Enhanced Follicular Dendritic Cell-B Cell Interaction in HIV and SIV Infections and Its Potential Role in Polyclonal B Cell Activation

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INTRODUCTION

In addition to the well-documented changes in the level and function of T cells, HIV infections also lead to dramatic qualitative and quantitative changes in the B cell compartment in terms of both function and the specificity of the antibody molecules produced. In vivo abnormalities in B-cell function include polyclonal B-cell activation, hypergammaglobulinaemia, increased levels of autoantibodies, decreased ability to mount antigen (Ag)-specific responses, and premature loss of specific anti-HIV antibodies (Zolla-Pazner, 1984; Mizuma et al., 1988; Amadori and Chieco-Bianchi, 1990; Fenouillet et al., 1992). Accordingly, cultured peripheral blood mononuclear cells (PBMC) from seropositive patients secrete antibodies to HIV- and non-HIV-related antigens, spontaneously or following culture with HIV, and interleukins (IL), and exhibit decreased responsiveness to mitogens (Amadori et al., 1989; Edelman and...
Zolla-Pazner, 1991; Delfraissy et al., 1992; Shirai et al., 1992). Although the observed polyclonal B-cell activation occurs early and persists throughout infection, it is thought to be independent of the specific humoral anti-HIV response, which clearly predominates during asymptomatic infection, as evidenced by large number of B cells spontaneously secreting anti-HIV antibodies in patient blood (Amadori et al., 1989; Shirai et al., 1992), bone marrow (BM) (Cosentino et al., 1994), and duodenum (Eriksson et al., 1995). In addition, antibody-secreting cells (ASC) specific for HIV proteins and peptides have also been detected around follicles and in the medulla in HIV+LN biopsies (Laman et al., 1989). In vitro, the generation of HIV- and cytokine- (e.g., IL-6, IL-2 plus interferon-α [IFN-α]) induced anti-HIV ASC in PBMC cultures derived from HIV-seropositive but not -seronegative individuals further illustrates extensive in vivo activation and expansion of HIV-specific B cells (Amadori et al., 1991; Delfraissy et al., 1992; Chirmule et al., 1993).

During the course of disease, which varies amongst individuals, the number of anti-HIV ASC in blood and the levels of serum antibody specific for particular HIV determinants decline. Although the loss of certain specificities, for example, anti-p24, has been associated with disease progression, the duration of specific responses usually has not been shown to correlate with clinical, immunological, or virologic parameters (Yarchoan et al., 1986; Shirai et al., 1992; Binley et al., 1997). In contrast, the increase in polyclonal B-cell activation has been shown to have statistical significance among patients with advanced disease. Thus, in studies using PBMC by Shirai et al. (1992), high HIV envelope gp160- and gag p24-specific B-cell activation decreased, whereas polyclonal B-cell activation, as measured by the appearance of B-cell secreting anti-TNP and anti-DNA IgG antibodies increased during late-stage disease (CDC class IV). Likewise, the in vitro generation of HIV-ASC from PBMC from late-stage patients was also greatly reduced (Delfraissy et al., 1992). How this transition relates to the process by which ASC are generated and selected from B-cell precursors in lymphoid organs and how this loss in ASC reflects similar changes in tissues are critical to our understanding of AIDS pathogenesis.

In this context, histopathological studies on HIV/SIV organs have revealed dramatic changes in the follicles (B-cell area) of lymphoid organs that represent a dynamic process progressing from follicular hyperplasia to follicular involution (Biberfeld et al., 1986; Racz et al., 1986; Vago et al., 1989; Persidsky et al., 1995). During this time, follicular dendritic cells (FDC), which normally create the environment in germinal centers (GC) for B cell somatic mutation, affinity maturation, and isotype switch following exposure to antigen (Kraal et al., 1982; Jacob et al., 1991; Liu et al., 1992; Apel and Berek, 1996; Liu and Banchereau, 1996), also themselves undergo initial hypertrophy and subsequent fragmentation of their reticular networks. Although these alterations are not specific for HIV/SIV infections (Janossy et al., 1991; Rideout et al., 1992), they are highly characteristic of HIV/SIV-related lymphadenopathy and are closely associated with both increased trapping of viral antigens by FDC and infiltration into the GC of CD45RAloCD8+ cells known to produce potentially lytic molecules. (Tenner-Racz et al., 1987; Vago et al., 1989; Devergne et al., 1991; Tenner-Racz et al., 1993; Rosenberg et al., 1994b). Since FDC are critical to the induction of specific ASC precursors and the generation and maintenance of B-cell memory (Klaus et al., 1980; MacLennan and Gray, 1986; Tew et al., 1992), the simplest assumption is that the B-cell abnormalities observed during infection directly result from virus-induced changes in the GC environment. For practical reasons, such studies cannot be easily performed in humans and our laboratory has therefore focused on examining the ability of mesenteric LN FDC derived from SIV-infected macaques to support GC formation, lymphocyte growth, and antibody formation in vitro. The findings demonstrated that such FDC have enhanced function when compared to FDC from control cultures. The purpose of this paper is to describe the possible mechanisms underlying the decline in certain specific anti-HIV/SIV titers and polyclonal expansion of P cells secreting non-HIV-related antibodies in the context of increased FDC function.
RESULTS AND DISCUSSION

The ability of FDC derived from SIV-infected LN to induce cluster formation, activate and induce proliferation of B and CD8+ cells, and to induce Ig and Ab production has been examined (Rosenberg et al., 1997). To this end, FDC-enriched mesenteric LN cell cultures from SIV-infected macaques were functionally compared to two controls: (1) FDC-enriched mesenteric LN cultures from uninfected macaques and (2) non-FDC-containing LN-cell suspensions derived from the same LN. The findings indicated:

1. Greatly enhanced spontaneous cluster formation in FDC-enriched LN cultures from SIV-infected macaques. In the absence of exogenous cytokines, such cultures contained many large clusters comprising thousands of cells in addition to smaller clusters of 10-100 cells containing predominantly B cells. By contrast, uninfected monkey donors contained mostly small clusters, although occasional large clusters were also observed in uninfected macaques, suggesting ongoing environmental stimulation leading to GC formation in vivo. The increased size of the clusters also corresponded with prolonged cell survival. For example, FDC-enriched cultures derived from the LN of the SIV-251-infected macaque 93 days post infections (pi) supported >40% B cells for 10 days compared to death of control cultures by 5-7 days. In the presence of IL-2, CD8+ and CD56+ cells were also increased.

2. Enhanced ability of FDC-enriched cultures from LN of chronically SIV-infected to induce lymphocyte proliferation. Theses studies indicated that at peak time points (usually days 2-3), FDC-enriched LN cultures from infected macaques consistently incorporated 3-10 times higher cpm than control non-FDC-containing cell suspensions prepared from the same LN or control FDC-enriched cells from LN of uninfected monkeys. The level of incorporation also appeared to be associated with the CD4/CD8 ratio of the LN and the degree to which GC were infiltrated with CD8+ lymphocytes. It should be noted that the addition of SIV-251 to fresh LN cells in vitro did not result in cluster formation or proliferation and may actually suppress anti-CD3-induced proliferation (unpublished observations).

3. Exogenous IL-2 at the initiation of culture resulted in greatly elevated lymphocyte proliferation at D4 in the FDC-enriched population from SIV-positive LN. For example, the [3H-TdR] incorporation in FDC-enriched cultures was five fold higher than that in control non-FDC-containing suspension cultures.

4. High background proliferation in the non-FDC-containing cell cultures, occasionally observed in tissues of SIV-infected macaques is rapidly lost in the absence of FDC and IL-2.

5. Increased production of IgG1 immunoglobulin and anti-SIV Ab by FDC-enriched cultures from SIV-infected LN. ELISA results indicate that, in addition to their requirement for B-cell growth, FDC play an important role in the high-level production of IgG1 and specific anti-SIV Ab by B cells from infected LN. No anti-SIV Ab was observed in either of two FDC-enriched LN preparations derived from uninfected monkeys. The high levels of Ab specific for SIV do indicate the commitment of GC in SIV-infected macaques toward the production of anti-SIV Ab.

6. FDC-dependent clusters predominantly contained activated CD45RAlo CD8+ cells and CD56+ cells. Unlike clusters from immunized mice or uninfected human tonsil (Koopman et al., 1991; Petrasch et al., 1991; Kosco et al., 1992) where CD8+ cells are rarely found, FDC-enriched cultures from infected macaques maintained 10-20% CD8+ cells that were predominantly CD45RAlo. In keeping with this activated phenotype, the addition of IL-2 to cultures at day 12 appeared to further select for the expansion of CD8+ cells. In control cultures, CD8+ cells were almost totally CD456RAhi.

GC Formation and HIV/SIV Infections

High-affinity IgG and IgA antibody synthesis and the generation of memory B cells involves complex cellular interactions among FDC, B cells, and T cells at several levels (Klaus et al., 1980; MacLennan and Gray, 1986; Tew et al., 1992). FDC, which initially “present” unprocessed Ab-complexed antigen to B
cells in association with the adhesion molecular pairs lymphocyte function-associated antigen-1 (LFA-1) — intercellular adhesion molecule-1 (ICAM-1) and very late antigen-4 (VLA-4) — vascular adhesion molecule-1 (VLA-1) (Koopman et al., 1991; Kosco et al., 1992). They also play a subsequent role in selecting the high-affinity mutants from among the pool of rapidly dividing centrocytes. Each of these FDC-B cell interactions involves the delivery of a costimulatory signal(s) from the FDC to the B cell in addition to the antigen-specific interaction (Burton et al., 1993; Kosco-Vilbois et al., 1993). Although not yet identified, possible candidates for the costimulatory signals include the ligand for CD19, complement fragments on the FDC surface, other interactions involving CD23, adhesion molecules or chemokines. The CD40+ B7 high B cells thus selected then present processed antigen to GC T cells inducing CD40 ligand (CD40L) expression and the synthesis of cytokines such as IL-4 and IL-10, required for further B-cell proliferation, isotype switching, and Ab secretion. Normally, any B cell that expresses low-affinity Ig receptors or receptors specific for autoantigens do not normally receive the appropriate signals for further maturation, undergo programmed cell death (PCD) (Liu et al., 1991), and are taken up by tingible body macrophages.

Following HIV/SIV infections, virus-induced changes occur in LN GC that may enhance normal FDC-B cell interactions leading to polyclonal B cell activations and relative decreases in humoral HIV/SIV specific responses. During the period of follicular hyperplasia, GC increase in size and become irregular in shape and exhibit increased expression of adhesion molecules. The expanded area within the dark zone shows greatly increased FDC expression of CD23, rapidly proliferating KI-67+ centroblasts, and an accumulation of infiltrating CD45RO+ CD8+ cells. In addition the expansion of the light zone, where complexed viral antigens are deposited and CD4+ T cells are found, results in a much reduced mantle zone (Janossy et al., 1991; Joling et al., 1992; Persidsky et al., 1995). It is not currently known how an increase in the volume of the reticular network and increased FDC function relates to quantitative increases in FDC number. FDC-dependent and T cell-dependent mechanisms that may account for this observed GC B-cell hyperactivity consistent with these immunohisto-logical changes are described below and depicted in Figure 1.

**Increased Survival of Low-Affinity and Autoreactive B Cells**

(i) gp120 Cross-Linking of Ig Receptors on Low-Affinity B Cells

As HIV/SIV infections progress, virus particles become bound to FDC complement receptors (CR) either directly via C3b, iC3b, and C3d on the virus (Marschall et al., 1993; Thielen et al., 1993) or via antibody bridges. Studies in mice with the MAIDS virus (Masuda et al., 1995) indicate that during the infection, previously bound soluble nonviral antigens are also displaced, presumably because virions bearing C3 components dislodge and replace the preexisting Ag-Ab complexes from the complement receptors on FDC (CR1, CD2, and CR3). In this situation, presentation of FDC-bound virions expressing membrane gp120 molecules in a spacially ordered arrangement could induce sufficient cross-linking of B-cell Ig receptors to activate B cells. The increased avidity resulting from high levels of multipoint binding may thus overcome affinity considerations and result in the activation of cells with very low specificity for viral antigens, including some high-frequency autoreactive B cells. Such binding by B cells in the presence of costimulation signals from FDC might serve to prevent the downregulation of Bel-2 and up regulation of CD95 (fas) (Garrone et al., 1995) expression and render the cells susceptible to T-cell-derived signals, rescuing them from PCD that would normally have been their fate. Several predictions can be made according to this scenario: (1) Initially, depending on the B-cell repertoire of the individual, less stringent selection might result in an overall decrease in the percentage of total effective anti-HIV ASC, whereas that for other B-cell specificities might increase; and (2) whereas anti-gp120 antibody responses would be maintained at relatively high levels, those specific for monomeric soluble proteins, would remain low or be lost due to displacement from the FDC or an inability
of the specific B cells to gain physical access to these antigens on FDC coated with virus. In line with these predictions, published findings have indicated (1) a loss of HIV-specific ASC in blood concomitant with an increase in anti-DNA and anti-TNP ASC in late-stage individuals (Shirai et al., 1992); (2) a loss of serum p24 antibodies in patients with advanced disease in the face of high-gp120 antibodies titers

FIGURE 1 Interactions among FDC, B, and T cells accounting for enhanced FDC function and B-cell hyperactivity during HIV/SIV infections. For simplicity, different areas of the dendritic reticular network display distinct cellular interactions even though each B cell clearly undergoes several interactions simultaneously by virtue of the fact that each expresses Ig; CD21; MHC class II; CD40; LFA-1; VLA-4; B7 receptors for IL-4, IL-10, IL-6; and probably TNFR-1 or 2. In addition to activation of high-affinity B (Bh) cells, increased survival of low-affinity (Bh) or autoreactive B (Bh) cells could occur as a result of enhanced presentation by FDC-bound Ag and costimulation by T cells and FDC (arrows). The former FDC-dependent processes include: (1) multipoint (high-avidity) binding of B-cell Ig to viral particles (via gp-120). These may displace many preexisting and newly formed monomeric soluble Ag-Ab complexes from complement receptors or render them inaccessible to B cells; (2) cytokine (e.g., IFN-γ) induced increases in ICAM-1 and VCAM-1 integrins on FDC and their ligands, LFA-1 and VLA-4, on B cells; and (3) increased expression of CD23 on FDC in the expanded GC “dark zone” and subsequent increased interaction with its ligand CD21 on B cells. Although FDC stimulation of resting B cells is known to induce both B7-BB1 and MHC Class II molecules (Kosco-Vilbois et al., 1993), the extent to which each of these interactions are independently capable of providing sufficient signals to induce B-cell activation in vivo is still unclear. Together they may facilitate binding of FDC to low affinity or non-HIV-reactive B cells in the presence of FDC costimulation and T-cell-derived maturation factors sufficient to attain an activation threshold and avoid deletion. In the GC, cytokines produced by T cells, for example, IL-2, IL-4, and IL-10, require cognate recognition of Ag presented by activated B (B') cells or interdigitating dendritic cells (IDC) in addition to costimulatory signals delivered via B7-CD28 and CD40-CD40L binding. By contrast, synthesis and release of TNF-α, which is also known to induce plasma cells generation from activated B cells, appear to require virus infection of T cells rather than antigen-specific stimulation. Binding of CD4+ and CD8+ cells to FDC-bound virus leading to new rounds of infection or damage to FDC dendrites is also indicated. (See color plate V)
(Binley et al., 1997); and (3) in studies using duodenal biopsies from AIDS patients, a marked decrease in the numbers of ASC specific for the soluble antigens keyhole limpet hemocyanin and dog serum albumin in the absence of any decline in the percent anti-gp160 ASC (Eriksson et al., 1995).

(ii) Increased Expression of Adhesion and Activation Molecules on B Cells and FDC

Cytokines play a critical role in mediating immune function; the pattern of cytokine production determining the type of immune response generated. In addition, cytokines such as IFN-γ and tumour necrosis factor-α (TNF-α) are known to induce chemokines (Ebnet et al., 1996) and regulate lymphocyte migration and sequestration by virtue of their ability to induce adhesion molecules, eg, ICAM-1, VCAM-1, LFA-1 on lymphocytes, antigen-presenting cells, and vascular endothelium (Anderson and Reynolds, 1979; Mackay, 1991; Colditz and Watson, 1992; May and Ager, 1992; Adams et al., 1994). Since the ability of FDC-enriched LN cells to form clusters in vitro is known to be dependent on LFA-1-ICAM-1 and VLA-4-VCAM-1 interactions (Koopman et al., 1991; Kosco et al., 1992), it is highly likely that CD8+ cell-derived IFN-γ production in GC (Emilie et al., 1990; Graziosi et al., 1994) (unpublished observation) can induce increased expression of ICAM-1 and VCAM-1 on FDC and facilitate their binding to B cells. Enhanced VCAM-1 expression has been demonstrated in reactive human LN (Ruco et al., 1992) and more recently on FDC in GC of SIV-infected LN (Persidsky et al., 1995). Although increases in cells producing IL-1, IL-4, IL-6, TNF-α, and IFN-γ have been reported in LN and blood from HIV and SIV-infected individuals (Emilie et al., 1990; Fan et al., 1993; Graziosi et al., 1994; Rosenberg et al., 1994a; Persidsky et al., 1995), a comparison with the appropriate control LN or blood from uninfected monkeys or from humans with HIV-unrelated hyperplasia has indicated to date that only IFN-γ production is consistently increased throughout infection.

Another alteration observed in GC of HIV-infected LN is the greatly increased expression of CD23 antigen in the expanded part of the “dark” zone: CD23 being expressed normally only on FDC and B cells in the apical light zone. This CD23 positivity, which may result from IL-4 synthesis or increased numbers of immune complexes or FDC (Maeda et al., 1992) may increase signalling to B cells via interaction with CD21 (Bonnefoy et al., 1993) and may skew the maturation of B cells into plasma cells rather than memory cells (Liu and Banchereau, 1996). Regardless of whether binding of integrins with their ligands or CD23 with CD21 is able to generate maturation signals in vivo (Liu et al., 1992; Bonnefoy et al., 1993; Adams et al., 1994; Lub et al., 1995; Liu and Banchereau, 1996), it is possible that such interactions may augment low-affinity binding of FDC-bound antigen to membrane Ig sufficient to allow the costimulation and/or differentiation of low-affinity B cells that would normally have been destined to die.

Increase in T-Cell-Derived Maturation and Differentiation Signals

Maturation and differentiation of activated B cells into IgG and IgA ASC or memory cells require the participation of T cells either via cognate CD40-CD40L or B7-CD28 interactions and the elaboration of cytokines eg. IL-4 and IL-10 (Liu and Banchereau, 1996), or via nonspecific T-cell-derived signals, eg., TNF-α and IL-2 (Macchia et al., 1993). In the former case, LN GC from HIV+ individuals have been shown to have increased expression of CD40 ligand (Janossy, personal communication). In addition, although different studies have published variable findings with respect to IL-10 secretion/expression (Montaner, 1994), the evidence to date shows no obvious reduction in IL-10 levels in LN or blood during HIV infection. The high levels of IgG produced during HIV/SIV infections indicate that sufficient T-cell help is present to induce isotype switching.

In the case of nonspecific signaling, TNF-α, produced by CD4+ cells from HIV patients or infected
cells in vitro, is able to induce IgG production by syngeneic (presumably activated) B cells (Macchia et al., 1993). In this way, any B cell of varying affinity for HIV antigens that are rescued by interaction with HIV-coated FDC and express TNF receptors 1 or 2 (TNFR-1 or 2) may be induced to secrete Ig. The TNF-α and IL-6 families of molecules are important at several levels. Thus, in addition to the critical role of TNF-α, lymphotoxin-α (LT-α), and TNFR-1 on FDC generation, GC formation and the levels of IgG production as recently demonstrated using knockout mice (Le Hir et al., 1996; Matsumoto et al., 1996), IL-6 is produced by FDC (M. K.-V., unpublished observation) and is important in spontaneous anti-HIV production by B cells (Amadori et al., 1991; Delfraissy et al., 1992). Furthermore TNF-α and IL-6 cytokines have also been closely linked with increased HIV and SIV replication (Birx et al., 1993; Scala et al., 1994; Virelizier, 1994), thereby increasing antigen levels.

Finally, it is noteworthy that whereas B cells constitute roughly 30% of total lymphocytes in the body, usually only 10-20% of the 1-2% of lymphocytes that circulate in the blood at any time are B cells (Westermann and Pabst, 1992). Thus the usual sampling of PBMC may not be an accurate means of assessing overall B-cell function. Accordingly, (1) the number of peripheral blood B cells secreting anti-gp-160 antibody shows no significant correlation with serum anti-HIV gp-160 antibody concentrations (Schwartz et al., 1994); (2) BM contains >30-fold more anti-gp160 ASC than blood following immunization with gp160 (Cosentino et al., 1994); and (3) levels of HIV-specific ASC in duodenal biopsies and BM are present in large numbers throughout infection when numbers in blood are very low (Eriksson et al., 1995). These results emphasize the need to examine tissues and not just blood in order to elucidate the mechanisms underlying the observed hyperactivity. In this context, another caveat arises because during the budding process, HIV virions become enveloped by the lipid bilayer of the host cell and simultaneously by many host-cell proteins (Arthur et al., 1992; Moerloot et al., 1993; Dierich, unpublished observation). The extent to which such molecules may be responsible for the increased expression of adhesion and activation molecules and receptors as well as enhanced effector function observed in HIV+ GC are currently only beginning to be understood.

MATERIALS AND METHODS

Animals and Infections

Rhesus macaques were infected with 1-10 tissue culture infectious doses 50 (TCID50) of SIV-251 and sacrificed between days 93 and 535, at which time mesenteric LN were removed for assaying. Control LN were collected from rhesus and pig-tailed macaques (Regional Primate Center, Seattle, WA).

Analysis of Lymphocyte Populations by Flow Cytometry (FCM)

Fresh or cultured cells were double or triple stained with fluorescein isothiocyanate (FITC)-coupled CD45RA mAb (Gentrak, Wayne, PA), anti-CD4, and CD56 coupled to phycoerythrin (PE) and CD20 and CD8 mAbs coupled to either FITC or peridinin chlorophyll protein (PerCP; Becton Dickinson, Mountain View, CA). Analysis was done on a FACScan (Becton Dickenson).

Isolation of Cells and Culture Conditions

FDC-enriched cultures were prepared using mesenteric LN from uninfected or SIV-infected macaques according to the method of Schnizlein et al. (1985) with modifications (Kosco et al., 1992; Rosenberg et al. 1997). Cultured cells were analyzed phenotypically by FCM and for their proliferative ability by the incorporation of [3H]-TdR following a 6-8-hr pulse. In some cases, 1-ml cultures (2.5 × 10⁶ cells/well) were set up and 100-μl aliquots from each of three wells were pulsed with [3H]-TdR before harvesting producing similar results. Other control cultures were comprised of single-cell suspensions produced by passing a portion of the same un fractionated LN through a sieve; such mechanical disruption results in the destruction of the FDC (Schnizlein et al., 1985). Although the latter controls may be suboptimal due to
a loss of plasma cells and blasts noted using this method, the rapid loss of LN cells with high-background incorporation in some of these FDC-deprived cultures served to indicate the important role of FDC in sustaining proliferation.

**Immunoglobulin and Anti-SIV Antibody Assays**

IgG1 levels and anti-SIV antibody levels were measured using commercial ELISA kits, which cross react with macaque immunoglobulin obtained from The Binding Site (Birmingham, UK) and Genetic Systems (Seattle, WA).

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