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Intestinal CD4 Depletion in HIV / SIV Infection

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Abstract

Among the most significant findings in the pathogenesis of HIV infection was the discovery that almost total depletion of intestinal CD4+ T cells occurs rapidly after SIV or HIV infection, regardless of the route of exposure, and long before CD4+ T cell losses occur in blood or lymph nodes. Since these seminal discoveries, we have learned much about mucosal and systemic CD4+ T cells, and found several key differences between the circulating and intestinal CD4+ T cell subsets, both in phenotype, relative proportions, and functional capabilities. Further, specific subsets of CD4+ T cells are selectively targeted and eliminated first, especially cells critically important for initiating primary immune responses, and for maintenance of mucosal integrity (Th1, Th17, and Th22 cells). This simultaneously results in loss of innate immune responses, and loss of mucosal integrity, resulting in mucosal, and systemic immune activation that drives proliferation and activation of new target cells throughout the course of infection. The propensity for the SIV/HIV to infect and efficiently replicate in specific cells also permits viral persistence, as the mucosal and systemic activation that ensues continues to damage mucosal barriers, resulting in continued influx of target cells to maintain viral replication. Finally, infection and elimination of recently activated and proliferating CD4+ T cells, and infection and dysregulation of Tfh and other key CD4+ T cell results in hyperactive, yet non-protective immune responses that support active viral replication and evolution, and thus persistence in host tissue reservoirs, all of which continue to challenge our efforts to design effective vaccine or cure strategies.

Keywords

HIV-1; SIV; mucosa; gut; CD4; T-cell; cytokine; transcription factors

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CONFLICT OF INTEREST

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1. INTRODUCTION

Since the beginning of the epidemic, loss of CD4+ T cells in blood has been a defining hallmark of the Acquired Immune Deficiency Syndrome (AIDS). In fact, loss of CD4+ T cells in blood was recognized as a feature of AIDS even before the etiology was identified. Once the cause of AIDS was discovered to be HIV, and that CD4 was the primary co-receptor for viral attachment, it was evident that direct viral infection of CD4+ T cells was playing a major role in this loss. However, neither the infection rate, nor the rate of CD4+ T cell loss in the blood could explain the pathogenesis of the disease, which led to much confusion. The “gradual” loss of CD4+ T cells in blood, and seemingly long incubation period from the time of HIV exposure to the manifestation of AIDS defining illnesses was usually years, leading many to consider the virus had a “dormant” stage, or at least a prolonged period of “clinical latency” [1, 2]. Later, the discovery of HIV infected cells in much larger numbers in lymph nodes indicated the virus was not “latent” but simply not replicating in blood [3]. Further, once investigators began examining tissues other than blood, and examining the *earliest* events in infection, particularly in nonhuman primate models, it was soon shown that HIV, and its recent ancestor SIV replicated rapidly in the host from the time of infection, resulting in a high burst of viral replication within days of exposure, supported by the large numbers of activated, CD4+CCR5+ T cells normally residing in mucosal tissues that serve as “fuel” for the virus [4]. Further, this initial burst of viral replication is accompanied by the generation of numerous viral mutations that “decoy” the immune system with a plethora of viruses having tremendous antigenic variation, which thwart the initial antibody responses. It is now apparent the virus also produces large amounts of proteins that seem to serve little else but to further decoy the initial cellular and humoral response to antigens generated by the transmitted founder virus [5, 6]. Subsequent mutations in the envelope thus continuously fool and deflect the immune response to non-essential antigens while preserving its core antigens which are necessary for viral infection and dissemination. Tfh cells (CD4+ T cells that have matured and migrated to lymphoid germinal centers) become pre-occupied with multiple responses resulting in evasion of effective antibody (or cellular) immune responses. The vast reservoir of activated CD4+ T cells residing in mucosal tissues thus plays a major role in the early pathogenesis of HIV pathogenesis, in particular by permitting a massive early burst in viral replication, mutation, and protein production which it uses to escape from both cellular and humoral immune responses.

Further studies focusing on the mucosal immune system have revealed much more insights into the early events and pathogenesis of infection, and the mechanisms involved in immune evasion, dysregulation, and disease progression. In fact, emerging and converging evidence suggests mucosal CD4+ T cells may also be the key to effective immune control of pathogenic SIV/HIV infection. In parallel, evolving immunology research shows that mucosal CD4+ T cells are highly varied, and consist of several different subsets that can be distinguished by cell surface markers, gene expression (transcription factors), and functionality (lymphokine secretion). Importantly, these varied CD4+ T cell subsets normally provide help for maintaining mucosal barrier integrity, eliciting CD8+ T cell responses, tempering overactive immune responses, and in organized gut-associated

lymphoid tissues (GALT), they provide major “help” for generating effective mucosal (and possibly even systemic) antibody responses. Although we have known for decades that mucosal CD4+ T cells differ drastically from those in peripheral blood or tissues, we are finally beginning to understand the many roles and subsets of CD4+ T cells, and how they are induced to differentiate. These subsets have unique roles in balancing protective intestinal immune responses against microbial pathogens, while maintaining immune homeostasis and tolerance to symbiotic resident bacteria and benign food proteins that could potentially trigger adverse or unnecessary immune responses if this balance is altered. Accumulating evidence demonstrates imbalances between regulatory and effector CD4+ T cell immune responses and the intestinal microflora may play a previously unsuspected role in HIV infection as well as a number of diseases including inflammatory bowel disease (IBD), diabetes, obesity [7] and even neurologic diseases [8]. It is increasingly clear that HIV/SIV selectively infects, and either destroys, or dysregulates, specific CD4+ T cell subsets that in a myriad of ways, affect all of these effector functions and alter the homeostatic mechanisms of mucosal immune regulation. Finally, hints are emerging that perhaps the mucosal and systemic immune systems may not be as distinct as once suspected, as antibody therapy directed against mucosal homing markers has shown promising results in reducing viral replication in systemic and mucosal lymphoid tissues as well [9]. Here we discuss the various types of mucosal CD4+ T cells, and their role in maintaining immune homeostasis in mucosal tissues, as well as their suspected roles in the pathogenesis of SIV/HIV infection and AIDS.

2. MUCOSAL CD4+ T CELLS IN HIV INFECTION

Notably, and in hindsight, the first recognized cases of HIV induced AIDS involved opportunistic infections that were considered diseases of “mucosal” tissues including cytomegalovirus infection (CMV), herpes simplex 1 and 2 (HSV), thrush (candidiasis), and pneumocystis infections [10–12]. Interestingly, and also in hindsight, even in the first described cases of AIDS in SIV-infected macaques, intestinal amyloidosis was among the most common pathologic finding, indicating that chronic intestinal inflammation was also a major feature of the disease [13]. Soon after, Kotler *et al.* suggested a specific enteropathy was occurring in HIV patients long prior to the onset of significant CD4+ T cell declines in blood, or establishment of opportunistic infections (OI’s) which was the definition of AIDS. Biopsies taken 6 months to a few years after HIV exposure showed marked reductions in intestinal CD4+ T cells of HIV-infected patients, before there were notable depletions in blood [14]. This, combined with the fact that HIV enteropathy was a well-known syndrome in HIV patients, suggested to a few that the intestine played a key role in HIV pathogenesis [15]. Despite these early findings, the role, and relationships between mucosal CD4+ T cells in tissues in the intestine, vagina, and lung in early HIV / SIV infection remained essentially unexplored for several years into the HIV epidemic.

In 1998, using nonhuman primate models in which intestinal tissues could be collected within days of viral exposure, it was first shown that SIV infection of macaques resulted in massive early depletion of intestinal CD4+ T cells within days of infection, independent of the route of inoculation [16, 17]. Subsequent studies showed CD4+ T cells were also rapidly depleted from the vaginal mucosa, again independent of the route of inoculation [18]. This

rapid and marked infection and loss of intestinal mucosal CD4+ T cells was eventually confirmed in HIV-infected humans [19–21]. Direct examination of infected cells in intestinal tissues indicated most of the early CD4+ T cell loss was attributed to direct viral infection and lysis of these cells [22, 23]. Further, early viral replication in mucosal tissues was consistently accompanied by marked levels of viral RNA in the plasma (peak viremia), further reflecting the large numbers of activated, CD4+CCR5+ T cells supporting high levels of viral replication in primary infection [4, 17, 19, 23]. In addition, marked mucosal inflammation in HIV patients was also being recognized as a key feature of HIV infection [24]. In summary, the initial and subsequent investigations into the immunopathogenesis of HIV and SIV infection revealed mucosal CD4+ T cells were the major early target for viral replication and amplification, and that this loss was likely responsible for early, marked defects in mucosal immune responses, leading many to conclude that HIV infection was primarily a disease of the mucosal immune system, at least in the acute stage of infection [17].

In adult SIV infected macaques, and HIV infected adult humans, following the acute phase of infection, viral levels in plasma decline to a relatively stable “set point” coinciding with the marked loss of mucosal CD4+ T cells. This stabilization of viremia may be concurrent with the rate of remaining CD4+ T cell turnover, and/or activation levels in mucosal and systemic tissues as discussed below. This is further supported by data from neonatal infants infected with SIV, which have a much higher rate of intestinal CD4+ T cell turnover, resulting in a prolonged, sustained high level of viremia with no apparent “peak” in neonatal HIV/SIV infection [25]. This higher turnover rate of CD4+ T cells in mucosal tissues of infants may also lead to more rapid exhaustion of the CD4+ T cell “progenitor” pool, which combined, suggests the sustained “peak” viremia and more rapid progression to AIDS in HIV infected infants may be directly attributed to this higher rate of CD4+ T cell turnover in the pediatric host. Conceivably, the development of AIDS may simply be due to exhaustion of the regenerative capacity of naïve CD4+ T cells, but whether this could simply be due to a loss of precursors (“stem cells”) or dysregulation of CD4+ T cell maturation and development pathways remains unknown.

From birth, CD4+ T cells are present in organized and inductive lymphoid tissues of primates, particularly in the intestine [26]. Moreover, many mucosal CD4+ T cells in fetal and newborn humans [27] and macaques already have an “activated memory” phenotype, and high CCR5 expression [28], suggesting there is some type of T cell priming in utero, resulting in ample target cells supportive of viral infection in the intestinal tract of infants from the day of birth [25, 26, 28]. Transcriptomics also suggest there are bacterial antigens in placental tissues and meconium of newborn infants [29, 30], yet recent advances in sequencing technology and sensitivity also brings the potential for detecting bacterial RNA contamination of even the media and laboratory reagents currently used in laboratories, so this remains debated. Nonetheless, it is inarguable that intestinal CD4+ T cells are abundant throughout the length of the intestinal tract at birth, which are turning over at a much higher rate in infants than adults, and many co-express memory markers and CCR5, making the intestinal tract a potential site of initial transmission in newborns or infants who ingest HIV-infected maternal blood or milk. In support of this, the frequency of sufficient numbers of CD4+CCR5 target cells in intestinal tissues correlates with mother to infant transmission

rates of SIV in nonhuman primates [31]. Regardless of the route or source of infection, the intestinal mucosal of infants and adults provides an ample “feeding ground” rich in activated and/or “tissue resident memory” CD3+CD4+ T cells co-expressing CCR5 to support HIV infection and replication from the moment of birth and throughout pediatric HIV infection [25, 27]. However, and in addition to CCR5 expression, it is increasingly apparent that other factors are important for viral infection and replication in the various CD4+ T cell specific cell subsets as described in more detail below.

In addition to simply serving as “fuel” for the virus, various mucosal CD4+ T cell subsets are being recognized as key players in the varied functional roles of maintaining mucosal integrity and immune responses, Further, the markers we typically associated with memory, activation, or other properties are continually changing, and altering the way we view the mucosal immune system. From the moment of birth, CD4+ T cells provide help for initiating, and maintaining CD8+ T cell responses, antibody responses, and even arming and activating macrophages and other myeloid cells through direct, and indirect mechanisms. How mucosal CD4+ T cells are affected by, and even “exploited” by HIV to avoid immune responses, perpetuate viral replication, and persist in patients for a lifetime, all the while evading and thwarting all attempts at an effective immune response are the subject of intense research. Here we describe the latest advancements in identifying the differences in phenotype and function of intestinal CD4+ T cell subsets, and how their selective and possibly even sequential deletion and/or dysregulation result in viral persistence, and eventually result in the disease we know as AIDS.

3. TYPES AND FUNCTIONS OF MUCOSAL CD4+ T CELLS

3.1. Naïve, Resting, and “Activated”, Memory CD4+ T Cells in Mucosal Tissues

Defining the quiescence and activation state of CD4 T cells in tissues is critical for better understanding the mechanisms HIV pathogenesis, and the persistence of HIV reservoirs in tissues [32]. In both mice and humans, CD4+ T cells are clearly the most abundant lymphocytes in the body, as they predominate in both systemic and mucosal lymphoid tissues [33]. In fact, the vast majority of T cells in infants at birth are CD4+ T cells, as it seems most CD8+ T cells and CD20+ B cells develop after birth, likely in response to external antigenic stimuli. In addition, most of the bodies lymphocytes reside in the intestinal tract, arguably making the intestinal tract the largest reservoir of CD4+ T cells in mammalian hosts. Notably, a large percentage of these T cells reside within the “organized lymphoid tissues” a term which includes lymph nodes and spleen, yet it is often overlooked that there are vast numbers of solitary to coalescing lymphoid follicles (a.k.a. Peyer’s patches) in the terminal small intestine (especially the ileum), and throughout the large intestine (cecum, appendix, colon, and rectum) of all primates. Development and generation of T cells is beyond the scope of this review, but at birth, most CD4+ T cell in primates (including humans) are naïve, resting cells characterized by the absence of CD95, expression of CD45RA, and an absence of “activation” or memory markers such as CD25, CD69 and HLA-DR [32]. It is also thought the vast majority of these naïve CD4+ T cells in infants are recent thymic emigrants, based on CD31 expression [34]. Throughout the neonatal period however, CD4+ T cell proliferation occurs at a much higher rate, most likely in response to

the plethora of environmental antigens the infant is exposed to [25]. From the time an infant is born, it encounters a plethora of bacteria, viruses, and other microbes first in the birth canal, and subsequently by dirt or objects transferred by fingers to its mouth, and even the antigens in milk or food, which must be carefully discriminated by the immune system to determine whether they are potential pathogens, worthy of eliciting an inflammatory response, or nutritional antigens, which must be "tolerated" by the immune system so as not to provoke an unnecessary immune response. Subsequently, the phenotype and function of CD4+ T cells undergoes rapid and dynamic changes within the first days or weeks of age as these cells transition from a truly naïve state, to varied stages of activation and "memory" status in the host. Importantly, the local cytokine/chemokine milieu and their effects on cellular transcription factors appear to determine the fate of CD4+ T cell differentiation.

Basically, activation of both naïve and "resting" memory CD4+ T lymphocytes occurs in organized lymphoid tissues, generating diverse subsets of effector T cells with the capacity to migrate to multiple tissue sites [35]. Once activated, "activated effector" cells are short lived, but a subset develop into long-lived memory T cells which persist as heterogeneous populations in both peripheral and mucosal lymphoid tissues. Once they reach their target tissue, studies in mice have shown these mucosal "tissue-resident memory" $CD4^+$ and $CD8^+$ T cells provide rapid recall responses to site-specific pathogens [36, 37]. However, defining these tissue resident memory cells, or defining the states of cellular activation and memory status based on the surface expression of specific antigens has been challenged, and changing, especially in recent years with the advances in RNA transcriptomics, which is literally changing the way research is conducted and interpreted. Cell surface expression of various molecules we previously used to describe activated and resting memory CD4+ T cells as well as those identifying Tfh, Treg, and Th17 cells is more dynamic and "regulated" than previously understood, and is largely regulated by the effects the local cytokine/chemokine environment, and its downstream effects on transcription factors. We are also finding some subsets previously considered distinct lineages may transform to other lineages and functions depending on the local tissue environment, and in fact many markers we use for defining such cells are often receptors for cytokines and chemokines that regulate local tissue responses. For example, once considered useful for discriminating T regulatory cells, CD25 is an IL-2 receptor that may be up or down regulated depending on the level of IL-2 in tissues. Similarly, CD69, once believed to be specific for cell activation, is now considered a marker for "tissue resident memory" cells which may or may not be in a "classical" state of activation.

Patterns of chemokine receptor expression are especially useful for characterizing intestinal T cell subsets. Naive T cells, which preferentially migrate to organized lymphoid tissues (*i.e.*, lymph nodes and Peyer's patches), express CXCR4, the receptor for stromal cell-derived factor1/CXC ligand 12, CCR7, the receptor for Epstein-Barr virus-induced molecule 1 ligand chemokine/C-C chemokine ligand (CCL) 19, and secondary lymphoid organ chemokine/CCL21 [38]. Memory T cells can be further distinguished into two functionally distinct subsets based on CCR7 expression [39]. Both CCR7 and the "lymph-node homing" receptor CD62L have been shown to be important for recruitment to lymph nodes. However, CCR7 is more useful for distinguishing effector *versus* central memory functions, as CD62L is rapidly shed upon activation, or possibly even during delays in processing cells, and thus

subject to interpretation [40]. In general $CCR7^+$ central memory T cells home to lymphoid organs, whereas CCR7 negative effector memory T cells migrate to sites of tissue inflammation [38, 39]. CCR7 negative memory cells also express other chemokine receptors important for migration to inflamed tissues (*i.e.*, CCR1, CCR3 and CCR5) and demonstrate effector functions following stimulation, whereas CCR7+ memory cells can express CD62L, CCR4, CCR6 and CXCR3 and seem to lack immediate effector functions. However, these cells efficiently stimulate dendritic cells in vitro, and can rapidly differentiate into CCR7 negative effector cells upon secondary stimulation [39]. Thus central memory (TCM) and effector memory (TEM) T cells are often distinguished by CCR7+ and CCR7− expression, respectively, especially when used in conjunction with general markers for naive (CD45RA) or memory cells (CD45RO and/or CD95) but the caveats above should be considered. Surface expression of most of these proteins is not only dynamic, but in certain cells where differentiation has been carefully examined, some of these markers and cytokines may be transiently expressed at different stages of differentiation.

Chemokine receptors are especially useful for identifying CD4+ T cells as some are preferentially expressed by different Th subsets; CCR3 and CCR4 are preferentially expressed by Th2 cells, and CCR5 and/or CXCR3 are expressed by Th1 and Th17 cells [41–43]. CXCR5 expression on memory CD4+ T cells, in conjunction with other markers (see below) defines T follicular helper CD4+ T cells (Tfh) that are homing to germinal centers in the B cell follicles of lymph nodes and GALT which support B cells in the generation and maturation of high affinity antibodies [44–46]. In addition, CCR4 and CCR8 are highly expressed by human $CD4^+CD25^+$-regulatory CD4+ T cells, or “Tregs” [47]. Combined, these data demonstrate a major role for chemokines and their receptors in both identifying key subsets in blood and tissues, and in regulating the homing of specific T cell subsets into specific tissue compartments of both systemic (peripheral) and mucosal lymphoid tissues.

Although useful, the previous paradigm of naïve cells (CD45RA+CCR7+CD27+) converting to “central memory” (CM: CD45RAnegCCR7+CD27+), effector-memory (EM: CD45RA-CCR7-CD27-) transitional memory (CD45RACCR7-CD27+) or terminally-differentiated effector cells (TEMRA: CD45RA+CCR7-CD27-) subsets is also undergoing revision, as new subsets with different phenotypical and functional properties are being recognized, especially in mucosal tissues [48]. Clearly, naıve CD4 T cells differ phenotypically from all other memory subsets [32]. However, memory T cells in the intestine have a distinct phenotype compared with cells in secondary lymphoid tissues [48, 49]. Specifically, some intestinal CD4+ and CD8+ T cells co-express CD103, previously considered a marker of cells homing to intestinal epithelia (intraepithelial lymphocytes). Now CD103 is considered a “tissue resident memory” marker [48, 50]. Similarly, mucosal CD4+ T cells, especially those in intestinal effector sites, were once considered to be in a state of activation based on high CD69 expression [51]. CD69 is typically upregulated on peripheral blood lymphocytes once stimulated, and thus this has long been considered an early marker for cell activation [51, 52]. However, CD69 is also now recognized as another marker of tissue resident memory cells [50]. Although originally described in mucosal tissues, CD69+CD103+ T cells are now considered tissue “resident” memory T cells (T_{RM}), since they do not equilibrate in tissues, nor are they replenished from the blood [48, 50]. Further, these cells have been described in many tissues other than the mucosa, especially

after an infection including the skin, lung, brain, sensory ganglia, salivary gland and female reproductive tract [48–50]. It is now believed continued expression of CD69 may not necessarily reflect activation, but intead a signal to remain in tissues. Previous studies in SIV-infected macaques showed these (CD69+CD4+) T cells, almost exclusively residing in the gut, were major targets for direct viral infection and depletion in SIV infection of macaques [4, 51]. Thus, infection and elimination of large numbers of these resident memory CD4+ T cells likely plays a key role in the early pathogenesis of HIV infection, and dysregulation of mucosal immune responses in general. Additional studies are showing that these cells may also be further subdivided into subsets with differing functions and viral susceptibilities, and their selective depletion may play an even larger role in the pathogenesis of HIV infection and AIDS than previously estimated.

3.2. Cytokine Signals, Transcription Factors and Mucosal CD4+ T Cell Differentiation

It is increasingly clear that CD4+ T cell differentiation is much more dynamic and malleable than previously recognized. Naïve CD4+ T cells released from the thymus can be induced to differentiate into different CD4+ T cell subsets with similar or changing surface marker expression, yet with strikingly different functional qualities. The cytokines and molecular transcription factors that lead to differentiation of specific CD4+ T cell subsets has been fairly well established in mice, but less so in humans or nonhuman primates. After clonal selection in the thymus, naive CD4+ T cells obtained from blood or lymph nodes can be programmed to differentiate into a variety of T helper subsets with different functional roles mimicking those of mature mucosal CD4+ T cells. It is also clear that concentrations and/or combinations of cytokines are responsible for initiating transcription factors that distinguish this differentiation. However, we will refrain from using the term “terminal differentiation”, as increasing evidence suggests individual T helper cells (Th) programmed for one role/function may later be “reprogrammed” to de-differentiate into other subsets with different phenotypic and functional qualities [53]. However, this remains difficult to prove *in vivo*, especially on an individual cell basis. Nonetheless, our understanding of the types, functions, and roles of CD4+ cells have markedly expanded since the original seminal description of the Th1 and Th2 paradigms established in 1986 by Coffman and Mosmann [54].

It is now known that naïve CD4+ T cells are stimulated to differentiate into distinct regulatory subsets through specific cytokines which elicit transcription factors that further regulate the development of specific T cell subsets with functional characteristics designed for antimicrobial defense or immune regulation. For examples, Th1 cell development is driven by IFN-γ, IL-18 and IL-12, Th2 cell development through IL-4 stimulation, whereas Th17 cell differentiation is largely dependent on IL6 (or IL-21), and TGF-β. However, there seem to be redundant and autocrine pathways involved in perpetuating specific cellular responses. For example, and although less potent than IL-6 in promoting Th17 differentiation, IL-2 is autologously produced by Th17 cells and plays a role in continued Th17 expansion/amplification [55]. Similarly, IL-23 also seems to plays a role in maintaining or “stabilizing” Th17 cells [55]. Although cytokines appear to responsible for the differentiation, it is the initiation of specific transcription factors that determine the fate of individual cells.

T cell differentiation is accomplished by upregulation of master lineage specific transcription factors. For example, Th1 cells are activated/induced by T-bet and Th2 cells require GATA3 and c-Maf, which activate the hallmark Th1 and Th2 cytokine genes IFN-γ and IL-4, respectively, at least in mice [56, 57]. Among the most significant findings in the last decade have included deciphering the role of specific regulation factors, especially the; retinoid-related orphan receptor gamma t (RORγt) transcriptional factor which upregulates the expression of the aryl hydrocarbon receptor (AhR), which is a highly conserved transcription factor important for responses to environmental antigens [58], especially the intestinal mucosa, which is continuously exposed to bacteria, viruses, and innocuous antigens.

Exposure of mouse CD4+ T cells to TGF-β combined with IL-6 results in induction of RORγt. In mice, RORγt expression induces expression of the IL-17 gene in naïve CD4+ T cells, and in the presence of IL-6 results in differentiation of CD4 cells into Th17 cells [55, 59, 60]. This is thought to largely be the result of IL-6 signaling and induction of another transcription factor STAT3 which induces RORγt [60]. Similarly, Th17 cell differentiation in humans is dependent onTGF-β and RORγ3τ. TGF-β in combination with IL-1β and IL-6, or IL-21 or IL-23 is sufficient to induce human Th17 cells, indicating the TGF-β / Th17 differentiation pathway is highly conserved among vertebrates [60].

Activation of AhR during Th17 cell development markedly increases the proportion of Th17 cells, and their production of cytokines [61]. Notably, expression of AhR is also required for expansion of intestinal RORγt(+) innate lymphoid cells, as well as the formation of isolated lymphoid follicles in the intestine [58](and see below). Thus, converging evidence indicates that although local cytokine levels are important, initiation of specific transcription factors plays the major role in determining the generation, and fate of individual CD4+ T cells, especially in mucosal tissues.

4. SPECIFIC CD4+ T CELLS DEPLETED IN SIV/HIV INFECTION:

4.1. Evolution of the Th1, Th2, Th3….Paradigm?

In mice, it was first shown that CD4+ T cells could be induced to differentiate into distinct subsets that either secrete IFN-γ and promite inflammatory and cell mediated immune responses (T helper 1 cells or Th1) or CD4+ T cells that secrete IL-6 and promote humoral immune responses (Th2). In fact, the production of effector cytokines underlies the term “effector helper T cells” [54, 56].

We now characterize Th1 cells as pro-inflammatory cells that produce IFN-γ, TNF-α, and TNF-β which primarily stimulate and promite cellular immune responses [62]. Many of these also express granzyme B indicating cytolytic potential, and excessive Th1 responses can promote tissue damage. These cells also stimulate macrophages and dendritic cells, and are critical for combatting many intracellular pathogens [55, 56]. Further, Th1 cells co-express CCR5, and are susceptible targets for direct viral infection, and are major contributors to the global mucosal CD4+ T cell depletion in early SIV/HIV infections [6, 23, 51, 63, 64].

In contrast, Th2 cells are induced upon exposure to IL-4, which upregulates transcription factors GATA3 and c-Maf, and these cells subsequently produce IL-4, IL-5, and IL-13 [56, 65, 66]. Th2 cells have significantly lower levels of CCR5 expression and are primarily involved in allergic reactions as they target eosinophils and basophils. Th2 cells are also important for generating IgA responses and allergic inflammatory responses especially in mucosal tissues, including the intestine and lung. As noted above, Th1 cells are clearly targets for HIV infection and lytic destruction, and their high activation state also makes them particularly susceptible to destruction in HIV infection [64]. The massive loss of these cells, especially in mucosal tissues results in massive dysregulation of mucosal immune responses in acute, and throughout HIV infection. HIV infection has been shown to impair Th1 responses in favor of Th2 responses in blood [67]. This is also supported by findings that mucosal cytokine profiles change from a Th1 to a Th2 environment in vaginal fluids of HIV patients [68]. The marked loss of Th1 cells, and the corresponding loss of feedback regulation by these and other cells (Treg – see below) could promote excessive Th2 responses in mucosal tissues, which may be at least partially responsible for the hypergammaglobulinemia described in early HIV infected patients [69]. Further, an overactive Th2 response, combined with the absence of Th1 or other regulatory cells that normally modulate Th2 responses has also been implicated in the lymphoproliferative disorders including lymphomas in HIV patients [67]. Thus, massive loss of Th1 cells through direct infection, and sparing and dysregulation of Th2 cells may play a critical role in the early lymphadenopathy and hypergammaglobulinemia and even the lymphomas that are common in untreated HIV and SIV infections [67].

In addition to Th1 and Th2 cells, there are several subsets of intestinal CD4+ T cells with unique cell surface markers, functional capacities (cytokine production) and distinct gene transcription profiles that play major roles in the pathogenesis of SIV/HIV. Soon after the original description of the Th1/Th2 paradigm, Th3 cells were described as a unique set of CD4+ T cells obtained from the mesenteric lymph nodes that secrete a large amount of TFG-β, and showed immunosuppressive properties including induction of oral tolerance, which clearly distinguished them from Th1 and Th2 subsets [70]. However, it soon became apparent that there were further subdivisions of “suppressor” cells. Notably, early studies were performed prior to the discovery to transcription factors [71] and subsequent studies have shown that most of these “Th3” cells express the nuclear transcription factor forkhead box protein P3 (FoxP3) and are indeed Treg cells. However, a fraction of $CD4^+$ TGF-$\beta1^+$ producing cells that lack Foxp3 expression may still exist as pure “Th3” cells [72]. In addition, other mucosal cells have been shown to have similar cytokine (TGF-β) expression and suppressive activity yet without CD3 or CD4 expression (innate lymphoid cells or ILC). For now, the term Th3 cell is restricted to a minor subset of the immunosuppressive or regulatory CD4+ T cells now known as Treg cells, which modulate or suppress cellular immune responses [73, 74]. Since both Th3 and Treg secrete large amounts of the suppressive cytokine TGF-β, they are thought to play a major role in the intestinal CD4 depletion, and perhaps more importantly, the chronic immunopathogenesis of SIV/HIV infections.

4.2. Intestinal "Suppressor" CD4+ T Cells: Th3 *Versus* Treg Cells

In general, Treg are currently defined as T cells (CD3+) that express CD4 and high levels of IL-2 receptor alpha (IL-2Ra), a.k.a CD25, and FoxP3 [75]. FoxP3 expression is also dependent on STAT 5 signaling mediated through the IL-2 / CD25 pathway thus these cells are mainly regulated by local levels of IL-2 [75]. However, even these definitions are changing as FoxP3 and/or CD25 may be not be specific for Treg cells, and co-expression of the Helios transcription factor are now being used to discriminate functional Treg [76, 77].

Treg exert their immunosuppressive activity mainly by secretion of the inhibitory cytokines IL-10 and TGF-β [55]. In fact, Treg are difficult to study as they have poor growth characteristics since the large amounts of TGF-β they secrete both inhibit their growth, as well as induce other cells to differentiate into CD25+FoxP3+ Tregs [78].

Foxp3+ Treg cells have been shown to regulate specific effector T cell responses and control inflammation at specific tissue sites [79]. However, Treg can be further subdivided into different subpopulations [80]. Treg are now subdivided into "natural" Treg (nTreg), and adaptive or "induced" Treg (iTreg) [81]. Natural Tregs develop in the thymus, and are thought to be generated by negative selection, whereas induced Tregs, can be derived from naïve $CD4^+$ T cells in the peripheral and mucosal immune systems [82]. Induced Treg may also be distinguished by upregulation of Helios [83]. Natural Treg mediate their suppressive functions by cell to cell contact with the CD3 antigen receptor and MHC interactions. Although TCR binding is required for nTreg to suppress T cell proliferation, once activated, their continued immunosuppressive effects are non-specific, as they are mostly exerted through production of IL-10 and TGF-b [73].

In contrast, iTreg are even further subdivided based upon the cytokines that cause their induction, and the effects they mediate. Type 1 regulatory T cells (Tr1), are induced by IL-10 yet they do not express FoxP3 [84]. IL-10 was first described as a suppressive factor and has since been associated with numerous immunologic effects including downregulation of MHC and co-stimulatory molecules, inhibition and prevention of cytokine and inflammatory mediator secretion [73]. Tr1 cells also produce TGF-β but few other cytokines [73]. Interestingly, it has also been proposed that Treg subsets can be distinguished by their expression of the mucosal homing marker α4β7. Treg that co-express high levels of α4β7+ nTreg induce conventional CD4+ T cells to produce IL-10 and become Tr1 cells, whereas nTreg that express α1β7 generate TGF-β producing Th3 cells [85]. Interestingly, α4β7 has recently become of major importance to HIV pathogenesis as discussed below. Th3 cells may play a role in maintaining Tregs, and may also contribute to the local induction of iTregs [86]. Finally, different Treg subsets may also be distinguished by their different levels of CCR5 expression [87] but at least a subset of these cells are clearly targets for SIV/HIV infection.

4.3. Treg and Th3 Cells in HIV Infection

Tregs suppress immune responses and thus limit collateral tissue injury in response to infections, or down regulate inflammation after antigen clearance. In HIV however, the suppressive function of Treg is critical because of its potential for preventing the immune

hyperactivation associated with HIV infection, even though it may also have a detrimental effect by suppressing HIV-specific immune responses [75]. In fact, this has been described as a “Janus faced” cell subset, referring to the ancient god of transitions or new beginnings, suggesting that the face of the immune system may change, and/or have both detrimental and beneficial effects in response to different stimuli [75, 88].

Several studies have shown that HIV-1 can directly infect Treg, altering their phenotype and function *via* different mechanisms including down regulation of Foxp3, CD25 and impairment of suppressive capacity [75, 89]. Decreased absolute counts of Treg have also been described in HIV-infected patients [90] supporting the hypothesis that Treg deregulation might be related to the immune hyperactivation present in HIV-infected patients [75]. On the other hand, other papers have described an increase in Treg in HIV patients [75, 91, 92]. However, part of this discrepancy may be in the way Treg were (and are) defined, and/or by examining percentages of CD4+ T cells expressing Treg markers in the face of declining absolute counts of total other subsets of CD4+ T cells [75]. Alternatively or in addition, monitoring Treg in peripheral blood alone is not be reflective of what is occurring in tissues, especially the intestinal mucosa [93].

In macaques, Treg are more abundant in intestinal tissues of infants, as it is suspected they play a major role in the suppression of immune responses to food, or other harmless environmental antigens encountered shortly after birth [94]. Treg may be necessary to suppress potentially overactive immune responses to food antigens in tissues, preventing unnecessary, or even potentially damaging immune responses. On the other hand, high levels of Treg activation may also result in an inadequate response to potential pathogens. When IL-2 levels are high, Treg consume the IL-2 resulting in their suppressive activities that include disruption of metabolic pathways, induction of antigen presenting cell (APC) tolerance, generation of anti-inflammatory cytokines, and induction of apoptosis of other cells *via* various pathways [75]. In other words, the local tissue environment may dictate the “phenotype” and particularly the lineage commitment of at least some subsets of mucosal CD4+ T cells. Surprisingly, subtle differences in cytokine signals can trigger differentiation of very different CD4+ T cell subsets having opposite functions. For example, signaling with TGF-β1 is required for both Th17 and Treg development in mice (and humans) but TGF-β in the presence of low levels of IL-6 induces Th17 cells, and as above, Treg development requires IL-2 co-stimulation [59]. Importantly, experiments have shown that naïve CD4+ T cells may be induced to either become Th17 cells (IL-17 producing) or Treg cells depending on stimuli applied. In addition, Treg can be induced to become Th17 cells by addition of TGF-β and IL-6, and transitional cells that simultaneously co-express FoxP3 and other important transcriptional factors have been identified, suggesting significant plasticity of the immune response, depending on the local environmental mileau [55]. Thus, mucosal CD4+ T cell subsets are highly regulated by the dynamic mucosal cytokine/chemokine environment, which plays a major role in programming cells through initiation of specific transcription factors that dictate the fate of individual cells.

4.4. Th17 and Th22 Cells, and Intestinal Barrier Permeability

Since the original descriptions of the Th1/Th2 paradigm, several CD4+ T cells produced cytokines that could not be classified according to the Th1/Th2 scheme, and in 2005, Th17 cells were described that produced IL-17 but not IL-4 or IFN-γ [95, 96]. Notably, some Th17 cells can also produce IL-22 [97], but a different subset of CD4+ T cells has been described that secrete IL-22 and never IL-17, and these are now termed Th22 cells [98]. Although the cytokines and transcriptional factors necessary to induce Th22 cells are not clear, they are further characterized by the surface expression of chemokine receptors CCR4, CCR6, CCR10, and AhR [99]. In addition, and similar to Th17 cells, they also appear to have the capacity to acquire functional features of Th1 cells [100].

Clearly, Th17 cells have critical functions for maintaining mucosal antimicrobial immune defenses through the production of IL-17. These cells also express CCR6, and develop in response to cytokines and specific transcription factors [101]. In humans and macaques, Th17 cells are abundant in mucosal tissues, including the female reproductive tract and throughout the intestine [102, 103]. Th17 cells are a major line of defense in mucosal tissues, especially in the intestine where the balance between inflammatory (Th1 and Th17) and suppressive (Th3, Treg) cells and responses is critical for maintaining normal homeostasis [104]. Both IL-17 and IL-22 are important for maintenance of intestinal mucosal barrier integrity. IL-22, also produced by innate lymphoid cells [105] is important in host resistance to extracellular pathogens, as demonstrated by its ability to restrict commensal bacteria from the systemic circulation in mouse models [106]. IL-17A blockade results in increased intestinal permability, increased susceptibility to gram negative bacteria, and has been shown to be important for producing and stabilizing/positioning tight junction proteins that maintain mucosal epithelial integrity [55, 107]. Further, Th17 cells isolated from intestinal tissues are much more functional that those isolated from blood, as the former spontaneously produce IL-17, and may co-express multiple cytokines simultaneously after stimulation compared to those from blood [108]. In summary, both IL-17 and IL-22 are critical for maintaining mucosal immunity through a variety of mechanisms. Several lines of evidence suggest that Th17 cells also represent long-lasting lymphocytes that can proliferate in a homeostatic, stem cell-like fashion with a high grade of developmental plasticity that allows them to serve as precursor cells for more differentiated regulatory CD4+ T cells of the same, or different polarization [101].

4.5. Mucosal Th17/Th22 Cells and SIV/HIV Infection

Both Th17 and Th22 cells have been implicated in the intestinal pathology associated with multiple inflammatory bowel diseases [109]. Infection and loss of these cells in mucosal tissues is increasingly implicated in the pathogenesis of HIV infection [108, 110–113]. Th17 and Th22 cells in the intestine are markedly and selectively depleted in SIV-infected macaques [97, 105, 108, 114] and HIV patients [111]. Compared with Th1 and other Th subsets, Th17 cells are much more susceptible to HIV *in vitro* and are severely depleted during infection [115]. Further, recent studies have shown that SIV/HIV selectively targets Th17 cells immediately after mucosal viral exposure [116]. Although these represent a minority of the total CD4+ T cell population in vaginal tissues, more than 85% of the first cells infected following SIV challenge were RORγt+CCR6+ T cells, indicating that these

are the first cells infected by SIV/HIV. Further, Th17 cells have multiple transcription factors that support HIV replication compared to other subsets [117]. This is further supported by studies of HIV patients demonstrating persistence of HIV in Th17 cells even on ART [104].

Notably, the Th17 loss is more prominent in intestinal tissues than in blood or bronchoalveolar lavage from HIV-infected subjects [103]. Further, Th17 cells in patients on ART have disproportionally higher levels of HIV DNA suggesting they are major reservoirs for viral persistence [118, 119]. The mechanisms behind the increased infection rate, and greater viral production by Th17 cells is likely may be multifactorial. The greater susceptibility of Th17 cells is associated with the higher expression of HIV receptors CD4, CCR5, CXCR4, and α4β7 [102, 103, 115, 119]. Further, lower intracellular expression of HIV-inhibitory RNases may render Th17 cells more permissive to HIV infection and persistence [120]. Due to the critical role of IL-17 in maintaining epithelial tight junctions in intestinal tissues, the Th17 loss is thought to cause destruction of the gut mucosal barrier, and increased leakage of luminal contents into the systemic circulation (microbial translocation) that leads to generalized HIV-associated immune hyperactivation. The IL-17 depletion also leads to alterations of the Th17/Treg balance in the GALT, which results in microbial translocation of both commensal and pathogenic bacterial products into the intestinal lamina propria, and the blood stream, resulting in a generalized and persistent systemic immune activation [121, 122].

Th17 and even other IL-17 producing cells that do not co-express CD4 are markedly depleted in the intestinal mucosal of macaques infected with pathogenic SIV, likely due to apoptosis induced through bacterial products in the lamina propria engaging TLR receptors [97, 114]. However, partial restoration of Th17 cells by administration of IL-21 alone or with probiotic therapy has been reported to alleviate the immune hyperactivation and systemic bacterial translocation associated with HIV disease [123, 124]. Nonetheless, the reasons for the higher susceptibility of Th17/Th22 cells to SIV/HIV-mediated depletion are not fully understood. A better understanding of the mechanisms and factors contributing to Th17 infection and depletion may result in better targets for treatment and prevention strategies [115].

4.6. T Follicular Helper Cells (Tfh)

Another important T cell subset recently implicated in the pathogenesis of HIV infection and AIDS are the T follicular helper cells (Tfh) which specifically reside within germinal centers of organized lymphoid tissues. These cells are critical for primary antibody responses, especially for moderating affinity maturation and antibody production by B cells. Tfh cells in lymph nodes have also been implicated as a major persistent reservoir for HIV in patients on suppressive anti-retroviral treatment (ART)[125]. In fact, it has been hypothesized that infection and dysregulation of Tfh cells is at least in part responsible for the hypergammaglobulinemia in HIV patients, and the inability to generate effective HIV neutralizing antibodies in a timely manner [46, 126].

Tfh cells were first described in 2000, and are characterized by high expression of the B cell follicle homing molecule CXCR5 and the costimulatory molecules CD40L and ICOS [127]. Later studies defined these cells as distinct subsets identified by the transcription factor Bcl6

which is required for their differentiation but this is not entirely specific for Tfh cells. Bcl-6 promotes the Tfh transcriptional program at least in part by suppressing the expression of other transcriptional regulators including T-bet (Th1), RORγt (Th17), GATA3 (Th2), and Blimp-1 [128]. Although the initiators of Tfh development are not fully understood, several additional transcription factors have been identified that are involved in their regulation [127, 128]. Further, there is significant heterogeneity of Tfh subsets reported in the literature. Those that reside specifically in germinal centers have higher PD-1 and CXCR5 expression, and those in the mantle zones have lower PD-1 expression, and may represent an intermediate stage of development as they migrate towards the germinal centers where they support B cell development and function, mostly by secreting IL-21 [129, 130]. These cells co-express CXCR5 and are localized in the germinal center of lymph node B cell follicles, but populations of circulating CD4 T cells with phenotypic and functional characteristics similar to those of Tfh have also been described [101, 131]. In fact, there is confusion in the literature as as to the nature and phenotype of Tfh cells based on phenotyping for Bcl6 and/or CXCR5 levels alone, and recent studies suggest there are distinct subsets of Tfh cells partly based on their susceptibiltity to HIV [132]. Those that highly co-express CCR5, are permissible to HIV infection whereas those that do not co-express CCR5 are less susceptible, despite the presence of Bcl6 and CXCR5 [132]. Alternatively, we have proposed that CXCR5+CCR5+ CD4+ T cells migrating towards germinal centers may be an intermediate or precursor stage for bona fide Tfh cells restricted to germinal centers, and CCR5 may be downregulated once they reach this target destination [130]. This could explain why Tfh cells in germinal centers may be infected with SIV/HIV, despite the absence of CCR5 expression [133]. In SIV-infected macaques and HIV-infected humans, Tfh cells abnormally accumulate in germinal centers, and many of these are productively, and latently infected [46, 125, 133]. Once within this germinal center “sanctuary site” HIV infected Tfh cells have been proposed to be “out of reach” of cytotoxic CD8+ lymphocyte responses (CTL) although this remains controversial. While some studies suggest that $CD8^+$ CTL do not enter GC, other studies have shown that CTL are dramatically increased in lymph nodes and can be observed in follicles [133].

In summary, Tfh cells are infected and dysregulated in SIV infected macaques [46], which may underlie the hypergammaglobulinemia, follicular hyperplasia and lymphadenopathy characteristic of HIV and SIV infections. Tfh cells are directly responsible for B cell maturation and development in lymphoid follicles. Although Tfh signals are not necessary to induce B cell differentiation, Tfh cells are definitely required for production and release of high affinity plasma cells [134]. The fact that most HIV patients have abnormalities in antibody production, and fail to develop effective antibodies to control HIV, may also be linked to Tfh dysregulation. Since Tfh cells, as well as most of the target cells for initial infection (activated CD4+ T cells, Th17, Treg, Th1 *etc.*) reside in the intestine, it is little wonder that the intestine has been proposed to be the major reservoir for SIV in macaques controlling infection [135]. Since the major reservoir for persistent HIV in patients on ART has also been shown to be Tfh cells in lymphoid tissues [125], the intestine is also likely the major reservoir for HIV persistence in HIV patients on ART. In support of this, in SIV-infected macaques, the large intestine (containing abundant GALT containing numerous germinal centers and Tfh), has been shown to be the largest reservoir of latently infected

cells compared to small intestine or other lymphoid tissues [135]. Whether productively or latently inected cells can be targeted for eradication in the gut with novel treatment strategies are the focus of several ongoing investigations.

4.7. α4β7+ Cells and Mucosal Homing CD4+ T Cells

Of particular importance to mucosal immunology and HIV infection was the discovery that HIV can attach to and bind the mucosal homing molecule α4β7. Intestinal-specific homing markers such as the β7 integrins and the chemokine/chemokine receptor pair CCL25/CCR9 direct cells to specifically home to the intestine. The β7 integrins can associate either with the α4 or with the αE integrin subunit, and the resulting heterodimers have specific roles in the recruitment and retention of lymphocytes in gastrointestinal tissues [136]. For example, effector T-cell migration to the lamina propria is highly dependent on α4β7 interactions with the mucosal addresin cell adhesion molecule-1 (MAdCAM-1) expressed on lamina propria endothelium [137] whereas αEβ7 (CD103) is expressed by intraepithelial lymphocytes (IELs), and binds epithelial cell expressed E-cadherin, resulting in their migration to intestinal epithelium [138] which is why CD103 was originally considered a marker for intraepithelial lymphocytes. The GALT produces retinoic acid naturally through dendritic cells and likely other sources that prime T cells to express high levels of the gut homing receptors α4β7 and CCR9, predisposing the migration of recently activated T cells from the blood to the effector sites in the gut mucosa [139, 140]. Through this mechanism, homing of lymphocytes stimulated in inductive sites of the intestine (GALT) migrate to the mesenteric lymph nodes, then the circulation, but are directed to specifically home back to the intestinal tract [141]. Thus, HIV binding to α4β7 may result in efficient transport of virions to this target cell rich region where it may either infect the transporting cell itself, or other susceptible cells in the gut [142]. In addition, binding of HIV to α4β7 has been shown to induce cellular activation, which may result in upregulation of HIV receptors, and promoting viral transcription in infected cells. More recently, α4β7 has been shown to be incorporated into the envelope of HIV, which may serve as an additional mechanisms by which cell free HIV/SIV may home and target specifically to intestinal tissues [143]. Thus, the mucosal homing marker α4β7 may play a critical role in the mucosal pathogenesis of HIV infection and AIDS [142], findings that were strongly supported by subsequent studies in SIV-infected macaques treated with α4β7 blocking antibodies [144, 145]. α4β7-exressing CD4+ T cells are clearly major targets for early SIV and HIV infection, and the loss of α4β7 cells in the blood in primary SIV infection parallels the overall loss of intestinal CD4+ T cells in SIV-infected macaques }[146]. In fact, the rate of loss of α4β7+ CD4+ T cells is even greater than the rate of CD4+CCR5+ T cell depletion indicating gut homing cells are more permissive to infection and destruction [146, 147].

Studies have shown that blockade of α4β7 expression can reduce viral loads in SIV infected macaques [144, 145], and in a recent landmark study, treatment of SIV-infected macaques on ART with monoclonal antibodies against α4β7 was shown to result in complete control of viremia even months after withdrawal of all treatments [9]. Notably, virus was cleared from tissues examined, and most subsets of CD4+ T cells were eventually restored in the blood and peripheral lymph nodes even though this treatment was considered to be specific for mucosal homing cells [9]. Although the mechanisms of this “protection” are still under

investigation, conveivably, blocking the migration of infected cells (or virus) to and from the intestinal tract may have been sufficient to prevent re-seeding of systemic reservoirs, and eventual clearance of virus from tissues. Alternatively, or in addition, combined ART with blockade of α4β7 may have given the immune system time to develop effective responses to control the infection, although definitive immune correlates of protection remain to be discovered. If verified, these studies could have profound significance for future treatment and potential cure strategies in HIV patients.

5. INTESTINAL CD4+ T CELL RECONSTITUTION AFTER HIV INFECTION

When susceptible hosts become infected with pathogenic strains of SIV or HIV, marked and rapid intestinal CD4+ T cell depletion ensues. In SIV-infected macaques that will progess to AIDS, intestinal CD4+ T cells are never fully restored, resulting in opportunistic infections, usually in mucosal tissues. Although this depletion undoubtedly results in marked dysregulation of the immune system, this alone does not result in AIDS, as the few macaques that eventually become long term nonprogressors (LTNP), and certain species of non-progressing hosts show similar levels of early intestinal CD4 depletion yet they do not progress to disease [148, 149]. In fact, intestinal CD4+ T cell reconstitution has been shown to be the best prognostic indicator of whether an SIV-infected macaque will become a LTNP [149] although “cause and effect” have yet to be determined. In contrast, most macaques and HIV patients never fully restore intestinal CD4+ T cell levels to those of baseline controls, even when on highly effective ART that results in effective control of viremia.

Whether CD4+ T cells can be fully restored to basal levels, especially with the appropriate proportions of CD4+ Th cells reflective of the normal homestatic intestinal environment remains a subject of debate. Undoubtedly, restoration of CD4+ T cells in the blood occurs much faster than that of the intestine which may reflect different rates of reconstitution for specific cell subsets. In addition, early initiation of treatment clearly results in better restoration of mucosal CD4+ T cells than initiating treatment in the chronic stage of disease [150–152]. With rare exceptions, intestinal CD4+ T cell restoration in HIV patients on ART takes several years to accomplish, as several studies have demonstrated an inability to reconstitute intestinal CD4+ T cells to baseline levels even after 5 years of treatment [19, 153]. In addition, even though some CD4+ T cells may recover in the intestine, it appears that Th17 cells in particular have the least capacity to recover [123] resulting in persistent Th17 / Treg imbalance which sets the stage for abberant intestinal immune responses supportive of HIV persistence and replication [154, 155]. Interestingly, addition of probiotics, especially when combined with IL-2 in HIV patients on ART resulted in lower levels of intestinal inflammation and improved intestinal CD4+ T cell recovery, especially within the Th17 cell subset [156]. Thus, alterations in the intestinal microbiota may be beneficial in contributing to the restoration of mucosal CD4+ T cell subsets.

Alhough ART can raise peripheral CD4+T cell counts to the “normal” range, it is much less certain that ART can completely restore CD4+T cells in the gut [157]. While many studies have shown delayed or incomplete restoration after ART [153], other studies have suggested that complete restoration can be achieved, but only in experimental NHP models in which

treatment was initiated very early [152, 158, 159], or in rare HIV patients pre-selected for elite control of viremia on ART [160, 161].

The inability to fully restore mucosal CD4+ T cells on ART may be due to inadequate suppression of HIV replication and persistent immune activation in the gut [153]. Other explanations for the inability of the intestine to fully reconstitute CD4+ T cells include permanent damage to mucosal barriers such as fibrosis and collagen deposition, which may disrupt the ability of GALT to support normal cell-cell interactions, trafficking, and survival [162]. CD4+ T cell depletion in the terminal ileum, including Peyer's patches, is accompanied by extensive fibrotic damage and collagen deposition in chronic infection. This may be due to the fact that mucosal barrier dysfunction and activation are not restored by ART despite virologic suppression in the blood [155].

Clearly early initiation of ART improves responses to treatment and results in better restorartion of mucosal CD4+ T cells. Although some of this may be due to irreversible damage to the mucosal barriers and tissues, it also may be due to the loss of key precursor "stem" cells necessary to generate new or naive CD4+ T cell subsets. Determining the mechanisms behind the inabiltity to reconstitute specific subsets of mucosal CD4+ T cells in HIV patients may lead to improved treatment strategies.

5.1. Mucosal "Stem" Cells and Intestinal CD4+ T Cell Reconstitution: Is the Primate Gut a Primary Lymphoid Tissue?

Primary lymphoid tissues are where lymphocytes are formed and mature. These provide an environment for stem cells to undergo gene rearrangements and positive and negative selection to generate new T and B cells "de novo". For decades, only the bone marrow and thymus were considered to be primary lymphoid tissues in mammals. In contrast, lymph nodes, tonsils, spleen, Peyer's patches (GALT) and other mucosal associated lymphoid tissues were considered "secondary" lymphoid tissues, where naïve, yet mature lymphocytes are recruited and become "activated" or programmed upon antigen encounter. However, increasing evidence suggest that some mucosal tissues, at least the intestinal tract, may also function as a primary lymphoid tissue. If proven in humans, this would have important implications for understanding the hosts ability to reconstitute intestinal CD4+ T cells, as well as implications for new treatment strategies.

Evidence for the intestine as a primary lymphoid organ was first demonstrated in birds which have the bursa of Fabricus in the cloaca (equivalent to rectum), which is responsible for their generation of B cells (reviewed in [163]. Similarities between the sacculus rotundus of rabbits and the bursa of birds originally prompted some to consider this massive B cell rich region of the rabbit intestine to be a primary lymphoid organ as well [163, 164]. More recently however, and with new technologies, the concept that GALT may contain primary lymphoid tissues capable of replenishing B cell follicles and possibly T cells is re-emerging. The ileal Peyer's patches in sheep, cattle, swine, horse, and rabbits have now been shown to be a primary site of lymphocyte generation, at least for B cells and some of these species populate their entire peripheral B-cell compartment primary using the GALT [165]. Thus, at least the ileal Peyer's patches of several mammalian species have now been shown to be primary sites of lymphocyte generation, suggesting that at least for B cells, key stem cells

and precursors reside in the gut which need to be protected for proper immune reconstitution after injury or assault. Notably, HIV has been shown to rapidly induce GALT follicular damage and germinal center loss especially in the terminal ileum Peyer's patches of HIV patients in the earliest stages of HIV infection [69]. Conceivably, such rapid targeting of ileal Peyer's patches may be responsible for the rapid polyclonal hyperactivation, as well as the delay in plasma antibody responses to HIV [69].

Whether CD4+ T cells can also be generated from stem cells in the gut of primates remains debated. Intestinal stem cells that re-generate mucosal epithelial cells have been well characterized [166]. However, accumulating evidence indicates that GALT or isolated lymphoid follicles containing T and B cells can be created in the intestine of mice by "cryptopatches" which are small clusters of lymphocytes which include key stem cells termed lymphoid tissue inducer (LTi) cells. These Lti are a distinct population of innate lymphoid cells subtype 3 (ILC3) that develop into solitary B-cell containing lymphoid follicles, complete with the Tfh cells that regulate antibody development and maturation. It remains to be shown how they originate, persist, and to what extent they can develop into larger aggregates (Peyer's patches). In is also of interest to determine if these can re-generate primary and secondary intestinal lymphoid tissues, and especially CD4+ T cells following the massive intestinal CD4+ T cell depletion characteristic of SIV/HIV infections.

Clearly, intestinal CD4+ T cells play a central role in the pathogenesis of HIV infection, and their preservation or replenishment after HIV infection will likely prove fundamental for viral clearance in treatment and cure strategies. Understanding the mechanisms by which the intestine can regenerate mucosal CD4+ T cells is thus of critical importance. In mice, cryptopatches (CP) were shown to be responsible for the generation of organized lymphoid tissues in the intestine, and were first identified as potential site of extrathymic T cell differentiation [167]. However, this remains controversial, as some data suggest this process occurs only under the setting of significant immunodeficiency [168]. Nonetheless, lineage negative, $ckit^+$ cells have been found in the lamina propria of human [169] and macaque intestine [6], indicating primates do have intestinal cryptopatches containing Lti cells.

Interestingly, Lti cells are lineage negative, c-kit+ cells which, like Th17 cells, are regulated by RORγt expression. In other words, the same stimuli that promote Th17 cell differentaion and proliferation may also induce Lti cells to generate new lymphoid tissues. This is further evidence that the mucosal immune system may replenish key structures and cells independently of systemic immune signals. Since infection and depletion of Th17 cells appears to be fundamental to the pathogenesis of HIV infection and AIDS, preserving or repopulating these cells, as well as the organized lymphoid tissues in the intestine, may be an effective adjunct strategy for new HIV therapies.

Finally, a subset of memory T cells with stem cell–like properties (T_{SCM}) has been identified [170] that appears to correlate with rates of CD4+ T cell reconstitution [171]. These cells are the least differentiated of all distinct memory populations, expressing multiple naive markers but also the memory antigen CD95 [170]. Functionally, T_{SCM} cells can generate into multiple memory CD4+ T cell populations, and they are capable of self-renewal [170, 172]. Further, these cells also co-express CCR5 and are susceptible to HIV infection, and may

play a role as a long term reservoir in HIV patients on ART [172]. Direct infection and loss of Tscm occurs in SIV-infected macaques, compared to nonprogressing host species in which Tscm display relative resistance to infection, suggesting that their infection and loss may play a major role in the pathogenesis of AIDS [172]. Additional studies are needed to determine whether the depletion of these cells in intestinal tissues is largely responsible for the failure to replenish mucosal CD4+ T cells, and/or whether intestinal LTi and/or Tscm cells may be stimulated by specific modulators to promote effective CD4+ T cell reconsitituion after HIV infection.

CONCLUSIONS AND FUTURE DIRECTIONS

In summary, SIV and HIV selectively infects, and destroys several key subsets of CD4+ T cells in the intestinal tract within days of infection, resulting in a massive depletion of intestinal CD4+ T cells. The first cells to be targeted and eliminated appear to be Th17/Th22 cells, resulting in rapid damage to mucosal barriers which permits intestinal luminal antigens and bacteria to enter the lamina propria, and subsequently the systemic circulation, resulting in continued mucosal damage, and the generalized systemic immune activation that drives HIV persistence and replication [121].

The selective loss of Th17, Th22, Th1, and other key subsets also results in alteration of the fine tuned Th17/Treg balance that normally limits overactive immune responses to innocuoous antigens, which further drives mucosal inflammation and local viral replication [99, 112, 113]. Restoration of the Th17 and/or Th22 cells in particular may be the key to restoring the normal homeostatic functions of the gut, and possibly the immune system in general in HIV patients [112]. Other key regulatory CD4+ T cells are infected and depleted in SIV/HIV infection including most of the activated, CCR5-expressing, α4β7+, and pro-inflammatory Th1 cells. The propensity for HIV to infect and eliminate activated, functional CD4+ T cells may be one of the major difficulties in designing an affective vaccine. Since HIV “prefers” activated CD4+ T cells for infection and replication, standard vaccine strategies that proved successful against other viruses sometimes induced inflammatory responses favorable to viral transmission [173, 174].

Although HIV-induced intestinal CD4+ T cell depletion is not necessarily irreversible, treatment with ART that completely suppresses viral replication in blood for years does not result in complete restoration of intestinal CD4+ T cells, or restore the mucosal and systemic environments to baseline levels, suggesting active ongoing viral replication or mucosal damage may persist in mucosal tissues despite suppressive therapy. Initiating treatment very early after infection clearly results in better reconstitution of mucosal CD4+ T cells. Thus, irreversible damage to mucosal tissues, and/or loss of key stem cells necessary to reconstitute CD4+ T cells in tissues may underlie the inability to replenish CD4+ T cells in HIV patients if treatment is substantially delayed.

However, a better understanding of the intricacies of the systemic and mucosal immune system, and specifically the mechanisms by which specific cells are induced or suppressed, may provide new drug targets and strategies for HIV treatments and vaccine design. For example, treatments with anti- α4β7 or anti-HIV antibodies may prevent trafficking of

infected cells or intact virions from entering the intestine, and possibly prevent the reseeding of tissue reservoirs that are less accessible to drug treatments, such as the germinal centers of lymphoid follicles and Peyer's patches. Similarly, treatments designed to stimulate specific transcription factors such as RORγt or Bcl6 to promote Th17 and Tfh cell reconstitution, or function may also improve patient outcomes. In additon, specific transcription factor activators might be used to re-activate latently HIV-infected cells in intestinal reservoirs for cure strategies [175]. The rapid advancements in sequencing and data analysis, combined with our increasing understanding of the complexities of the immune system in animal models, are poised to result in unprecedented scientific advances, and improved preventions for HIV and many other infectious diseases.

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