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Innate Gamma/Delta T-Cells during HIV Infection: *Terra* Relatively *Incognita* in Novel Vaccination Strategies?

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Abstract

Despite a long-lasting global effort, the Holy Grail quest for a protective vaccine, able to confer prevention to HIV infection, did not reach the hoped for results, nor seems able to do so in the near future. Since mucosal surfaces of the host serve as the main entry point for HIV, it seems now logical to switch from a systemic to a localized view of events, in order to reveal critical steps useful in designing new and different vaccination strategies. In this context, the recent description of the very early phases of infection, from the eclipse to the viremia peak phase, seems to define a point-of-no-return threshold after which the main HIV infection steps, i.e. the massive destruction of the $CD4^+CCR5^+$ cell pool, the destruction of the mucosal physical barrier, and the establishment of reservoir sanctuaries, have already been accomplished. Nevertheless, the underlying mechanisms, the timing, and the consequences of evasion mechanisms exploited by HIV are still under scrutiny.

Innate immunity, as part of a rapid lymphoid stress surveillance system, is known to play a central role in host responses to many infectious agents. In particular, $V\gamma9V\delta2$ T-cells are able to quickly respond to danger signals without the need for classical major histocompatibility complex presentation, and may act as a bridge between innate and acquired arms of immune response, being able to kill infected/transformed cells, release antimicrobial soluble factors, and increase the deployment of other innate and acquired responses.

Many experimental evidences suggest a direct role of circulating $V\gamma9V\delta2$ T-cells during HIV disease. They may exert a direct anti-HIV role by secreting chemokines competing for HIV entry coreceptors as well as other soluble antiviral factors, and by killing infected cells by cytotoxic natural killer-like mechanisms. Moreover, they were found progressively depleted and anergic in advanced stages of HIV disease, this effect being directly linked to uncontrolled HIV replication.

Scarce evidences are available on the involvement of mucosal gamma/delta T-cells during the early phases of HIV infection. In particular, the relative cause/effect links between HIV infection, destruction of the mucosal physical barrier, nonspecific activation of the immune system, and mucosal innate cell activation and effector functions, are still not completely defined.

In order to attain an effective manipulation of innate immune cells, aiming at the induction of an effective adaptive immunity against HIV, any information on the role of mucosal antiviral factors and innate immune cells will be very important. The aim of this review is to summarize the information on

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the role of gamma/delta T-cells during HIV infection, from the general circulating population to mucosal sites, in order to better describe areas deserving increased attention. In particular, strategies enhancing gamma/delta T-cell functions may open the possibility to formulate new immunotherapeutic regimens, which could impact the improvement of immune control of HIV disease. **(AIDS Rev. 2011;13:3-12)**

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Key words

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Introduction

A much needed and intensely performed effort, aiming at the development of a protective vaccine able to confer prevention of HIV infection, gave disappointing[1] or, more recently, modest results[2], underlining the need for the application of a coordinated research approach.

It is now clear that at host mucosal surfaces, which are the main entry point for HIV, a decisive confrontation occurs between pathogen and host defenses. Indeed, it is now clear that the very early phases of infection, from the eclipse phase to the viremia peak phase, define a point-of-no-return threshold after which the main HIV infection steps, i.e. the massive destruction of the $CD4^+CCR5^+$ cell pool, the destruction of the mucosal physical barrier, and the establishment of reservoir sanctuaries, have already been accomplished[3].

Innate immunity, as part of a rapid lymphoid stress surveillance system, is known to play a central role in early host responses to many infectious agents by coordinating the full deployment of antimicrobial defenses[4]. Among innate immune cells, gamma/delta T-cells bearing the Vγ9Vδ2 T-cell receptor (TCR) are believed to play a critical role, being able to link innate and acquired immune responses[5,6]. Even though Vγ9Vδ2 T-cells represent a major constituent of gut-associated lymphoid tissue, particularly among lamina propria lymphocytes, scarce evidences are available on their involvement during the early phases of HIV infection[7]. In particular, the relative cause/effect links between HIV infection, destruction of the mucosal physical barrier, nonspecific activation of the immune system, and mucosal innate cell activation and effector functions, are still not completely defined.

Understanding the role of innate immunity in the induction of a protective immune response to HIV could allow the definition of new vaccination strategies[8,9].

Gamma/delta T-cells as bridges between innate and acquired arms of immune response

Possible clues about the presence of a different class of antigen-specific T-cells besides alpha/beta T-cells went from studies of TCR genes in mice, where a third V-gene segment cluster, described as Vγ after Vα and Vβ, was found[10]. Shortly after that, a fourth TCR gene cluster (Vδ) was found in humans; moreover, T-cells bearing the Vγδ heterodimer TCR were identified as displaying it in association with the CD3 complex, but with no CD4 or CD8 molecules[11,12]. Thereafter, many different subtypes (Vδ1-5) of gamma/delta T-cells were found among circulating gamma/delta T-cell pools, associated with many different Vγ chains. Under normal conditions, Vδ2 is the most represented subtype, invariably associated to Vγ9 chain, followed by Vδ1 subtype, and associated to many different Vγ chains[13].

In humans and other primates, antigen-specific $CD3^+$ T-cells using a Vγ9Vδ2 TCR represent a small percentage among peripheral blood lymphocytes (< 5%). With respect to $CD3^+$ T-cells using alpha/beta chains, they display unique characteristics: alpha/beta T-cells mostly respond to protein-derived peptides, recognized on antigen-presenting cells within major histocompatibility complex (MHC) class I or class II presentation; in contrast, Vγ9Vδ2 T-cells respond to non-protein stress-related molecules, apparently without the need for antigen-presenting cell

presentation[4,14]. Moreover, while the alpha/beta T-cell pool is composed by many different antigen-specific clones, gamma/delta T-cells in primates expand during early life in response to autologous triggering signals, driving limited clonotype variability; as a result, cells displaying a Vγ9Vδ2 TCR represent a vast majority within circulating gamma/delta T-cell pool[15].

Upon activation, Vγ9Vδ2 T-cells are able to mediate many different effector functions. By secreting T-helper type 1 (Th1) cytokines, such as tumor necrosis factor alpha (TNF-α) and gamma interferon (IFN-γ), activated Vγ9Vδ2 T-cells were found able to directly induce monocyte-derived dendritic cell maturation and activation, suggesting a potential adjuvant role of this cross-talk in enhancing antigen-specific alpha/beta T-cell responses[16,17]. By releasing monocyte chemotactic protein-2 (MCP-2), they may recruit and activate neutrophil phagocytes able to release antimicrobial factors such as alpha-defensins, thus contributing to a local activation of innate immunity mechanisms[18]. They release many different cytokines/chemokines, able to drive other immune cells' activation[19]. Finally, they are able to mediate natural killer (NK)-like cytotoxicity functions against infected or transformed autologous cells[20-22]. Interestingly, their NK-like activity was found regulated by the very similar mechanisms, including the "missing self" strategy, used by classical NK cells[23-25].

Shortly after being identified, Vγ9Vδ2 T-cells were found expanded after M. tuberculosis[26] and ehrlichiosis[27] infections. The non-proteic nature of the phosphoantigens present in M. tuberculosis, able to trigger Vγ9Vδ2 T-cells, was rapidly defined[28-30], and the recognition mechanism was found very different from alpha/beta T-cells, as Vγ9Vδ2 T-cells directly recognize phosphoantigens, without any antigen processing and MHC class I or II presentation[31]. Recognized antigens are small phosphorylated molecules, such as isopentenyl pyrophosphate, normally present within eukaryotic cells as an intermediate of the mevalonate-cholesterol metabolic pathway, that could be used as danger signals by stressed-transformed or infected cells[14].

As recently proposed[4], Vγ9Vδ2 T-cells may constitute a relevant part of the lymphoid stress-surveillance system: they are an "activated yet resting" circulating lymphocyte population, able to quickly and effectively respond to danger signals without the need for antigen processing and presentation. Stress surveillance is not compatible with a delayed response following clonal expansion. Indeed, Vγ9Vδ2 T-cells outnumber among circulating lymphocytes any single alpha/beta T-cell clone, thus making clonal expansion or *de novo* differentiation unnecessary; their huge number, and the possibility to be polyclonally activated by stress signals, makes them key actors of immune response by linking innate and acquired arms of the immune system[13].

Gamma/delta T-cell involvement during HIV infection

Many experimental evidences suggest a direct role of Vγ9Vδ2 T-cells in HIV disease. Shortly after the discovery of gamma/delta T-cells, an inversion in the Vδ2 to Vδ1 ratio was found in peripheral blood mononuclear cells from HIV-infected persons in comparison to healthy donors[32]. This inversion was due to a loss of circulating Vγ9Vδ2 T-cells in AIDS and p24-antigenemic HIV-infected patients[33], related to an apoptosis mechanism[34]. On analyzing HIV-infected patients at different disease stages, it was found that the circulating $CD4^+$ T-cell count was inversely correlated with the activation capability of Vγ9Vδ2 T-cells, evaluated by human leukocyte antigen D-related expression[35]. Recent data on subjects infected by HIV through blood transfusions confirmed the significant correlation between Vγ9Vδ2 T-cell numbers and disease progression from both immunological and virologic points of view[36]. Moreover, the remaining Vγ9Vδ2 T-cells were found anergic and unable to expand after TCR stimulation[37,38]. This anergy was defined as one of the first HIV-related immune evasion events, being present also in asymptomatic patients, preceding any $CD4^+$ T-cell loss[39]. Since Vγ9Vδ2 T-cells are very similar in humans and other primates, similar data were obtained in simian immunodeficiency virus (SIV)-infected rhesus monkeys[40,41].

The Vγ9Vδ2 T-cells unable to proliferate upon phosphoantigen stimulation were also shown unable to perform their effector function, with a reduced production of IFN-γ and TNF-α[5,42,43]. Moreover, anergy was greater in advanced AIDS patients, and was at least partially recovered after HAART[44]. Interestingly, anergy was associated to a loss of specific effector cell subpopulations, and could be due to a differentiation block in an immature stage[45]. The molecular mechanisms causing anergy to TCR triggering are

still under scrutiny; however, a decreased expression of CD3ζ TCR-associated molecule was recently shown in HIV-infected patients[46], similar to what is known on CD4 and CD8 alpha/beta T-cells during HIV infection[47]; differently from alpha/beta T-cells, however, CD3ζ expression could not be restored by overnight incubation with interleukins IL-2 or IL-15, while it could be restored by direct protein kinase C activation[46]. An exposure to proinflammatory cytokines inducing CD3ζ down-modulation has been proposed as a possible mechanism of T-cell anergy[48]; since one of the earliest consequences after HIV infection is a cytokine storm including IL-15, IFN-α and TNF-α release[49], it is reasonable that this mechanism could also induce Vγ9Vδ2 T-cell anergy. Finally, an explanation for Vγ9Vδ2 T-cell loss and anergy may include "negative co-stimulators" such as PD-1, known to control alpha/beta effector CD8 T-cell expansion in chronic virus infections that could also regulate gamma/delta T-cell expansion after HIV infection[4].

The dynamics of Vγ9Vδ2 T-cells in relation to HIV replication were studied in the HAART interruption model. Vγ9Vδ2 T-cell loss and anergy were found to be strictly dependent on acute HIV replication, and could be partially recovered after stopping virus replication with HAART reintroduction[50]. Moreover, HIV infection was found able to deeply affect the Vγ9Vδ2 T-cell repertoire, since loss was specific for Jγ1.2 subset, known to include most phosphoantigen-reactive cells[51]. How or if HAART could induce Vγ9Vδ2 Jγ1.2 repertoire recovery is still unresolved[52,53]. Interestingly though, in a group of HIV-infected patients who suppressed HIV replication without antiretroviral therapy, increased Vγ9Vδ2 Jγ1.2 cells were very recently found[54].

Vγ9Vδ2 T-cells can exert direct and bystander antiviral activity[55,56]. Notably, IL-2 expanded gamma/delta T-cells from primates were able to lyse SIV-infected target cells by NK-like mechanisms[57], and this antiviral protective capability was found also in Vγ9Vδ2 T-cells from healthy humans[58]. The close similarity between Vγ9Vδ2 T-cells and NK cells was underlined by a similar use of activator and inhibitory receptors, controlling their cytotoxic effector function[23]. More importantly, activated Vγ9Vδ2 T-cells may directly suppress HIV replication by releasing CC-chemokines, competing with HIV for CCR5 entry coreceptor, and other soluble antiviral factors[56,59]. Similar data were obtained on mucosal gamma/delta T-cells from macaques infected by SIV by a rectal mucosa route[55]. Interestingly, an increased ability of Vγ9Vδ2 T-cells to secrete Th1 cytokines was found after non-pathogenic SIV infection of sooty mangabey monkeys, suggesting a direct relationship between this capability and the absence of clinical manifestations[60].

May gamma/delta T-cells be targeted as immunomodulating agents during HIV infection?

Gamma/delta T-cells emerged as playing a major role in immuno-protection and immunoregulation, as summarized in figure 1. Their potential as a target for immunomodulating strategies was fully recognized when aminobiphosphonates, drugs already in use to reduce osteoclastic bone resorption, were found able to induce *in vivo* Vγ9Vδ2 T-cell expansion[61], and when safe and effective ways aiming to induce their activation *in vivo* by phosphoantigens or aminobiphosphonates, with or without cytokines, were developed[62-65]. This approach has recently been used against myeloma, carcinoma, and lymphoma tumors[66,67]. Interestingly, gamma/delta T-cell reconstitution was found as the major determinant of graft-versus-leukemia activity in patients receiving T-depleted bone marrow grafts[68]. Two different strategies were defined as new immune-based treatment strategies for tumors by Vγ9Vδ2 T-cell activation. Drugs could be given *in vivo*, able to induce Vγ9Vδ2 T-cell activation[69]. Differently, Vγ9Vδ2 T-cells could be expanded by *in vitro* stimulation and adoptively transferred[70]. In effect, a relevant antitumor effect of Vγ9Vδ2 T-cell activation *in vivo* has been demonstrated, confirming the feasibility of a new therapeutic approach for translation into clinical settings[71].

Vγ9Vδ2 T-cell antimicrobial reactivity, and the ability to produce inflammatory cytokines involved in protective immunity against intracellular pathogens, suggested their use also for infectious diseases as a target for new immuno-mediated treatment strategies[72,73]. Different studies were designed, aiming at the study of immune correlates of protection induced by Vγ9Vδ2 T-cell *in vivo* stimulation in HIV infection. In a study on simian HIV-infected macaques[74], phosphoantigen plus IL-2 was able to induce Vγ9Vδ2 T-cell expansion *in vivo*, correlated to HIV Env-specific antibody titres, as well as CD4 and CD8 alpha/beta T-cell activation, driving the release of antimicrobial cytokines. In a different study on HIV-infected humans[75], zoledronate plus IL-2 treatment was able to induce *in vivo* Vγ9Vδ2

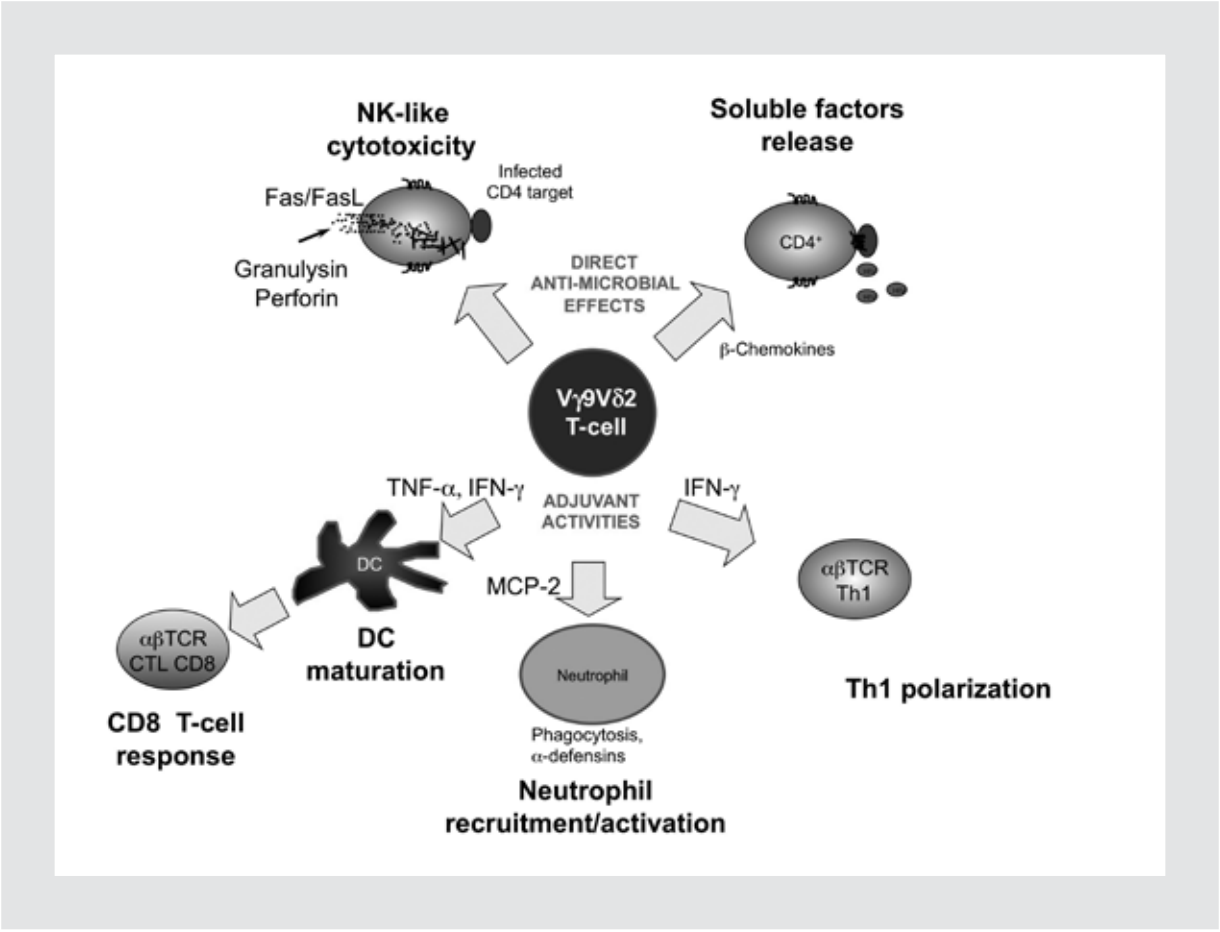

Figure 1. *Pleiotropic immune activities of Vγ9Vδ2 T-cells. NK: natural killer; TNF: tumor necrosis factor; IFN: interferon; MCP: monocyte chemoattractant protein; FasL: Fas ligand; CTL: cytotoxic T-lymphocyte; DC: dendritic cell; Th1: T-helper type 1.*

T-cell expansion and differentiation. Moreover, paralleling Vγ9Vδ2 T-cell activation, increased dendritic cell maturation and HIV-specific CD8 T-cell responses were found, suggesting a possible adjuvant role of zoledronate and IL-2 treatment in restoring both innate and specific competence in HIV-infected persons. Thus, effective tools allowing increased immune competence through the enhancement of Vγ9Vδ2 T-cell function during HIV infection are now available[76-80].

Do mucosal gamma/delta T-cells play a role in mucosal sites during very early steps of HIV infection?

As a major component in the mucosal immune system, gamma/delta T-cells deserve major attention as possible key players in early HIV-induced events. Despite the fact that gamma/delta T-cells, and particularly Vγ9Vδ2 T-cells, represent a major component among lamina propria lymphocytes, scarce evidences are available on their role in this early, decisive phase of infection. A contraction of the mucosal Vγ9Vδ2 T-cell population was found in parallel to a circulating pool in chronically infected treated patients despite successful treatment[7,81]. Interestingly, a lower level of mucosal gamma/delta T-cells was found associated to short survival expectancy in advanced AIDS patients[82]. The mechanism and the timing of mucosal Vγ9Vδ2 T-cell loss, and particularly its possible link with mucosal $CD4^+CCR5^+$ T-cell loss, are not yet clear. Vγ9Vδ2 T-cell specific loss has been attributed to activation-induced cell death by apoptosis[83], which in this case could be secondary to massive HIV-induced activation, and migration to secondary lymph nodes. On the

other hand, reports on productive infection of Vγ9Vδ2 T-cells by HIV have been only anecdotal[84]; a secondary effect, concomitant to human herpes virus 6 coinfection, was proposed[85], which in any case cannot be described as an explanation for the loss in the early phases of HIV infection.

Given their multiple capabilities, mucosal Vγ9Vδ2 T-cells could play a relevant role in the early phases of infection. Interestingly, a relevant role of mucosal gamma/delta T-cells shortly after oral SIV infection in macaques was found, since this induced a rapid and significant increase of mucosal gamma/delta T-cells, paralleled by an increase of lymphoid homing receptors, and preceding any change in CD4 T-cells, thus suggesting a rapid change in the mucosal innate compartment at the earliest times post infection[86]. Moreover, by studying macaques immunized with SIV gp120 and p27 in alum, and challenged with live SIV by the rectal mucosal route, protected animals showed an increased frequency of mucosal gamma/delta T-cells in comparison to infected animals. Moreover, gamma/delta T-cells produced antiviral factors, such as Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES), Macrophage Inflammatory Protein (MIP)-1α and MIP-1β, which could prevent SIV infection in mucosal sites by competing for the CCR5 coreceptor[55].

One of the first damages at mucosal sites after HIV infection affects T-helper type 17 (Th17) cells, since they are preferentially lost in HIV/SIV-infected hosts with progressive disease[87-89], but not in non-progressing hosts such as sooty mangabeys[88] or African green monkeys[87]. Numerous lines of evidence suggest that the mucosal barrier function is orchestrated by a subset of cytokines (IL-17 and IL-22), which belong to the Th17 family. Both IL-17 and IL-22 induce expression of antimicrobial peptides and neutrophil chemoattractant at mucosal sites, thus playing an important role in controlling mucosal infections[90]. Moreover, Th17 cells were shown depleted after SIV infection, thereby impairing mucosal barrier resistance to Salmonella typhimurium dissemination[91].

Interestingly, in HIV-infected patients, increased serum levels of neopterin and β2-microglobulin, reflecting immunostimulation, were found inversely related to duodenal gamma/delta intraepithelial lymphocytes, suggesting a possible role of these cells in limiting nonspecific immune stimulation[82]. Indeed, a rapid cross-talk between activated Vγ9Vδ2 T-cells and monocytes was found able to induce Th17 cell activation, which in turn recruit and activate neutrophils; this suggests a direct link between invading pathogens, microbe-responsive Vγ9Vδ2 T-cells, and monocytes in the inflammatory infiltrate, which could play a crucial role in early response and in the generation of microbe-specific immunity[92]. Since Vγ9Vδ2 T-cells, similarly to Th17 cells, are able to directly activate neutrophils through MCP-2 release[18], their improvement could play a major role in the mucosal immune response by preventing systemic microbial dissemination from the gastrointestinal tract in the early phases of HIV infection.

Open questions and conclusions

Early facts associated to mucosal transmitted HIV infection are of paramount importance in defining the following course of events[3,9,93]. Natural antiviral factors and cells, such as Vγ9Vδ2 T-cells, are now recognized as critical in these very early events, given the high number of these cells in mucosa and how easily they can be activated. Recent data on the confrontation between HIV and the immune system in the first days after infection show that anti-HIV CD8 T-cell responses are causally linked to the decline of viral load in acute infection by killing productively infected cells, suggesting that a quick, wide breadth CD8 response could possibly limit HIV infection and still avoid the emergence of virus escape variants[94]. Interestingly, mucosal polyfunctional CD8 T-cell responses have been described as a potential correlate of immune control in elite controller patients, able to spontaneously control HIV replication in the absence of therapy[95]. The unique capability of Vγ9Vδ2 T-cells to be activated by quickly recognizing stress signals, and to mediate both an adjuvant role on innate response recruitment and CD8 T-cell activation, and a direct role in secreting antiviral molecules and in killing infected cells, makes them very relevant in the early events course[76].

Any possibility to strengthen host responses relies on the precise depicture of host-pathogen confrontation in order to overcome pathogen-induced evasion strategies. Unfortunately, many different questions about the role of Vγ9Vδ2 T-cells during early HIV infection are still not answered, as summarized in table I.

First of all, it is not clear where and when the massive loss of Vγ9Vδ2 T-cells occurs, and if circulating Vγ9Vδ2 T-cell loss and anergy precedes, is concomitant, or is secondary to the massive $CD4^+CCR5^+$

Table 1. Open questions on the role of Vγ9Vδ2 T-cells in mucosal early events associated to HIV infection

- Where and when does Vγ9Vδ2 T-cell loss occur?
- Which HIV-related mechanisms are responsible for Vγ9Vδ2 T-cell depletion?
- Is Vγ9Vδ2 T-cell depletion related to lamina propria CD4 T-cell loss?
- Could Vγ9Vδ2 T-cell antiviral action be enhanced, and how could this affect the early phases of HIV infection by targeting HIV-infected cells and interfering with virus dissemination?
- Could adjuvant action of Vγ9Vδ2 T-cells allow the generation of protective antiviral CD8 T-cell response?
- Can Vγ9Vδ2 T-cells exert a protective role against bacterial translocation process by inducing neutrophil recruitment and activation?

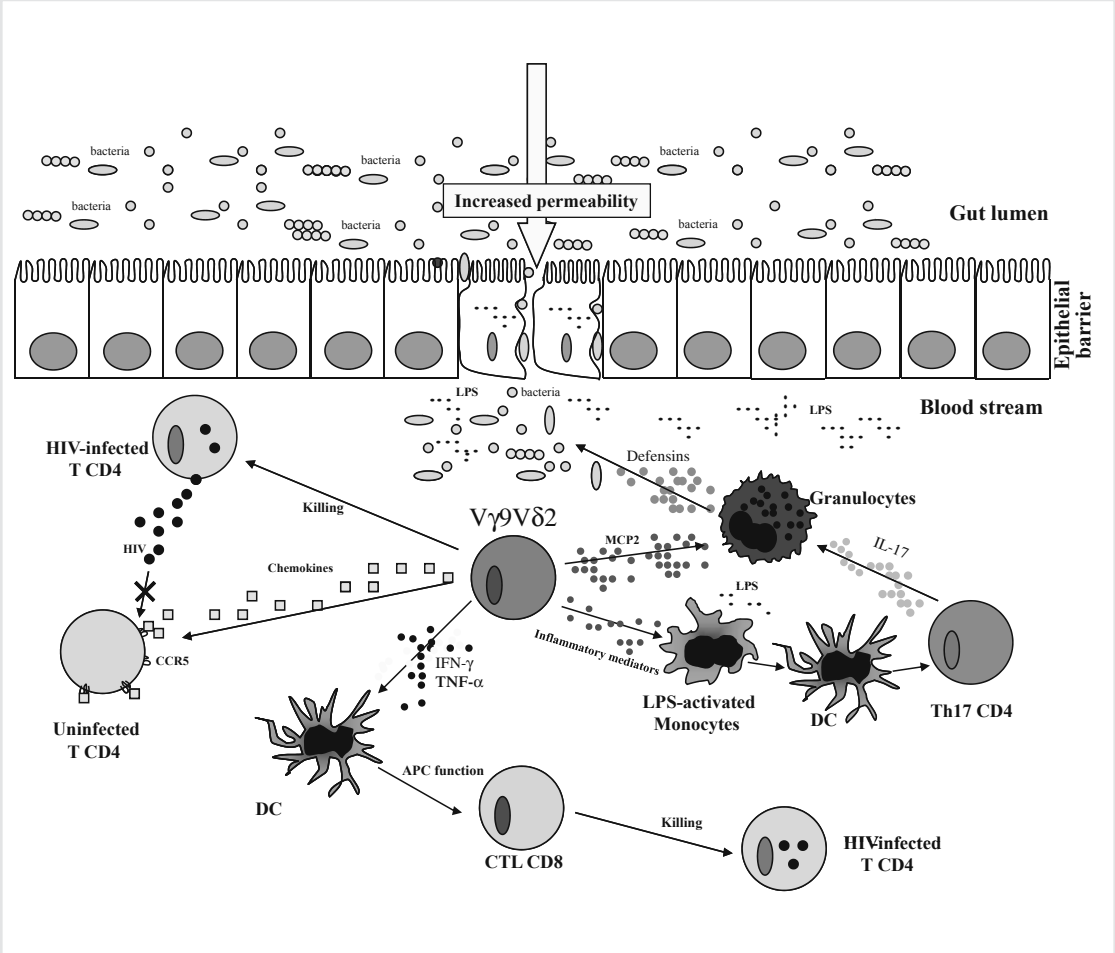

Figure 2. *How Vγ9Vδ2 T-cells at mucosal sites could affect the early phases of HIV infection. DC: dendritic cell; CTL: cytotoxic T-lymphocyte; APC: antigen-presenting cell; LPS: lipopolysaccharide; TH17: T-helper type 17.*

T-cell loss in lamina propria[96]. Moreover, the HIV-driven mechanism(s) responsible for anergy and loss are not clearly defined. How Vγ9Vδ2 T-cell activation in mucosal sites could affect the early phases of HIV infection is not known, and neither is their effect in enhancing mucosal protective CD8 T-cell response. Finally, the relation between Vγ9Vδ2 T-cell activation and damage to gastrointestinal mucosal integrity, leading to local generalized activation of the immune system and increased susceptibility to infection, is not known[97].

Regarding this, several different possible roles of Vγ9Vδ2 T-cells may be depicted, as shown in figure 2. First of all, Vγ9Vδ2 T-cells could quickly recognize HIV-infected cells by stress-surveillance mechanisms, driving a directly protective antiviral role by quickly releasing chemokines, interfering with HIV entry, and killing infected cells by NK-like mechanisms. Secondly, Vγ9Vδ2 T-cells could improve innate antimicrobial responses by recruiting and activating neutrophils by MCP-2 release[18], or by indirectly inducing Th17 cell activation[92], thus limiting the microbial translocation-immune activation pathogenesis loop[93]. Finally, Vγ9Vδ2 T-cells could allow the fast generation of an increased protective anti-HIV CD8 response to transmitted/founder virus, strong enough to avoid the emergence of escape virus mutants[94].

It is now clear that the very early phases of HIV infection in mucosal sites are decisive in defining a point of no return threshold[3,93]. In this scenario, the fast innate response of Vγ9Vδ2 T-cells could tip the balance between virus escape mechanisms and protective host immune response by directly targeting infected cells, by enhancing acquired CD8 T-cell response, and by limiting generalized immune activation caused by microbial translocation[9].

A better depiction of the role of Vγ9Vδ2 T-cells could therefore give new weapons to correct the disadvantaged equilibrium between HIV and the host immune system.

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