ T cells are still being investigated.

Figure 1-2. Models of $CD8^+$ T cell differentiation. Differentiation of $CD8^+$ T cells from naïve to effector or memory $CD8^+$ T cells has yet to be elucidated, but several models have been hypothesized. Models have been adapted from the following articles: Sallusto and Lanzavecchia, 2001; Sallusto et al., 2004; and Kaech and Wherry, 2007. (A) Linear model of differentiation. (B) Uniform potential model of differentiation. (C) Signal strength model of differentiation. (D) Decreasing potential model of differentiation. (E) Fixed lineage model of differentiation. (F) Fate commitment with progressive differentiation model.


A
Effector
Memory
B
MPECs
Transitional T_{EM}
T_{CM}
C
T_{CM}
Effector
T_{EM}
D
MPECs
T_{CM}
SLECs
T_{EM}
E
Memory
T_{CM}
SLECs
T_{EM}
F
MPECs
SLECs
Effector
T_{CM}
T_{EM}

1.4.3 Maintenance of the memory $CD8^+$ T cell pool

Memory $CD8^+$ T cells are maintained in the body for many years by cytokine signaling and cellular interactions, and protect against re-infection. There are two hypotheses for the requirements of memory $CD8^+$ T cell maintenance, which are antigen-dependent and antigen-independent maintenance. Antigen-dependent memory $CD8^+$ T cell maintenance requires stimulation through MHC/TCR interactions (Tanchot et al., 1997; Markiewicz et al., 1998) Many studies have shown that stimulation through the TCR or co-stimulatory molecules is not required for memory $CD8^+$ T cell maintenance (Lau et al., 1994; Mullbacher, 1994; Murali-Krishna et al., 1999), but antigen may be required for preserving cellular function (Jameson, 2002). Antigen-independent maintenance of memory $CD8^+$ T cells is governed by the cytokine microenvironment, specifically by IL-7 and IL-15 (Surh et al., 2006). IL-7 and IL-15 both mediate survival of memory $CD8^+$ T cells by inducing proliferation and enhancing expression of anti-apoptotic molecules on the $CD8^+$ T cells. T_{CM} cells circulate through the blood and lymph nodes, and require predominantly IL-7 for homeostatic proliferation, whereas T_{EM} circulate through the blood and require mainly IL-15 for homeostatic proliferation (Boyman et al., 2009). Memory $CD8^+$ T cells are important for ongoing immune system surveillance and are essential for secondary immune responses.

1.4.4 $CD8^+$ T cells during a secondary immune response

Upon re-infection with a pathogen, memory $CD8^+$ T cells are stimulated by their specific antigens and rapidly exhibit effector functions or differentiate into T_{EMRA} $CD8^+$ T cells. A secondary immune response is typically more efficient than a primary immune response, and pathogens are cleared more quickly (Meng et al., 2006; Veiga-Fernandes et al., 2000). Both T_{CM} and T_{EM} subsets exhibit effector function by producing cytotoxic molecules after

antigenic stimulation (Williams and Bevan, 2007). Memory $CD8^+$ T cells are polyfunctional, and each individual $CD8^+$ T cell is able to produce a variety of cytotoxic cytokines and effector molecules including IFN-γ, granzymes and perforin. T_{EM} cells can also differentiate into T_{EMRA} cells, and their cytotoxic function increases as they differentiate (Romero et al., 2007). Memory $CD8^+$ T cells can also proliferate to replenish the memory $CD8^+$ T cell pool, and the T_{EMRA} cells become apoptotic and die after the infection has been cleared. Cytokines in the cellular environment, in particular IL-7, may also be required for an efficient secondary immune response, however the role of cytokines in the enhancement of recall responses is not well understood.

1.5 The role of IL-7/IL-7Rα in the immune system

1.5.1 IL-7 and IL-7R are required for T cell development and survival

IL-7 is an essential cytokine for $CD4^+$ and $CD8^+$ T cell survival and function. IL-7 knock-out mice are highly lymphopenic, have reduced numbers of thymocytes as well as T and B cells, and exhibit impaired thymic development (von Freeden-Jeffery et al., 1995; Moore et al., 1996). IL-7 receptor (IL-7R) knock-out mice have decreased immature B cells and thymocytes, and have reduced cellularity of peripheral $CD4^+$ and $CD8^+$ T cells and B cells (Sudo et al., 1993; Peschon et al., 1994). IL-7 is produced by epithelial cells in the thymus, and is required for the proper growth and maturation of thymocytes (Plum et al., 1996). IL-7 is required during the early stages of thymopoiesis, and in its absence, thymocyte development halts at an immature thymocyte stage (Hofmeister et al., 1999). It has been recently shown that IL-7 is also expressed in hepatocytes, and this source of IL-7 is important for peripheral $CD8^+$ T cell survival and cytotoxic function (Sawa et al., 2009).

Signaling results from IL-7 ligation to its receptor, which consists of the IL-7-specific chain, IL-7Rα (CD127), and the common gamma chain, IL-2Rγ (CD132), which is shared by other cytokines in the common gamma chain family (IL-2, -4, -7, -9, -15, and -21) (Ozaki and Leonard, 2002). CD127 is predominantly expressed on thymocytes, immature B cells, and peripheral $CD4^+$ and $CD8^+$ T cells (Sudo et al., 1993). Once IL-7 ligates its receptor, intracellular signaling begins through phosphorylation of the Janus family kinases (Jaks), Jak1 and Jak3, and subsequent activation of the signal transducer and activator of transcription 5 (STAT5) molecule. Other signaling pathways are also activated, including the phosphoionositide 3-kinase (PI3K)/ protein kinase B family proteins (AKT) pathway and the extracellular signal-regulated kinase (ERK)/ mitogen-activated protein kinase (MAPK) pathway (Hofmeister et al., 1999). In $CD8^+$ T cells, IL-7 predominantly signals through the Jak/STAT and PI3-K pathways. These signals are transduced to the nucleus, and leads to the transcription of genes required for many aspects of $CD4^+$ and $CD8^+$ T cell survival and function. IL-7 promotes proliferation and glucose uptake of recent thymic emigrants (RTEs) (Swainson et al., 2007). In peripheral T cells, IL-7 induces expression of survival and anti-apoptotic molecules, including B-cell lymphoma 2 (BCL-2)-family proteins in T cells (Mazzucchelli and Durum, 2007). IL-7 also induces proliferation in T cells and is required for homeostatic expansion of T cells in circulation (Grabstein et al., 1990; Schluns et al., 2000). IL-7 can also enhance expression of functional proteins, including IFN-γ and perforin, in $CD4^+$ and $CD8^+$ T cells (Borger et al., 1996; Smyth et al., 1991). IL-7 is a critically important cytokine for survival, maintenance, and function of $CD4^+$ and $CD8^+$ T cells.

1.5.2 IL-7 is required by $CD8^+$ T cells for effective immune responses

During the contraction phase of a primary immune response, IL-7 promotes the survival of effector $CD8^+$ T cells that will differentiate into memory cells (Schluns and Lefrancois, 2003). CD127 expression decreases as $CD8^+$ T cells become activated, but is up-regulated on some cells as they enter the contraction phase (Schluns and Lefrancois, 2003). Expression of CD127 on effector $CD8^+$ T cells is required for memory $CD8^+$ T cell development (Hand et al., 2007). IL-7 may also enhance $CD8^+$ T cell responses early during an immune response. It has recently been shown in mice that there is a requirement for IL-7 in T-cell homeostasis and activation in response to antigen *in vivo*, but not *in vitro* (Saini et al., 2009). Memory $CD8^+$ T cell survival is also regulated by IL-7 and the presence of this cytokine allows for homeostatic proliferation and maintenance of the memory $CD8^+$ T cell pool. Since it is know that IL-7 is necessary for T cell survival, it is currently being used in human clinical trials primarily to enhance immune reconstitution. In patients with non-heamtologic and non-lymphoid cancer, IL-7 treatment resulted in an expanded T cell pool, specifically in recent thymic emigrants and T_N and T_{CM} cell populations, increased TCR repertoire diversity and preservation of T cell function (Sportes et al., 2008). Results from clinical trials have shown IL-7 is a promising candidate to increase cell numbers and enhance immune function.

1.5.3 HIV and IL-7/IL-7Rα

During HIV infection, cytokine production becomes disrupted, leading to impaired immune function and enhanced viral replication. Plasma IL-7 levels are increased in HIV^+ individuals, and these levels are correlated with $CD4^+$ T cell depletion and can be used as a marker of disease progression (Llano et al., 2001; Napolitano et al., 2001; Sasson et al., 2006). The increase in circulating IL-7 levels may occur in an effort to increase proliferation

of T cells in response to $CD4^+$ T cell lymphopenia (Napolitano et al., 2001). However, IL-7 has been shown to enhance viral replication in peripheral blood mononuclear cells and macrophages from HIV^+ individuals, (Smithgall et al., 1996; Zhang et al., 2006), suggesting IL-7 can regulate HIV replication and the increased levels of IL-7 may enhance disease progression. Furthermore, while IL-7 levels are increased, the $CD8^+$ T cells do not fully respond to IL-7 stimulation (Colle et al., 2006; Rethi et al., 2005). IL-7 signaling is impaired in $CD4^+$ T cells during HIV infection (Bazdar et al., 2009; Camargo et al., 2009), and this defect may also occur in $CD8^+$ T cells. Expression of CD127 is significantly decreased on $CD8^+$ T cells during HIV infection, and is also decreased on $CD4^+$ T cells (MacPherson et al., 2001; Koesters et al., 2006). HIV-specific $CD8^+$ T cells have been shown to have decreased CD127 expression, and the down-regulation of this molecule may be a reason for improper differentiation of memory $CD8^+$ T cells and impaired cytotoxic function (Wherry et al., 2006). CD127 expression is down-regulated to different extents on the $CD8^+$ T cell subsets, and this may also result in impaired function (Colle et al., 2006; Mercier et al., 2008). It is also possible that IL-7 activity is inherently impaired during HIV infection, leading to reduced responsiveness to IL-7 by $CD8^+$ T cells. IL-7 activity may be restored with effective treatment, and clinical trials using IL-7 are also being conducted in ART-treated HIV^+ individuals, as therapy alone does not result in full reconstitution of the immune system. In effectively-treated HIV^+ individuals, the administration of IL-7 led to an increase in T_N and T_{CM} cells, increased the number of $CD127^+$ $CD4^+$ and $CD8^+$ T cells, but did not significantly affect T cell activation (Levy et al., 2009; Sereti et al., 2009). Understanding the effect of IL-7 on $CD8^+$ T cells in health and HIV disease and determining potential defects in the signaling pathway may lead to the development of new therapeutic targets.

1.6 Rationale

It has been observed that IL-7 is important during a primary immune response, and it is critical for maintenance of the $CD8^+$ T cell pool in the periphery. CD127 expression is required for memory $CD8^+$ T cell generation, and it is necessary for the survival of these cells. It is possible that IL-7 can also enhance a secondary immune response by increasing antigen-mediated proliferation or function of memory $CD8^+$ T cells. During HIV infection, $CD8^+$ T cell function and differentiation is impaired, and this may lead to an ineffective memory response. The dysfunction of $CD8^+$ T cells may be due to a defect in IL-7 signaling, and IL-7 activity in memory $CD8^+$ T cells may be impaired as a result of this.

HYPOTHESIS

IL-7 enhances the survival and function of $CD8^+CD45RA^-CD127^+$ T cells in response to antigen, and this effect will be impaired in HIV infection.

OBJECTIVES

1. To evaluate the proliferation and function of $CD8^+CD45RA^-CD127^+$ T cells in response to IL-7 in the presence of antigen.
2. To evaluate whether IL-7 activity in $CD8^+CD45RA^-CD127^+$ T cells in HIV^+ individuals is impaired compared to healthy individuals.

CHAPTER 2: MATERIALS AND METHODS

2.1 Patient sample information and isolation of peripheral blood mononuclear cells

Blood was drawn from HIV^- and HIV^+ individuals as approved by the Ottawa Hospital Research Ethics Board, and written consent was obtained from donors. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque Plus (GE Healthcare Biosciences, Baie d'Urfe, Quebec, Canada) density gradient, and centrifuged in a Megafuge 1.0 centrifuge (Heraeus Instruments) for 30 minutes at 1600 rpm. The buffy coat was isolated and washed twice in phosphate-buffered saline (PBS; Invitrogen, Burlington, ON, Canada) for 10 minutes at 1600 rpm. The supernatant was discarded, the cell pellet was resuspended in PBS and cells were counted on a hemocytometer with 0.4% Trypan blue solution (Sigma Aldrich, Oakville, ON, Canada) at a 1:10 dilution of cells to Trypan blue solution. The cells were washed in PBS and $CD8^+$ T cells and $CD8^+$ T cell-depleted PBMC were subsequently isolated.

2.2 Isolation of $CD8^+$ T cells and $CD8^+$ T cell-depleted PBMC

PBMC isolated from whole blood were counted as above and centrifuged for 10 minutes at 1600 rpm. The supernatant was removed, and cells were resuspended in 40 ul MACS buffer (25 mmol ethylenediaminetetraacetic acid (EDTA, Sigma Aldrich), 0.5% bovine serum albumin (BSA, (Sigma Aldrich) in PBS) per 10^7 cells. $CD8^+$ T cells were isolated using the $CD8^+$ T cell isolation kit (Miltenyi Biotec, Auburn, CA, USA). Briefly, a cocktail containing biotin-conjugated monoclonal antibodies against CD4, CD15, CD16, CD19, CD34, CD36, CD56, CD123, TCR γ/δ, and CD235a was added at a concentration of 10 ul/10^7 PBMC. The cells were then incubated at 4°C for 20 minutes, after which MACS buffer was added at a concentration of 30 ul/10^7 cells, as well as anti-biotin microbeads (mircrobeads conjugated to monoclonal antibodies against human CD14 and biotin) at a

concentration of 20 ul/10^7 cells. Cells were incubated another 15 minutes at 4°C, and then washed with MACS Buffer for 5 minutes at 1600 rpm. The supernatant was removed and cells were resuspended in 500 ul MACS buffer per 10^8 cells. $CD8^+$ T cells were isolated by negative selection, and this was carried out by magnetic separation using the Deplete program on an autoMACS Separator (Milenyi Biotec). The resulting cell populations after sorting were untouched $CD8^+$ T cells, with a purity of >98%, and $CD8^+$ T cell-depleted PBMC. These cells were washed in PBS and cultured overnight in RPMI (Invitrogen) containing 20% fetal calf serum (FCS; Invitrogen) and 1% penicillin/streptomycin (pen/strep; Invitrogen) (complete RPMI media) at 1x10^6 cells/mL at 37°C and 5% CO_2.

2.3 Isolation of $CD8^+CD45RA^-CD127^+$ T cells

After overnight incubation the $CD8^+$ T cells were washed with PBS and counted. Cells were then resuspended in 80 ul MACS buffer per 10^7 cells, and incubated with anti-CD45RA microbeads (Miltenyi Biotec) for 20 minutes at 4°C. Cells were then washed with MACS buffer, and resuspended at a concentration of 500 ul/10^8 cells in MACS buffer. $CD8^+CD45RA^-$ T cells were obtained from the negative fraction, which was isolated using the Possel program on an autoMACS separator (Miltenyi Biotec). The $CD8^+CD45RA^-$ fraction was then washed with PBS and resuspended with 80 ul MACS buffer per 10^7 cells, and incubated with 65 ul of PE-conjugated monoclonal antibodies against CD127. Cells were incubated at 4°C in the dark for 20 minutes. After the incubation, cells were washed with MACS buffer, and resuspended in 80 ul MACS buffer per 10^7 cells, and incubated with anti-PE microbeads at 4°C in the dark for 15 minutes. $CD8^+CD45RA^-CD127^+$ T cells were isolated using the Possels program on an autoMACS separator (Miltenyi Biotec).

$CD8^+CD45RA^-CD127^+$ T cells were resuspended in complete RPMI media at a concentration of $1x10^6$ cells/ml.

2.4 Peptide pulsing of $CD8^+$ T cell-depleted PBMC

$CD8^+$ T cell-depleted PBMC were washed with PBS at 1600 rpm for 5 minutes. Cells were counted, and resuspended at $7x10^6$ cells/ml in serum-free RPMI. The cells were then pulsed with a CEF peptide pool (PANATecs GmbH, Tuebingen, Germany) using a concentration of 2 ug of each peptide/ml, and were incubated at 37°C for 90 minutes. The CEF peptide pool contains 23 HLA-I class restricted antigens from human cytomegalovirus (CMV), Epstein Barr virus (EBV), and Influenza virus (Flu). After the incubation, the $CD8^+$ T cell-depleted PBMC were washed with PBS and resuspended in complete RPMI media at a concentration of $1x10^6$ cells/ml.

2.5 Proliferation of memory $CD8^+$ T cells

To evaluate proliferation of memory $CD8^+$ T cells, carboxyfluorescein diacetate succinimidyl ester (CFSE; Invitrogen) dilution was analyzed by flow cytometry. A 5 mM CFSE stock solution in dimethylsulfoxide (DMSO; Sigma Aldrich) was used, and a 12 uM CFSE working solution was prepared containing 0.1% BSA in PBS. $CD8^+CD45RA^-CD127^+$ T cells were washed in PBS and resuspended in the CFSE working solution at 10^7cells/ml, and incubated at 37°C for 10 minutes. After the incubation, the staining was quenched by adding 5 times the labelling volume with cold RPMI, and incubated on ice for 5 minutes. Cells were then centrifuged for 5 minutes at 1600 rpm, and resuspended at $1x10^6$ cells/ml in complete RPMI media. The CFSE-labelled memory $CD8^+$ T cells were then co-cultured with peptide-pulsed or un-pulsed $CD8^+$ T cell-depleted PBMC at a 1:5 ratio of $CD8^+CD45RA^-$ $CD127^+$ T cells to $CD8^+$ T cell-depleted PBMC in 96-well plates at a concentration of $2x10^6$

resuspended in 100 ul of fixative solution (Caltag Laboratories, Carlsbad, CA, USA) and incubated in the dark for 15 minutes. After the incubation, cells were washed twice with PBS, and resuspended in 100 ul permeabilization solution (Caltag Laboratories). FITC-conjugated monoclonal antibodies to IFN-γ (Beckman Coulter, Mississauga, ON, Canada), phycoerythrin-Texas red (ECD)-conjugated monoclonal antibodies to CD45RA (Beckman Coulter), phycoerythrin-Cy5 (PC5)-conjugated monoclonal antibodies to CD8 (Beckman Coulter), and phycoerythrin-Cy7 (PC7)-conjugated monoclonal antibodies to CCR7 (BD Biosciences) were added to the cells and incubated in the dark for 20 minutes. After the incubation cells were washed twice with PBS for 5 minutes at 1600 rpm, and the samples were resuspended in 100 ul of PBS and analyzed by flow cytometry. As a positive control for both CD107a/b expression and IFN-γ production, cells were incubated with phorbol 12-myristate 13-acetate (PMA; 0.005 ug/ml) Sigma Aldrich) and ionomycin (0.5 ug/ml; Sigma Aldrich) and analyzed as above.

2.7 Flow cytometric analysis

To analyze proliferation of $CD8^+$ T cells, 3-colour flow cytometry was performed using CFSE, CD45RA-PC5, and CCR7-PC7. To analyze $CD8^+$ T cell function, 4-colour flow cytometry was performed using CD107a/b-FITC or IFN-γ-FITC, CD45RA-ECD, CD8-PC5, and CCR7-PC7. Flow cytometry was performed using an Epics ALTRA flow cytometer (Beckman Coulter).

2.8 Statistical Analysis

Data generated from flow cytometry were analyzed using FCS Express. Statistical analysis was performed using GraphPad Prism v.4.0 software (Graph Pad Software, San Diego, CA, USA), and significance was determined by performing one-tailed paired

Student's *t*-tests or a one-way ANOVA followed by Dunnett's post-test on data ($p \leq 0.05$) as appropriate. ANOVA was used to analyze the effect of increasing concentrations of IL-7 on each measure, while Student's *t*-test was used for all other comparisons. Error bars indicate the standard error of the mean (SEM), and the N value represents the number of individual donors.

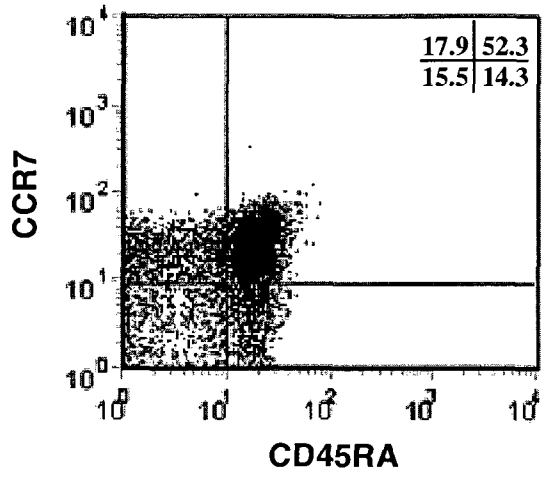
CHAPTER 3: RESULTS

3.1 $CD8^+$ T cell subset distribution in peripheral blood of HIV^- individuals

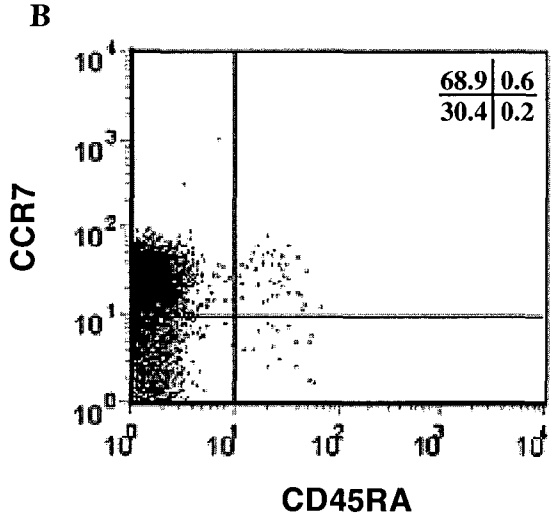
Memory $CD8^+CD45RA^-CD127^+$ T cells were isolated from PBMC as described above and the subset distribution was evaluated. For all experiments, the starting population of $CD8^+$ T cells was assumed to be exclusively memory cells selected on the basis of the lack of CD45RA expression. By isolating the $CD45RA^-$ population from the $CD8^+$ T cells, the direct responses of memory $CD8^+$ T cells to antigen and IL-7 could be evaluated. The bulk $CD8^+$ T cells were analyzed for CD45RA and CCR7 expression to determine the proportions of $CD8^+$ T cell subsets in peripheral blood (Figure 3-1A). In bulk $CD8^+$ T cells, the largest proportion of cells were T_N (45.35 $\pm$ 4.3%), followed by T_{CM} (21.45 $\pm$ 3.6%) and T_{EM} (19.00 $\pm$ 2.6%), and the smallest $CD8^+$ T cell subset was T_{EMRA} (14.2 $\pm$ 2.2%; Table 3-1). After sorting for memory $CD8^+CD45RA^-CD127^+$ T cells, it was found that approximately 70% of the population were T_{CM}, while the remainder were T_{EM} $CD8^+$ T cells based on CCR7 expression (Figure 3-1B). In all experiments, total memory $CD8^+$ T cells were gated on the basis of CD45RA and CCR7 expression to evaluate the effects of antigen and IL-7 within the individual memory cell subsets. The T_{EMRA} $CD8^+$ T cells were not present in the initial population, as they were sorted out on the basis of CD45RA expression. Over time in culture, the T_{EMRA} population emerged from the T_{CM} or T_{EM} subsets, and represent memory $CD8^+$ T cells that have differentiated to gain effector phenotype and function.

Figure 3-1. $CD8^+$ T cell subset distribution. (A) $CD8^+$ T cells from healthy individuals were isolated from PBMC, and CD45RA and CCR7 expression was analyzed to determine cell subsets. (B) $CD8^+CD45RA^-CD127^+$ T cells were isolated from bulk $CD8^+$ T cells and stained for CD45RA and CCR7 to determine initial memory $CD8^+$ T cell subsets.

A



B



$CD8^+$ T cell Subset	HIV^- Individuals Average ± SD	HIV^+ Individuals Average ± SD
T_N ($CD45RA^+CCR7^+$)	45.35 ± 4.3	19.83 ± 0.4
T_{CM} ($CD45RA^-CCR7^+$)	21.45 ± 3.6	10.57 ± 3.0
T_{EM} ($CD45RA^-CCR7^-$)	19 ± 2.6	23.47 ± 6.4
T_{EMRA} ($CD45RA^+CCR7^-$)	14.2 ± 2.2	46.18 ± 9.5

Table 3-1. $CD8^+$ T cell subsets from HIV^- donors and HIV^+ individuals. $CD8^+$ T cells were isolated from PBMC and analyzed for CD45RA and CCR7 expression by flow cytometry to determine $CD8^+$ T cell subsets. Percentage is expressed as mean values with the indicated range. N=10.

3.2 Antigen-specific proliferation of memory $CD8^+$ T cells is enhanced by IL-7

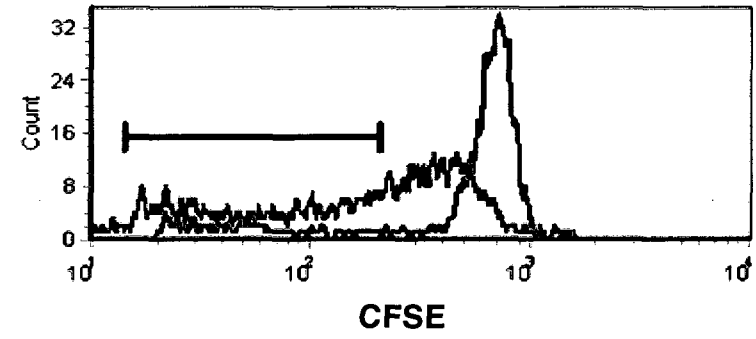
The effect of antigen and IL-7 on proliferation of memory $CD8^+$ T cells was evaluated by flow cytometry. $CD8^+CD45RA^-CD127^+$ T cells were labelled with CFSE and co-cultured with peptide-pulsed or un-pulsed PBMC and IL-7 at increasing concentrations (1, 10, or 50 ng/ml) for 6 days. The concentrations of IL-7 used were chosen based on in vitro proliferation assays in which concentrations less than 1 ng/ml did not elict any proliferation, and 5-10 ng/ml were able to induce proliferation of $CD8^+$ T cells (Liu et al., 2007; Gagnon et al., 2008; Rosenthal et al., 2009). Following culture, CFSE dilution, and expression of CD45RA and CCR7 on memory $CD8^+$ T cells were measured by flow cytometry (Figure 3-2A, 3-2B, and 3-2C). When the memory $CD8^+$ T cells were analyzed, it was found that antigen alone induced proliferation of $CD8^+CD45RA^-CD127^+$ T cells as expected ($p=0.012$; Figure 3-3A), specifically in the T_{CM} and T_{EM} cell subsets ($p=0.025$ and $p=0.033$, respectively; Figure 3-3B). The memory $CD8^+$ T cells in the T_{EMRA} subset had the largest percentage of $CFSE^{lo}$ cells in the absence of any stimulation.

When cultured with IL-7 alone (10 and 50 ng/ml), the proliferation of $CD8^+CD45RA^-CD127^+$ T cells increased (ANOVA $p=0.002$, Dunnett's $p<0.01$; Figure 3-4A), and this was observed in the T_{CM} and T_{EM} subsets (T_{CM} and T_{EM}: ANOVA $p<0.0001$, Dunnett's $p<0.01$; Figure 3-4B), while IL-7 had no effect on proliferation in the T_{EMRA} subset. When $CD8^+CD45RA^-CD127^+$ T cells were cultured with antigen plus IL-7, IL-7 enhanced antigen-mediated proliferation at 10 and 50 ng/ml (ANOVA $p=0.004$, Dunnett's $p<0.05$; Figure 3-5A), and this was also predominantly seen in the T_{CM} and T_{EM} subsets (ANOVA $p<0.0001$

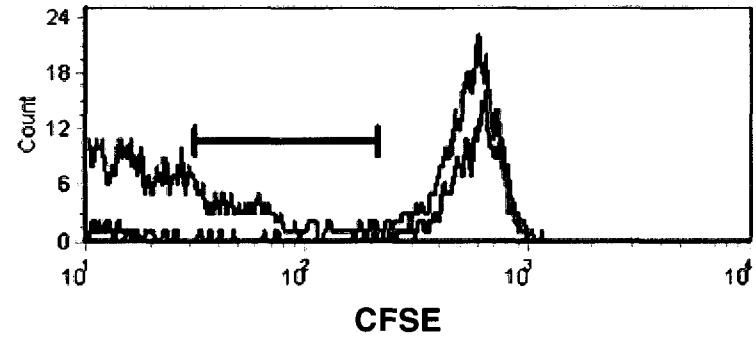
and p=0.0005, respectively, Dunnett's $p<0.01$; Figure 3-5B). IL-7 alone can enhance proliferation of memory $CD8^+$ T cells at concentrations higher than 1 ng/ml.

Figure 3-2. Representative flow cytometry histogram of $CD8^+CD45RA^-CD127^+$ T cell proliferation. (A) Proliferation of $CD8^+CD45RA^-CD127^+$ T cells was controlled for using colchicine (negative control; black line) and PHA (positive control; red line). (B) Proliferation of unstimulated (black line) and antigen-stimulated $CD8^+$ T cells (red line) after 6 days of PBMC co-culture. (C) Proliferation of antigen-stimulated (black line) and antigen plus IL-7 (10 ng/ml) stimulated $CD8^+$ T cells (red line). Cells were gated on $CFSE^+$ cells.

A


32
24
16
8
0
Count
10¹
10²
10³
10⁴
CFSE

B


24
18
12
6
0
Count
10¹
10²
10³
10⁴
CFSE

C

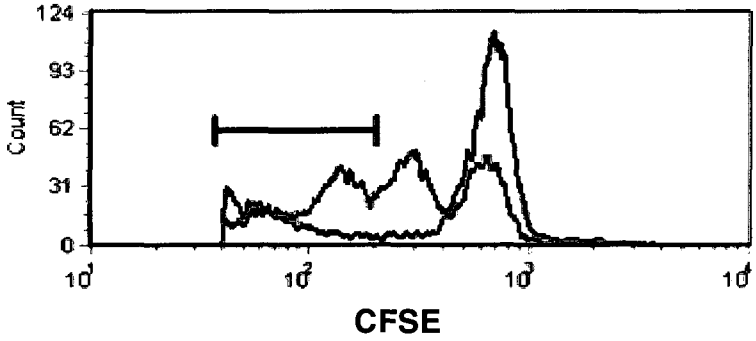
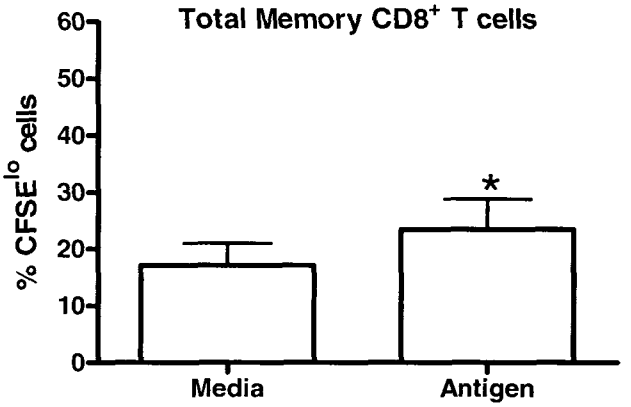

124
93
62
31
0
Count
10¹
10²
10³
10⁴
CFSE

Figure 3-3. Antigen enhances proliferation of $CD8^+CD45RA^-CD127^+$ T cells. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC for 6 days, and CFSE dilution, CD45RA expression, and CCR7 expression were analyzed by flow cytometry. Proliferation is indicated by % $CFSE^{lo}$ cells. Antigen enhanced proliferation of (A) total memory $CD8^+$ T cells (*p=0.012 by Student's *t* test) and (B) the $CD8^+$ T cell subsets, specifically in the T_{CM} and T_{EM} subsets (**p=0.022 and ***p=0.033, respectively, by Student's *t* test). Graph shows mean of samples $\pm$ SEM. N=6.

A


Total Memory CD8+ T cells
% CFSE lo cells
60
50
40
30
20
10
0
*
Media
Antigen

B

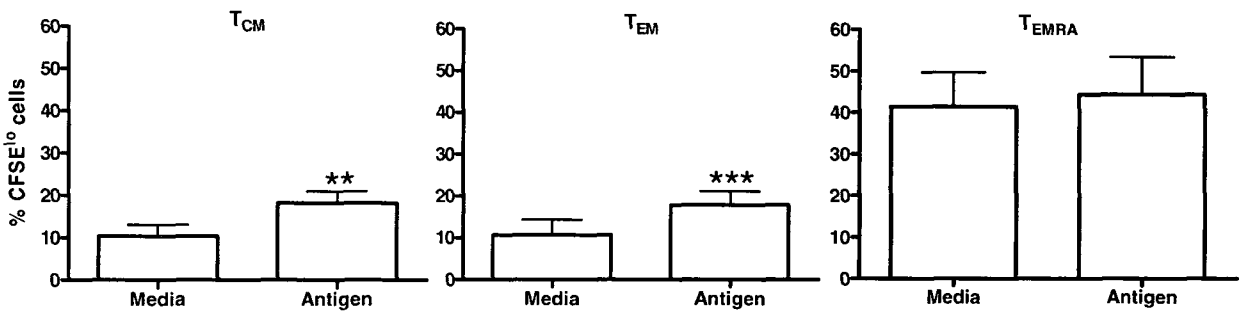

% CFSE lo cells
TCM
TEM
TEMRA
**
***
Media
Antigen

Figure 3-4. IL-7 enhances proliferation of $CD8^+CD45RA^-CD127^+$ T cells. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with $CD8^+$ T cell-depleted PBMC and IL-7 (1, 10, or 50 ng/ml) for 6 days, and CFSE dilution, CD45RA expression, and CCR7 expression were analyzed by flow cytometry. Proliferation is indicated by % $CFSE^{lo}$ cells. IL-7 enhanced proliferation of (A) total memory $CD8^+$ T cells (*p=0.002 by ANOVA and p<0.01 by Dunnett's post-test) and (B) the $CD8^+$ T cell subsets within the total memory $CD8^+$ T cell population, specifically in the T_{CM} and T_{EM} subsets (**p<0.0001 by ANOVA and p<0.01 by Dunnett's post-test; ***p<0.0001 by ANOVA and p<0.01 by Dunnett's post-test, respectively). Graph shows mean of samples $\pm$ SEM. N=6.

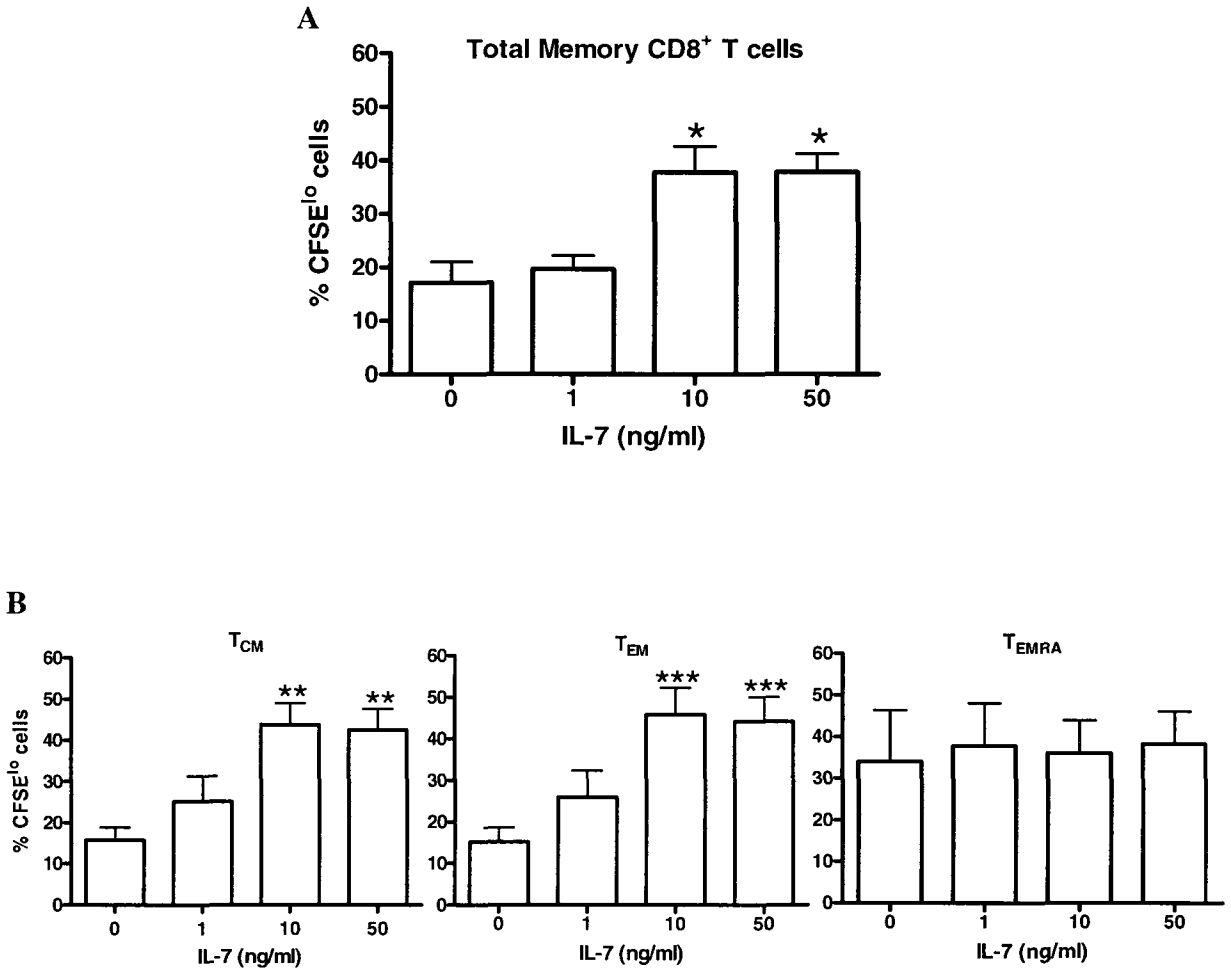
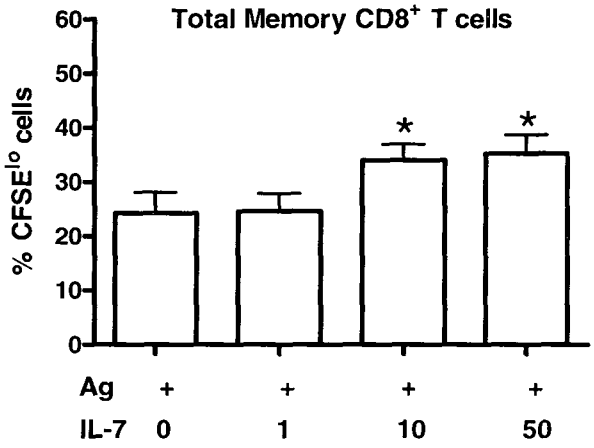
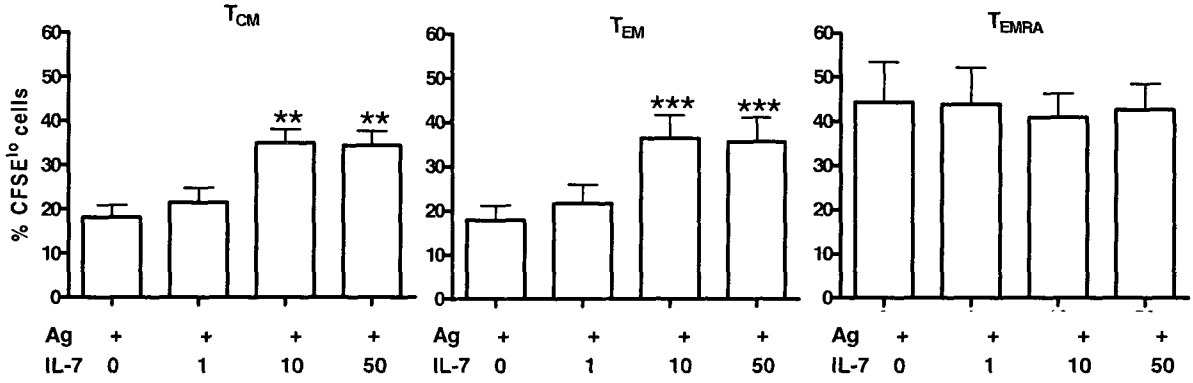

A
Total Memory CD8+ T cells
% CFSE^lo cells
60
50
40
30
20
10
0
*
*
0
1
10
50
IL-7 (ng/ml)
B
TCM
TEM
TEMRA
% CFSE^lo cells
**
**
***
***
0
1
10
50
IL-7 (ng/ml)

Figure 3-5. IL-7 enhances antigen-mediated proliferation of $CD8^+CD45RA^-CD127^+$ T cells. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC and IL-7 (1, 10, or 50 ng/ml) for 6 days, and CFSE dilution, CD45RA expression, and CCR7 expression were analyzed by flow cytometry. Proliferation is indicated by % $CFSE^{lo}$ cells. IL-7 enhanced antigen-mediated proliferation of (A) total memory $CD8^+$ T cells (*$p=0.004$ by ANOVA and $p<0.05$ by Dunnett's post-test) and (B) the $CD8^+$ T cell subsets specifically the T_{CM} and T_{EM} subsets (**$p<0.0001$ by ANOVA and $p<0.01$ by Dunnett's post-test; ***$p=0.0005$ by ANOVA and $p<0.01$ by Dunnett's post-test, respectively). Graph shows mean of samples $\pm$ SEM. N=6.

A


Total Memory CD8+ T cells
% CFSElo cells
60
50
40
30
20
10
0
*
*
Ag + + + +
IL-7 0 1 10 50

B


TCM
TEM
TEMRA
% CFSElo cells
**
**
***
***
Ag + + + +
IL-7 0 1 10 50

3.3 Memory $CD8^+$ T cell function in response to antigen and IL-7 in health

To evaluate functions of $CD8^+CD45RA^-CD127^+$ T cells after culture with antigen and IL-7, IFN-γ production and CD107a/b expression were analyzed. These functional readouts were chosen to determine whether the cells exhibited increased cytolytic activity, through IFN-γ production, and degranulation, through CD107a/b expression, upon stimulation with antigen and IL-7. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured for 6 hours, based on previous experiments in which IFN-γ production and CD107a/b expression was maximal at this time point (Betts et al., 2003), with pulsed or un-pulsed-PBMC, IL-7 at increasing concentrations (1, 10, or 50 ng/ml), and αCD28/αCD49d. After incubation, cells were fixed and permeabilized, and analyzed for IFN-γ production and expression of CD8, CD45RA, and CCR7 by flow cytometry (Figure 3-6A-D). In response to antigen alone IFN-γ production was increased ($p=0.029$; Figure 3-7A), specifically in the T_{EM} and T_{EMRA} cell subsets ($p=0.001$ and $p=0.031$, respectively; Figure 3-7B). The T_{CM} subset had the greatest proportion of IFN-γ^+ cells however antigen did not enhance IFN-γ production in this population.

When $CD8^+CD45RA^-CD127^+$ T cells were cultured with IL-7 alone, IFN-γ production was not increased at any concentration of the cytokine (Figure 3-8A and 3-8B). Within the memory $CD8^+$ T cell subsets, the T_{CM} subset had the highest proportion of IFN-γ^+ cells compared to the T_{EM} and T_{EMRA} subsets (Figure 3-8B). Similarly, when $CD8^+CD45RA^-CD127^+$ T cells were cultured with antigen plus IL-7, IL-7 did not lead to an increase in antigen-mediated IFN-γ production at any cytokine concentration (Figure 3-9A and 3-9B).

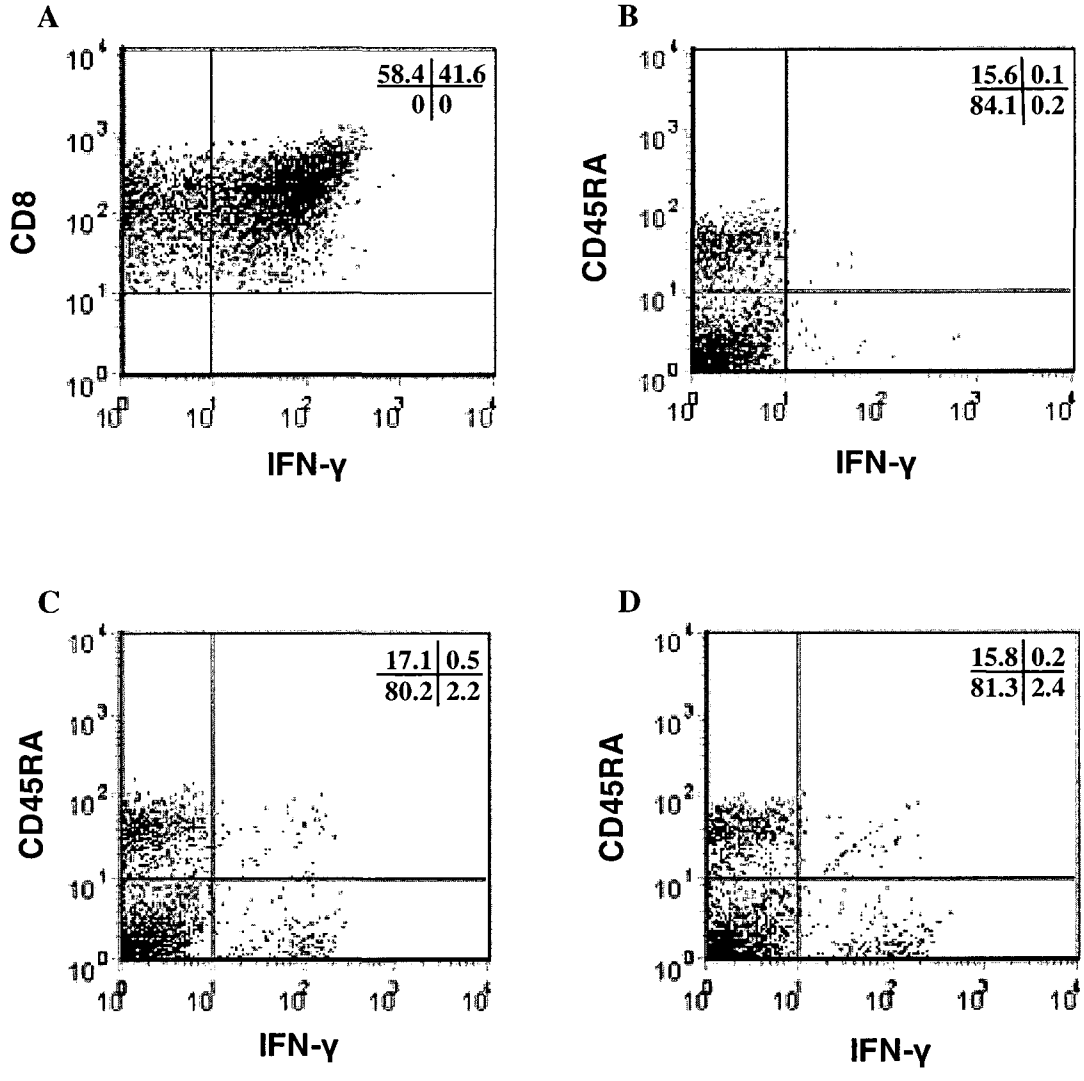

A
58.4 | 41.6
0 | 0
CD8
IFN-γ
B
15.6 | 0.1
84.1 | 0.2
CD45RA
IFN-γ
C
17.1 | 0.5
80.2 | 2.2
CD45RA
IFN-γ
D
15.8 | 0.2
81.3 | 2.4
CD45RA
IFN-γ

Figure 3-7. Antigen induces IFN-γ production by $CD8^+CD45RA^-CD127^+$ T cells. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC for 6 hours and analyzed by flow cytometry for IFN-γ production and CD8, CD45RA, and CCR7 expression. Antigen induced IFN-γ production in (A) total memory $CD8^+$ T cells (*p=0.023 by Student's *t* test) and (B) the memory $CD8^+$ T cell subsets, specifically the T_{EM} and T_{EMRA} subsets (**p=0.0008 and ***p=0.031 by Student's *t* test). IFN-γ production is expressed as a percentage of IFN-γ^+ cells. Graph shows mean of samples ± SEM. N=6.

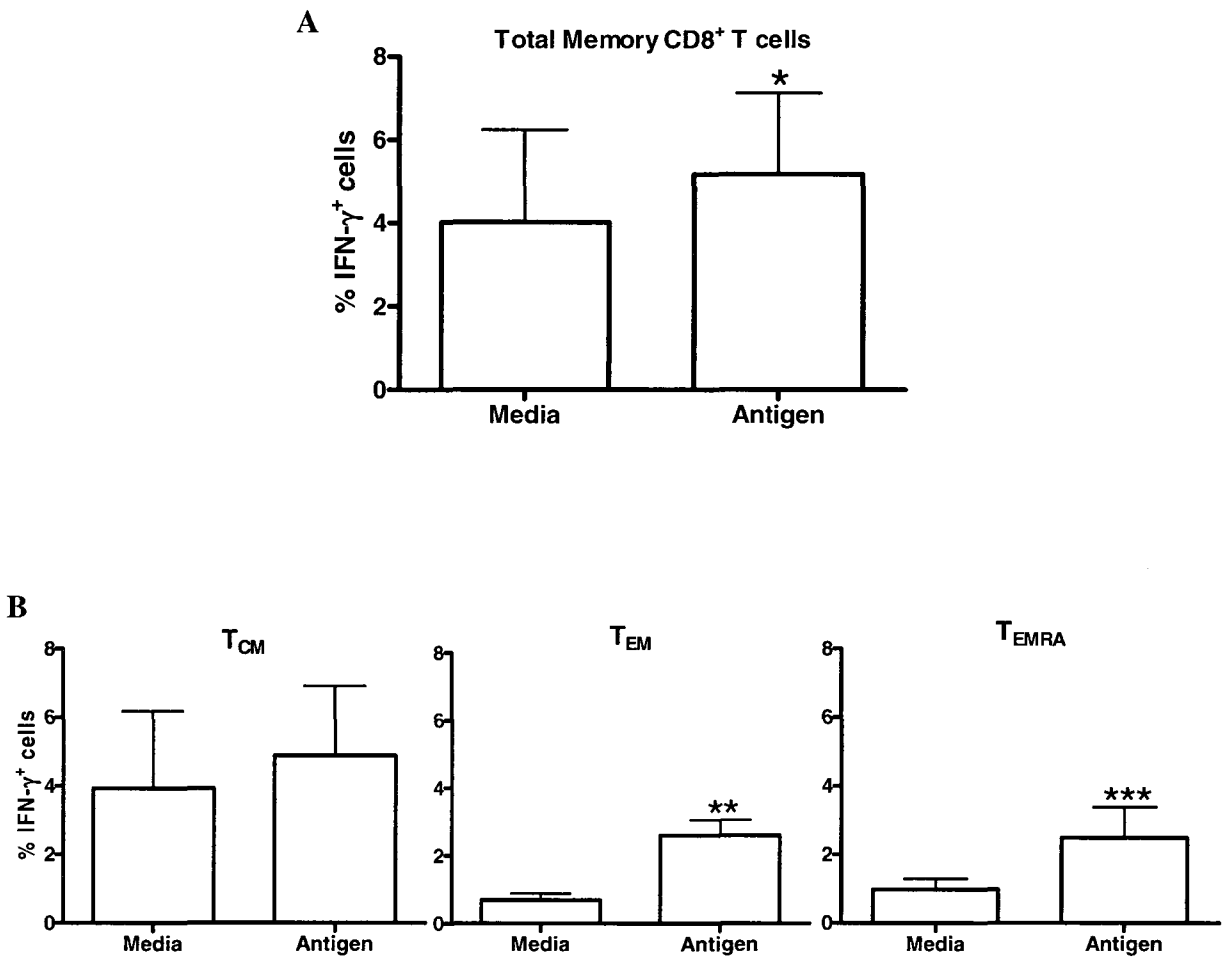

A
Total Memory CD8+ T cells
% IFN-γ+ cells
0
2
4
6
8
*
Media
Antigen
B
TCM
TEM
TEMRA
% IFN-γ+ cells
**
***
Media
Antigen

Figure 3-8. IL-7 does not enhance IFN-γ production by $CD8^+CD45RA^-CD127^+$ T cells. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with $CD8^+$ T cell-depleted PBMC and IL-7 (1, 10, or 50 ng/ml) for 6 hours and analyzed for IFN-γ production and CD8, CD45RA, and CCR7 expression. IL-7 did not enhance IFN-γ production in (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. IFN-γ production is expressed as a percentage of IFN-γ^+ cells. Graph shows mean of samples ± SEM. N=6.

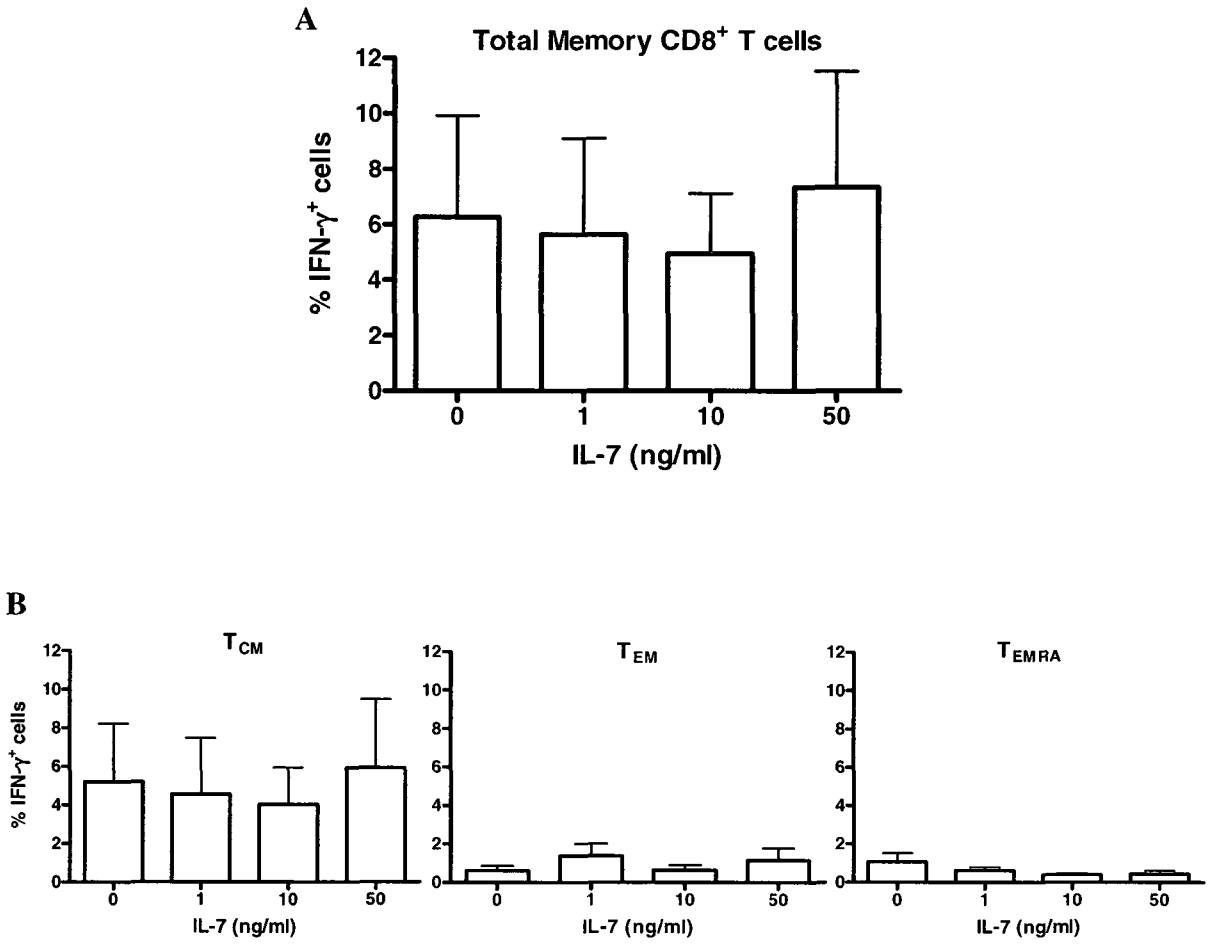

A
Total Memory CD8+ T cells
% IFN-γ+ cells
12
10
8
6
4
2
0
0
1
10
50
IL-7 (ng/ml)
B
TCM
TEM
TEMRA
% IFN-γ+ cells
IL-7 (ng/ml)

Figure 3-9. IL-7 does not enhance antigen-mediated IFN-γ production by $CD8^+CD45RA^-CD127^+$ T cells. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC and IL-7 (1, 10, or 50 ng/ml) for 6 hours and analyzed for IFN-γ production and CD8, CD45RA, and CCR7 expression by flow cytometry. IL-7 did not enhance antigen-mediated IFN-γ production in (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. IFN-γ production is expressed as a percentage of IFN-γ^+ memory cells. Graph shows mean of samples ± SEM. N=6.

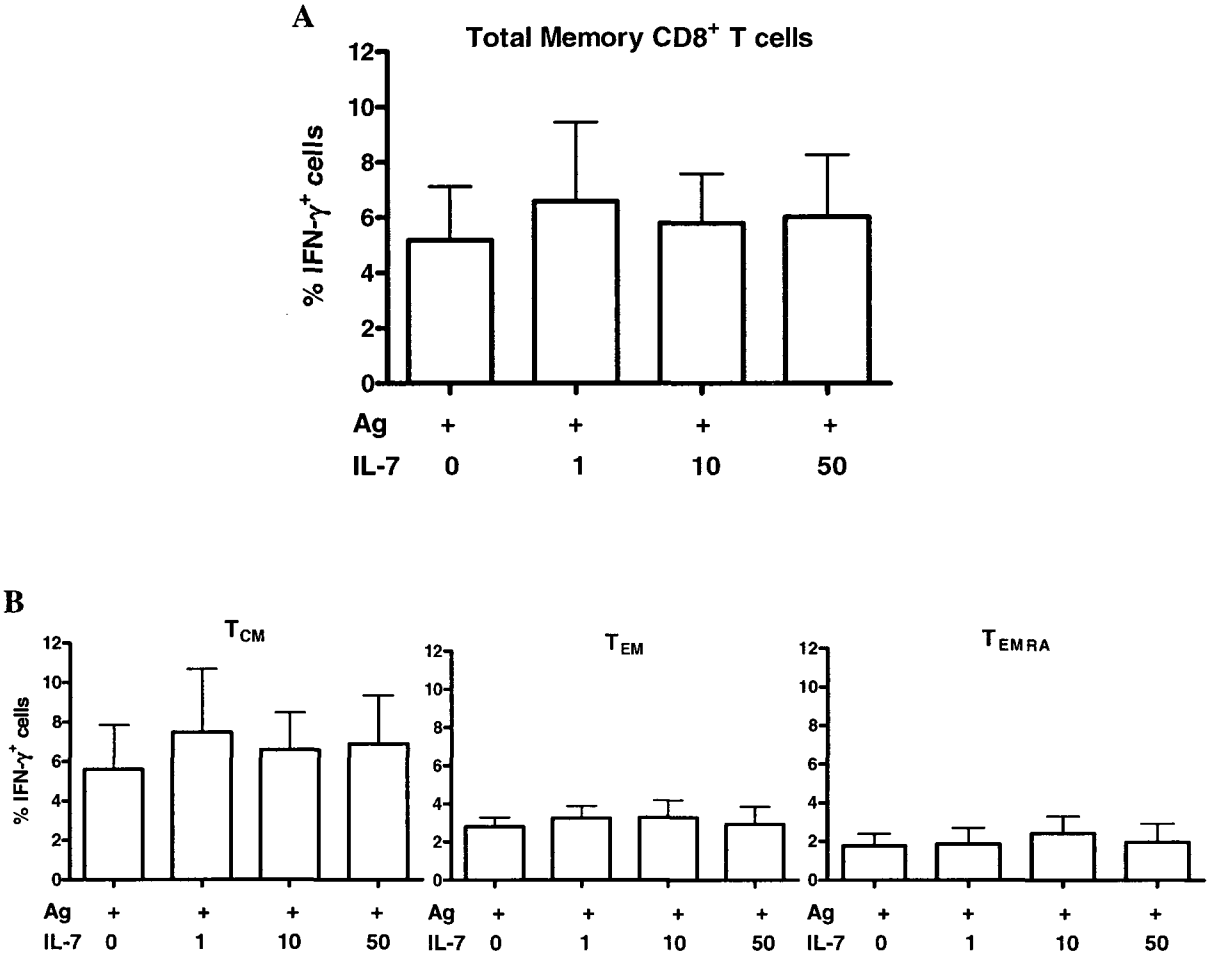

A
Total Memory CD8+ T cells
% IFN-γ+ cells
12
10
8
6
4
2
0
Ag + + + +
IL-7 0 1 10 50
B
TCM
TEM
TEMRA
% IFN-γ+ cells
Ag + + + +
IL-7 0 1 10 50

Figure 3-10. Representative flow cytometry dot plot of CD107a/b expression on $CD8^+CD45RA^-CD127^+$ T cells. (A) $CD8^+CD45RA^-CD127^+$ T cells were stimulated with PMA and ionomycin as a positive control for CD107a/b expression. CD107a/b expression by $CD8^+CD45RA^-CD127^+$ T cells (B) in unstimulated cells, (C) in response to antigen, and (D) in response to antigen plus IL-7 (10 ng/ml).

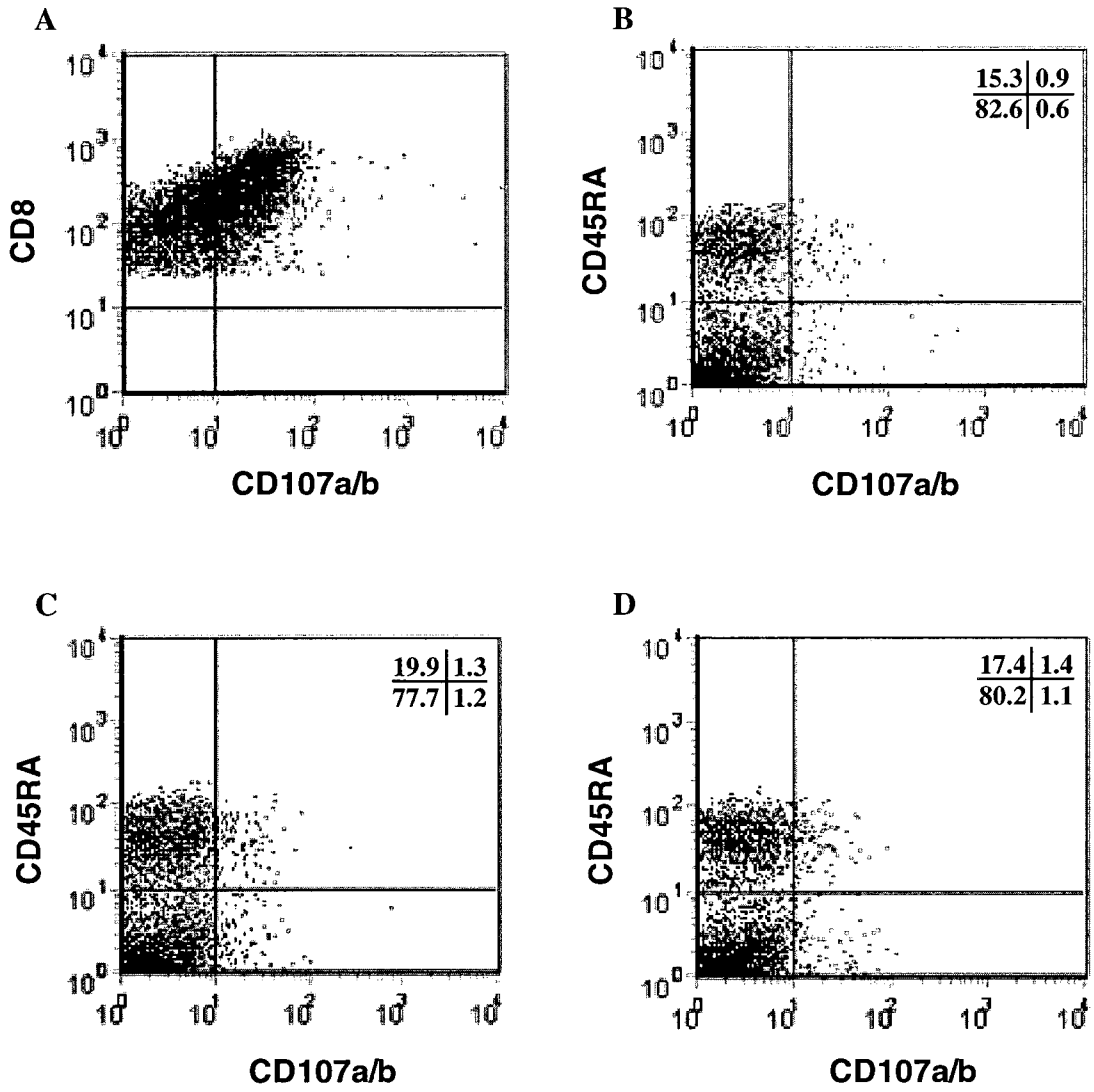
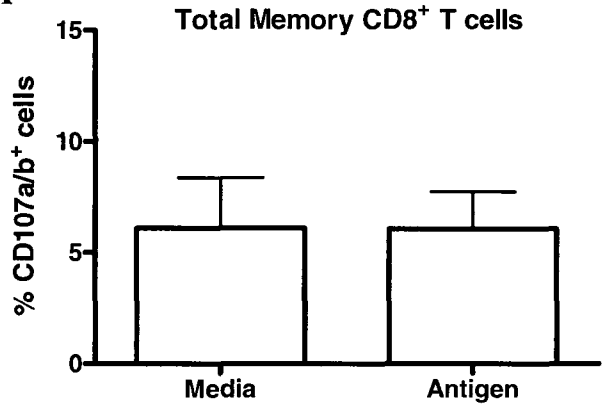

A
CD8
CD107a/b
B
CD45RA
CD107a/b
15.3 0.9
82.6 0.6
C
CD45RA
CD107a/b
19.9 1.3
77.7 1.2
D
CD45RA
CD107a/b
17.4 1.4
80.2 1.1

Figure 3-11. The effect of antigen on CD107a/b expression by $CD8^+CD45RA^-CD127^+$ T cells. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC for 6 hours and analyzed for CD107a/b expression and CD8, CD45RA, and CCR7 expression by flow cytometry. Antigen did not enhance CD107a/b expression in (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. CD107a/b expression is expressed as a percentage of $CD107a/b^+$ cells. Graph shows mean of samples $\pm$ SEM. N=6.

A


Total Memory CD8+ T cells
% CD107a/b+ cells
15
10
5
0
Media
Antigen

B

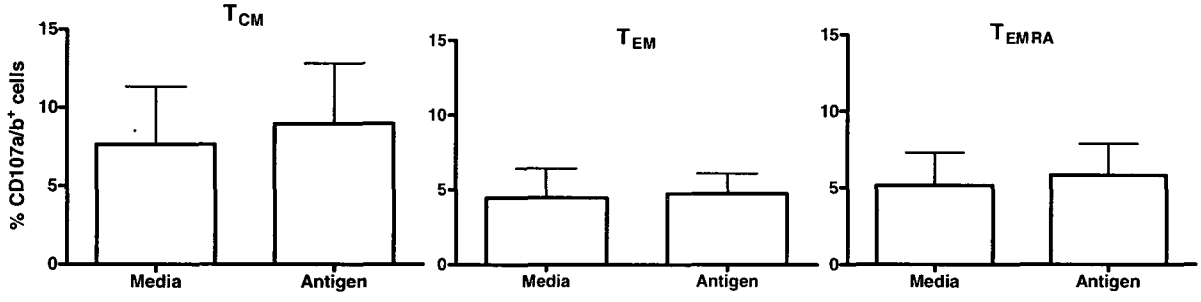
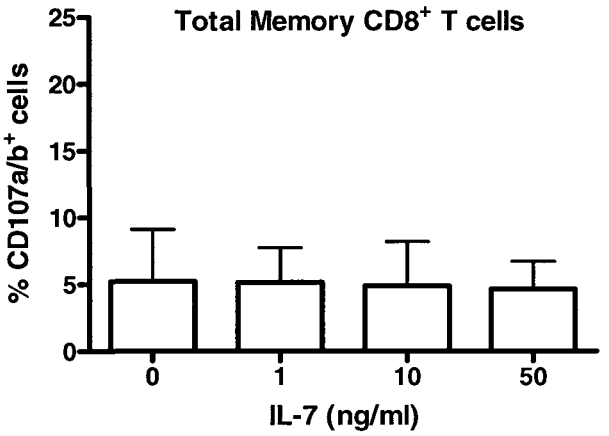

TCM
TEM
TEMRA
% CD107a/b+ cells
15
10
5
0
Media
Antigen

Figure 3-12. IL-7 does not enhance CD107a/b expression by $CD8^+CD45RA^-CD127^+$ T cells. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with $CD8^+$ T cell-depleted PBMC and IL-7 (1, 10, or 50 ng/ml) for 6 hours and analyzed for CD107a/b expression and CD8, CD45RA, and CCR7 expression, by flow cytometry. IL-7 did not enhance CD107a/b expression in (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. CD107a/b expression is expressed as a percentage of $CD107a/b^+$ cells. Graph shows mean of samples $\pm$ SEM. N=6.

A


Total Memory CD8+ T cells
% CD107a/b+ cells
25
20
15
10
5
0
0
1
10
50
IL-7 (ng/ml)

B

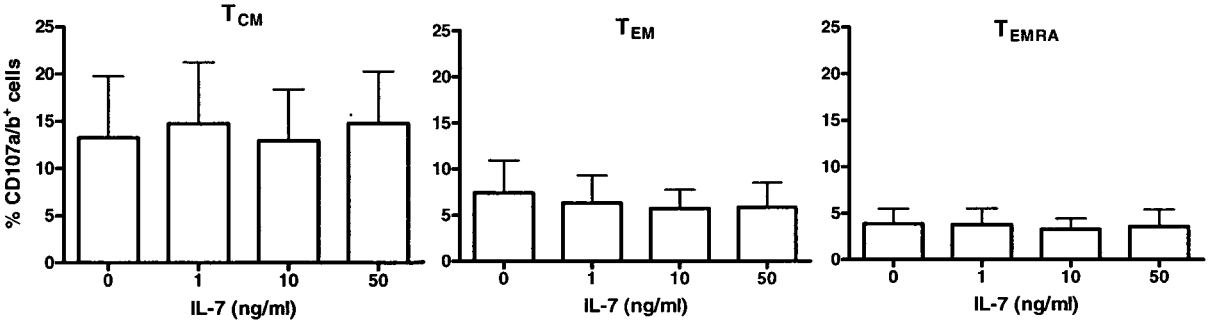
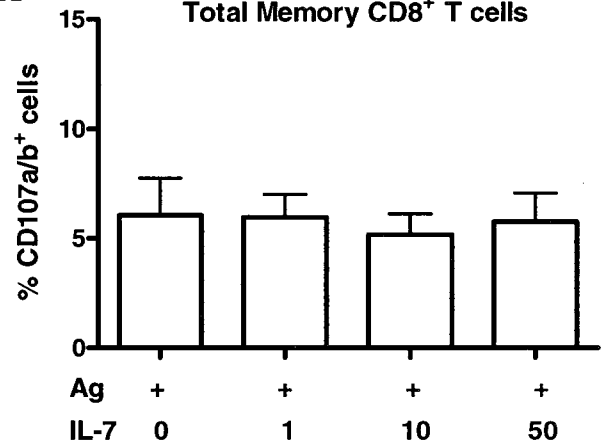
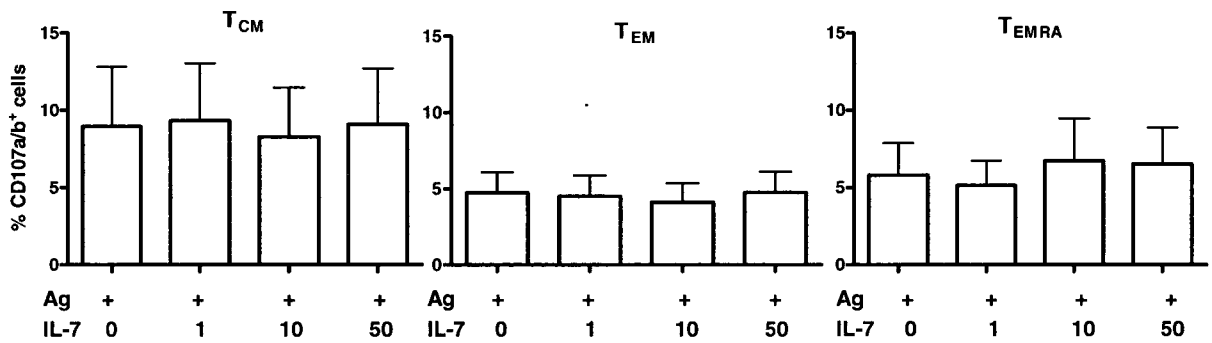

TCM
TEM
TEMRA
% CD107a/b+ cells
25
20
15
10
5
0
0
1
10
50
IL-7 (ng/ml)

Figure 3-13. IL-7 does not enhance antigen-mediated CD107a/b expression by $CD8^+CD45RA^-CD127^+$ T cells. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted and IL-7 (1, 10, or 50 ng/ml) for 6 hours and analyzed for CD107a/b expression and CD8, CD45RA, and CCR7 expression by flow cytometry. IL-7 did not enhance antigen-mediated CD107a/b expression in (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. CD107a/b expression is expressed as a percentage of $CD107a/b^+$ cells. Graph shows mean of samples $\pm$ SEM. N=6.

A


Total Memory CD8+ T cells
% CD107a/b+ cells
15
10
5
0
Ag + + + +
IL-7 0 1 10 50

B


TCM
TEM
TEMRA
% CD107a/b+ cells
15
10
5
0
Ag + + + +
IL-7 0 1 10 50

3.4 $CD8^+$ T cell subset distribution during HIV infection

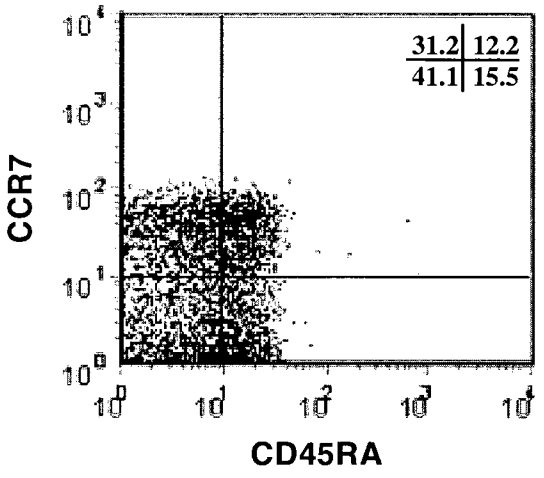
To evaluate the effects of HIV infection on memory $CD8^+$ T cell responses to antigen and IL-7, HIV^+ individuals recruited were either naïve to therapy or off ART for more than one year and were asymptomatic. The HIV^+ individuals had mean $CD4^+$ T cell counts of 259.2 cells/ul and mean viral loads of 4.53 log copies/ml (Table 3-2). By isolating $CD127^+$ T cells, responses from HIV^+ individuals could be compared to HIV^- individuals, and the difference in the level of CD127 expression between these groups would not be a confounding factor in the analysis of the data. To determine the effect of antigen and IL-7 on the proliferation and function of memory $CD8^+$ T cells during HIV infection, $CD8^+$ T cells were isolated from PBMC from HIV^+ individuals. Bulk $CD8^+$ T cells were analyzed by flow cytometry for CD45RA and CCR7 expression to determine the proportion of $CD8^+$ T cell subsets in peripheral blood in HIV^+ individuals (Figure 3-14A). In bulk $CD8^+$ T cells, the subset distribution was as follows: T_{EMRA} (46.18%), T_{EM} (23.47%), T_N (19.83%), and T_{CM} (10.57%) (Table 3-2). The $CD8^+$ T cell subset distribution is skewed when compared to that from HIV^- individuals, where T_N is the largest population, and T_{EMRA} is the smallest (Table 3-2). After sorting for $CD8^+CD45RA^-CD127^+$ T cells, the subset distribution was approximately 60% T_{CM} and 40% T_{EM} (Figure 3-14B), which is similar to that seen in HIV^- individuals (Figure 1B).

Age	CD4 number (cells/ul)	CD4 number (%)	Viral load (log copies/ml)	Treatment History
42.5 (33-60)	259.2 (65-499)	15.6% (8.5-28.5%)	4.53 (3.91-5.28)	8 Naïve; 2 off ART>1 year

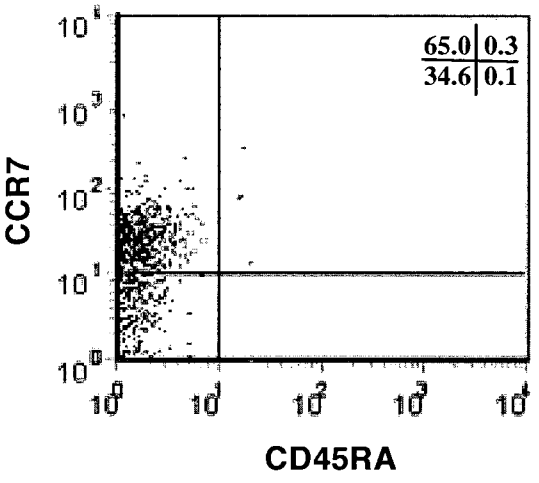
Table 3-2. HIV^+ patient characteristics. Age, $CD4^+$ T cell count and percentage, and viral load are expressed as mean values with the indicated range. N=10.

Figure 3-14. $CD8^+$ T cell subsets during HIV infection. (A) $CD8^+$ T cells from HIV^+ individuals were isolated from PBMC, and CD45RA and CCR7 expression was analyzed to determine cell subsets. (B) $CD8^+CD45RA^-CD127^+$ T cells were isolated from bulk $CD8^+$ T cells and stained for CD45RA and CCR7 to determine initial memory $CD8^+$ T cell subset distribution.

A



B

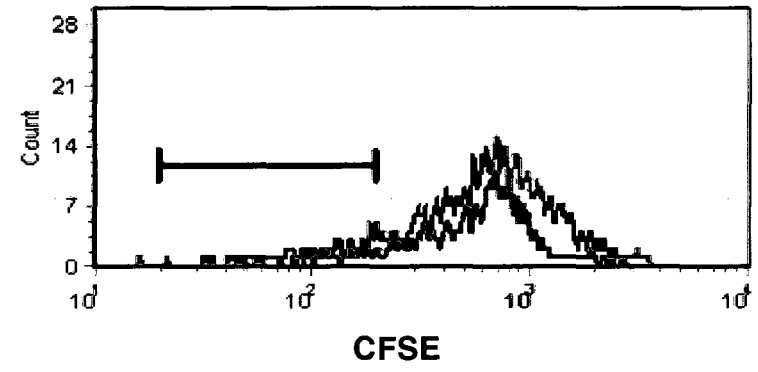


3.5 Antigen and IL-7 do not enhance proliferation of memory $CD8^+$ T cells during HIV infection

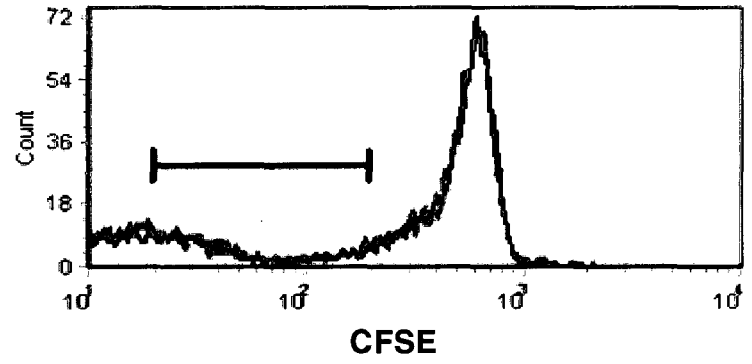
During HIV infection, $CD8^+$ T cells progressively lose cytolytic functions. To determine if memory $CD8^+$ T cell responses, and in particular whether $CD8^+CD45RA^-CD127^+$ T cells are impaired, proliferation, IFN-γ production, and CD107a/b expression were evaluated in memory $CD8^+$ T cells from HIV^+ individuals. Proliferation of $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals in response to antigen and IL-7 was evaluated by flow cytometry (Figure 3-15A and 3-15B) as in HIV^- donors, however only one concentration of IL-7 (10ng/ml) was used, as a limited number of cells were available from each donor, and this concentration consistently elicited responses in HIV^- individuals. It was found that the $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals did not proliferate in response to antigen in any subset (Figure 3-16A and 3-16B). Also, IL-7 (10 ng/ml) did not induce the proliferation of $CD8^+CD45RA^-CD127^+$ T cells in any subset (Figure 3-17A and 3-17B) in contrast to that observed in HIV^- individuals (Figure 3-4A and 3-4B). It was also found that IL-7 (10 ng/ml) did not enhance antigen-mediated proliferation of $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals in any subset (Figure 3-18A and 3-18B).

Figure 3-15. Representative flow cytometry histogram of $CD8^+CD45RA^-CD127^+$ T cell proliferation from HIV^+ individuals. (A) Proliferation of $CD8^+CD45RA^-CD127^+$ T cells was controlled for using colchicine (black line) and PHA (red line). (B) Proliferation of unstimulated (black line) and antigen-stimulated $CD8^+$ T cells (red line) after 6 days of PBMC co-culture. (C) Proliferation of antigen-stimulated (black line) and antigen plus IL-7 (10 ng/ml) stimulated $CD8^+$ T cells (red line). Cells were gated on $CFSE^+$ cells.

A


28
21
14
7
0
Count
CFSE

B


72
54
36
18
0
Count
CFSE

C

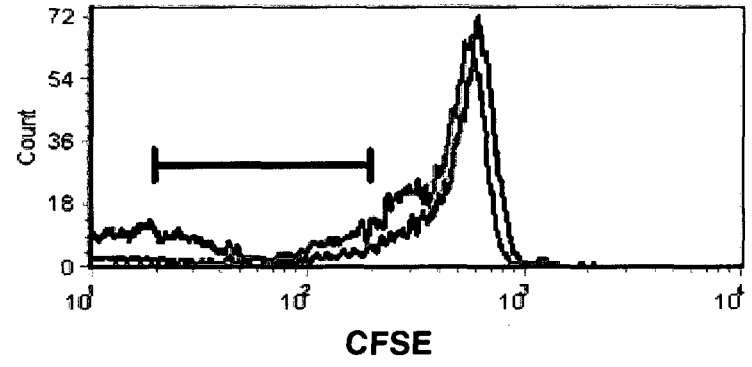
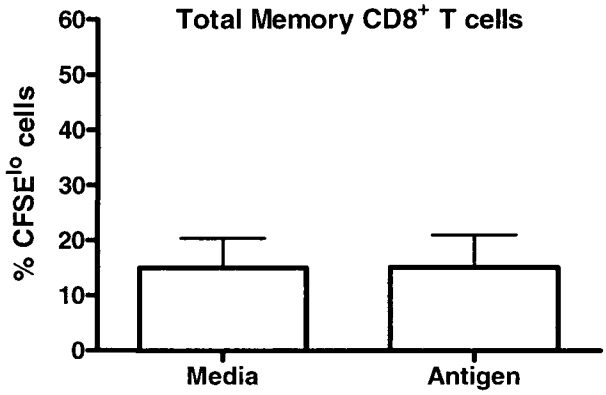

72
54
36
18
0
Count
CFSE

Figure 3-16. Antigen does not enhance proliferation of $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC for 6 days and CFSE dilution, CD45RA expression, and CCR7 expression were analyzed by flow cytometry. Proliferation is indicated by % $CFSE^{lo}$ cells. Antigen did not enhance proliferation in (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. Graph shows mean of samples $\pm$ SEM. N=5.

A


Total Memory CD8+ T cells
% CFSElo cells
60
50
40
30
20
10
0
Media
Antigen

B

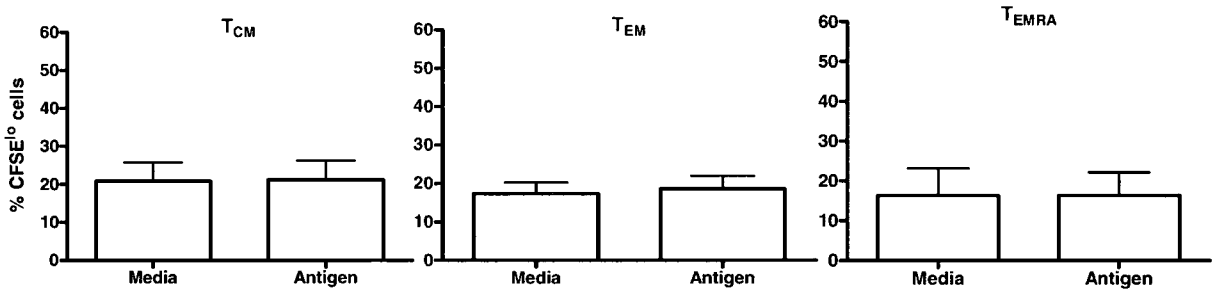
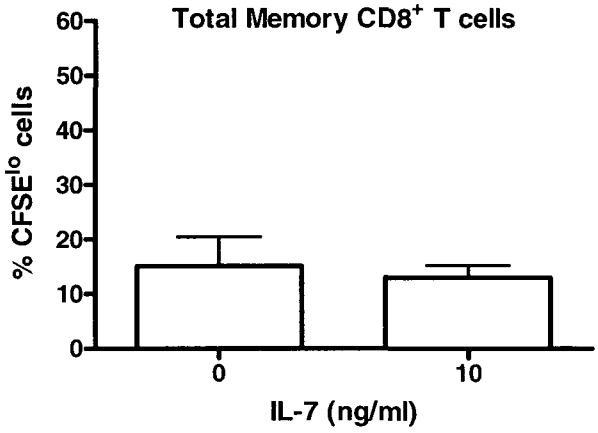

TCM
TEM
TEMRA
% CFSElo cells
60
50
40
30
20
10
0
Media
Antigen

Figure 3-17. IL-7 does not enhance proliferation of $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with $CD8^+$ T cell-depleted PBMC and IL-7 (10 ng/ml) for 6 days and CFSE dilution, CD45RA expression, and CCR7 expression was analyzed by flow cytometry. Proliferation is indicated by % $CFSE^{lo}$ cells. IL-7 did not enhance proliferation in (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. Graph shows mean of samples $\pm$ SEM. N=5.

A


Total Memory CD8+ T cells
% CFSElo cells
60
50
40
30
20
10
0
0
10
IL-7 (ng/ml)

B

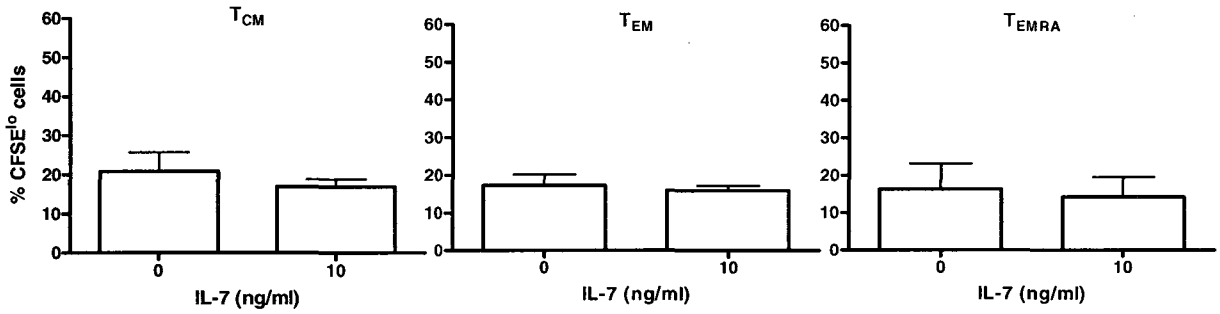
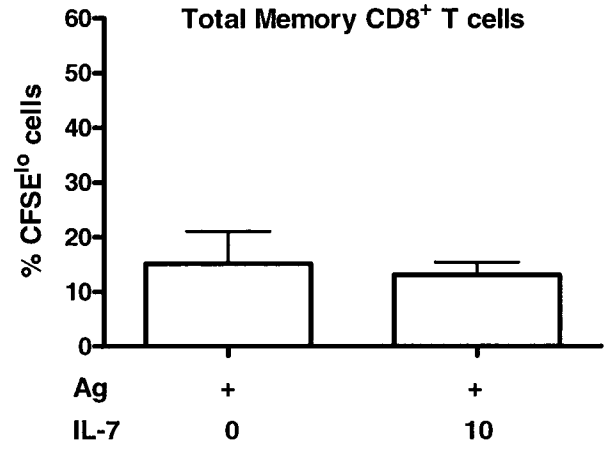
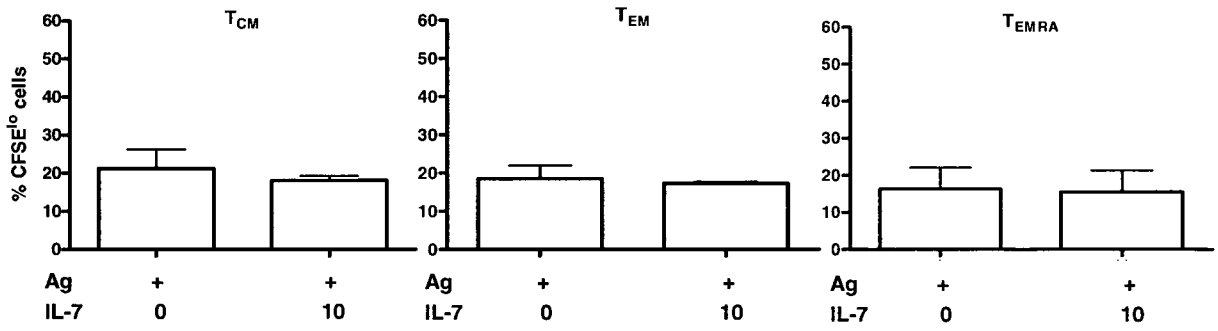

TCM
TEM
TEMRA
% CFSElo cells
60
50
40
30
20
10
0
0
10
IL-7 (ng/ml)

Figure 3-18. IL-7 does not enhance antigen-mediated proliferation of $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC and IL-7 (10 ng/ml) for 6 days and CFSE dilution, CD45RA expression, and CCR7 expression were analyzed by flow cytometry. Proliferation is indicated by % $CFSE^{lo}$ cells. IL-7 did not enhance antigen-mediated proliferation of (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. Graph shows mean of samples $\pm$ SEM. N=5.

A


Total Memory CD8+ T cells
% CFSElo cells
60
50
40
30
20
10
0
Ag + +
IL-7 0 10

B


% CFSElo cells
TCM
TEM
TEMRA
60
50
40
30
20
10
0
Ag + +
IL-7 0 10

3.6 Memory $CD8^+$ T cell function in response to antigen and IL-7 during HIV infection

IFN-γ production by memory $CD8^+$ T cells from HIV^+ individuals was analyzed after a 6 hour culture with IL-7 and αCD28/αCD49d by flow cytometry (Figure 3-19A-D) as in HIV^- individuals. Antigen enhanced IFN-γ production by $CD8^+CD45RA^-CD127^+$ T cells ($p=0.030$; Figure 3-20A), specifically in the T_{EM} and T_{EMRA} subsets ($p=0.050$, $p=0.024$, respectively; Figure 3-20B), however the proportion of IFN-γ^+ cells was extremely low compared to that seen in HIV^- individuals (Figure 3-7A and 3-7B). The T_{EMRA} subset had the highest percentage of IFN-γ^+ cells compared to the other memory $CD8^+$ T cell subsets. IL-7 did not enhance IFN-γ production on its own (Figure 3-21A and 3-21B), nor did it enhance antigen-mediated IFN-γ production (Figure 3-22A and 3-22B).

Figure 3-19. Representative flow cytometry dot plot of IFN-γ production by $CD8^+CD45RA^-CD127^+$ T cells. (A) $CD8^+CD45RA^-CD127^+$ T cells were stimulated with PMA and ionomycin as a positive control for IFN-γ production. IFN-γ production by $CD8^+CD45RA^-CD127^+$ T cells (B) in unstimulated cells, (C) in response to antigen, and (D) in response to antigen plus IL-7 (10 ng/ml).

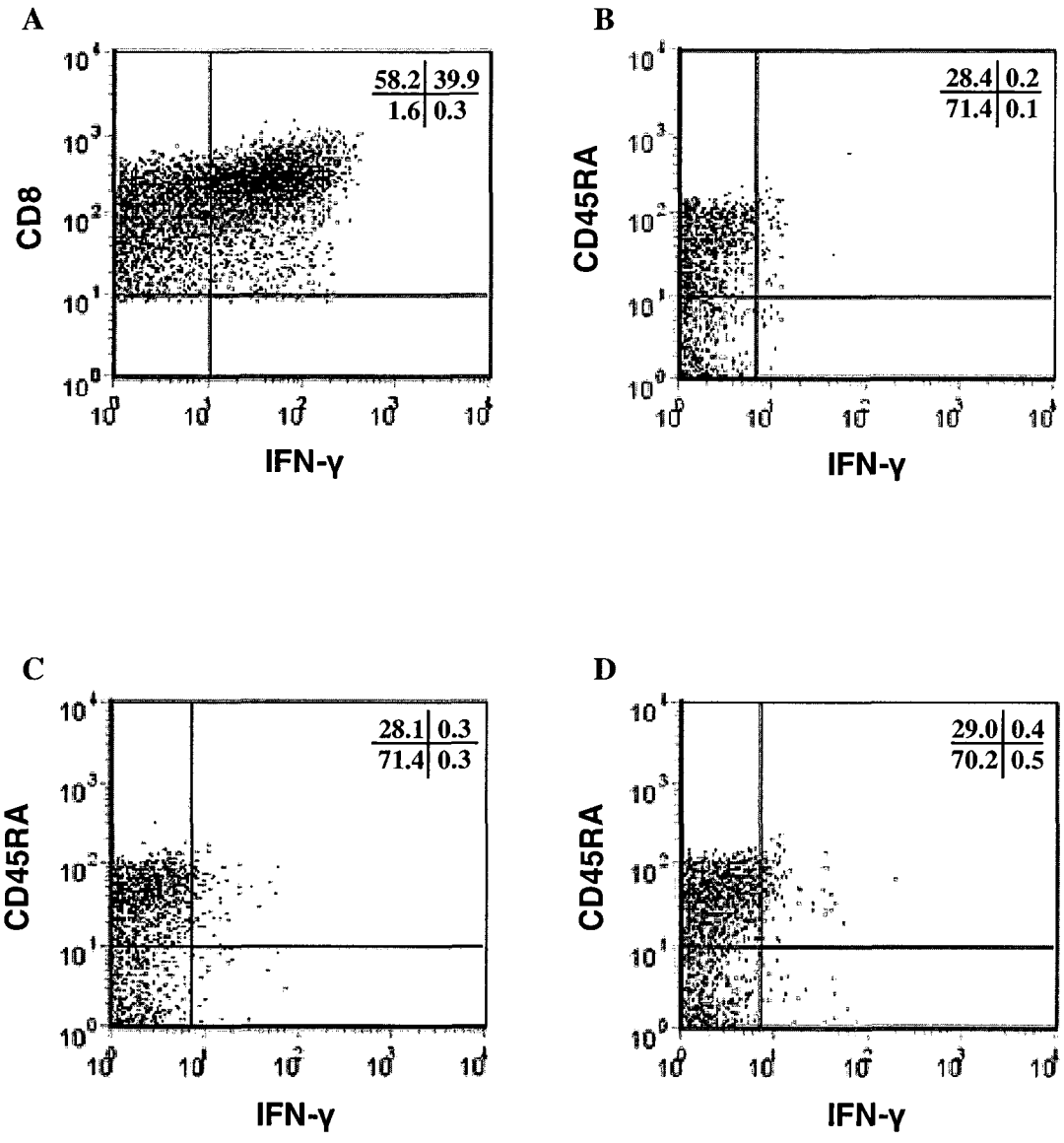

A
58.2 39.9
1.6 0.3
CD8
IFN-γ
B
28.4 0.2
71.4 0.1
CD45RA
IFN-γ
C
28.1 0.3
71.4 0.3
CD45RA
IFN-γ
D
29.0 0.4
70.2 0.5
CD45RA
IFN-γ

Figure 3-20. Antigen causes a slight increase in IFN-γ production by $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC for 6 hours and analyzed for IFN-γ production and CD8, CD45RA, and CCR7 expression by flow cytometry. Antigen enhanced IFN-γ production in (A) total memory $CD8^+$ T cells (*p=0.030 by Student's *t* test), and (B) the memory $CD8^+$ T cell subsets specifically the T_{EM} and T_{EMRA} subsets (**p=0.050 and ***p=0.024, respectively, by Student's *t* test). IFN-γ production is expressed as a percentage of IFN-γ^+ cells. Graph shows mean of samples ± SEM. N=5.

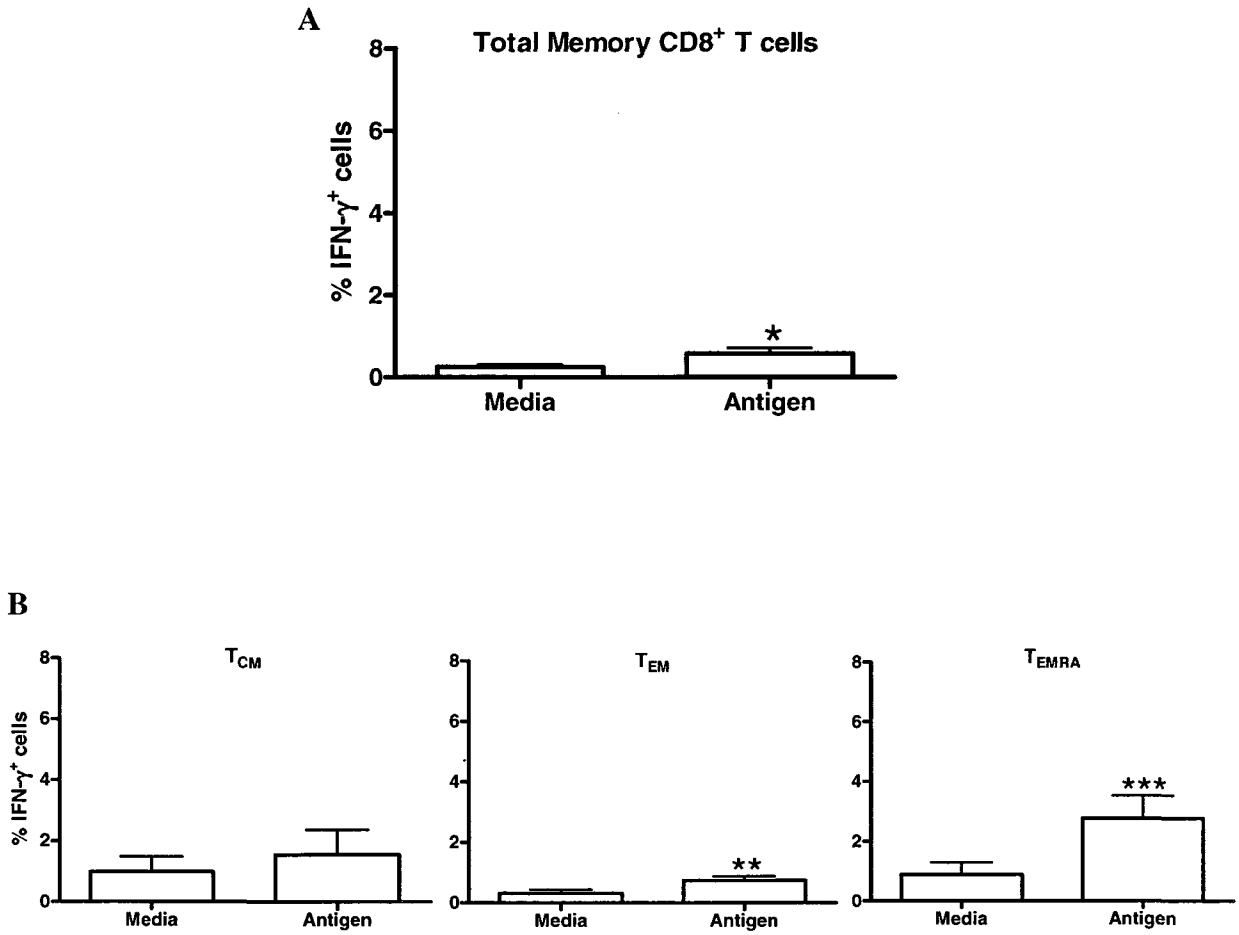
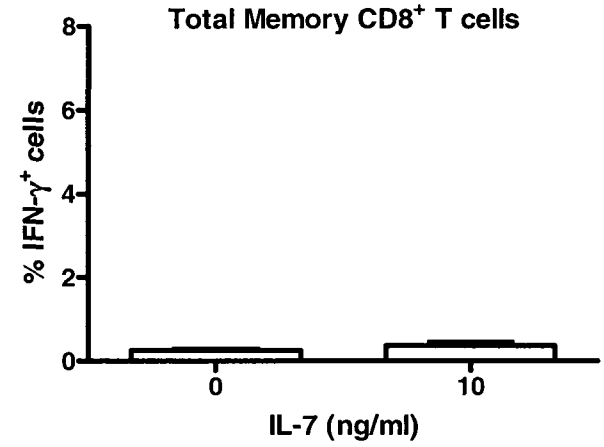

A
Total Memory CD8+ T cells
% IFN-γ+ cells
8
6
4
2
0
*
Media
Antigen
B
TCM
TEM
TEMRA
% IFN-γ+ cells
**
***
Media
Antigen

Figure 3-21. IL-7 does not enhance IFN-γ production by $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with $CD8^+$ T cell-depleted PBMC and IL-7 (10 ng/ml) for 6 hours and analyzed for IFN-γ production and CD8, CD45RA, and CCR7 expression by flow cytometry. IL-7 did not enhance IFN-γ production in (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. IFN-γ production is expressed as a percentage of IFN-γ^+ cells. Graph shows mean of samples ± SEM. N=5.

A


Total Memory CD8+ T cells
% IFN-γ+ cells
8
6
4
2
0
0
10
IL-7 (ng/ml)

B

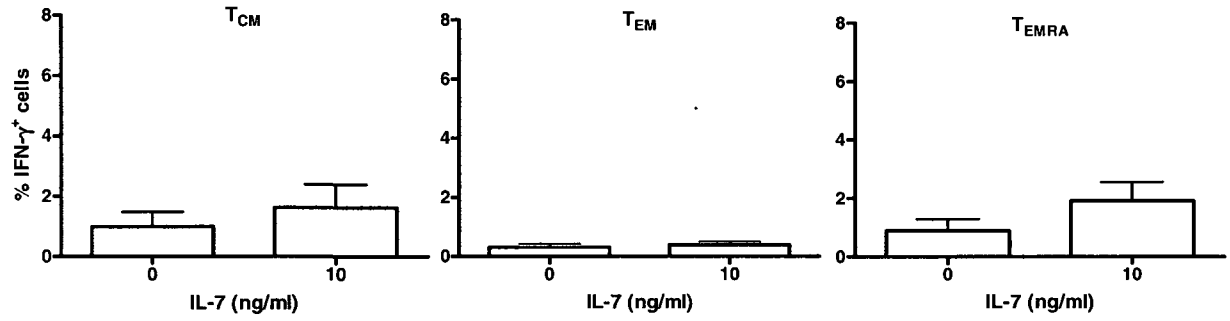
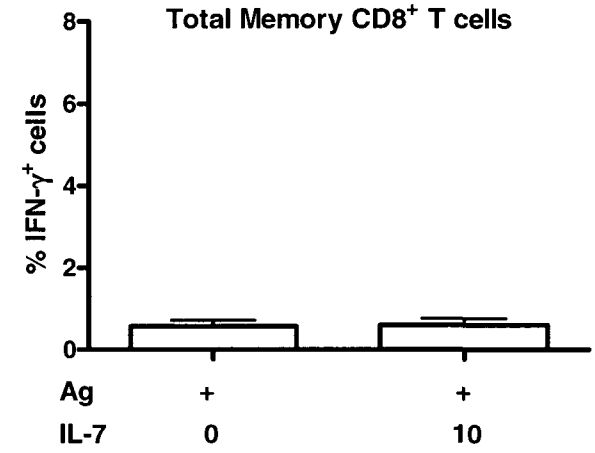
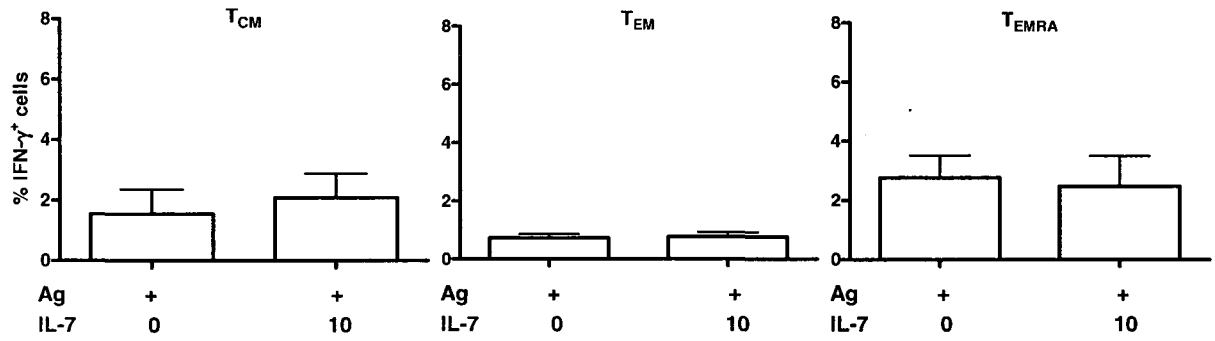

TCM
TEM
TEMRA
% IFN-γ+ cells
8
6
4
2
0
0
10
IL-7 (ng/ml)

Figure 3-22. IL-7 does not enhance antigen-mediated IFN-γ production by $CD8^+CD45RA^-CD127^+$ T cells. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC and IL-7 (10 ng/ml) for 6 hours and analyzed for IFN-γ production and CD8, CD45RA, and CCR7 expression by flow cytometry. IL-7 did not enhance antigen-mediated IFN-γ production in (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. IFN-γ production is expressed as a percentage of IFN-γ^+ cells. Graph shows mean of samples $\pm$ SEM. N=5.

A


Total Memory CD8+ T cells
% IFN-γ+ cells
8
6
4
2
0
Ag + +
IL-7 0 10

B


TCM
TEM
TEMRA
% IFN-γ+ cells
8
6
4
2
0
Ag + +
IL-7 0 10

CD107a/b expression on $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals was also analyzed after a 6 hour culture with IL-7 and αCD28/αCD49d by flow cytometry (Figure 3-23A-D) as in HIV^- individuals. It was observed that CD107a/b expression on memory $CD8^+CD45RA^-CD127^+$ T cells was not induced by antigen (Figure 3-24A and 3-24B), and CD107a/b expression was lower than that seen in HIV^- individuals (Figure 3-11A and 3-11B). IL-7 did not induce CD107a/b expression (Figure 3-25A and 3-25B) or enhance antigen-mediated CD107a/b expression (Figure 3-26A and 3-26B).

Figure 3-23. Representative flow cytometry dot plot of CD107a/b expression on $CD8^+CD45RA^-CD127^+$ T cells. (A) $CD8^+CD45RA^-CD127^+$ T cells were stimulated with PMA and ionomycin as a positive control for CD107a/b production. CD107a/b expression by memory $CD8^+$ T cells (B) in unstimulated cells, (C) in response to antigen, and (D) in response to antigen plus IL-7 (10 ng/ml).

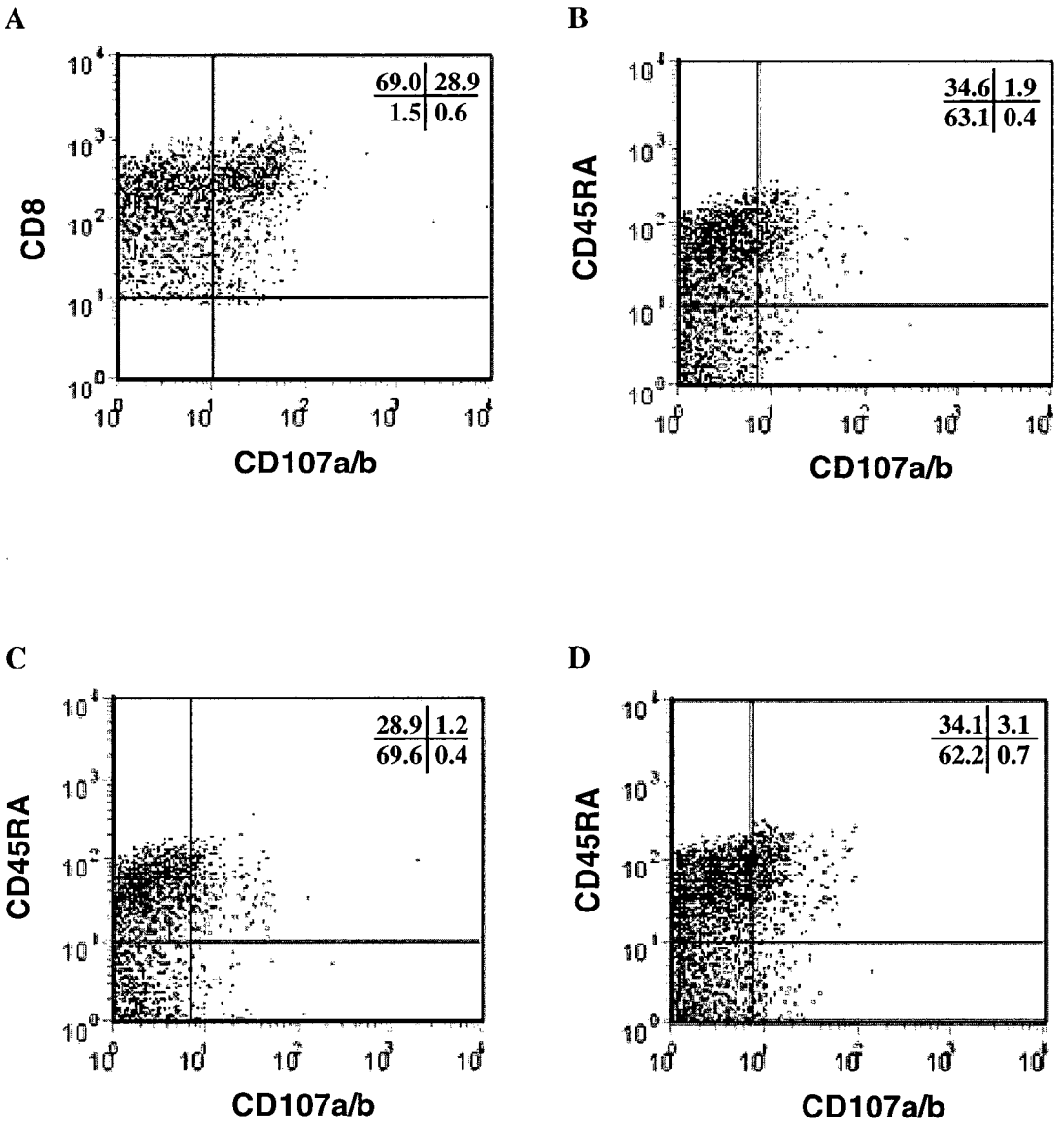
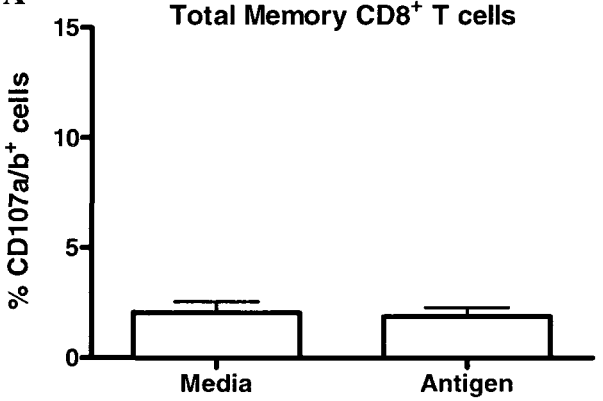

A
69.0 28.9
1.5 0.6
CD8
CD107a/b
B
34.6 1.9
63.1 0.4
CD45RA
CD107a/b
C
28.9 1.2
69.6 0.4
CD45RA
CD107a/b
D
34.1 3.1
62.2 0.7
CD45RA
CD107a/b

Figure 3-24. Antigen does not enhance CD107a/b expression by $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC for 6 hours and analyzed for CD107a/b expression and CD8, CD45RA, and CCR7 expression. Antigen-enhanced CD107a/b expression in (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. CD107a/b expression is expressed as a percentage of $CD107a/b^+$ cells. Graph shows mean of samples $\pm$ SEM. N=6.

A


Total Memory CD8+ T cells
% CD107a/b+ cells
15
10
5
0
Media
Antigen

B

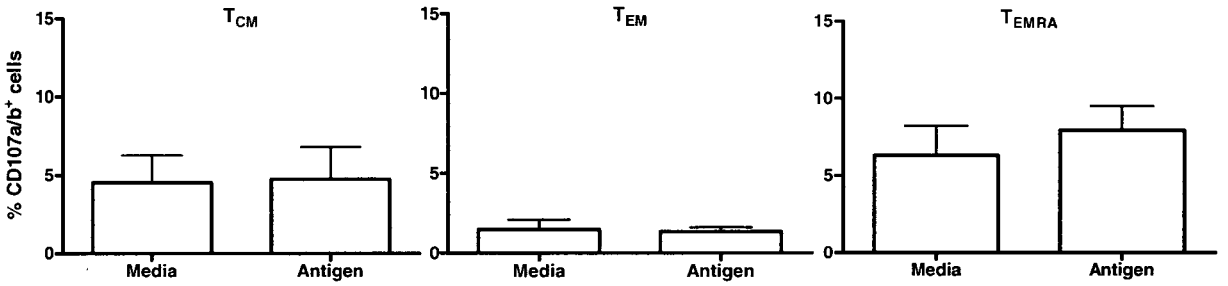
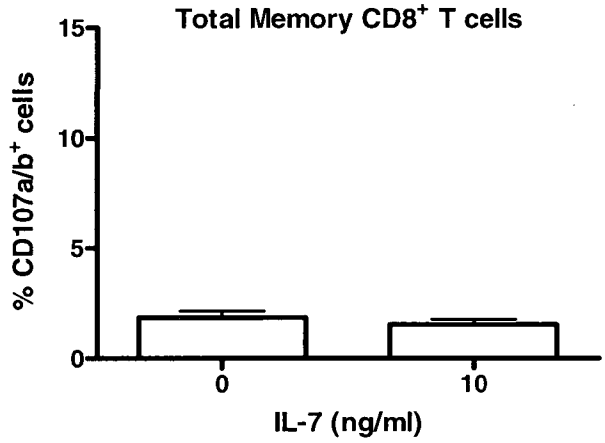

% CD107a/b+ cells
TCM
TEM
TEMRA
15
10
5
0
Media
Antigen

Figure 3-25. IL-7 does not enhance CD107a/b expression by $CD8^+CD45RA^-CD127^+$ T cells. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with $CD8^+$ T cell-depleted PBMC and IL-7 (10 ng/ml) for 6 hours and analyzed for CD107a/b expression and CD8, CD45RA, and CCR7 expression by flow cytometry. IL-7 did not enhance CD107a/b expression in (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. CD107a/b expression is expressed as a percentage of $CD107a/b^+$ cells. Graph shows mean of samples $\pm$ SEM. N=6.

A


Total Memory CD8+ T cells
% CD107a/b+ cells
15
10
5
0
0
10
IL-7 (ng/ml)

B

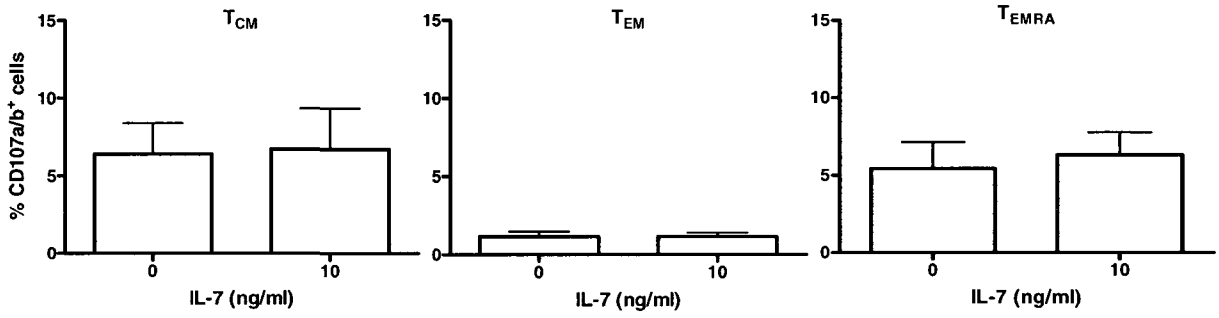
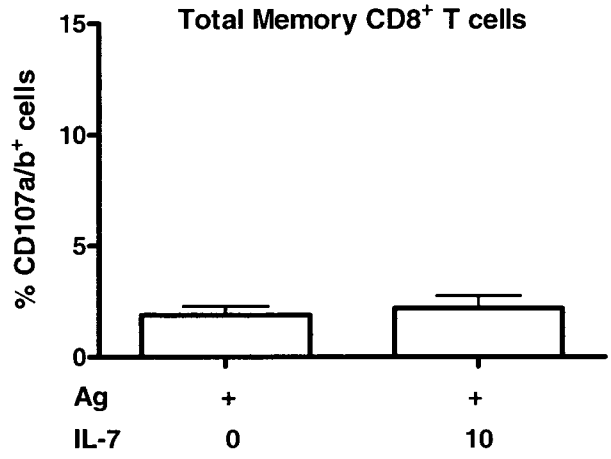
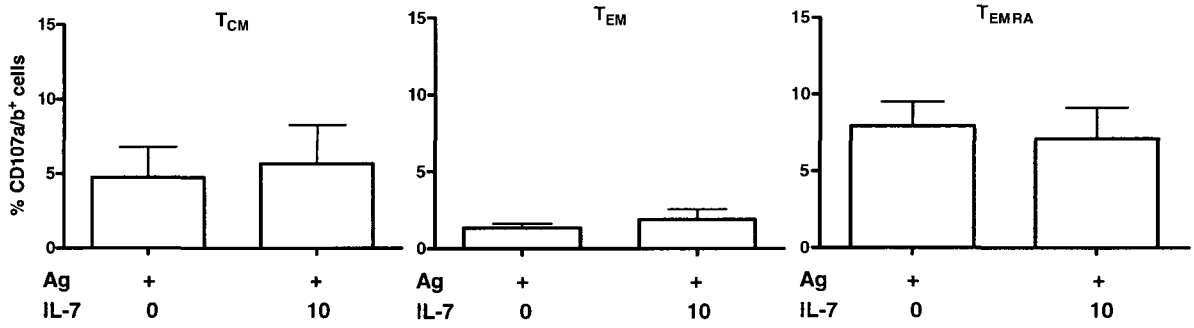

% CD107a/b+ cells
TCM
TEM
TEMRA
15
10
5
0
0
10
IL-7 (ng/ml)

Figure 3-26. IL-7 does not enhance antigen-mediated CD107a/b expression by $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals. $CD8^+CD45RA^-CD127^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC and IL-7 (10 ng/ml) for 6 hours and analyzed for CD107a/b expression and CD8, CD45RA, and CCR7 expression by flow cytometry. IL-7 did not enhance antigen-mediated CD107a/b expression in (A) total memory $CD8^+$ T cells or (B) the memory $CD8^+$ T cell subsets. CD107a/b expression is expressed as a percentage of $CD107a/b^+$ cells. Graph shows mean of samples $\pm$ SEM. N=6.

A


Total Memory CD8+ T cells
% CD107a/b+ cells
15
10
5
0
Ag + +
IL-7 0 10

B


TCM
TEM
TEMRA
% CD107a/b+ cells
15
10
5
0
Ag + +
IL-7 0 10

CHAPTER 4: DISCUSSION

4.1 $CD8^+$ T cell subsets in health

A secondary immune response mediated by $CD8^+$ T cells is initiated by re-exposure to a previously encountered antigen, and it is possible that other signals, such as cytokines, may be involved in enhancing recall responses. IL-7 is required for an efficient primary immune response and is important for overall immune function. During HIV infection, $CD8^+$ T cells lose their function, and this may be due to impaired IL-7 activity in these cells. It was hypothesized that IL-7 would enhance antigen-mediated $CD8^+$ T cell function, and that the ability of IL-7 to enhance antigen-mediated $CD8^+$ T cell function would be impaired during HIV infection. The purpose of this work was to evaluate the ability of IL-7 to enhance antigen-mediated proliferation, IFN-γ production, and CD107a/b expression in $CD8^+CD45RA^-CD127^+$ T cells in health and in HIV disease. It was found that in health, antigen induced proliferation and IFN-γ production, and IL-7 enhanced antigen-mediated $CD8^+$ T cell proliferation however it did not enhance IFN-γ production or CD107a/b expression (Table 4-1). It was also found that in HIV disease, antigen did not induce $CD8^+$ T cell proliferation, and only had a limited impact on IFN-γ production. Additionally, IL-7 did not enhance proliferation, IFN-γ production, or CD107a/b expression in subpopulations of $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals (Table 4-2).

	T_{CM}			T_{EM}			T_{EMRA}		
	Ag	IL-7	Ag+IL-7	Ag	IL-7	Ag+IL-7	Ag	IL-7	Ag+IL-7
Proliferation	+	++	++	+	++	++	-	-	-
IFN-γ	-	-	-	++	-	++	++	-	++
CD107a/b	-	-	-	-	-	-	-	-	-

Table 4-1. Summary of $CD8^+CD45RA^-CD127^+$ T cell responses in HIV^- individuals. Response of $CD8^+CD45RA^-CD127^+$ T cells within the memory $CD8^+$ T cell subsets to antigen, IL-7, and antigen plus IL-7 are summarized based on a minimal response (+), a strong response (++), or no response (-).

	T_{CM}			T_{EM}			T_{EMRA}		
	Ag	IL-7	Ag+IL-7	Ag	IL-7	Ag+IL-7	Ag	IL-7	Ag+IL-7
Proliferation	-	-	-	-	-	-	-	-	-
IFN-γ	-	-	-	+	-	+	+	-	+
CD107a/b	-	-	-	-	-	-	-	-	-

Table 4-2. Summary of $CD8^+CD45RA^-CD127^+$ T cell responses in HIV^+ individuals. Response of $CD8^+CD45RA^-CD127^+$ T cells from HIV^+ individuals within the memory $CD8^+$ T cell subsets to antigen, IL-7, and antigen plus IL-7 are summarized based on a minimal response (+) or no response (-).

Memory $CD8^+$ T cells make up approximately 30% of the circulating $CD8^+$ T cells (Figure 3-1A), and this population is important for protection against re-infection with a pathogen. T_{CM} $CD8^+$ T cells are predominantly found in the lymph nodes and proliferate rapidly while the T_{EM} $CD8^+$ T cells are predominantly found in the periphery and are quick to gain effector function (Geginat et al., 2003; Romero et al., 2007). By isolating the $CD8^+CD45RA^-$ T cells, the initial population studied here consisted of only memory $CD8^+$ T cells. Upon isolation of $CD8^+CD45RA^-CD127^+$ T cells it was found that the highest proportion of cells were T_{CM} (Figure 3-1B), and this is likely because CD127 is expressed to a higher level on this subset compared to the T_{EM} $CD8^+$ T cell subset (van Leeuwen et al., 2005). The response of the $CD8^+CD45RA^-CD127^+$ T cells varied considerably between each individual, and this biological variability was likely due to the proportion of memory $CD8^+$ T cells that were antigen-specific for peptides in the CEF peptide pool. The CEF peptide pool contains common antigens to CMV, EBV, and Flu for a variety of HLA-haplotypes, and the memory $CD8^+$ T cell response by each individual is governed by the number of antigen-specific $CD8^+$ T cells in circulation at the time of blood collection. If an individual had previous exposure to several viral antigens from the CEF peptide pool, they would be expected to have a more robust response than an individual who had less antigen-specific $CD8^+$ T cells or had not been exposed to as many viral antigens.

4.2 Memory $CD8^+$ T cell proliferation in response to antigen and IL-7 in health

When memory $CD8^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC, proliferation of memory $CD8^+$ T cells was enhanced (Figure 3-3A). Proliferation is a central function of memory $CD8^+$ T cells, and it has also been observed that while there is a large amount of proliferation after antigenic stimulation, it requires approximately three days

for proliferation to begin, while effector function can be induced after several hours of stimulation (Whitmire et al., 2008). By analyzing CFSE dilution after six days of co-culture, the memory $CD8^+$ T cells were able to respond to antigen from the CEF peptide pool and proliferate. Within the memory $CD8^+$ T cell subsets, the T_{CM} and the T_{EM} subsets exhibited enhanced proliferation in response to antigen (Figure 3-3B). $CD8^+$ T cells in the T_{EMRA} subset proliferated to the greatest extent, but exhibited a similar degree of proliferation regardless of antigenic stimulation (Figure 3-3B). The T_{EMRA} subset consists of $CD8^+CD45RA^+CCR7^-$ T cells that likely differentiated from the initial memory $CD8^+$ T cell population upon antigenic stimulation, and they represented the smallest proportion of cells. Proliferation is coupled with differentiation during a primary immune response (Kaech et al., 2002), and the memory $CD8^+$ T cells may need to proliferate in order to gain effector function and differentiate into the T_{EMRA} subset. It is possible that the proliferation seen in the T_{EMRA} subset represents proliferation of T_{CM} or T_{EM} cells that have differentiated into T_{EMRA}, and not proliferation of T_{EMRA} $CD8^+$ T cells.

It has been previously shown that IL-7 is required for a primary immune response, and it can enhance proliferation of $CD8^+$ T cells both *in vitro* and *in vivo* (Kittipatarin and Khaled, 2009; Moniuszko et al., 2004; Levy et al., 2009). When IL-7 was added to the memory $CD8^+$ T cell co-cultures, it was found that proliferation was enhanced at 10 and 50 ng/ml of IL-7 (Figure 3-4A), and this occurred in the T_{CM} and T_{EM} $CD8^+$ T cell subsets (Figure 3-4B). The T_{EMRA} $CD8^+$ T cells differentiated from the memory $CD8^+$ T cells and were not part of the starting population, and therefore could not respond to IL-7. Concentrations higher than 1 ug/ml of IL-7 were required to enhance proliferation of memory $CD8^+$ T cells, and this may be due to the decreased expression of CD127 after activation of these cells. When memory

$CD8^+$ T cells were cultured with IL-7 at 1 ng/ml, proliferation was not enhanced, however 10 and 50 ng/ml of IL-7 enhanced proliferation to the same extent (Figure 3-4A and 3-4B). It has been previously shown that the lowest concentration of IL-7 that can enhance proliferation of memory $CD8^+$ T cells *in vitro* was 5 ng/ml (Rosenthal et al., 2009). The observed result, in conjunction with these previous findings, suggests there may be a threshold for IL-7-mediated proliferation *in vitro*, and this is between 5-10 ng/ml of IL-7. While these concentrations are significantly greater than concentrations seen in the plasma, concentrations in tissue may be considerably higher than that in plasma, and the concentrations of IL-7 used for these experiments may be similar to tissue concentrations.

To evaluate the ability of IL-7 to enhance antigen-mediated proliferation, memory $CD8^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC and increasing concentrations of IL-7. It was found that IL-7 could enhance proliferation at 10 and 50 ng/ml in the T_{CM} and T_{EM} subsets (Figure 3-5B) as with IL-7 alone. However, the relative increase in antigen-induced proliferation of memory $CD8^+$ T cells due to IL-7 tended to be less than the relative increase in proliferation with IL-7 alone. Antigen alone led to increased proliferation compared to unstimulated cells, and the addition of IL-7 may have only been able to increase proliferation to a small extent before reaching the maximum amount of proliferation possible by memory $CD8^+$ T cells in this culture system. Alternatively, antigen stimulation causes a down-regulation of CD127 expression (Hammerbeck and Mescher, 2008), and IL-7 activity may have decreased as a result. Since IL-7 enhanced antigen-mediated proliferation of $CD8^+CD45RA^-CD127^+$ T cells, it may be required for an effective secondary immune response.

4.3 Memory $CD8^+$ T cell function in response to antigen and IL-7 in health

The function of $CD8^+$ T cells is characterized by the production and release of several cytotoxic cytokines and molecules including IL-2, IFN-γ, perforin, and granzymes (Harari et al., 2009). Antigen stimulation is the primary stimulus of memory $CD8^+$ T cells and production of IFN-γ is an important aspect of their cytotoxic function. After a six hour co-culture with antigen, there was an increase in the proportion of memory $CD8^+$ T cells that expressed IFN-γ (Figure 3-7A). Within the memory $CD8^+$ T cell subsets, it was found that the T_{CM} subset had the highest proportion of IFN-γ^+ cells regardless of stimulation, however the T_{EM} and the T_{EMRA} subsets had the greatest response to antigen (Figure 3-7B). The T_{CM} $CD8^+$ T cells did not respond to antigen, and this may be because the T_{CM} subset is slower to gain effector function than the other memory $CD8^+$ T cell subsets, and may require more time to produce IFN-γ in response to antigen (Romero et al., 2007). However, since the cells within the T_{CM} subset did produce IFN-γ, which is not a functional property of this subset, they could contain memory $CD8^+$ T cells that are transitioning into effectors. By only using two phenotypic markers, it is possible that the responding cells in this subset are phenotypically T_{CM} by CD45RA and CCR7 expression, but their function suggests that they are more differentiated. The T_{EMRA} $CD8^+$ T cell subset produced IFN-γ in response to antigen, suggesting that the cells that differentiated from the initially isolated memory $CD8^+$ T cell pool were functional.

When memory $CD8^+$ T cells were cultured with IL-7 at increasing concentrations without antigen, it was found that IFN-γ production was not enhanced (Figure 3-8A and 3-8B). IL-7 has been shown to enhance IFN-γ mRNA transcripts in a mixed lymphocyte population and IFN-γ protein secretion was increased after 12 hours of culture with IL-7, suggesting that

under certain experimental conditions IL-7 can enhance IFN-γ production (Borger et al., 1996). IL-7 has also been shown to enhance IFN-γ production in the T_{CM} and T_{EM} $CD8^+$ T cell subsets after 7 days of culture (Wallace et al., 2006).

The primary stimulus of memory $CD8^+$ T cells is antigen, and this may be required for these cells to gain effector functions. Although in the present experimental conditions IL-7 by itself does not increase the proportion of IFN-γ^+ cells, it is possible that IL-7 can expand the number of antigen-specific $CD8^+$ T cells that have responded to antigen stimulation and would lead to greater IFN-γ production. While IL-7 alone may not induce IFN-γ production, it may prime cells to be more responsive to other stimuli and enhance effector function of antigen-specific memory $CD8^+$ T cells. It has been previously shown that IL-7 can lower the threshold for the activation of the IL-12/IL-18 IFN-γ pathway (Smeltz, 2007). To evaluate the effect of IL-7 on antigen-mediated IFN-γ production, $CD8^+CD45RA^-CD127^+$ T cells were cultured with peptide-pulsed PBMC and increasing concentrations of IL-7. IFN-γ production was not enhanced with IL-7 compared to antigen alone (Figure 3-9A and 3-9B). Stimulation of $CD8^+CD45RA^-CD127^+$ T cells with antigen alone resulted in significant IFN-γ production and IL-7 may not have been able to further enhance IFN-γ production. IL-7 may be insufficient by itself and may require the presence of other cytokines to enhance IFN-γ production during a recall response to antigen. Previous studies have found that TCR-stimulated $CD8^+$ T cells produced more IFN-γ when IL-7 and IL-21 were cultured with the cells compared to IL-7 alone after seven days of culture (Liu et al., 2007). Alternatively, IL-7 may not play an important role in the production of IFN-γ during a memory $CD8^+$ T cell response.

A major pathway of $CD8^+$ T cell killing is granule-dependent, and cells degranulate and release perforin and granzymes to kill infected cells (Trapani and Smyth, 2002). Degranulation is another aspect of memory $CD8^+$ T cell function, and can be measured by expression of the CD107a and CD107b molecules on the surface of the $CD8^+$ T cells. $CD8^+$ T cell function can be evaluated by a number of methods, including chromium (^{51}Cr) release assays, perforin production, or cytokine production as measured by ELISPOTs (Betts et al., 2003). Expression of CD107a/b was chosen as an additional functional readout to IFN-γ because it could be easily measured and evaluated within the memory $CD8^+$ T cell subsets by multi-colour flow cytometry. After six-hour culture, it was found that antigen did not significantly enhance CD107a/b expression on the total bulk memory $CD8^+$ T cells (Figure 3-11A), or within the memory $CD8^+$ T cell subsets (Figure 3-11B). The lack of significant CD107a/b expression could be due to the length of culture. It has previously been reported that cytotoxic granules, as measured by granzyme B expression, required approximately three days of proliferation before they could be induced (Meng et al., 2006). Other studies have shown that CD107a/b expression can be induced and measured in $CD8^+$ T cells after 5 hours (Betts et al., 2003). CD107a/b expression could be induced when $CD8^+CD45RA^-CD127^+$ T cells were cultured with the positive control of PMA and ionomycin, indicating that the culture times were sufficient, however the antigen stimulus from the CEF peptide pool may not have been strong enough to enhance CD107a/b expression.

When $CD8^+CD45RA^-CD127^+$ T cells were cultured with IL-7, CD107a/b expression was not significantly enhanced (Figure 3-12A). However, there may be a signaling pathway between IL-7 and degranulation as it has previously been shown that IL-7 can enhance perforin expression (Smyth et al., 1991; Wallace et al., 2006), and this could be coupled with

degranulation. There is very little information on the effect of IL-7 on $CD8^+$ T cell degranulation, and more studies are required to determine if there is a signalling pathway. When $CD8^+CD45RA^-CD127^+$ T cells were cultured with antigen and IL-7, it was observed that IL-7 did not enhance antigen-mediated CD107a/b expression in these cells (Figure 3-13A and 3-13B). Since IL-7 alone did not cause significant enhancement of CD107a/b expression, this suggests that IL-7 may not be an important stimulus for degranulation, and therefore would not expected that IL-7 would enhance antigen-mediated CD107a/b expression.

4.4 $CD8^+$ T cell subsets during HIV infection

During HIV infection, $CD8^+$ T cell subset distribution is altered compared to that observed in HIV^- individuals (Figure 3-1A). The largest subset of $CD8^+$ T cells were T_{EMRA} and the smallest was T_N (Figure 3-14A), and this difference could be due to the presence of more activated HIV-specific $CD8^+$ T cells, which would have a more effector-like phenotype, or the previously described skewed differentiation of $CD8^+$ T cells (Appay et al., 2000; Champagne et al., 2001). It has been shown that HIV-specific $CD8^+$ T cells are predominantly T_{EM} cells, and these subsets are required at higher proportions to combat the infection (Ellefsen et al., 2002). If the differentiation of $CD8^+$ T cells is significantly altered, they may not function optimally and therefore fail to efficiently kill infected cells. After isolating $CD8^+CD45RA^-CD127^+$ T cells, the proportions of cells in the T_{CM} and T_{EM} subsets (Figure 3-14B) were comparable to proportions seen in HIV^- individuals (Figure 3-1B). During HIV infection, CD127 expression is decreased on $CD8^+$ T cells, predominantly in the T_{CM} and T_{EM} subsets (Boulassel et al., 2007; Mercier et al., 2008). Despite this, the proportion of $CD127^+$ T cells is still higher in the T_{CM} subset compared to the T_{EM} subset

(Mercier et al., 2008), which could be why the subset distribution was similar in HIV^+ and HIV^- individuals after isolating $CD8^+CD45RA^-CD127^+$ T cells. Since all memory $CD8^+$ T cells express CD127 in these experiments, IL-7 activity during HIV infection can be evaluated and accurately compared to that in the absence of HIV infection.

4.5 Memory $CD8^+$ T cell proliferation in response to antigen and IL-7 during HIV infection

To determine if memory $CD8^+$ T cell responses are impaired during HIV infection, CFSE-labelled memory $CD8^+$ T cells were co-cultured with peptide-pulsed $CD8^+$ T cell-depleted PBMC and proliferation was analyzed. It was found that memory $CD8^+$ T cells from HIV^+ individuals did not proliferate in response to antigen (Figure 3-16A and 3-16B). The lack of proliferation in response to antigen indicates that memory $CD8^+$ T cell responses to CMV, EBV, and Flu are impaired during HIV infection. During early stages of HIV infection, HIV-specific $CD8^+$ T cells expand in an effort to control infection (Pantaleo et al., 1994). As HIV infection persists, $CD8^+$ T cells lose proliferative capacity after re-encounter with antigen, and is referred to as anergy (Lieberman et al., 2001). Also, the diminished response to antigen by the $CD8^+CD45RA^-CD127^+$ T cells may be due to impaired antigen presentation by the APCs as opposed to impaired memory $CD8^+$ T cell responses.

The effect of IL-7 on proliferation of memory $CD8^+$ T cells from HIV^+ individuals was analyzed by CFSE dilution after 6 days of co-culture. During HIV infection, CD127 expression is downregulated on $CD8^+$ T cells (MacPherson et al., 2001; Koesters et al., 2006). Previous studies have demonstrated impaired IL-7 activity on T cells during HIV infection however this may have been due to decreased expression of CD127 in the cells studied (Rethi et al., 2005; Colle et al., 2006). By isolating $CD8^+CD45RA^-CD127^+$ T cells,

the decreased proportion of $CD8^+$ T cells that express CD127 during HIV infection was no longer a factor, and IL-7 activity could be properly compared to that observed in HIV^- individuals. It was found that IL-7 did not enhance proliferation of memory $CD8^+$ T cells (Figure 3-17A and 3-17B), whereas in HIV^- individuals, proliferation was increased at the concentration of IL-7 used in these experiments. This is a significant finding and indicates an impairment of IL-7 activity in memory $CD8^+$ T cells during HIV infection. It has also been found that IL-7 activity is impaired in $CD4^+$ T cells during HIV infection, which indicates a potential IL-7 signaling defect in these cells (Bazdar et al., 2009; Camargo et al., 2009). It appears that CD127 receptor density is decreased on $CD8^+$ T cells from HIV^+ individuals compared to HIV^- individuals, and this could also lead to a decreased response to IL-7. The minimum required CD127 expression for IL-7 stimulation is not know, and decreased receptor density observed during HIV infection may influence the IL-7 response.

When memory $CD8^+$ T cells were stimulated with antigen and IL-7, it was found that IL-7 did not enhance antigen-mediated proliferation of the memory $CD8^+$ T cells (Figure 3-18A and 3-18B). The effect of IL-7 on memory $CD8^+$ T cells during HIV infection has not been previously studied, and the lack of proliferation by the memory $CD8^+$ T cells in response to antigen and IL-7 shows a major impairment of these cells. However, IL-7 has also been shown to enhance certain functions of $CD8^+$ T cells during HIV infection. IL-7 was found to enhance proliferation and effector function of mitogen-stimulated $CD8^+$ T cells from HIV-infected individuals (Carini and Essex, 1994). The addition of IL-7 to PBMC cultures from HIV^+ individuals also resulted in decreased spontaneous apoptosis of the cells, which could prevent the massive depletion of $CD4^+$ T cells (Vassena et al., 2007). Recent clinical trials using IL-7 treatment in addition to highly-active antiretroviral therapy (HAART) have shown

IL-7 can increase the number of T_N and T_{CM} $CD8^+$ T cells, and these cells can respond to antigenic stimulation, and suggests with suppression of viral replication, IL-7 activity may be returned (Levy et al., 2009).

4.6 Memory $CD8^+$ T cell function in response to antigen and IL-7 during HIV infection

To determine if other functions of memory $CD8^+$ T cells from HIV^+ individuals were impaired, IFN-γ production and CD107a/b expression were analyzed. Treatment with peptide-pulsed PBMC induced IFN-γ production by total $CD8^+CD45RA^-CD127^+$ T cells (Figure 3-20A), and was primarily observed in the T_{EM} and T_{EMRA} subsets (Figure 3-20B). This supports previous findings in HIV^+ individuals, where it was seen that CMV-specific $CD8^+$ T cells were predominantly T_{EMRA} and could produce IFN-γ in response to antigenic stimulation (Ellefsen et al., 2002). The CEF peptide pool used to stimulate the $CD8^+CD45RA^-CD127^+$ T cells in these experiments contains common antigens to CMV, and this is likely why the T_{EMRA} subset had the greatest IFN-γ response to antigen. The T_{CM} and T_{EM} $CD8^+$ T cell subsets exhibited fewer IFN-γ^+ cells compared to these subsets in HIV^- individuals, suggesting an impairment in these subsets. HIV-specific $CD8^+$ T cells are predominantly in the T_{EM} subset (Appay et al., 2002), and the low IFN-γ production seen in this subset supports previous work that HIV-specific $CD8^+$ T cells are functionally impaired (Appay et al., 2000; Betts et al., 2006; Lichterfeld et al., 2004; Rehr et al., 2008). The functional impairment of $CD8^+$ T cells during HIV infection is a major factor why disease progression is poorly controlled.

Expression of CD107a/b was also decreased in memory $CD8^+$ T cells from HIV^+ individuals compared to HIV^- donors (Figure 3-24A and 3-24B). None of the $CD8^+$ T cell subsets showed increased CD107a/b expression in response to antigen, and the expression of

CD107a/b was lower than that observed in HIV$^-$ individuals. CD107a/b expression in the T_{EM} subset was low compared to healthy HIV$^-$ donors, again suggesting a functional impairment of these cells. Memory CD8$^+$ T cell responses could also be impaired due to the lack of CD4$^+$ T cell help. Previous studies have shown that proliferation of HIV-specific CD8$^+$ T cells was enhanced when cultured with autologous CD4$^+$ T cells compared to CD8$^+$ T cells cultured alone (Lichterfeld et al., 2004). HIV infection causes a massive depletion of CD4$^+$ T cells, and this could be why the proliferative and IFN-γ responses by CD8$^+$CD45RA$^-$CD127$^+$ T cells to antigen were impaired in this experimental system.

The effect of IL-7 on memory CD8$^+$ T cell responses was also evaluated in HIV$^+$ individuals. CD8$^+$CD45RA$^-$CD127$^+$ T cells were co-cultured with peptide-pulsed or un-pulsed PBMC and IL-7. It was found that IL-7 did not enhance IFN-γ production or CD107a/b expression on its own (Figure 3-21 and Figure 3-25, respectively) or with antigen (Figure 3-22 and Figure 3-26, respectively). Since IL-7 did not enhance these CD8$^+$ T cell functions during health, it was not expected to increase antigen-mediated IFN-γ production or CD107a/b expression in memory CD8$^+$ T cells from HIV-infected individuals. IL-7 may work synergistically with other cytokines to enhance some aspects of CD8$^+$ T cell function during HIV infection. Previous studies have shown that IL-7 and IL-15 together were able to enhance IFN-γ production in CD8$^+$ T cells from HIV-infected individuals, and these cytokines were also able to enhance GAG-mediated IFN-γ production (Gu et al., 2007). IL- 2 has also been shown to increase the number of IFN-γ producing CD8$^+$ T cells (Shankar et al., 2000). IL-15 was also found to enhance proliferation of memory CD8$^+$ T cells from HIV$^+$ individuals, and this cytokine may be important for the cytotoxic function of CD8$^+$ T cells (Pahwa et al., 2006). IL-21, another common gamma chain cytokine, can enhance perforin

and granzyme B production by $CD8^+$ T cells from HIV-infected individuals, both on its own and synergistically with IL-15 (White et al., 2007). These findings suggest that the regulation of secondary immune responses by $CD8^+$ T cells is very intricate, and it may become more complicated during HIV infection, since $CD8^+$ T cells do not function properly in response to antigen and cytokines.

There is a large body of work focusing on the impairment of HIV-specific memory $CD8^+$ T cells, but the impairment of non-HIV specific memory $CD8^+$ T cells during HIV infection has still not been thoroughly investigated. HIV-specific $CD8^+$ T cells can proliferate during acute HIV infection but lose this function as disease progresses (Lichterfeld et al., 2004). It has been found that progressors and LTNPs have comparable numbers of HIV-specific $CD8^+$ T cells, but there is a higher proliferative capacity in LTNPs, suggesting that $CD8^+$ T cell proliferation is important for control of infection (Migueles et al., 2002). Most HIV-specific $CD8^+$ T cells respond to HIV antigens with only one cytotoxic function (for example CD107a/b expression, IFN-γ, MIP1-β, IL-2, or TNF-α production) (Betts et al., 2006). It has been shown that with prolonged antiretroviral therapy and suppression of viral load, $CD8^+$ T cells can become polyfunctional (Rehr et al., 2008). It has also been shown that HIV-specific $CD8^+CD127^{hi}$ T cells can become functional, long-lived memory $CD8^+$ T cells if viral load is suppressed early during infection with antiretroviral treatment (Sabbaj et al., 2007). A high proportion of polyfunctional $CD8^+$ T cells may lead to better control of HIV infection and could result in less severe disease progression.

While it is known that HIV-specific $CD8^+$ T cells are impaired during HIV infection, there are conflicting reports about the functional impairment of non-HIV-specific memory $CD8^+$ T cells during HIV infection. It has been previously shown that $CD8^+CD127^{hi}$ T cells

from HIV^+ individuals produced less IFN-γ in response to a CEF peptide pool compared to HIV^- donors (Sabbaj et al., 2007). Other studies have found that HIV-specific $CD8^+$ T cells from HIV^+ donors have impaired IFN-γ production compared to CMV-specific $CD8^+$ T cells, but are able to produce other cytotoxic cytokines (Appay et al., 2000). In HAART-treated HIV^+ individuals, it was found that there were an increased proportion of IFN-γ producing $CD8^+$ T cells when pulsed with a CEF peptide pool, but this effect was not seen when cells were cultured with HIV-GAG peptides which suggests the functional impairment is limited to HIV-specific $CD8^+$ T cells (Arrode et al., 2005). While some studies have shown that non-HIV-specific $CD8^+$ T cell responses are functional, the lack of proliferation and decreased IFN-γ production in response to antigen demonstrated here indicates that memory $CD8^+$ T cell responses are impaired to some extent in HIV^+ individuals. The impaired proliferative and functional response to antigen and IL-7 observed in these experiments have shown that non-HIV-specific $CD8^+$ T cells are impaired to some extent, and are clinically relevant since the experiments were conducted using $CD8^+$ T cells from untreated HIV^+ individuals.

CONCLUSION

These experiments have shown that IL-7 may be an important factor for memory $CD8^+$ T cells during a recall response to antigen, however it likely works in conjunction with other signals or cytokines to produce effector functions. While IL-7 enhances antigen-mediated proliferation of memory $CD8^+$ T cells, it did not enhance antigen-mediated IFN-γ production or CD107a/b expression. It was also found that memory $CD8^+$ T cell responses are impaired in untreated HIV^+ individuals, as they had a decreased proliferative capacity and IFN-γ production in response to antigen. The activity of IL-7 on memory $CD8^+$ T cells is also impaired during HIV infection, as it was not able to enhance proliferation of the memory $CD8^+$ T cells. These are significant findings, as the impairment of non-HIV specific $CD8^+$ T cell function and IL-7 activity has not been thoroughly described. These results further our understanding of the signals required during a secondary immune response, and provides insight into HIV immunopathogenesis which may lead to the development of novel immune based therapeutics or vaccines.

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