Brief Definitive Report

The Efficiency of Acute Infection of CD4$^+$ T cells Is Markedly Enhanced in the Setting of Antigen-specific Immune Activation

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Summary

Human immunodeficiency virus (HIV) disease is sub-Saharan Africa generally differs from that observed in the United States and other developed countries in that the risk of seroconversion after exposure is greater and the rate of disease progression to AIDS and death is faster. One theory that could in part explain this difference is the increased state of immune activation associated with a relatively high rate of parasite infestation and other infections among inhabitants of these regions. Using a model based on the cellular microenvironment of lymphoid organs, the role of exposure to HIV during a state of antigen-specific immune activation was investigated. Dendritic cells and CD4$^+$ T cells are the major cellular components of the paracortical region of lymphoid tissue, the primary site of HIV replication. We analyzed cocultures of HIV-pulsed dendritic cells that had matured in the presence of tetanus toxoid and CD4$^+$ T cells before and after inducing an antigen-specific response by in vivo immunization with tetanus toxoid. During antigen-specific immune activation, 100 times less HIV was needed to initiate a productive infection. These findings provide a model system to further delineate the relationship between immune activation and the propagation of HIV infection and suggest a mechanism for the epidemiologic observations of an increased ease of developing HIV infection and faster progression for HIV disease in geographic areas where immune activation is prevalent.

Cellular activation is an important component of the pathogenesis of HIV disease (1, 2). It has been demonstrated in in vitro experiments that propagation of HIV in culture depends to varying degrees on the state of activation of the target cells (3–7). With in vivo correlates, markers of cellular activation have been demonstrated to be closely associated with the level of HIV disease activity (8–10). Furthermore, it has been observed that in sub-Saharan Africa, individuals have an increased risk of acquiring HIV infection on exposure to the virus, and disease progresses more rapidly after infection as compared with individuals in developed countries (11–13). It has been proposed that these differences can be explained, at least in part, by the heightened level of activation of the immune system in Africans, which is related to the chronically active immune responses to parasitic infestations and infection with other pathogenic microbes (13, 14).

Recently, other lines of evidence have supported the association of immune activation and HIV pathogenesis. In has been demonstrated that replication of HIV occurs predominantly in the activated population of CD4$^+$ T cells that are rapidly turning over and recently infected (15, 16). Other studies have demonstrated that in the spleen, HIV replication occurs in T cells in the white pulp that is driven to divide in an antigen-specific manner (17). We and others have demonstrated that HIV replication occurs predominantly in the lymphoid tissues throughout the entire course of HIV disease (18, 19). Dendritic cell (DC)–T cell interactions have been shown to be important in the initiation and propagation of HIV infection (20–23). In this regard, DC populate the paracortical regions of lymph nodes (24) where they come into close proximity with CD4$^+$ T cells. DC are the major APC to T cells, and in the process of an antigen-specific immune response DC–T cell interaction, they result in T cell activation (24). In the setting of an HIV-infected lymph node, an ongoing antigen-specific response would be a favorable milieu for the initiation or propagation of HIV infection.

In the present study, we use an in vitro model system of presentation of antigen by DC to CD4$^+$ T cells in the presence of HIV to directly address the question of the role of an ongoing antigen-specific immune response in the initiation and propagation of HIV infection; this system mimics the in vivo microenvironment of lymphoid tissue. We immunized healthy volunteers with tetanus toxoid, and 2 wk after immunization, exposed their DC in vitro to tetanus antigen and allowed the DC to present the antigen to CD4$^+$ T cells in the presence of HIV. We found that up to 100 times less virus was required to initiate HIV infection in cultures from individuals after immunization compared

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with before immunization, indicating the potential contribution of the cellular activation associated with an ongoing antigen-specific immune response to the pathogenesis of HIV disease.

Materials and Methods

Reagents. RPMI 1640 (Biowittaker, Walkersville, MD) was supplemented with glutamine (2 mM) (Biofluids, Rockville, MD), penicillin–streptomycin (Biofluids), Hepes (15 mM) (Biofluids), and 10% normal human AB^+ serum (NHS) (Advanced Biotechnologies, Inc., Columbia, MD) or 10% FCS (Hyclone Laboratories Inc., Logan, UT). No difference in the proliferation in autologous MLRs between DC and $CD4^+$ T cells or $CD4^+$ T cells alone, or in the HIV infection assays was observed between NHS and this lot of FCS such that the two sera were used interchangeably.

DC Purification. DC were purified (Fig. 1) either before or 2 wk after tetanus toxoid immunization from leukopaks obtained from healthy volunteers under an Institutional Review Board–approved protocol. PBMC were obtained by Ficoll–Hypaque density gradient centrifugation. To simulate an antigen-specific activated immune state, DC matured in the presence of antigen were prepared from PBMC by first depleting T cells with 2-aminoethylisothiouronium bromide–treated SRBC–T cell rosetting as previously described (25). The DC precursors present in the T cell–depleted PBMC were cultured overnight in the presence of tetanus toxoid (Wyeth-Ayerst Laboratories, Marietta, PA), mumps antigen (Connaught Laboratories, Swiftwater, PA), or no antigen (control). The DC were then purified as described previously, using metrizamide density gradient centrifugation and negative selection (20, 25).

HIV Infection. B cells and $CD4^+$ T cells were purified from PBMC and SRBC-rosetted T cells after overnight culture to allow dissociation of mature DC from T cells using CD19 and CD4 magnetic beads (Dynal, Lake Success, NY), respectively. The beads were mixed with cells at a 3:1 ratio for 30 min on ice. Cells bound to beads were separated using a magnet and detached from the beads with the Detach-a-Bead product (Dynal). Macrophages were depleted on human Ig–coated plates (20). $CD4^+$ T cells were 99% $CD4^+$ and contained no $CD3^-$ $HLA\text{-}DR^+$ contaminants. Purified populations of DC and B cells were incubated with decreasing multiplicities of infection (MOI) of HIV_{IIIB} (Advanced Biotechnologies, Inc.) for 1.5 h at 37°C followed by two washes to remove unbound virus. Experiments with HIV-pulsed DC worked equally well with primary isolates and laboratory strains; however, since primary viral isolates were obtained from PHA-stimulated T cell blasts or monocyte-derived macrophages, which contained cytokines that could alter DC function, purified HIV_{IIIB} was used. The HIV-pulsed cells (5×10^3 cells/well) were mixed with autologous $CD4^+$ T cells (10^5 cells/well) in 96-well flat-bottom microtitier plates (Costar Corp., Cambridge, MA). Fresh medium was replenished twice weekly. Supernatants for reverse transcriptase (RT) activity were removed every 2–3 d and frozen at −70°C. RT activity was measured as described previously (25).

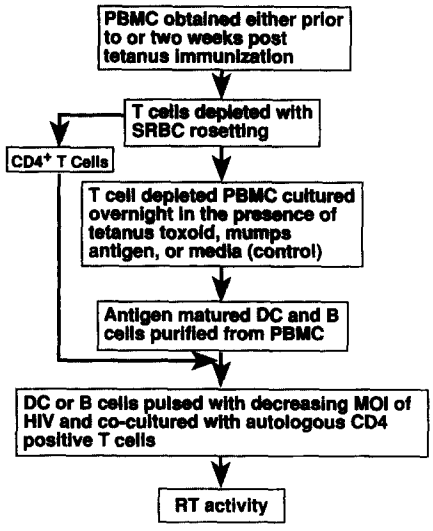

Figure 1. Protocol for the purification of DC that have matured in the presence of tetanus toxoid, mumps, or media control and coculture with $CD4^+$ T cells for measurement of [^{3}H]thymidine incorporation, cellular activation, and HIV infection.

Measures of Cellular Proliferation and Activation. Cocultures of DC or B cells and $CD4^+$ T cells (5×10^4) were performed in triplicate. Cells were pulsed after 5 d with [^{3}H]thymidine (0.5 μCi/well) (Amersham Corp., Arlington Heights, IL) for 16 h, harvested (Tomtec Harvester; Wallac, Gaithersburg, MD), and counted in a counter (Betaplate model 1205; Wallac). Expression of HLA-DR on $CD4^+$ T cells was analyzed by staining 3-d cocultured (DC or B and/or $CD4^+$ T) cells with anti–CD3–fluorescein and anti–HLA-DR–PE mAb (Becton Dickinson & Co., Mountain View, CA) and analyzing in a flow cytometer (Coulter Corp., Hialeah, FL).

Statistical Analysis. Values from triplicate cultures were averaged, and SEM were calculated using the Excel™ (Microsoft, Redmond, WA) software package. Typically, SE errors ranged from 5 to 15%.

Results

The Effect of Tetanus Toxoid Immunization on Cellular Activation and Proliferation in Cocultures of $CD4^+$ T Cells and DC That Had Been Matured in the Presence of Tetanus Antigen. It has been previously demonstrated that DC can bind HIV to their surface and induce infection in co-cultured autologous $CD4^+$ T cells in the absence of added mitogen (20). This infection can be modulated by exogenously added and endogenously produced cytokines and is dependent on activation of the $CD4^+$ T cells (20; Weissman, D., T. D. Barker, and A. S. Fauci, unpublished observations).

In individuals before tetanus immunization (date of last tetanus shot, 2.5 to >10 yr), maturation of DC in the presence of tetanus toxoid, 95 lymphocyte flocculation unit (LfU)/ml (60 μg/ml), did not enhance the proliferation of $CD4^+$ T cells cocultured with these DC when compared with $CD4^+$ T cells cocultured with DC that were matured in the presence of mumps antigen or media (control) (Fig. 2 *A*). However, all DC–T cell cocultures showed significant levels of proliferation compared with B cell–T cell cocultures demonstrating an active autologous MLR. Analysis of proliferation in tetanus toxoid–stimulated unfractionated PBMC also demonstrated minimal stimulation, less than twofold above control (data not shown) before tetanus immunization. It has been demonstrated that 2 wk

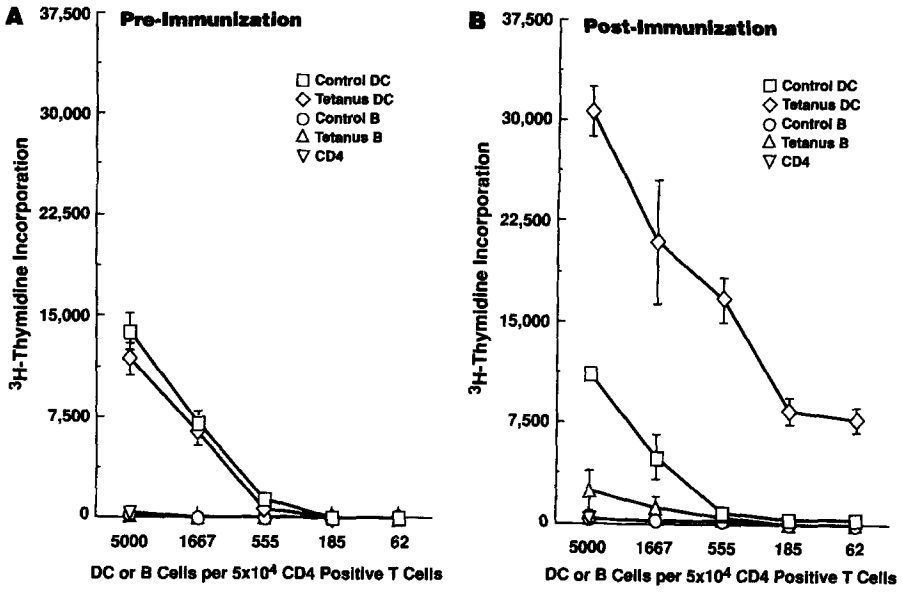

Figure 2. Maturation of DC in the presence of tetanus toxoid does not increase proliferation in $CD4^+$ T cells in preimmunized individuals but markedly increases proliferation 2 wk after tetanus immunization. (*A*) Before tetanus immunization, DC were matured in the presence of tetanus toxoid, 95 LfU/ml, and purified as described in Fig. 1 and Materials and Methods. When these cells were cocultured with $CD4^+$ T cells, they induced a substantial autologous MLR but did not increase proliferation above that induced by DC matured in the presence of no antigen or mumps antigen. (*B*) 2 wk after tetanus immunization, DC matured in the presence of tetanus toxoid induced a significant increase in $CD4^+$ T cell proliferation compared with that induced by DC matured in the presence of mumps antigen or no antigen. Decreasing numbers of DC or B cells were added to 50,000 $CD4^+$ T cells and pulsed with [^{3}H]thymidine for 16 h on day 5, harvested, and counted. DC or B cells alone did not proliferate above background. $CD4^+$ T cells typically incorporated 150 cpm. Data are from one individual that is representative of seven (*A*) and three (*B*) others tested.

after tetanus immunization, there is a peak of tetanus-specific T cells in PBMC (26). We chose this time point to obtain a population of $CD4^+$ T cells that were enriched for tetanus-specific cells capable of responding to an in vitro challenge with tetanus (26). DC, obtained 2 wk after immunization and matured in the presence of tetanus toxoid, induced a significant increase in proliferation of cocultured $CD4^+$ T cells obtained at the same time compared with DC that were matured in the presence of mumps antigen or control (Fig. 2 *B*).

Activation of $CD4^+$ T cells by DC was measured by determining the expression of HLA-DR on T cells after 3 d of coculture. The in vitro maturation of DC in the presence of tetanus toxoid in subjects before immunization did not increase the level of HLA-DR expression (data not shown). In cells obtained 2 wk after tetanus immunization, the DC that were matured in the presence of tetanus toxoid induced an increase in the percentage of activated $CD4^+$ T cells. Variability among donors in the percentage of $CD4^+$ T cells activated in coculture with DC was observed (ranging from 10 to 38% for control DC); however, in all cases, DC obtained 2 wk after immunization and matured in the presence of tetanus toxoid induced a 50–75% increase in the expression of HLA-DR on $CD4^+$ T cells (data not shown).

The Effect of Immunization on the Infectability with HIV of $CD4^+$ T Cells Cocultured with DC That Were Matured in the Presence of Tetanus Toxoid. It has previously been demonstrated that two populations of DC were present after purification of overnight-cultured PBMC. One population was DC derived from DC precursors, and the second population was de novo mature DC that were bound to T cells and released during overnight culture (25). These mature DC, which are depleted by SRBC rosetting before overnight culture because of their conjugation with $CD4^+$ T cells, were necessary for efficient induction of infection in autologous, unstimulated $CD4^+$ T cells (20). In the next set of experiments, no mature DC were added, which resulted in no infection in the HIV-pulsed control DC–$CD4^+$ T cell cocultures.

Before tetanus immunization, the pulsing of DC that had matured in the presence of tetanus with decreasing MOI of HIV followed by culturing with unstimulated $CD4^+$ T cells and measurement of RT activity demonstrated no enhancement of infection compared with control DC (Fig. 3 *A*). 2 wk after immunization, no infection was observed for the control-treated DC, even when pulsed with a MOI = 0.1 (Fig. 3 *B*). DC matured in the presence of tetanus toxoid–induced efficient infection when pulsed with as little as 0.001 infectious units of virus per DC (Fig. 3 *D*). B cells exposed to tetanus antigen 2 wk after immunization were ineffective in transferring infection to $CD4^+$ T cells (data not shown). DC matured in the presence of mumps antigen also induced no infection 2 wk after immunization (Fig. 3, *B–D*). Similar results were obtained when tetanus was added to cocultures of DC and $CD4^+$ T cells, although less sensitivity and greater variability were noted.

Similar experiments were performed with much lower concentrations of tetanus, 1 LfU/ml (0.12 mg/ml), and the addition of small numbers of mature DC, which resulted in infection in the control-treated DC–$CD4^+$ T cell cocultures. After immunization, 25 times less HIV was required to initiate a productive infection in cocultures of tetanus-matured DC and $CD4^+$ T cells (data not shown). Thus, HIV-pulsed DC that are in the process of interacting with and presenting antigen to antigen-specific $CD4^+$ T cells are much more efficient at inducing a productive infection in the $CD4^+$ T cells.

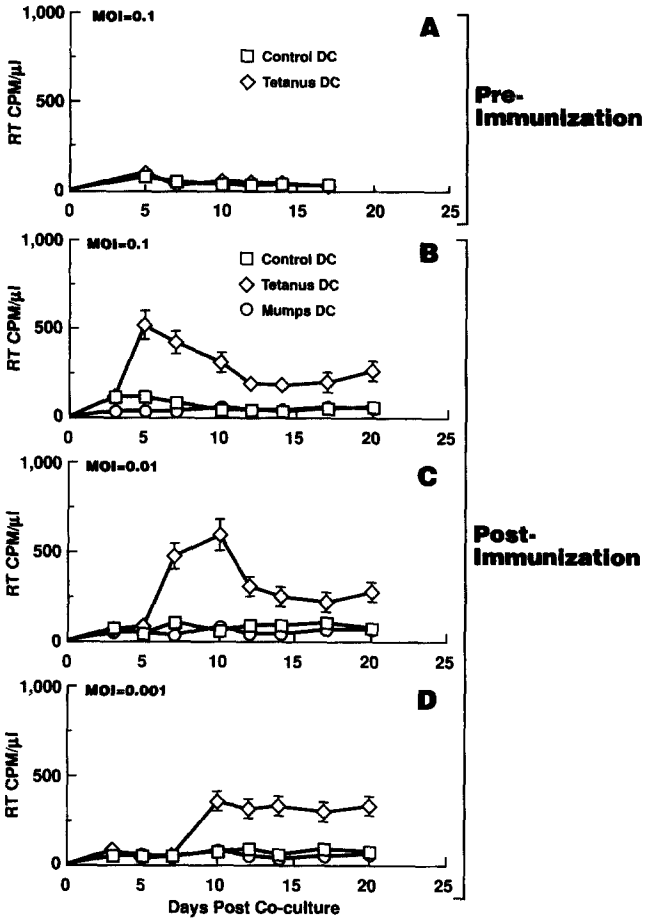

Figure 3. 100 times less HIV is required to induce a productive infection in cocultures of DC matured in the presence of tetanus toxoid and CD4+ T cells obtained 2 wk after immunization compared with similar cultures before immunization. (*A*) Before tetanus immunization, no infection was observed in cocultures of HIV-pulsed DC matured in the presence or absence of tetanus toxoid and CD4+ T cells. (*B*) 2 wk after immunization, DC matured in the presence of mumps antigen or media (control) induced no infection even when pulsed with a MOI = 0.1, whereas DC matured in the presence of tetanus toxoid induced productive infection when pulsed with a MOI of 0.001. All cocultures were run in culture triplicates. Data are representative of three (*A*) and four (*B–D*) experiments.

Discussion

Using a protocol of in vivo immunization with a common recall antigen (tetanus toxoid) and a physiologically based in vitro model system of DC presentation of HIV to CD4+ T cells, this study has demonstrated that, after immunization, up to 100 times less HIV compared with before immunization is required to be pulsed onto DC to allow them to initiate a productive infection in CD4+ T cells when the DC are in the process of presenting antigen to these cells. These studies directly address the question of the potential pathophysiologic role of cellular activation associated with ongoing antigen-specific responses in the initiation and propagation of HIV infection.

DC–CD4+ T cell interactions have been proposed as a model for the initiation and propagation of HIV infection (20–23). The paracortical or T cell region of lymphoid organs is a major site of HIV replication (19; Weissman, D., and A. S. Fauci, unpublished observations). DC are the most efficient APC to CD4+ T cells and are present in abundance in the paracortical region of the lymph node (24). Presentation of any antigen by DC to CD4+ T cells provides a potentially important source of cellular activation to CD4+ T cells, which, when in proximity to infectious virus favors efficient virus replication.

The role of cellular activation in the initiation and propagation of HIV infection of CD4+ T cells in vitro has been firmly established (3–7). A similar in vivo phenomenon has been suggested in reports of an increase in HIV replication in HIV-infected humans or chimps who were immunized with specific antigen or infected with pathogenic microbes (27–30). In addition, it has been reported that immune activation at the time of acute infection of monkeys with simian immunodeficiency virus led to an accelerated course of disease (31). Furthermore, recent studies have indicated that the cellular activation associated with the rapid CD4+ T cell turnover associated with high levels of virus replication (15, 16) and the antigen-specific activation of CD4+ T cells in the splenic white pulp (17) are important contributors to the high degree of virus production observed in HIV infection. Parallel with these findings are the observations that treatment of HIV-infected individuals with agents that might decrease cellular activation, under certain circumstances, could have a transient beneficial effect on surrogate markers of HIV disease (32, 33).

It has recently been hypothesized that the observed higher rate of HIV disease progression as well as susceptibility to infection on exposure to HIV in individuals in sub-Saharan Africa might be due, at least in part, to the chronic and persistent immune activation associated with the ongoing immune response to parasitic infestation and other infections that are common among individuals living in this region (13, 14). This study provides experimental support for this hypothesis and should provide a useful model to further delineate the precise mechanisms of the enhancement of HIV infection associated with antigen-specific immune responses. Furthermore, it may contribute to the development of public health strategies for the suppression and elimination of microbes that induce these antigen-specific immune responses.

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