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Role of Dendritic Cells in Immunopathogenesis of Human Immunodeficiency Virus Infection

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INTRODUCTION

Dendritic cells (DC) are the most potent antigen-presenting cells (APC) of the immune system. They are critical in the initial activation and recruitment of T cells during immune responses (17, 43, 76, 135). Although most APC can present antigen to and activate memory T cells, DC almost exclusively initiate primary immune reactions involving naive T cells (18, 49, 67, 78, 79, 135, 140). They populate most tissues in the body, especially the skin and squamous mucosal epithelium, the sites that are exposed to the environment. Because DC represent a very small percentage of the cells at a given site (e.g., epidermis, 2 to 4%; blood, 0.1 to 0.5%), the study of them has been extremely difficult (reviewed in references 7 and 35). Much of the modern knowledge about the antigen-presenting function of the DC family comes from the pioneering work of Ralph Steinman and his colleagues (136). Multiple populations of DC have been identified, including lymphoid interdigitating DC; Langerhans cells (LC), the prototypic nonlymphoid, immature DC; and veiled cells of afferent lymph. The main function of DC is to transport antigens, likely both self and nonself, from their site of entrance into the body to the paracortical (T-cell) region of the draining lymphoid organ. DC then initiate an immune response through the activation of antigen-specific T cells which continually recirculate through lymphoid organs. It is this function that makes DC potentially important players in the pathogenesis of human immunodeficiency virus (HIV) infection. Other reviews have discussed the potential role of DC dysfunction in the pathogenesis of HIV disease, which we will only briefly address (9, 14, 22, 27, 60). In this review, we concentrate on the role of DC in the initiation and propagation of viral replication, particularly in the context of future directions of research and therapeutic strategies.

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LIFE CYCLE OF DC

DC are derived from bone marrow progenitor cells (19, 54, 120, 138, 144). In the presence of cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 4 (IL-4), tumor necrosis factor alpha (TNF-α), and stem cell factor, $CD34^+$ cells, which can be isolated from bone marrow, cord, or peripheral blood, are capable of developing into functional DC. They may be identified by the expression of CD1a (a marker of LC and thymocytes); CD83 (a marker found on lymphoid, thymic, and peripheral blood DC; nonlymphoid DC; and LC); high levels of class II major histocompatibility complex (MHC) antigen, adhesion, and costimulatory molecules; and the ability to stimulate allogeneic cells in culture (16, 115, 126–128, 158, 159). At present, there are no totally specific antigens on DC that allow for easy identification. In many of the experimental systems used to study DC derivation from bone marrow progenitor cells, mixed colonies of DC, monocytes, and sometimes granulocytes were observed, suggesting a common precursor for all these cell types (16, 126–128). DC precursors have been isolated from peripheral blood. These cells are typically round and multinucleated, have moderate levels of human leukocyte antigen DR, are CD13 and CD33 (early myeloid markers) positive, and have low or absent expression of CD14. When left in culture, they differentiate into classic DC, with an increase in their expression of MHC class II, adhesion, and activation antigens, including CD80, CD86, and CD40; they express CD83 (158, 159); and they develop dendritic processes (48, 50, 141, 150). It has been theorized that these precursor cells have exited from the bone marrow and are migrating to tissues in order to become tissue DC. LC and other tissue DC may remain in the tissues for short periods (hours to days) or long periods (e.g., LC) (137, 146). An inflammatory reaction in response to a local antigenic challenge or other stimuli causes DC to migrate via the afferent lymph vessels, where they are known as afferent veiled cells, to draining lymph nodes (135); however, a proportion may also travel in the blood (66). Once in the lymph node, they become part of the network of interdigitating DC in the paracortical region, where they interact with and activate T cells (reviewed in references 135 and 137).

A second source of DC has also been identified. Peters and

coworkers originally reported that in the absence of serum or with adenosine modulation or IL-4 treatment, $CD14^+$ monocytes can develop a dendritic morphology and an increased allostimulatory potential (39, 85, 89, 100–103, 122, 154). Additional studies by Sallusto and Lanzavecchia and Weissman and Fauci demonstrated that $CD14^+$ monocytes treated with IL-4 and GM-CSF could become DC as defined by the loss of CD14 expression; development of a dendritic morphology; increases in MHC class II, adhesion, and costimulatory molecules; enhanced activation of allogeneic T cells; and development of CD1a (125) and CD83 expression (149). These DC are derived from monocytes, and this may represent an alternative method for the development of DC in vivo under conditions in which GM-CSF, IL-4, TNF, and/or possibly other cytokines are produced. Thymic DC have also been observed to be derived from the same precursor cells as T cells (1). Thus, DC appear to have multiple derivations, including the same precursor as monocytes and granulocytes, the same precursor as T cells, and derivation from monocytes. This variety may allow them to be produced both constitutively and, when needed in increased numbers or at specific sites, as an inducible population.

FUNCTIONS OF DC

A description of the entire spectrum of DC function is beyond the scope of this review (see references 135 and 153 for more information). Briefly, DC, excluding thymic DC, have at least four major functions: they (i) obtain foreign antigens from various tissues of the body, (ii) process the antigens into peptides that associate with MHC antigens on their surfaces, (iii) present these antigens to T cells, and (iv) activate the responding T cells. This activation occurs primarily but not exclusively in the paracortical regions of lymphoid organs. A number of mechanisms whereby DC obtain antigen have been identified; these include phagocytosis, macropinocytosis, fluid-phase pinocytosis, and mannose receptor-mediated internalization (43, 72, 86, 91, 116, 124, 129). It is believed that the functions of DC change as these cells progress through their life cycle. For example, LC and other tissue DC are very efficient processors of antigen but are less able to activate T cells. After DC leave their tissue sites and travel to the paracortical regions of draining lymphoid organs, the T-cell stimulatory action of DC increases, and there may be a decrease in their antigen-obtaining activity (62, 72, 117, 118). This increase in T-cell stimulatory activity has been attributed in part to increases in MHC, adhesion, and coactivation molecules on the surfaces of DC (reviewed in references 34, 62, 72), and 117) and is regulated in part by cytokines, such as IL-1, GM-CSF, and TNF (reviewed in reference 118). Fully mature DC are capable of directly activating naive and cord blood $CD4^+$ T cells (18, 67, 79) and of inducing the generation of antigen-specific $CD8^+$ cytotoxic T lymphocytes (5, 79, 121, 139).

IN VIVO INFECTION AND IN VITRO INFECTABILITY OF DC WITH HIV

The in vitro infectability of DC with HIV and the extent of infection of DC isolated from HIV-infected individuals were initially examined by purifying DC from the peripheral blood of healthy volunteers or HIV-infected individuals by a variety of methods, including in vitro culture and density gradient centrifugation. DC from healthy volunteers were initially found to be highly infectable in vitro, and cells from HIV-infected individuals were found to be infected in vivo with HIV; however, these studies used relatively impure populations of cells (56, 59, 69, 96). Follow-up studies by multiple groups were discordant in their results; some groups found that DC purified from peripheral blood of healthy volunteers by negative selection were easily and productively infected with multiple strains of HIV (21, 22, 55, 64, 65, 111); other groups found that peripheral blood DC isolated by similar methods were not infectable (12, 104, 150). Part of the discrepancy could be explained by the fact that multiple populations of cells with dendritic morphology could be identified in peripheral blood (90, 141, 150). When three of these cell populations were analyzed for infectability with HIV, only one was easily and productively infected (150). The study of DC from HIV-infected individuals yielded similarly conflicting results, with some authors finding high levels of infection of DC isolated from peripheral blood (69, 97) and others not finding significant levels of HIV infection (46, 53).

The degree of infection and infectability of DC in peripheral blood will likely remain controversial. Since the procedures used in the isolation of DC may introduce artifacts, and since the main function of the DC present in peripheral blood is to migrate to tissue, it may not be physiologically relevant to focus on peripheral blood DC with regard to HIV infection. A more relevant question may be whether DC are infected in the peripheral tissues or in lymphoid organs. We will not directly address thymic DC, since the study of the role of these cells in HIV disease has been limited. A number of studies have examined LC from the skin of HIV-infected individuals. The general agreement is that these cells can contain HIV DNA, which signifies infection; however, this situation occurs at a very low frequency, at most equal to the level of infection found in peripheral blood $CD4^+$ T cells and often 10 to 100 times less (8, 25, 29, 30, 38, 52, 84, 123, 130, 155, 157). Since these studies, for the most part, examined normal-appearing skin from HIV-infected individuals at different stages of disease, the data suggest that infection of DC, at least in the skin, occurs at very low levels. The study of DC infection in lymphoid organs has been more limited. In a study of spleen white pulp from HIV-infected individuals, the level of infection in the DC population as determined by DNA PCR was approximately 100 times less than that observed in the $CD4^+$ T cells (75). In an analysis of lymph node biopsy samples, tissue sections from HIV-infected individuals at various stages of disease were stained for DC by using the p55 (83) antibody that recognizes DC but no other lymphoid cells; the presence of HIV RNA was also determined by in situ hybridization (Fig. 1). No cells that stained for both p55 and HIV were noted, suggesting that none of the DC were productively infected with HIV at any stage of disease (Fig. 1). Thus, these data suggest that in the tissues in which DC reside for the purposes of obtaining antigen or in the lymphoid organs where they activate T cells, DC are not highly or productively infected. This is not to say that DC do not play a role in initiating or propagating HIV infection or that they may not become productively infected outside of the skin or lymphoid organs, as has been suggested and will be discussed below.

Many of the studies of LC have examined skin from individuals infected with HIV type 1. Other workers have attempted to study the infection of LC in vitro and have had various degrees of success owing to the lack of proliferation and short period of viability of these cells in culture; however, most of these studies have found that LC are infectable, although viral production is very low (2, 20, 28, 29, 32, 108, 113, 156). In a series of experiments which attempted to address the differences between the epidemiology of HIV infection in the United States and Europe and that in sub-Saharan Africa, Asia, and India, infection of LC using different subtypes (clades) of HIV-1 was studied. In the United States and Eu-

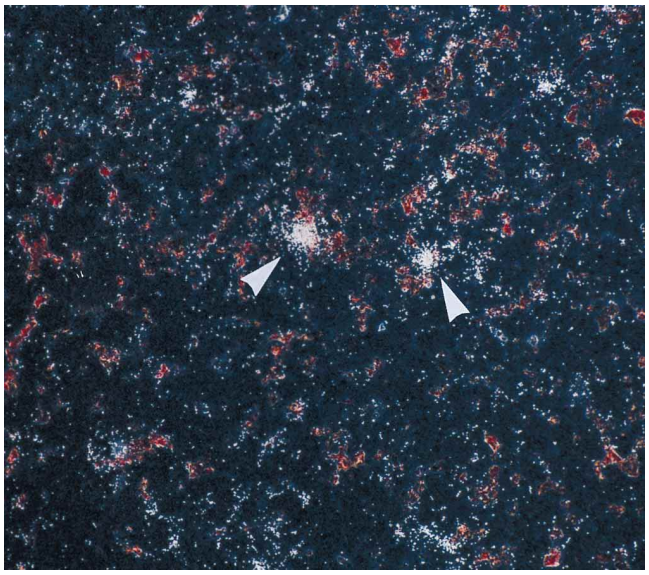

FIG. 1. Interdigitating DC in the paracortices of lymph nodes are not productively infected with HIV. HIV-infected cells (white) are located in the same microscopic field as p55-stained DC (red); however, the DC are not productively infected. Arrows indicate single-cell infection with HIV as detected by in situ analysis with labeled antisense HIV riboprobes. Lymph node sections from 30 HIV-infected individuals were examined, and no HIV RNA staining was observed overlying a p55-stained DC. When standard microscopy was used, HIV staining overlaid round lymphoid cells (not shown). Sections of lymph node were stained by immunohistochemical methods for p55 and then stained in situ for HIV RNA as described previously (33, 94). The slide was visualized by ×100 magnification with dark-field microscopy.

rope, the majority of HIV infections occur in homosexual populations and in persons who inject drugs; the dominant subtype of virus in these regions, particularly in the United States, is clade B. In sub-Saharan Africa, Asia, and India, more than 90% of infections are spread among sexually active heterosexuals, and clade B accounts for only a minor proportion of the viral subtypes involved in the epidemic in these regions (40, 105, 112, 152). Essex and coworkers presented data showing that a major difference in the clade B viruses in the United States and Europe and the clade E viruses in Thailand is that the clade E viruses replicate well in LC in vitro, whereas the clade B viruses do not (132). Follow-up studies to confirm these observations are needed. Thus, it is possible that an HIV subtype that can replicate well in LC, likely the major cell involved in the initiation of viral infection through mucosal contact (133), may have a greater ability to be transmitted heterosexually.

DEPLETION AND DYSFUNCTION OF DC IN HIV INFECTION

The question of depletion and dysfunction of DC in HIV disease is subject to the same controversy as that discussed above for infectability. Studies of the number of DC present in peripheral blood of HIV-infected individuals (reviewed in reference 14) have demonstrated a decrease (69), increase (114), or no change (12). Most studies of LC have found no decrease in the percentage of these cells when infected individuals were compared with uninfected individuals or when HIV-infected individuals at various stages of disease were compared (reviewed in reference 8). In a study of lymphoid organs, sections of lymph node tissue from HIV-infected individuals at various stages of disease were stained with p55 and examined by light microscopy. Visual analysis did not suggest a selective loss in the number of DC populating the paracortical regions of the lymph node (Fig. 2) in HIV-positive non-AIDS patients. The loss of DC from the lymph node occurs in parallel with the loss of lymphoid architecture and the development of fibrosis.

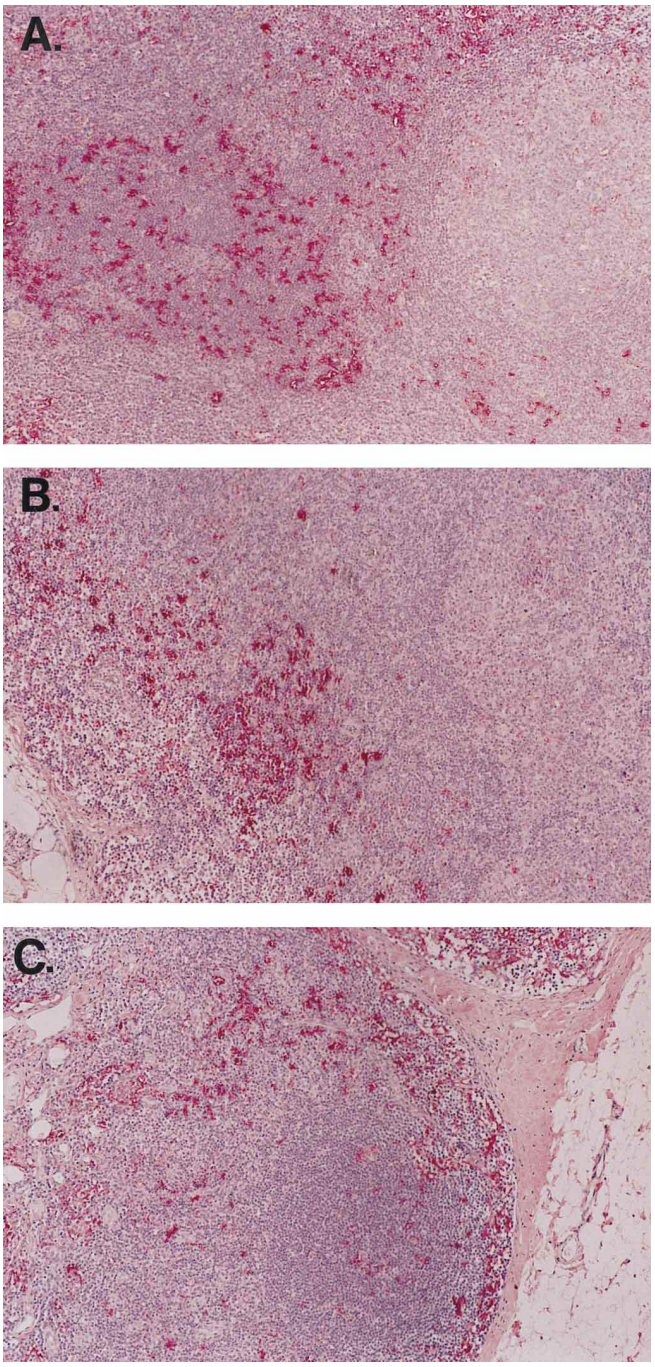

FIG. 2. DC are present in the paracortical regions of lymphoid organs in all stages of HIV disease. (A) A lymph node from an individual in the early stage of infection (CD4 count, 670/μl) demonstrates follicular hyperplasia. DC are seen (stained red with p55 antibody) in the surrounding paracortical regions. (B) A lymph node from an individual in the intermediate stage of infection (CD4 count, 392/μl) demonstrates follicular hyperplasia and involution, with DC staining in the surrounding paracortical regions. (C) A lymph node from an individual in the late stage of infection (CD4 count, 103/μl) manifests fibrosis and fatty infiltration, with DC staining seen in the remaining paracortical regions. Slides were stained with the p55 antibody and counterstained with alkaline phosphatase-labeled goat anti-mouse immunoglobulin G. A background stain of Giemsa was used. Magnification, ×100.

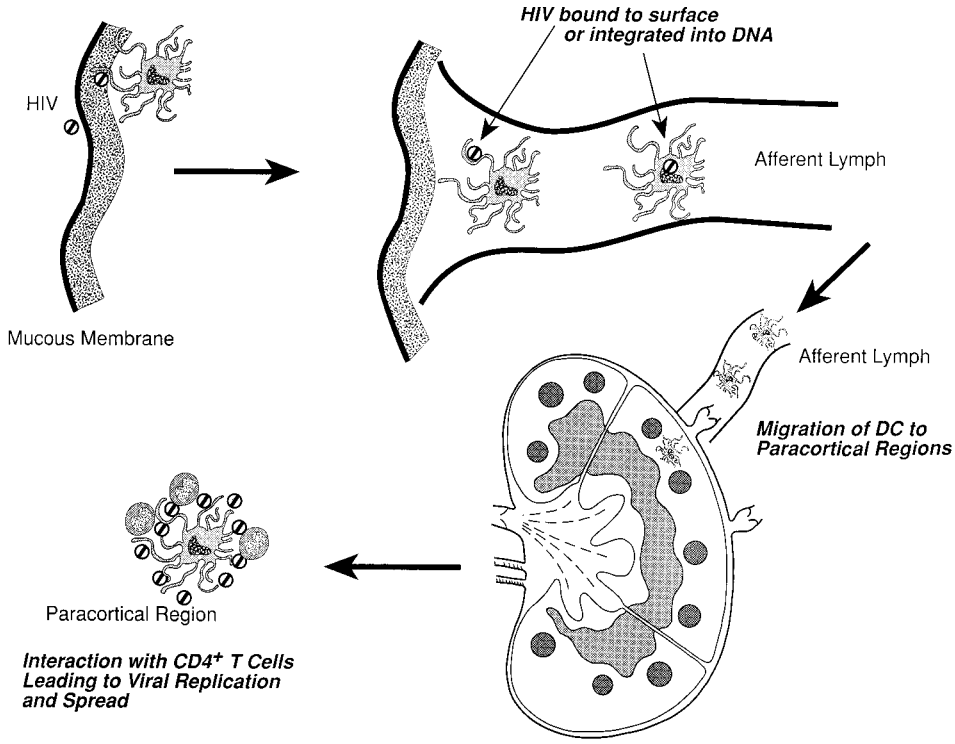

FIG. 3. Schematic diagram of the role of DC in the initiation of viral replication. HIV enters a mucous membrane (sexual spread) or skin (needle injury) and binds to or infects a tissue DC. Upon receiving the appropriate signal, the DC travels in the afferent lymphatics, enters a draining lymph node, and migrates through the subcapsular sinus to the paracortical region. Within the paracortical region, the DC interacts with and activates $CD4^+$ T cells, leading to productive infection and spread of HIV.

With regard to DC function, the effect of HIV infection on the ability of DC to activate T cells has been studied, and the results have been conflicting. Several studies found that peripheral blood DC from HIV-infected individuals or DC infected in vitro were much less efficient in activating T cells (22, 57, 58, 61, 68–70, 119), whereas another study found no difference in the abilities of peripheral blood DC to activate allogeneic $CD4^+$ T cells in HIV-infected versus uninfected individuals (12). All of these studies used peripheral blood DC, which again have the confounding variables of a requirement for in vitro maturation and long purification procedures and the presence of multiple populations of DC in blood. In a study of identical twins, in which one of each pair was infected with HIV, LC from each sibling were cocultured with his or her own $CD4^+$ T cells, the $CD4^+$ T cells of the sibling, or allogeneic $CD4^+$ T cells. When compared with DC from the uninfected sibling, DC from the HIV-infected sibling showed no defects in their ability to present antigen to the uninfected T cells. The only defect that was observed in the DC from the HIV-infected sibling was a decreased ability to activate allogeneic T cells (6, 7). Similar to the difficulties inherent in studies on infection of DC, analysis of depletion and dysfunction of DC in peripheral blood yielded conflicting results; however, as mentioned above, DC in peripheral blood are represented by multiple populations, and their isolation requires multiple in vitro purification steps. In studies using tissue sections or LC which are purified over a period of hours rather than days, there may not be significant DC dysfunction or depletion until the late stages of AIDS (6). Finally, because these studies were performed in vitro, potential problems of observing in vitro artifacts might render the extrapolation of results to in vivo disease states problematic.

ROLE OF DC IN THE INITIATION OF HIV INFECTION

DC are likely infected in vivo at relatively low levels compared to $CD4^+$ T cells, but DC have other roles in HIV infection independent of infection, dysfunction, and depletion. One of the main pathologic processes in which DC are involved appears to be the initiation of HIV infection following exposure to the virus. At areas of inflammation in mucous membranes, which are the major sites of initiation of HIV infection, DC are the first immune competent cells to encounter antigen (77). In this regard, DC are the main, if not the only, APC involved in the initiation of primary immune responses of T cells (18, 135). These observations have led to the belief that DC are important in primary HIV infection. A model has been proposed whereby HIV enters a mucous membrane and interacts with tissue DC, resulting in binding of the virus to the cell with or without infection. The cell then migrates to the paracortical region of the draining lymphoid tissue where virus is delivered, $CD4^+$ T cells become infected, and replication and spread of virus occur (Fig. 3) (10, 12, 28, 63, 80–82, 84, 87, 145, 151). In vitro data have demonstrated that DC can bind HIV for extended periods (151) and then induce infection in activated (2, 13, 15, 143) or quiescent (151) $CD4^+$ T cells. These data suggest that a DC, whether it simply carries HIV or is infected with it, is able to bring the virus to T cells and establish a productive infection. Recently, in vivo data in the macaque model have clarified certain of the pathogenic events associated with primary simian immunodeficiency virus (SIV) infection. SIV was placed in the vaginal vault of each animal, infected cells were identified, and the fate of the cells was followed by using in situ PCR technology. DC in the

lamina propria of the cervicovaginal mucosa were found to contain SIV DNA within 2 days after exposure to virus. The pattern of spread of infected cells observed in the subcapsular and paracortical regions of the draining lymph nodes mirrors the course that DC take upon receiving a signal to migrate from the tissues to lymphoid organs (133). Thus, in an animal model of HIV, DC appeared, but were not proven, to be responsible for bringing SIV from the site of inoculation, i.e., the cervicovaginal mucosa, to the paracortical regions of the draining lymphoid organs, leading to viral replication and systemic spread of infection.

If this model for the initiation of HIV infection (Fig. 3) ultimately proves to be correct, it could lead to a number of novel therapeutic strategies based on the likely mechanisms involved in viral binding to DC, transfer of HIV to T cells, and initiation of events leading to productive infection. HIV has been demonstrated to bind to or infect DC and LC in both a CD4-dependent and a CD4-independent manner (31; reviewed in reference 7). The CD4-independent binding occurs through unknown binding sites on the DC. Manca demonstrated that HIV and gp120 bind to mannose receptors on DC and monocytes (73). These receptors function by binding nonhost sugar residues present on invading organisms (51, 73). If specific binding between HIV and a receptor on DC is identified, blocking this interaction may offer an alternative treatment for needle stick victims or recently sexually exposed individuals. A second avenue of possible intervention involves the transfer of virus from the DC to $CD4^+$ T cells. As mentioned above, DC play multiple roles in the initiation of HIV infection. They transfer HIV to $CD4^+$ T cells, and this process can be blocked by anti-CD4 monoclonal antibodies or by soluble CD4 (149). In addition, DC activate the $CD4^+$ T cell, which allows HIV to replicate productively (151). Interference with activation of T cells through the blockade of the interaction between CD80 and CD28 or CD40 and CD40L (104) may offer a second approach for blocking viral infection after an exposure; however, such treatment will disrupt DC/$CD4^+$ T-cell interactions not involving HIV as well. In an experiment in which macaques were treated with cyclosporine or placebo at the time of acute SIV infection, a delay in viral replication and in trapping of virus in lymph node germinal centers was observed (74a). Clearly, further understanding of the interactions between DC and HIV and the transfer of HIV from DC to $CD4^+$ T cells may increase the repertoire of treatment strategies for HIV infection.

ROLE OF DC IN THE PROPAGATION OF HIV INFECTION

An additional role of DC in HIV infection involves their activity in the propagation or continuous daily production of HIV virions throughout the course of disease. The predominant cells that are infected with HIV in vivo are $CD4^+$ T cells and macrophages. Viral replication has been shown to occur in $CD4^+$ T cells that have recently become infected, and virions are produced at a high rate of turnover, ultimately leading to the death of the cell (45, 99, 147). It is unclear what percentage of these newly infected cells is not rapidly eliminated but rather enters the pool of chronically infected cells. These chronically infected cells can either be constitutive producers of HIV, such as brain macrophages, or can be "latent" and induced to produce HIV by a variety of stimuli (24, 98; reviewed in reference 106). Recent studies using potent antiretrovirus drugs have suggested that the source of more than 99% of HIV found in the plasma is acutely infected $CD4^+$ T cells. This virus has a short half-life (less than 6 h) and is replaced by new virions which are continually being produced from rapidly turning over, likely activated and replicating $CD4^+$ T cells (45, 99, 147). It is unclear what induces these cells to become activated. Current theories maintain that they are a differentiated or committed population that is being continually activated and expanded in an antigen-specific manner or that they are becoming infected as they are newly produced by the immune system, likely independent of any antigen stimulus.

A number of experiments suggest that HIV replication in vivo is dependent upon antigen-driven activation of $CD4^+$ T cells. In a series of experiments, HIV-infected individuals were infected with or immunized against another infectious organism. In both situations, immune system activation was observed and was correlated with induction of HIV replication (26, 37, 41, 44, 88, 131, 134). The amount of viral replication observed after vaccination with influenza or tetanus toxoid or during active infection with *Mycobacterium tuberculosis* correlated with the stage of HIV disease (41, 88, 134). Individuals with late-stage HIV disease had moderate increases in viral replication, whereas individuals with early-stage disease had much greater increases in viremia over their baseline levels. These data suggest a correlation between the increase in plasma viremia and the ability of the immune system to respond to antigen (88, 134). Furthermore, in an in vitro model with which antigen-specific activation and its effect on HIV replication is analyzed, peripheral blood mononuclear cells from HIV-infected individuals immunized with tetanus toxoid were stimulated with tetanus toxoid, and the increase in HIV replication observed correlated with the disease stage; i.e., early-stage individuals had much greater increases in viral replication than late-stage individuals (92, 134). Similar results were observed when purified protein derivative was used as a stimulus in HIV-infected, purified protein derivative-positive subjects (41). These studies suggest that the level of viral replication correlates with immune activation against an antigen.

In another type of experiment, lymphoid tissues from HIV-infected individuals were examined (23). The authors dissected splenic white pulp and found that within each specimen, a restricted number of individual antigen-specific immune responses was occurring, as defined by analysis of the T-cell receptor Vβ genes, and that each of the white pulp segments that contained a single immune response as defined by a restricted expansion of Vβ families also contained a single or limited number of HIV quasispecies (23). The data supported the theory that within each antigen-specific immune response (expansion of a single or limited number of T-cell clones), a single quasispecies of HIV, which was present at the initiation of the reaction, was spreading among the newly activated T cells. Thus, it is likely that the continuous daily production of HIV occurs in newly activated $CD4^+$ T cells that are being driven by antigen-specific activation and that are likely being amplified by the cytokine secretion associated with stimulation of the immune system.

It has also been demonstrated that HIV replication occurs in lymphoid organs and, in particular, the paracortical regions. This has been shown by comparing levels of HIV RNA in lymph node cells with those in peripheral blood mononuclear cells and finding that a much greater proportion of the lymph node cells express HIV RNA, particularly in the early stages of disease (94). In situ analysis for HIV RNA also demonstrated that most of the infected cells that are actively expressing viral RNA reside in the paracortical regions of the lymph nodes (Fig. 4). The paracortical region of a lymphoid organ is composed predominantly of DC and $CD4^+$ T cells (135). Given the importance of DC in the initiation of antigen-specific responses in $CD4^+$ T cells in the paracortical regions of lym-

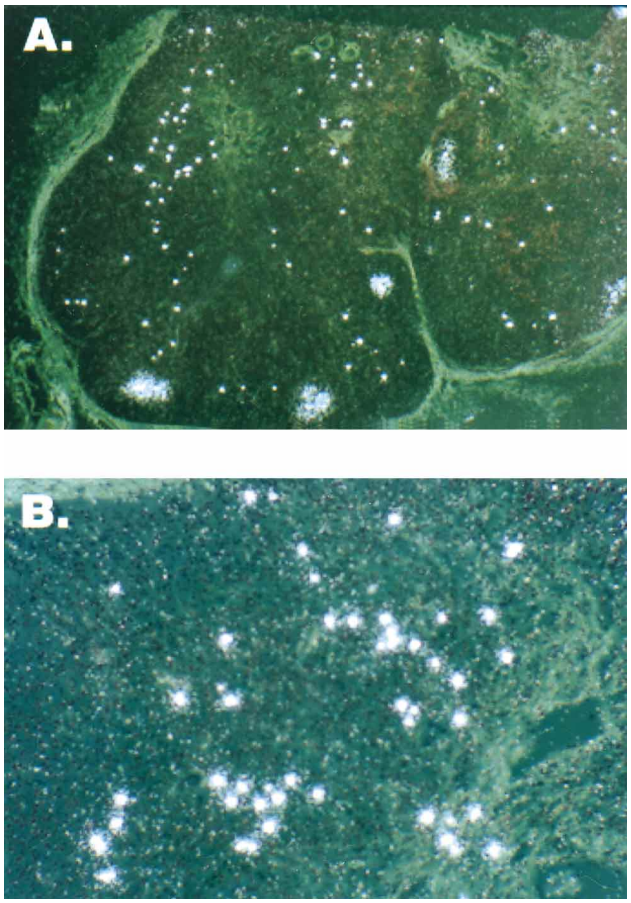

FIG. 4. The paracortical regions of lymphoid organs contain the productively HIV-infected cells. The figure shows dark-field microscopy of a lymph node from an HIV-infected individual that has been stained with radiolabeled HIV RNA riboprobes (white) as described previously (94, 95). (A) Diffuse, large patches represent extracellular HIV RNA (virions) bound to follicular dendritic cells in germinal centers, with surrounding single-cell infection in the paracortical regions. (B) Single-cell infection of lymphoid cells in the paracortical region is observed as small, densely staining regions overlying round cells. Slides were stained as described in the legend to Fig. 1. Magnifications: (A) ×10; (B) ×40.

phoid organs, it can be concluded that DC play an important role in driving HIV replication in $CD4^+$ T cells in lymphoid tissue.

ROLE OF NONLYMPHOID DC IN HIV REPLICATION

Another theory for a role for DC in the pathogenesis of HIV replication has been proposed by Steinman and coworkers, who isolated conjugates of DC and memory T cells that emigrated from skin explants that had been placed in culture (42, 107–110). These conjugates are easily and productively infected with HIV. When HIV is added to these conjugates, not only does infection ensue, but syncytia also develop (42, 107–110). Three lines of evidence indicate that such conjugates may exist in vivo. First, conjugates of DC and T cells have been directly isolated from peripheral blood and found to be capable of supporting HIV replication (150). Second, in a recent study, Frankel and coworkers identified in tonsil sections from HIV-infected individuals multinucleated cells that stained not only for DC and T cells but also for HIV antigens (36). Third, in a macaque model of acute SIV infection, SIV DNA-containing syncytia that resembled syncytia formed in vitro between DC and $CD4^+$ T cells were identified in deep mucosal regions (133). These data suggest that conjugates of DC and $CD4^+$ T cells exist in vivo and may be sites of viral replication; in addition, they may be important in the initiation of infection, the continuation of viral replication, and the observed depletion of $CD4^+$ T cells.

CONCLUSIONS AND FUTURE DIRECTIONS

The role of DC in the pathogenesis of HIV disease is just beginning to be understood. Difficulties in isolation, purification, and identification of their lineage and life cycle have made the study of DC difficult. Recent success in the ability to generate DC in vitro should allow further advances in the analysis of their role in HIV infection. Questions regarding the role of infection, depletion, and dysfunction of DC in HIV disease have been extensively addressed, and there is no universal agreement on the level or significance of the observed defects. Most current studies indicate that DC can be infected in vitro and are infected in vivo; however, the reported levels of in vivo infection vary. A major confounding issue of most studies of DC is that they examine the infectability or function of DC obtained from peripheral blood, which contains multiple populations of cells with dendritic morphology. The study of peripheral blood DC has led to important insights into their function; however, they may not be the most appropriate cells to study in order to delineate the significance of infection, dysfunction, and depletion of DC in HIV disease in vivo. Studies that examine DC in vivo in the tissues and lymphoid organs indicate that these cells are infectable with HIV but usually at levels much lower than that observed in $CD4^+$ T cells. Prior to end-stage HIV disease, there does not appear to be a significant depletion of LC (reviewed in reference 8) or interdigitating cells in the paracortical regions of lymphoid organs (Fig. 2). The cells appear to function normally, although not all reports agree with this finding. There is more general agreement regarding findings in end-stage HIV disease; DC are lost from the paracortical regions of lymph nodes as these regions become disrupted and fibrosed. There is also a loss of certain stimulatory functions of LC in individuals with advanced-stage HIV disease. Although these cells can stimulate T cells to respond to antigen, they become less able to do so in an allogeneic mixed lymphocyte culture (7).

Recent studies involving the role of DC in HIV infection concentrate on the physiologic functions of DC and how HIV might take advantage of these functions to promote its own replication. The main function of DC is to obtain foreign antigens and present them to T cells in order to initiate an immune response. As mentioned above, DC are the first immune competent cells to migrate to regions of inflammation in mucous membranes (77), the major site of viral entrance in the sexual spread of HIV. The primary site of HIV replication is in the paracortical regions of lymphoid tissue (Fig. 4). This is the same region in which DC initiate new immune responses and propagate ongoing ones. A recent study of the SIV macaque model demonstrated that tissue DC in the cervicovaginal mucosa are important in the initiation of viral replication after inoculation of SIV into the vagina (133). This finding strongly suggests that DC play an important role in the initiation of HIV infection. The selective ability of certain subtypes of HIV to infect and replicate in DC has been proposed as a potential explanation for the higher rate of heterosexual spread observed in Thailand, where clade E is prevalent, compared with rates in the United States and western Europe, where clade B is dominant (132).

The integration of data concerning the cells that are responsible for viral replication in vivo and the mechanisms that are responsible for driving this viral replication also points to an important role for DC in the propagation of HIV replication and disease. The amount of HIV produced each day has been reported to be approximately 10^{10} virions (45, 99, 147), and this viral replication occurs in acutely infected $CD4^+$ T cells that are rapidly expanding, likely in response to DC-driven, antigen-specific stimulation (see above). Analysis of multiple pathophysiologic events in HIV disease in cocultures of DC and $CD4^+$ T cells, a system that mimics the microenvironment in which HIV replicates in vivo, is providing new insight into the potential role of DC in the pathogenesis of HIV disease. The role of antigen-specific activation in the initiation and propagation of HIV infection has been studied. The observation has been made that in cultures in which an antigen-specific immune response is occurring, 100 times less HIV is required to initiate a productive infection than in cultures in which an antigen-specific response is not occurring (148). This observation could explain in part the increased ease of infection with HIV observed in certain parts of Africa, Asia, and India, where the level of immune activation in the population is high owing to chronic parasitic infestation and repetitious infection with a variety of microbes.

Systems employing DC–T-cell cocultures have also been used to study factors produced by $CD8^+$ T cells that can suppress HIV replication. This in vitro system of HIV replication allows discrimination among a number of $CD8^+$ T-cell-derived HIV suppressor activities (3) and may prove useful in the identification of the spectrum of $CD8^+$ T-cell-derived soluble factors that suppress HIV replication.

A recent application of DC research is in the field of vaccine development. DC loaded with small amounts of antigenic peptide, protein, or inactivated virus in vitro and then used to expand antigen-specific cytotoxic T lymphocytes or as a vaccine can induce potent immune responses (4, 11, 47, 71, 93, 139, 142). Antigen-loaded DC have been used to enhance responses against tumors (47, 93, 139, 142) and infectious organisms (4, 11, 71). A novel approach to the treatment of HIV-infected individuals may involve the priming of DC with HIV peptides or antigens in order to enhance CTL and other forms of protective immunity. A potential caveat to this approach is that the induction of this protective response may need to be strictly controlled because the generation of $CD4^+$ T cells specific for HIV antigens (74) may lead to an increase in viral replication as these cells become activated in the presence of virus.

Our understanding of the role of DC in the immunopathogenesis of HIV infection has increased greatly over the past few years. Further understanding of the complex roles that these extraordinary cells play in the initiation and propagation of HIV infection as well as of their role in the generation of HIV-specific immune responses will likely provide the scientific basis for the development of novel treatment and vaccine strategies.

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