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HIV-1 Envelope Triggers Polyclonal Ig Class Switch Recombination through a CD40-Independent Mechanism Involving BAFF and C-Type Lectin Receptors[1]

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Switching from IgM to IgG and IgA is essential for antiviral immunity and requires engagement of CD40 on B cells by CD40L on CD4$^+$ T cells. HIV-1 is thought to impair CD40-dependent production of protective IgG and IgA by inducing progressive loss of CD4$^+$ T cells. Paradoxically, this humoral immunodeficiency is associated with B cell hyperactivation and increased production of nonprotective IgG and IgA that are either nonspecific or specific for HIV-1 envelope glycoproteins, including gp120. Nonspecific and gp120-specific IgG and IgA are sensitive to antiretroviral therapy and remain sustained in infected individuals with very few CD4$^+$ T cells. One interpretation is that some HIV-1 Ags elicit IgG and IgA class switch DNA recombination (CSR) in a CD40-independent fashion. We show that a subset of B cells binds gp120 through mannose C-type lectin receptors (MCLRs). In the presence of gp120, MCLR-expressing B cells up-regulate the CSR-inducing enzyme, activation-induced cytidine deaminase, and undergo CSR from IgM to IgG and IgA. CSR is further enhanced by IL-4 or IL-10, whereas Ab secretion requires a B cell-activating factor of the TNF family. This CD40L-related molecule is produced by monocytes upon CD4, CCR5, and CXCR4 engagement by gp120 and cooperates with IL-4 and IL-10 to up-regulate MCLRs on B cells. Thus, gp120 may elicit polyclonal IgG and IgA responses by linking the innate and adaptive immune systems through the B cell-activating factor of the TNF family. Chronic activation of B cells through this CD40-independent pathway could impair protective T cell-dependent Ab responses by inducing immune exhaustion. *The Journal of Immunology,* **2006, 176: 3931–3941.**

The HIV type 1 infection impairs protective IgG and IgA responses against pathogens and vaccines by inducing progressive loss of CD4$^+$ T cells (1). Paradoxically, HIV-1-induced humoral immunodeficiency is associated with polyclonal B cell activation, hypergammaglobulinemia, and nonspecific switching from IgM to IgG, IgA, and IgE (2–4). Superimposed on these nonspecific Ab responses are strong, HIV-1-specific IgG and IgA responses to envelope (Env) glycoproteins gp41 and gp120 (3) and structural (Gag) proteins p17 and p24 (5–8). Gag-specific Ab responses decline in parallel with CD4$^+$ T cells, whereas both nonspecific and Env-specific Ab responses remain sustained throughout the course of the disease despite the progressive loss of CD4$^+$ T cells (9, 10). One interpretation is that certain HIV-1 Ags, including Env glycoproteins, can elicit IgH class switching and Ab production in the absence of T cell help to B cells.

Most Ags initiate Ab production by up-regulating CD40L on CD4$^+$ T cells (11). Engagement of CD40 by CD40L stimulates B cells to migrate in the germinal center (GC),[3] a specialized follicular compartment that fosters Ab diversification through Ig V(D)J somatic hypermutation and IgH class switching (12). Somatic hypermutation increases the Ab affinity for Ag by introducing point mutations within the V gene encoding the Ag-binding region of Igs, whereas class switching diversifies the Ab effector functions by substituting the C region of IgM with that of IgG, IgA, or IgE through class switch DNA recombination (CSR). Ultimately, GC B cells differentiate to plasma cells, which provide immune protection by releasing high-affinity IgG, IgA, and IgE both systemically and at portal sites of entry (13).

T cell-dependent Ab responses require 5–7 days, which is too much of a delay to control infections by quick-replicating pathogens. To compensate for this limitation, splenic marginal zone (MZ) and mucosal B cells rapidly undergo T cell-independent IgM production as well as switching to IgG, IgA, and IgE in response to pathogen-associated molecular patterns (PAMPs), including viral glycoproteins (14–17). Although unable to confer immune protection, low-affinity Abs generated through this T cell-independent pathway facilitate early Ag removal by innate immune cells. The mechanism underlying T cell-independent Ab production remains unclear. By stimulating innate immune cells through pattern-recognition receptors (PRRs) and type I IFN-α, PAMPs up-regulate B cell-activating factor of the TNF family (BAFF), which in turn elicits CD40-independent CSR and Ab production in B cells (18,

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[3] Abbreviations used in this paper: GC, germinal center; AID, activation-induced cytidine deaminase; AT-2$_{MN}$, aldrithiol-2-inactivated HIV-1 strain MN; BAFF, B cell-activating factor of the TNF family; MCLR, mannose C-type lectin receptor; CSR, class switch DNA recombination; DC, dendritic cell; DC-SIGN, DC-specific intercellular adhesion molecule-3-grabbing nonintegrin; SDF, stromal cell-derived factor; MFI, mean fluorescence intensity; MR, mannose receptor; MZ, marginal zone; PAMP, pathogen-associated molecular pattern; PRR, pattern-recognition receptor.

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19). In addition, PAMPs directly activate B cells through stereotyped BCRs, PRRs, and complement receptors such as CD21 (15, 17, 20). The relative contribution of these T cell-independent pathways to HIV-1-induced IgG and IgA production is unknown.

HIV-1 Ags play a key role in the activation of B cells as both nonspecific and virus-specific Ab responses subside upon initiation of antiretroviral therapy (21). Of viral Ags, Env accumulates in lymphoid follicles and can activate B cells without requiring $CD4^+$ T cell help (10, 22–27). Env-induced T cell-independent Ab responses might be further enhanced by BAFF because Env stimulates innate immune cells, including monocytes and macrophages, to up-regulate IFN-α and IL-10, two key cytokines endowed with powerful BAFF-inducing activity (18, 19, 28–31). Consistent with this possibility, HIV-1 infection is associated with increased BAFF production by monocytes and with elevated serum BAFF levels, which correlate with disease progression and $CD4^+$ T cell loss (32, 33).

HIV-1 Env could stimulate BAFF production by engaging specific receptors on the surface of innate immune cells. A functional HIV-1 Env complex comprises three surface gp120s, each noncovalently linked to a transmembrane gp41 subunit (34). By engaging the CD4 receptor on $CD4^+$ T cells, monocytes, macrophages, and dendritic cells (DCs), gp120 undergoes conformational changes that enable subsequent binding of a coreceptor, either the CCR5 (R5 isolates) or the CXCR4 (X4 isolates) chemokine receptor (35). Not only do CD4, CCR5, and CXCR4 mediate HIV-1 entry in target immune cells, but they can also deliver activation signals that alter immune cell functions, including cytokine production, at least in vitro (31, 36). These signals can be elicited by either virion-associated gp120 trimers or soluble gp120 monomers released by free virions or infected cells. HIV-1 Env would deliver additional signals through DC-specific intercellular adhesion molecule-3-grabbing nonintergrin (DC-SIGN), mannose receptor (MR), and langerin, three DC-associated mannose C-type lectin receptors (MCLRs) that bind high-order mannose saccharides on the glycosylated portion of gp120 (37, 38). The role of these PRRs in gp120-induced Ab responses is not known.

A better understanding of B cell responses to gp120 is necessary to elucidate HIV-1-induced B cell abnormalities, such as polyclonal hypergammaglobulinemia and humoral immunodeficiency, and to develop more effective vaccine strategies (2, 39). Given its ability to stimulate innate immune cells as well as B cells (22–24, 26, 31, 40), gp120 was hypothesized to initiate polyclonal CSR and Ab production through a CD40-independent mechanism linking the innate immune system with the adaptive B cell response. Our findings indicate that gp120 activates B cells and triggers IgG, IgA, and IgE class switching through an innate pathway involving BAFF and MCLRs. Chronic activation of this pathway could progressively compromise protective T cell-dependent Ab responses by inducing B cell exhaustion.

Materials and Methods

Cells

Buffy coats were purchased at the New York Blood Center (New York, NY). Tonsillar and splenic tissue specimens were obtained from patients undergoing tonsillectomy and splenectomy owing to tonsillitis and trauma, respectively. Tonsillar and spleen samples were obtained with the approval of the Institutional Review Board of Weill Medical College of Cornell University (New York, NY) and after parental or patient's informed consent. Blood, tonsillar, and splenic mononuclear cells were collected as previously described (18, 20, 41, 42) and enriched in $CD19^+$ B cells upon depletion of cells expressing CD3, CD11b, CD14, CD16, and CD56. IgD^+ B cells were sorted with a FITC-mAb to IgD (Southern Biotechnology Associates) and anti-FITC MicroBeads (Miltenyi Biotec). Microbeads are designed ad hoc to avoid significant receptor cross-linking. To further minimize sorting-induced signaling, all sorting procedures were performed on ice. In some experiments (data not shown), IgD^+ B cells were negatively selected through a commercially available kit (Miltenyi Biotec). Positively and negatively selected IgD^+ B cells displayed comparable size, CD69 expression, PI3K phosphorylation levels as well as CD40-dependent proliferation and Ig secretion. IgD^+ B cells were segregated into IgD^+CD1c^- and IgD^+CD1c^+ fractions with MultiSort Release Reagent (Miltenyi Biotec), PE-mAb to CD1c (BD Pharmingen), and anti-PE MicroBeads. In some experiments, IgD^+CD1c^+ B cells were segregated with biotin-mAb to CD1c (BD Pharmingen) and streptavidin-Microbeads. Monocytes were sorted with biotin-mAb to CD14 (BD Pharmingen) and streptavidin-Microbeads. The purity of sorted IgD^+ B cells, IgD^+CD1c^- B cells, IgD^+CD1c^+ B cells, and $CD14^+$ monocytes always exceeded 90%.

Binding assays

Fully glycosylated recombinant IIIB gp120 (ImmunoDiagnostics) and BaL gp120 (National Institutes of Health (NIH), AIDS Research and Reference Reagent Program, Rockville, MD) were prepared from Chinese hamster ovary and human embryonic kidney 293 cells, respectively. Both BaL and IIIB gp120s were biotinylated with a commercially available kit (Pierce). Naive B cells sorted with a FITC-mAb to IgD were preincubated for 40 min at 4°C in binding medium (i.e., RPMI 1640 medium with 1% albumin and 10 mM HEPES (pH 7.4), but without sodium carbonate) in the presence or absence of inhibitors. Then, biotin-gp120 was added at 5 μg/ml and labeled with 5 μg/ml PE-streptavidin (BD Pharmingen). Staining with 1 μg/ml allophycocyanin-mAb to CD1c (Miltenyi Biotec) was followed by measurement of gp120 binding on gated IgD^+CD1c^+ B cells by flow cytometry. Mean fluorescence intensity (MFI) was converted into a percentage of gp120 binding as follows: ((MFI of gp120 binding in the presence of inhibitors) − (background MFI in the absence of gp120)/(MFI of gp120 binding with no inhibitors) − (background MFI in the absence of gp120)) × 100. Competition experiments were performed with 100 μg/ml unlabeled gp120, 5 mg/ml mannan, 125 mM α-methyl-mannopyranoside, 100 μM bovine lactoferrin, 50 μM bovine β-lactoglobulin, 50 μM human albumin (Sigma-Aldrich), 5 mg/ml mannose-BSA (V-Labs), 20 μg/ml stromal cell-derived factor-1 (SDF)-1α, and 20 μg/ml RANTES (R&D Systems), respectively. Blocking experiments were performed with 30 μg/ml control MOPC21 mAb to an irrelevant epitope (Sigma-Aldrich) or with 30 μg/ml blocking mAbs B4 to CD4, 12G5 to CXCR4, 2D7 to CCR5 (NIH, AIDS Research and Reference Reagent Program), AZN-D1 to DC-SIGN (Beckman Coulter), eB-h209 to DC-SIGN (eBioscience), clone 19 to MR (BD Pharmingen), DCGM4 to langerin (Immunotech), MG38 to DEC-205, or B-Ly4 to CD21 (BD Pharmingen). Immobilized and soluble Abs to Ig κ and λ L chains (Caltag Laboratories) were used at 30 μg/ml for 2 h to induce extensive endocytosis-dependent BCR down-regulation.

Cultures

B cells were cultured in RPMI medium with 10% bovine serum with or without 500 ng/ml BAFF (ALEXIS Biochemicals), 200 U/ml IL-4, 200 ng/ml IL-10 (Schering-Plough), 2 μg/ml anti-μ (anti-BCR mimicking a soluble Ag) Ab (Caltag Laboratories), 2 μg/ml Immunobead Reagent (anti-$BCR^{TI\text{-}2}$ mimicking a T cell-independent type 2 Ag) against both H and L chains of human Igs (Irvine Scientific), 20 ng/ml PMA (Sigma-Aldrich), 1 mg/ml LPS (Sigma-Aldrich), or 5 mg/ml oligodeoxynucleotide 2006-type B CpG DNA (Operon Technologies). Preliminary dose-response assays, including proliferation and Ig secretion assays, showed induction of optimal B cell responses by each reagent at the concentration indicated. Recombinant JR-FL (Progenics Pharmaceutical), BaL (NIH, AIDS Research and Reference Reagent Program), and IIIB and MN (ImmunoDiagnostics) gp120s were used at 4 nM, unless otherwise stated. Aldrithiol-2-inactivated HIV-1 strain MN ($AT\text{-}2_{MN}$) virus from Dr. J. Lifson (National Cancer Institute, AIDS Vaccine Program, Frederick, MD) was used at a concentration equivalent to 30 ng/ml p24, unless otherwise stated. HUT78 microvesicles were used as control. Monocytes were incubated in RPMI medium with 2% bovine serum with or without 10 U/ml IFN-α (Sigma-Aldrich), 10 ng/ml IL-10, 20 ng/ml SDF-1α, or 50 ng/ml RANTES (R&D Systems). Blocking experiments were performed as described. Small molecules AD101, which antagonizes CCR5, and AMD3100, which antagonizes CXCR4, were used at 50 nM. Monocytes were also incubated with blood IgD^+ B cells (1:2 ratio) with or without a blocking mAb to BAFF (ALEXIS Biochemicals).

Flow cytometry

Binding of biotin-gp120 to B cells was evaluated by using streptavidin conjugated with FITC, PE, or allophycocyanin. B cells were also stained with different combinations of FITC-, PerCP-, PE-, or allophycocyanin-conjugated mAbs to IgD, IgM, IgG (Southern Biotechnology Associates), CD1c, CD4, CD10, CD11c, CD19, CD21, CD23, CD27, CD38, CD77,

CD83, CD95, CD123, CXCR4, CCR5, MR (BD Pharmingen), BAFF receptor, DEC-205 (eBioscience), DC-SIGN (Beckman Coulter), or langerin (Immunotech). Monocytes were stained with FITC-mAb to CD14 (Serotec) and PE-mAb to membrane-bound BAFF (eBioscience). Cells were acquired with a FACSCalibur analyzer (BD Pharmingen).

Immunofluorescence and immunohistochemistry

FACSorted IgD$^+$ gp120$^-$ and IgD$^+$ gp120$^+$ B cells were adhered to polylysine-coated glass slides for 30 min at room temperature, fixed, and washed as described (18). Nuclei were visualized with DAPI (4′,6-diamidine-2′-phenylindole dihydrochloride; Boehringer Mannheim), whereas IgD was restained with a second FITC-mAb (Sigma-Aldrich). A rat mAb to DC-SIGN (eBioscience) was labeled with a Cy3 anti-rat Ab (Jackson ImmunoResearch Laboratories). Acetone-fixed cryostat tonsillar and splenic sections (5 μm) were first incubated with goat biotin-mAb to IgD and rat mAb to DC-SIGN or a mouse Ab to MR or langerin, and then with Alexa Fluor 488-streptavidin and Cy3 anti-rat or anti-mouse Ab (Jackson ImmunoResearch Laboratories). Slides were analyzed with a microscope Zeiss Axioplan 2 (Atto Instruments).

Proliferation assays

A total of 10^5 B cells were cultured in 96-well microtiter plates and pulsed with 1 μCi [^{3}H]TdR after 4 days. Cells were harvested for the measurement of [^{3}H]TdR uptake after 18 h.

RT-PCR and Southern blots

CSR-related transcripts and activation-induced cytidine deaminase (AID) and β-actin transcripts were amplified as described (18). CSR-related transcripts were hybridized with radiolabeled oligoprobes encompassing Cγ, Cα, Cϵ, or Cμ (18). DC-SIGN, MR, langerin, IgM, TLR4, and CD14 transcripts were amplified using the following primers: DC-SIGN, forward 5′-AAATCTCTGGGACTGTTCTTTGTC-3′ and reverse 5′-TAGGTG GAAGTTGAACAGATGGTA-3′; MR, forward 5′-GCAGATGGAAAC ATCTAATGAACG-3′ and reverse 5′-ACCCGATATGACAGAAAAC TGGAT-3′; langerin, forward 5′-AGTTAAAAACCAGTGTGGAG AAGG-3′ and reverse 5′-CCGCTGTTTTATACAGAAACTCCT-3′; IgM, forward 5′-GACACGGCTGTGTATTACTGTGCG-3′ and reverse 5′-CCGAATTCAGACGAGGGGGAAAAGGGTT-3′; TLR4, forward 5′-ACCTCCCCTTCTCAACCAAGAACC-3′ and reverse 5′-ATTGTGA GCCACATTAAGTTCTTTC-3′; and CD14, forward 5′-AGACCTCAGC CACAACTCGCTGC-3′ and reverse 5′-GTTCCGACACCCCCACCGA CAGGG-3′.

ELISA and immunoblots

Total Igs were measured as reported (18). To detect gp120-reactive Igs, microplates were coated overnight at 4°C with BaL gp120. Microplates were blocked with PBS and 1% BSA for 2 h at room temperature and B cell supernatants were incubated for 2.5 h at 37°C. A peroxidase-conjugated goat polyclonal Ab to human IgG and IgA (Caltag Laboratories) was added at room temperature, and the peroxidase reaction was developed after 2 h with tetramethylbenzidine. Standard curves were generated using known concentrations of mAbs to gp120. Soluble BAFF was detected as reported (18).

Statistical analysis

Values were calculated as mean ± SD for at least three separate experiments performed in triplicate. The significant difference between experimental variables was determined by using the Student's t test, and a value of $p < 0.05$ was considered statistically significant.

Results

HIV-1 gp120 naturally binds to a subset of tonsillar B cells

HIV-1 gp120 binds to a subset of human B cells (24, 43). The phenotype of these B cells remains unclear. From 4 to 16% of tonsillar CD19$^+$ B cells bound BaL (R5) gp120 and IIIB (X4) gp120 (data not shown), but not p24 (Fig. 1*A*). Although B cells with no gp120-binding activity included naive IgD$^+$CD38$^-$, founder GC IgD$^+$CD38$^+$, GC IgD$^-$CD38$^+$, memory IgD$^-$CD38$^-$, and plasmacytoid IgD$^-$CD38^{2+} subsets, B cells with gp120-binding activity were mostly IgD$^+$CD38$^+$. Unlike transitional IgD$^+$CD38$^+$ B cells (44), gp120-binding B cells lacked the ectoenzyme CD10 and expressed the complement receptor CD21 (Fig. 1*B*). Compared with founder GC IgD$^+$CD38$^+$ B cells and full-blown IgD$^-$CD38$^+$ GC B cells (45–47), gp120-binding B cells were smaller and lacked CD10, the globotriaosyl ceramide CD77, and the death-inducing molecule CD95, also known as Fas. Compared with naive IgD$^+$CD38$^-$ B cells (45), gp120-binding B cells were equipped with the activation molecules CD27 and CD38 and expressed IgD and CD23 at lower density and CD21 at higher density. In addition, gp120-binding B cells were slightly larger than naive B cells.

Although equipped with CD27, gp120-binding B cells were different from memory IgD$^-$CD38$^-$ B cells (48, 49), as they expressed IgD and CD38 and lacked surface IgG. In addition, gp120-binding B cells differed from plasmacytoid IgD$^-$CD38^{high} B cells (50), which were larger and expressed more CD38, but little or no IgM, IgD, CD21, CD23, and BAFF receptor, which is a pan-B cell marker. Overall, gp120-binding B cells resembled IgM$^+$IgD^{low}CD21^{high}CD27$^+$CD38$^+$ subepithelial B cells, the tonsillar equivalent of splenic MZ B cells (51). Similarly to splenic MZ B cells (52–54), tonsillar gp120-binding B cells expressed the MHC class I-like molecule CD1c together with IgM, IgD^{low}, CD21^{high}, and CD27. In addition to CD1c, some of the gp120-binding B cells expressed CD11c, CD83, and CD123, all DC-associated molecules that can be also expressed by activated B cells, including DC-like B cells and MZ B cells (55–57). These data indicate that gp120 naturally binds to a tonsillar IgD$^+$CD38$^+$ B cell subset expressing an activated phenotype.

Tonsillar gp120-binding B cells express MCLRs and occupy both extrafollicular and follicular areas

Given their ability to bind gp120, tonsillar IgD$^+$CD1c$^+$ B cells were hypothesized to express canonical HIV-1 receptors. Tonsillar IgD$^+$CD1c$^-$ B cells with no gp120-binding activity and IgD$^+$CD1c$^+$ B cells with gp120-binding activity expressed CXCR4, but lacked CD4 and CCR5 (Fig. 2*A*). Because gp120 does not bind CXCR4 in the absence of CD4 (35, 58), IgD$^+$CD1c$^+$ B cells were hypothesized to bind gp120 through MCLRs (38). This hypothesis was consistent with prior studies showing that MCLR expression is not confined to DCs, but extends to B cells (59, 60). Tonsillar IgD$^+$CD1c$^-$ B cells with no gp120-binding activity lacked DC-SIGN, MR, and langerin, but partially expressed DEC-205. In contrast, gp120-binding IgD$^+$CD1c$^+$ B cells expressed DC-SIGN and to a lesser extent MR and langerin in addition to DEC-205. DC-SIGN could be visualized on sorted tonsillar IgD$^+$ B cells with gp120-binding activity, but not on sorted tonsillar IgD$^+$ B cells with no gp120-binding activity (Fig. 2*B*).

DC-SIGN, MR, and langerin could be detected on 65–77 ± 8% ($n = 3$), 45–56 ± 5% ($n = 3$), and 55–68 ± 7% ($n = 3$) of sorted IgD$^+$ B cells with gp120-binding activity, respectively. In contrast, sorted IgD$^+$ B cells with no gp120-binding activity were negative for DC-SIGN, MR, and langerin. Of note, IgD$^+$ DC-SIGN$^+$ B cells were identified within the subepithelial area outside lymphoid follicles (Fig. 2, *C* and *D*), which represents the tonsillar equivalent of the splenic MZ (51). In addition to IgD$^+$ DC-SIGN$^+$ B cells, the subepithelial area comprised IgD$^-$ DC-SIGN$^+$ DCs. Some IgD$^+$ DC-SIGN$^+$ B cells were detected in the follicular mantle, which comprises activated naive B cells and founder GC B cells (45). Virtually no IgD$^+$ DC-SIGN$^+$ B cells were in the GC, consistent with the IgD$^-$ B cell make-up of this region of the follicle (45–47). Thus, gp120 binds to MCLR-expressing B cells located within both extrafollicular and follicular regions of the tonsil.

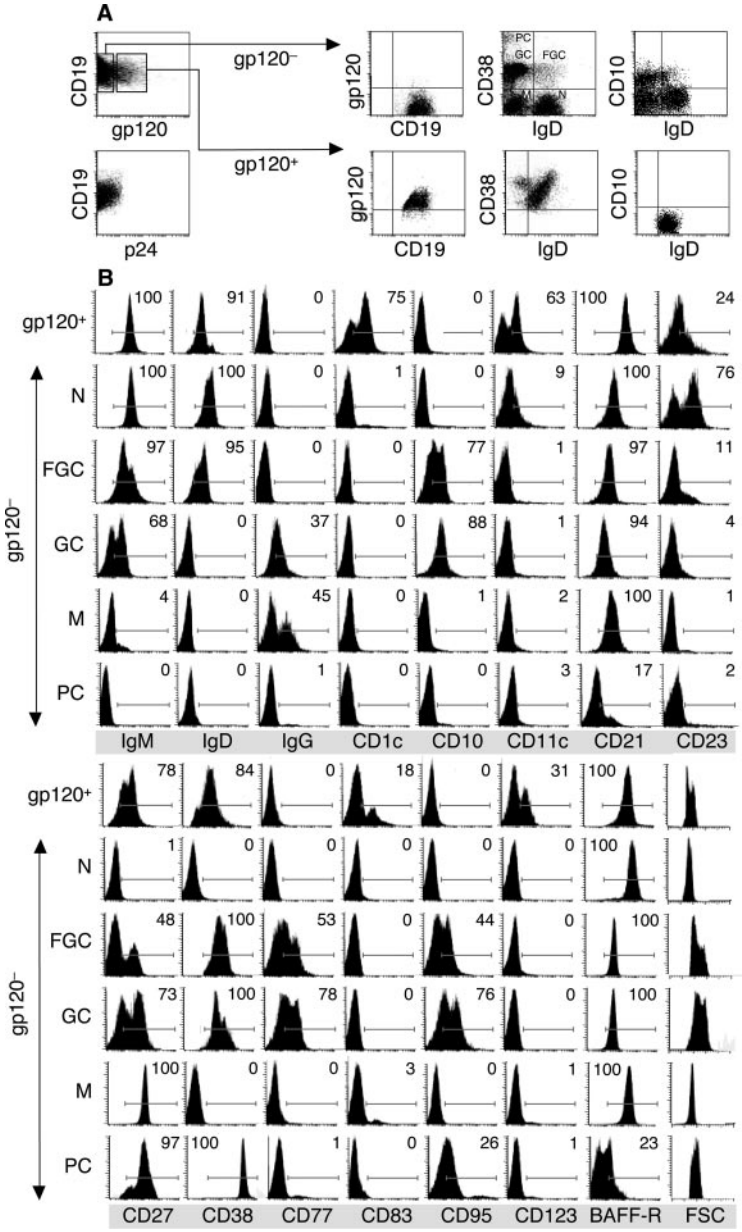

FIGURE 1. HIV-1 gp120 binds to a subset of tonsillar B cells. *A*, Binding of gp120 or p24 to tonsillar B cells and expression of IgD, CD10, CD19, and CD38 by tonsillar B cells with or without gp120-binding activity ($gp120^+$ and $gp120^-$, respectively). $gp120^+$ and $gp120^-$ fractions were purified by depleting total B cells from gp120-binding B cells. N, naive; FGC, founder GC; M, memory; PC, plasmacytoid B cells. *B*, IgM, IgD, IgG, CD1c, CD10, CD11c, CD21, CD23, CD27, CD38, CD77, CD83, CD95, CD123, BAFF receptor on tonsillar $gp120^+$ and $gp120^-$ B cells, and forward scatter (FSC, i.e., cell size) are shown. Values (*inset*) correspond to the percentage of positive B cells. Data depicted represent one of five experiments yielding similar results.

Splenic gp120-binding B cells express MCLRs and occupy extrafollicular areas

Tonsillar B cells with gp120-binding activity express an $IgD^{low}IgM^+CD1c^+CD21^{high}CD27^+$ phenotype similar to that of splenic MZ B cells (51–54). Because mouse splenic MZ B cells are capable of binding viral particles (16), it was verified whether human splenic IgD^+CD1c^+ B cells bind gp120 and express MCLRs. Splenic IgD^+CD1c^+ B cells accounted for 5–15% of total splenic B cells, expressed variable levels of DC-SIGN, MR, and langerin, and exhibited gp120-binding activity (Fig. 3*A*). Although partially expressing DEC-205, IgD^+CD1c^- B cells lacked DC-SIGN, MR, and langerin and did not bind gp120. At low magnification, IgD^+ B cells expressing CD1c, DC-SIGN, langerin and to a lesser extent MR could be detected within both para-MZ and MZ areas of the spleen (Fig. 3*B*). Compared with MZ and para-MZ B cells, follicular B cells expressed more IgD, but no or little CD1c, DC-SIGN, MR, and langerin. Of note, red pulp sinusoids were positive for MR and to some extent DC-SIGN. At higher magnification, MZ and para-MZ IgD^+ B cells expressing DC-SIGN, MR, and langerin were admixed with IgD^+ B cells completely negative for these MCLRs (Fig. 3*C*). These findings indicate that gp120 binds to MCLR-expressing B cells lodged within extrafollicular areas of the spleen.

Tonsillar and blood B cells up-regulate MCLRs upon activation by BAFF, CD40L, and cytokines

Because MCLR expression by tonsillar B cells correlates with an activated phenotype, it was verified whether resting blood B cells up-regulate MCLRs upon exposure to canonical B cell-activating stimuli, such as IL-4, IL-10, BAFF, and CD40L. Initial experiments were conducted with IL-4, a B cell-activating cytokine with well-defined MCLR-inducing activity (18, 59). In line with recent findings showing circulating MZ IgD^+CD1c^+ B cells (54), a variable proportion of blood B cells constitutively expressed CD1c together with DC-SIGN, MR, langerin, DEC-205, and CD11c and exhibited gp120-binding activity (Fig. 4*A*). A subset of tonsillar B cells displayed a similar phenotype (Fig. 4*B*). The proportion of blood and tonsillar B cells expressing DC-SIGN, MR, langerin,

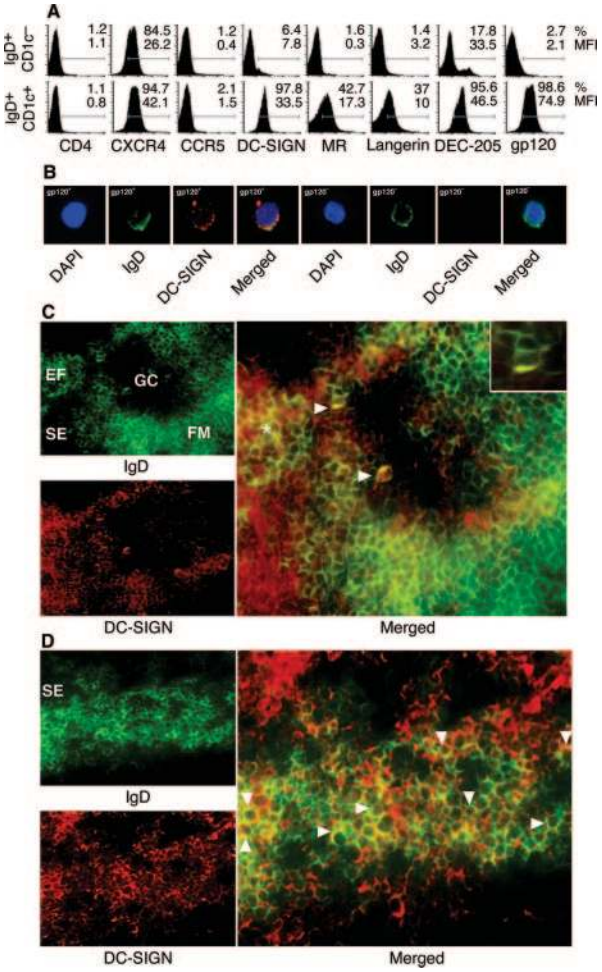

FIGURE 2. HIV-1 gp120-binding B cells express MCLRs and colonize both follicular and extrafollicular regions of tonsillar lymphoid tissue. *A*, CD4, CXCR4, CCR5, DC-SIGN, MR, langerin, DEC-205, and gp120-binding activity on tonsillar IgD$^+$CD1c$^-$ and IgD$^+$CD1c$^+$ B cells. Values (*inset*) indicate the percentage of positive cells (*top*) and of MFI (*bottom*). *B*, IgD (green) and DC-SIGN (red) in sorted IgD$^+$ gp120$^+$ and IgD$^+$ gp120$^-$ B cells from tonsils. Nuclei were visualized with DAPI (blue). *C* and *D*, IgD (green) and DC-SIGN (red) in tonsillar tissue sections (magnification, ×40). FM, follicular mantle; SE, subepithelial area. Extrafollicular B cell focus (∗) is indicated. Arrowhead shows IgD$^+$ DC-SIGN$^+$ B cells. One representative IgD$^+$ DC-SIGN$^+$ B cell is shown (*inset*) in *C* at higher magnification in the *top right*. Data depicted are from one of four experiments yielding similar results.

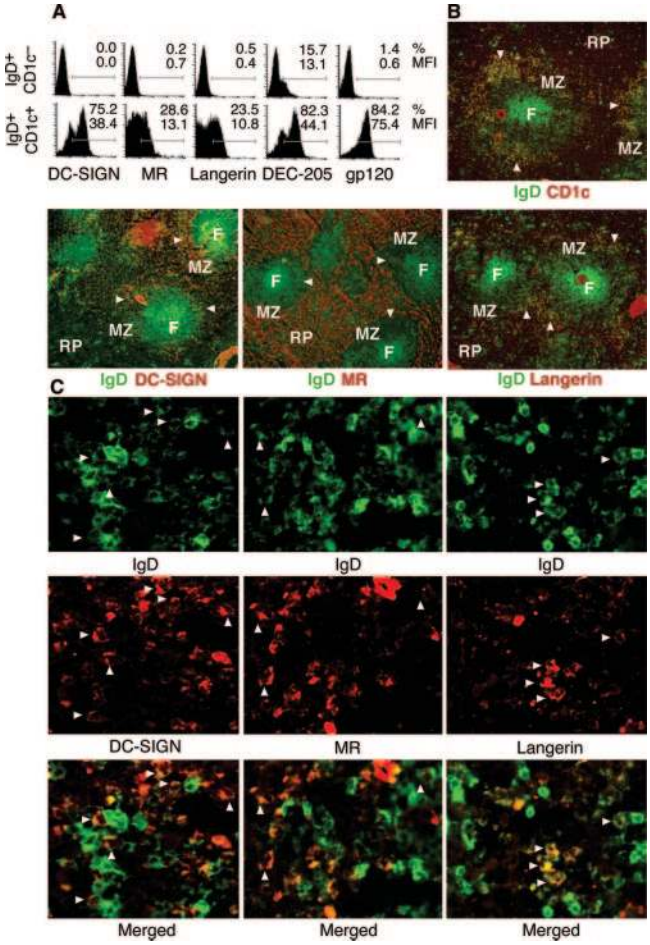

FIGURE 3. HIV-1 gp120-binding B cells express MCLRs and colonize extrafollicular areas of the spleen. *A*, DC-SIGN, MR, langerin, DEC-205, and gp120-binding activity on splenic IgD$^+$CD1c$^-$ and IgD$^+$CD1c$^+$ B cells. Values (*inset*) indicate the percentage of positive cells (*top*) and of MFI (*bottom*). *B*, IgD (green), CD1c, DC-SIGN, MR, and langerin (red) in splenic tissue sections (magnification, ×40). F, follicle; RP, red pulp. Arrowheads indicate areas containing IgD$^+$CD1c$^+$, IgD$^+$ DC-SIGN$^+$, IgD$^+$ MR$^+$, and IgD$^+$ langerin$^+$ B cells. *C*, IgD (green), CD1c, DC-SIGN, MR, and langerin (red) expression by splenic extrafollicular B cells (magnification, ×65). Arrowheads show IgD$^+$ DC-SIGN$^+$, IgD$^+$ MR$^+$, and IgD$^+$ langerin$^+$ B cells. Data depicted represent one of four experiments yielding similar results.

CD1c, CD11c, and gp120-binding activity increased upon exposure to IL-4 for 2 days. CD40L had a comparable effect, whereas IL-10 or BAFF up-regulated DC-SIGN and gp120 binding, but only marginally MR, langerin, CD1c, and CD11c. Of note, IFN-α did not up-regulate DC-SIGN, MR, langerin, CD1c, CD11c, and gp120 binding. Moreover, CD40L, BAFF, IL-4, and IL-10 either did not affect or down-regulated DEC-205.

When combined with IL-4 or IL-10, CD40L and BAFF up-regulated DC-SIGN, MR, langerin, CD1c, CD11c, and gp120 binding more effectively than CD40L and BAFF alone. In general, blood and tonsillar B cells exhibited comparable phenotypic responses to BAFF, CD40L, and cytokines. Up-regulation of MCLR expression did not simply result from proliferation and expansion of a discrete B cell subset constitutively expressing MCLRs because B cells depleted of any constitutive gp120-binding activity retained the capability of up-regulating DC-SIGN, MR, and langerin expression upon activation by IL-4 (data not shown). Expression of DC-SIGN, MR, and langerin by blood and tonsillar B cells was confirmed at a transcriptional level (Fig. 4*C*). This finding was specific because similar B cells contained VDJ-Cμ transcripts for the B cell-specific protein IgM as well as transcripts for the housekeeping protein β-actin, but lacked transcripts for the myeloid proteins CD14 and TLR4, which are usually expressed by DCs and monocytes (61). Thus, a fraction of B cells constitutively express DC-SIGN, MR, and langerin and further up-regulate them upon activation by T cell-independent and -dependent stimuli, including BAFF.

B cells can bind HIV-1 gp120 through MCLRs

Additional experiments were set up to evaluate whether tonsillar IgD$^+$CD1c$^+$ B cells bind gp120 through MCLRs. Nonlabeled JR-FL gp120 (R5) or IIIB gp120 (X4) abrogated binding of labeled BaL gp120 (R5) or IIIB gp120 (X4) (Fig. 5*A*). CCR5 and CXCR4 ligands, such as RANTES and SDF-1α, respectively, did not inhibit gp120 binding, whereas DC-SIGN ligands, such as

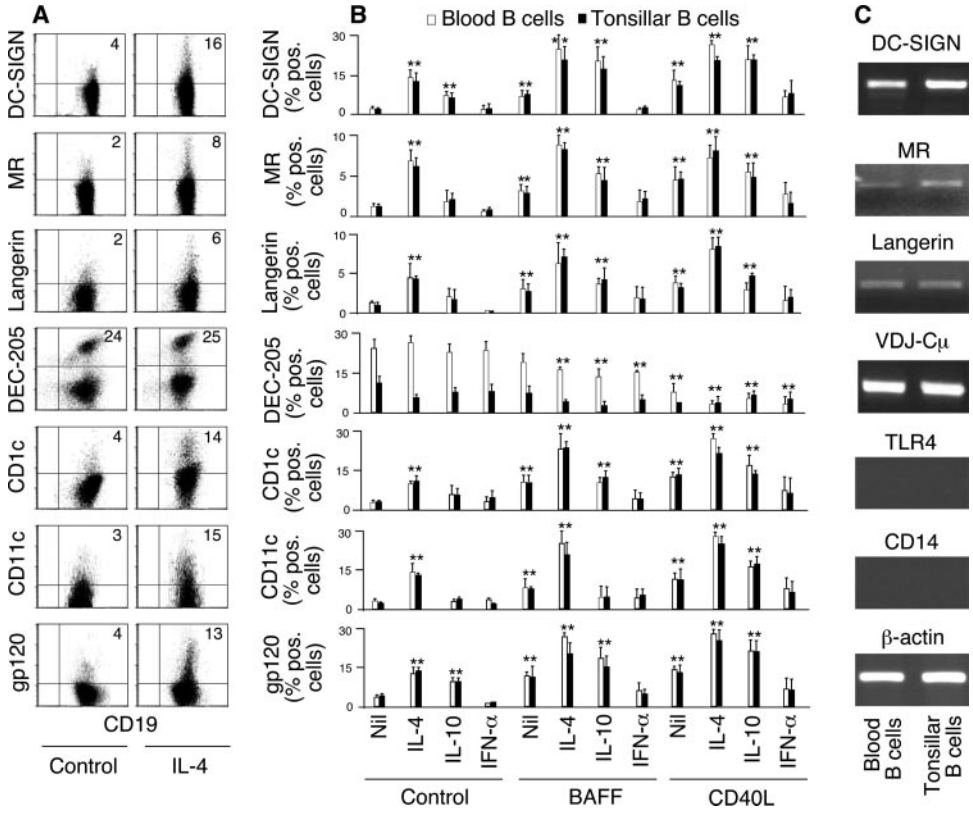

FIGURE 4. Blood and tonsillar B cells up-regulate MCLRs, including DC-SIGN, upon activation with BAFF and cytokines. *A*, CD19, DC-SIGN, MR, langerin, DEC-205, CD1c, CD11c, and gp120-binding activity on blood B cells incubated with or without IL-4 for 2 days. Values (*inset*) indicate the percentage of positive cells. *B*, Percentage of blood (□) and tonsillar (■) B cells expressing DC-SIGN, MR, langerin, DEC-205, CD1c, CD11c, and gp120-binding activity upon incubation with or without IL-4, IL-10, IFN-α, BAFF, and/or CD40L for 2 days. *C*, DC-SIGN, MR, langerin, VDJ-Cμ, TLR4, CD14, and β-actin transcripts in blood and tonsillar B cells. Data shown in *A* and *C* depict one of three experiments yielding similar results. Data in *B* summarize three experiments and bars indicate ± SD. ∗, $p < 0.05$ vs unstimulated B cells on *left*.

mannan, α-methyl-mannopyranoside, and the microbicidal protein lactoferrin (38, 62), inhibited gp120 binding. In contrast, proteins with no DC-SIGN-binding activity, such as β-lactoglobulin and albumin, did not affect gp120 binding. The MR ligand mannose-BSA attenuated gp120 binding, but less than DC-SIGN ligands. Two blocking mAbs to DC-SIGN decreased the binding of gp120 more than blocking mAbs to MR and langerin (Fig. 5*B*). Finally, blocking mAbs to CD4, CCR5, CXCR4, DEC-205, CD19, or CD21 did not affect gp120 binding, whereas Abs to BCR slightly did. Thus, gp120 binds to B cells through a CD4-independent mechanism involving DC-SIGN, MR, langerin, and to some extent, BCR.

HIV-1 gp120 cooperates with BAFF and cytokines to activate B cells

Prior studies indicate that gp120 activates a consistent proportion of human B cells (22–24, 26, 40). These B cells may comprise MCLR-expressing IgD^+CD1c^+ elements (54). Unlike p24, JR-FL and BaL (R5) and IIIB and MN (X4) gp120s up-regulated DNA synthesis in IgD^+CD1c^+ B cells from tonsils (Fig. 6*A*) and blood (data not shown). At high concentration, gp120 was almost as effective as well-defined T cell-independent B cell stimuli such as anti-BCR Ab, which mimics a soluble Ag, bead-conjugated anti-BCR Ab, which mimics a T cell-independent type 2 Ag, PMA, which mimics BCR signaling, or oligodeoxynucleotide 2006/type B CpG DNA, which mimics TLR9 binding by bacterial DNA. Due to their inability to express TLR4 (61), B cells did not respond to LPS, a type 1 T cell-independent Ag. The B cell-stimulating effect of gp120 was enhanced by MCLR-inducing stimuli, such as BAFF plus IL-4 and BAFF plus IL-10 (Fig. 6*B*). BAFF plus IL-10 increased also the B cell-stimulating activity of AT-2_{MN} virions (Fig. 6*C*), which retain Env glycoproteins in a native configuration. Activation of B cells by gp120 and AT-2_{MN} virions was reduced by a blocking mAb to DC-SIGN and, to some extent, by blocking mAbs to MR and langerin, whereas blocking mAbs to CXCR4 and DEC-205 had no effect (Fig. 6*D*). Thus, soluble or virion-bound gp120 stimulates B cells through a MCLR-dependent pathway that cooperates with BAFF and cytokines, including IL-4 and IL-10.

HIV-1 gp120 cooperates with cytokines to trigger IgG, IgA, and IgE CSR in B cells

The role of HIV-1 gp120 in CSR remains unknown. Switching from Cμ to a downstream C_H gene is preceded by germline I_H-C_H transcription. This process favors recruitment of the CSR machinery, including the B cell-specific enzyme AID, to the switch region, an intronic DNA element lying between the I_H exon and the targeted C_H gene (12). CSR leads to production of mature VDJ-C_H transcripts encoding IgG, IgA, or IgE proteins. By inducing looping-out deletion of the DNA between recombining switch regions, CSR yields a circular extrachromosomal DNA that actively transcribes a chimeric I_H-Cμ product known as circle transcript (12). Together with germline I_H-C_H transcripts and AID transcripts, I_H-Cμ circle transcripts constitute molecular by-products of ongoing CSR (18, 20, 42).

In the presence of soluble gp120, IgD^+CD1c^+ B cells from tonsils or blood (data not shown) up-regulated the expression of germline Iγ1-Cγ1, Iγ3-Cγ3, and Iα1-Cα1 transcripts, Iγ1-Cμ, Iγ3-Cμ, and Iα1-Cμ circle transcripts, mature VDJ-Cγ1, VDJ-Cγ3, and VDJ-Cα1 transcripts, and AID transcripts (Fig. 7*A*). This up-regulation was associated with increased production of the CSR-inducing cytokine IL-10 (data not shown), suggesting that gp120 may trigger polyclonal CSR with the help of autocrine IL-10. Consistent with this, gp120-induced CSR was augmented by exogenous IL-10. Thus, HIV-1 gp120 cooperates with IL-10 to initiate CD40-independent CSR from IgM to IgG and IgA in B cells. Induction of germline Iε-Cε transcripts required IL-4 and

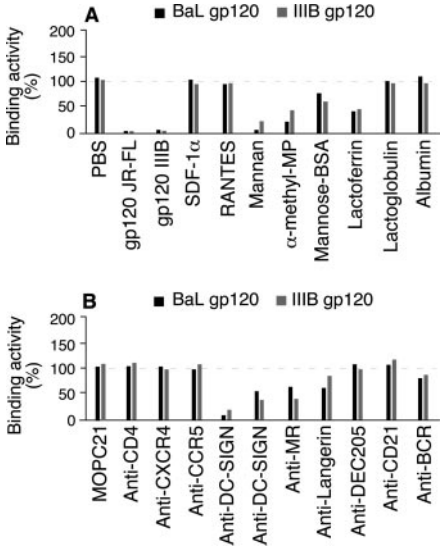

FIGURE 5. HIV-1 gp120 binds to tonsillar B cells through MCLRs, including DC-SIGN. *A*, Binding of gp120 to tonsillar IgD$^+$CD1c$^+$ B cells preincubated with PBS, gp120, RANTES, SDF-1α, mannan, α-methyl-mannopyranoside, mannose-BSA, lactoferrin, β-lactoglobulin, or albumin. MFI was converted into a percentage of gp120 binding as follows: ((MFI of gp120 binding in the presence of inhibitors) − (background MFI in the absence of gp120)/(MFI of gp120 binding with no inhibitors) − (background MFI in the absence of gp120)) × 100. The MFI of gp120 binding in B cells incubated without inhibitors was 71 ± 6%. *B*, Binding of gp120 to tonsillar IgD$^+$CD1c$^+$ B cells in the presence of a control mAb or a blocking mAb to CD4, CCR5, CXCR4, DC-SIGN (two different clones), MR, langerin, DEC-205, CD21, or BCR. Data depicted represent one of four experiments yielding similar results.

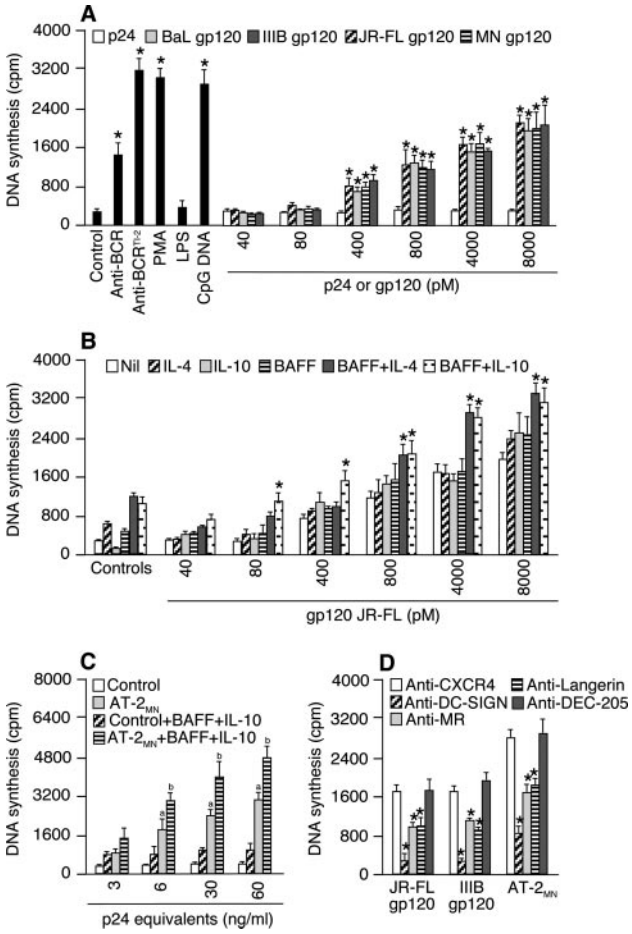

FIGURE 6. HIV-1 gp120 activates tonsillar B cells by cooperating with BAFF and cytokines. *A*, DNA synthesis in tonsillar IgD$^+$CD1c$^+$ B cells incubated for 4 days with medium alone (control), soluble anti-BCR, bead-conjugated anti-BCR^{TI-2} (which mimics a T cell-independent type 2 or Ag), PMA, LPS, CpG DNA, p24, R5 gp120 (JR-FL and BaL), or X4 gp120 (IIIB and MN). Bars indicate ± SD of four experiments. *, $p < 0.05$ vs control (*far left*). *B*, DNA synthesis in tonsillar IgD$^+$CD1c$^+$ B cells incubated for 4 days with gp120 JR-FL (4000 pM) with or without BAFF, IL-4, and/or IL-10. Bars indicate ± SD of three experiments. *, $p < 0.05$ vs B cells incubated with gp120 but not BAFF or cytokines (first histogram of each cluster). *C*, DNA synthesis in tonsillar IgD$^+$CD1c$^+$ B cells incubated for 4 days with control microvesicles or AT-2$_{MN}$ virions. Bars indicate ± SD of three experiments. *, $p < 0.05$ vs control microvesicles and control microvesicles plus BAFF plus IL-10, respectively. *D*, DNA synthesis in tonsillar IgD$^+$CD1c$^+$ B cells incubated for 4 days with AT-2$_{MN}$ virions (30 ng/ml) in the presence of a blocking mAb to CXCR4, CD4, DC-SIGN, MR, langerin, or DEC-205. Bars indicate ± SD of three experiments. *, $p < 0.05$ vs anti-CXCR4.

was enhanced by gp120, whereas induction of circle Iϵ-Cμ transcripts and mature VDJ-Cϵ transcripts occurred only in the presence of both IL-4 and gp120. Thus, HIV-1 gp120 cooperates with IL-4 to induce CD40-independent CSR from IgM to IgE in B cells.

HIV-1 gp120 cooperates with BAFF and anti-BCR to elicit IgG and IgA production in B cells

More experiments evaluated the role of HIV-1 gp120 in Ab production. Tonsillar IgD$^+$CD1c$^+$ B cells did not secrete IgG and IgA upon exposure to gp120 alone (Fig. 7*B*). In the presence of IL-10 and BCR engagement, gp120 stimulated IgD$^+$CD1c$^+$ B cells to secrete little IgG, but no IgA. Of note, gp120 augmented the secretion of both IgG and IgA by IgD$^+$CD1c$^+$ B cells incubated with IL-10, anti-BCR Ab, and BAFF. The latter was essential to trigger significant IgG and IgA secretion. In line with a prior report (43), a fraction of IgG and IgA secreted by IgD$^+$CD1c$^+$ B cells showed natural gp120 reactivity. Similar results were obtained with blood IgD$^+$CD1c$^+$ B cells (data not shown). Of note, in the presence of IL-4, anti-BCR, and BAFF, B cells secreted IgE and this secretion was augmented by gp120 (data not shown). Thus, HIV-1 gp120 cooperates with BCR engagement, BAFF, and IL-10 to trigger CD40-independent secretion of class-switched Abs.

HIV-1 gp120 up-regulates functional BAFF by stimulating monocytes through CD4, CCR5, and CXCR4

Given its ability to activate B cells in cooperation with BAFF, gp120 was hypothesized to up-regulate BAFF production in innate immune cells. In the presence of R5 or X4 gp120, the proportion of monocytes expressing membrane-bound BAFF increased (Fig. 8*A*). When combined with IL-10 or IFN-α (data not shown), which are two BAFF-inducing cytokines (18, 30), gp120 further up-regulated membrane-bound BAFF. In addition, IL-10 or IFN-α cooperated with gp120 to increase the release of soluble BAFF (Fig. 8*B*), a membrane-bound BAFF cleavage by-product (19).

More experiments evaluated the role of CD4, CCR5, and CXCR4 in BAFF induction by HIV-1 gp120. Induction of membrane-bound BAFF by R5 gp120 was attenuated by blocking mAbs to CD4 or CCR5 and by AD101 (Fig. 8*C*), a small molecule antagonist of CCR5 (63). In contrast, neither a blocking mAb to CXCR4 nor AMD3100, a small molecule antagonist of CXCR4 (64), inhibited membrane-bound BAFF up-regulation by R5 gp120. A blocking mAb to CCR5 and AD101 attenuated BAFF induction by RANTES, whereas blocking mAbs to CD4 or CXCR4 and AMD3100 did not. Blocking mAbs to CD4 or

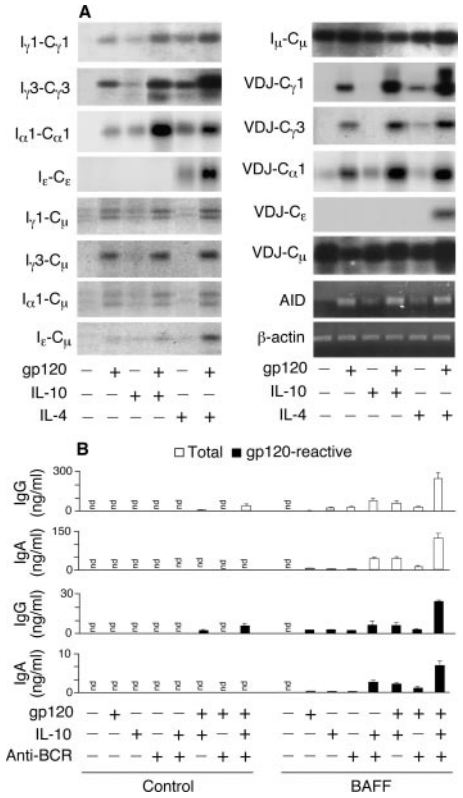

FIGURE 7. HIV-1 gp120 triggers IgM production as well as IgG, IgA, and IgE CSR by cooperating with BAFF and cytokines. *A*, Iγ1-Cγ1, Iγ3-Cγ3, Iα1-Cα1, Iε-Cε, Iγ1-Cμ, Iγ3-Cμ, Iα1-Cμ, Iε-Cε, VDJ-Cγ1, VDJ-Cγ3, VDJ-Cα1, VDJ-Cε and AID transcripts in tonsillar IgD^+CD1c^+ B cells incubated with or without JR-FL gp120, IL-4, and/or IL-10 for 4 days. Iμ-Cμ and VDJ-Cμ transcripts were used as loading controls together with β-actin. *B*, Secretion of total (□) and gp120-reactive (■) IgG and IgA by tonsillar IgD^+CD1c^+ B cells incubated with or without JR-FL gp120, IL-10, BAFF, and/or anti-BCR for 8 days. n.d., Not detected. Bars indicate ± SD of three experiments.

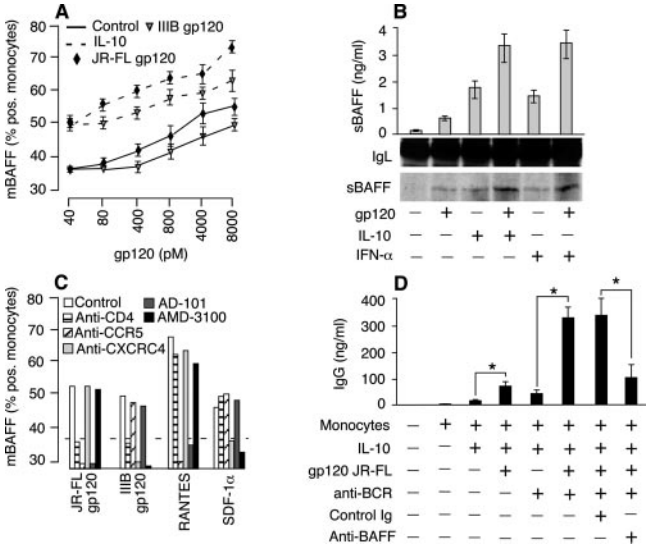

FIGURE 8. HIV-1 gp120 induces production of functionally active BAFF in monocytes. *A*, Membrane-bound BAFF (mBAFF) on monocytes incubated with gp120 (JR-FL or IIIB) in the presence (broken line) or absence (solid line) of IL-10 for 2 days. Control indicates medium alone (no IL-10). *B*, Soluble BAFF from monocytes incubated with or without gp120, IL-10, and/or IFN-α for 2 days. *C*, Membrane-bound BAFF (mBAFF) induction by gp120, RANTES, or SDF-1α in the presence of control MOPC21 mAb, blocking B4 (to CD4), 2D7 (to CCR5), or 12G5 (to CXCR4) mAb, small molecule AD101 (antagonizing CCR5), or small molecule AMD3100 (antagonizing CXCR4). Baseline level (broken line) of membrane-bound BAFF is shown. *D*, IgG production by blood IgD^+ B cells exposed to monocytes in the presence or absence of gp120 JR-FL, IL-10, anti-BCR, control mAb (MOPC21), and/or a blocking Ab to BAFF. Data depict one of three experiments (*A* and *B*) yielding similar results and bars indicate SEM. Data in *D* summarize three experiments with bars indicating ± SD. ∗, $p < 0.05$).

CXCR4 and AMD3100 each inhibited membrane-bound BAFF up-regulation by X4 gp120, whereas a blocking mAb to CCR5 and AD101 did not. Induction of BAFF by SDF-1α was attenuated by a blocking mAb to CXCR4 and by AMD3100, but not by blocking mAbs to CD4 or CCR5 or by AD101.

Finally, it was verified whether gp120-activated monocytes were capable of driving CD40-independent Ab production through BAFF. Blood IgD^+ B cells incubated with monocytes, IL-10, and gp120 produced more IgG than did IgD^+ B cells incubated with monocytes and IL-10 only (Fig. 8*D*). Similarly, IgD^+ B cells incubated with monocytes, IL-10, anti-BCR, and gp120 produced more IgG than did IgD^+ B cells incubated with monocytes, IL-10, and anti-BCR only. In IgD^+ B cells incubated with monocytes, IL-10, anti-BCR and gp120, IgG production was attenuated by a blocking mAb to BAFF, but not by a control mAb with irrelevant binding activity. These results indicate that HIV-1 gp120 cooperates with cytokines, including IL-10, to up-regulate production of functional BAFF by monocytes (Fig. 9). They also suggest that HIV-1 gp120 uses the CD4 receptor as well as CCR5 and CXCR4 coreceptors to up-regulate BAFF.

Discussion

In this study, we show that gp120 binds to a subset of IgD^+ B cells through MCLRs. In the presence of gp120, MCLR-expressing B cells proliferate, up-regulate AID, and undergo CSR from IgM to IgG and IgA. IL-4 and IL-10 further augment IgG and IgA CSR, whereas IL-4 elicits IgE CSR. Ab secretion requires BAFF, a polyclonal B cell-stimulating and MCLR-inducing factor produced by monocytes upon CD4, CCR5, and CXCR4 engagement by gp120. Our findings suggest that gp120 initiates polyclonal IgG, IgA, and IgE responses by linking the innate and adaptive immune systems via BAFF. Chronic activation of B cells through this CD40-independent pathway might impair protective T cell-dependent Ab responses by inducing immune exhaustion.

B cells can mount quick Ab responses to PAMPs, including viral Env glycoproteins, in a T cell-independent fashion (14–17, 54). Although unable to provide immune protection, this innate B cell response can blunt infections at an early phase. T cell-independent Ab production involves rapid differentiation of B cells into plasma cells and leads to the production of large amounts of opsonizing Abs with low affinity for Ag in extrafollicular areas of lymphoid organs (15). In mice, T cell-independent Ab production is driven by splenic MZ B cells, which recognize T cell-independent Ags through a panoply of polyreactive Ag receptors, including PRRs (e.g., TLRs), complement receptors, stereotyped BCRs, and MHC-like CD1 molecules (17, 65). Of note, mouse MZ B cells appear to mediate T cell-independent Ab responses against HIV-1 gp120. Consistent with this, *Env*-transgenic mice display an enlarged splenic MZ, and $CD4^+$ T cell-deficient mice produce specific IgG and IgA upon systemic or oral immunization with gp120 (25, 66).

Our findings indicate that a subset of human B cells can recognize gp120 through MCLRs, including DC-SIGN and, to a lesser

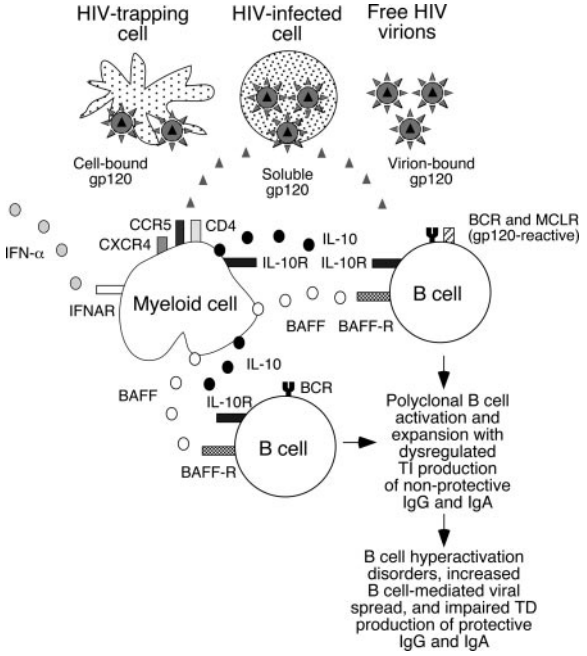

FIGURE 9. Pathways underlying T cell-independent B cell responses to HIV-1 gp120. Monomeric soluble gp120 released by HIV-1-infected cells and oligomeric gp120 on HIV-1-trapping cells, HIV-1-infected cells, and free HIV-1 virions stimulate BAFF production by engaging CD4, CCR5, and CXCR4 on myeloid cells. This gp120 would further increase BAFF production by stimulating myeloid cells to secrete IFN-α and IL-10, two BAFF-inducing cytokines. Together with IL-10, BAFF up-regulates gp120-binding MCLRs on a subset of IgD^+ B cells, including B cells expressing BCR with low affinity for gp120. In the presence of IL-10, MCLR engagement by gp120 triggers CD40-independent CSR. Subsequent polyclonal Ab secretion would require BAFF and BCR interaction with Ags from HIV-1 and/or HIV-1-associated opportunistic agents. By inducing BAFF and IL-10, gp120 would also trigger CD40-independent CSR in IgD^+ B cells with no MCLR expression. These B cells may undergo Ab secretion upon exposure to Ags, thereby further increasing polyclonal hypergammaglobulinemia. In addition to augmenting viral spread and favoring the onset of inflammatory and neoplastic disorders (38), chronic expansion and activation of B cells through gp120-initiated T cell-independent pathways could impair protective T cell-dependent Ab responses by eliciting functional exhaustion.

extent, MR and langerin. These gp120-binding B cells are distinct from transitional, naive, founder GC, full-blown GC, memory, and plasmacytoid B cells. They rather resemble subepithelial and MZ B cells, as they express IgM, IgD, CD1c, $CD21^{high}$, and CD27 (51–54). Consistent with this finding, IgD^+ B cells expressing DC-SIGN, MR, and langerin can be detected in the tonsillar subepithelium and splenic MZ. In these extrafollicular regions, B cell exposure to various immune stimuli might be implicated in the up-regulation of innate recognition and effector molecules, including MCLRs. Of note, some IgD^+ $DC\text{-}SIGN^+$ B cells were detected in tonsillar follicular mantles, which usually comprise naive and founder GC B cells, suggesting that, in the presence of appropriate stimuli, follicular B cells also express gp120-binding MCLRs. In this regard, we found that a variable proportion of B cells up-regulate DC-SIGN, MR, and langerin expression upon stimulation by BAFF, CD40L, and cytokines, including IL-4 and IL-10.

By showing that gp120 up-regulates AID and initiates IgG and IgA CSR in a CD40-independent fashion, our in vitro experiments extend to human B cells previous in vivo studies showing T cell-independent production of IgG and IgA by mouse B cells exposed to gp120 (25). This viral protein may trigger CSR through DC-SIGN, as blocking Abs to DC-SIGN attenuate B cell activation by gp120. DC-SIGN could enhance CSR by inducing B cells to secrete IL-10, a DC-SIGN-inducible cytokine with CSR activity (18, 20, 59, 67). IL-10 could further augment gp120-driven CSR by up-regulating DC-SIGN with the help of BAFF, an IL-10-inducible ligand (30). In addition to initiating T cell-independent Ab responses, DC-SIGN may modulate T cell-dependent Ab responses, as B cells up-regulate DC-SIGN as well as other MCLRs upon exposure to CD40L.

Although sufficient to up-regulate AID and induce IgG and IgA CSR, gp120 and IL-10 require BAFF and BCR engagement to elicit significant IgG and IgA secretion. Of note, a fraction of IgG and IgA secreted by gp120-binding B cells naturally reacts against gp120. This latter might bind a highly conserved domain outside the Ag-binding pocket of broadly represented Igs in a superantigen-like fashion (24, 43, 68, 69). Alternatively, gp120 could bind stereotyped Igs with multiple antigenic reactivity in a conventional fashion like other PAMPs do (14, 15, 17, 54). Surface Igs (i.e., BCRs) with natural gp120 reactivity might cooperate with MR, BAFF receptors, and cytokine receptors to initiate CSR and Ab production in gp120-binding B cells. This "natural" Ab response might provide an early T cell-independent foundation for subsequent T cell-dependent B cell responses against gp120.

In addition to activating B cells with the help of BAFF, gp120 up-regulates BAFF production in monocytes. This effect is attenuated by blocking mAbs to CD4, CCR5, and CXCR4 and by small molecule antagonists of CCR5 and CXCR4. Conversely, engagement of CCR5 and CXCR4 by RANTES and SDF-α, respectively, up-regulate BAFF production. Of note, monocytes up-regulate BAFF-inducing cytokines, such as IFN-α and IL-10, upon exposure to gp120 (18, 28–31). Thus CD4, CCR5, and CXCR4 signaling could augment BAFF production either directly or indirectly through a mechanism involving cytokines. Regardless of the mechanism underlying its production, gp120-induced BAFF may contribute to the initiation of polyclonal Ab responses as it stimulates IgG production in BCR-stimulated B cells exposed to IL-10.

The roles played by B cells in HIV-1 infection remain poorly understood. Prior work shows that B cells use the complement receptor CD21 to transport complement-bound HIV-1 virions (40). Our findings extend a recent study suggesting that B cells could bind HIV-1 virions through MCLRs as well (70). Activation signals originating from gp120-engaged MCLRs may contribute to HIV-1-induced B cell hyperplasia, thereby favoring B cell-mediated HIV-1 transportation throughout the body (26). Similar signals may also contribute to HIV-1-induced hypergammaglobulinemia owed to their ability to up-regulate AID and induce polyclonal IgG, IgA, and IgE CSR. In our study, the concentration of gp120 used in CSR-inducing assays is two or three orders of magnitude higher than that found in the plasma of HIV-1-infected patients (36). Nevertheless, infected individuals are likely to have larger amounts of gp120 in lymphoid organs, the main site of HIV-1 infection, deposition, and propagation (1, 27). In this microenvironment, oligomeric gp120 on HIV-1-trapping and HIV-1-infected cells would engage MCLRs on bystander B cells with higher avidity than monomeric soluble gp120.

Our data indicate that microenvironmental cues, such as BAFF, may lower the activation threshold of B cells for gp120. These stimuli would enhance B cell responses not only to virion-associated gp120, but also to gp120-based vaccines, which typically deliver high concentrations of gp120 in a broad area. Of note is our observation that gp120-reactive Abs, even those of multiple specificities from sera of HIV-1-infected individuals, do not interfere with the ability of gp120 to bind and activate B cells (data not shown). On

the contrary, anti-gp120 Abs against the CD4-binding site can augment B cell binding by increasing the exposure of CXCR4-binding sites on gp120, just as soluble CD4 does (data not shown).

By showing that gp120 and gp120-inducible cytokines cooperatively stimulate monocytes to up-regulate membrane-bound and soluble BAFF production, our study provides a novel mechanistic explanation for recent findings showing augmented BAFF levels in HIV-1-infected patients (32, 33). In these patients BAFF production could be further dysregulated by EBV, an opportunistic herpesvirus with B cell tropism and lymphomagenic activity (42, 71). In addition to down-regulating BAFF receptor on B cells (72), BAFF would trigger CSR, plasmacytoid differentiation, and Ab secretion in a CD40-independent fashion (18). This innate pathway may contribute to the pathogenesis of hypergammaglobulinemia, autoimmunity, and hyper-IgE syndrome in infected individuals with very few $CD4^+$ T cells (2–4, 21, 73). BAFF might also facilitate lymphomagenesis due to its ability to induce AID, a DNA-editing enzyme that promotes oncogenic chromosomal translocations by triggering aberrant CSR and somatic hypermutation (12, 18, 74). Thus, BAFF-blocking agents might be useful to treat aggressive B cell lymphomas associated with HIV-1 infection (75).

Paradoxically, hyperactivated B cells from HIV-1 viremic individuals respond poorly to T cell-dependent stimuli both in vivo and in vitro (4, 76). This humoral immunodeficiency is thought to stem from $CD4^+$ T cell depletion, GC disruption, CD40L down-regulation, and CD40 dysfunction (1, 66, 77). Protective T cell-dependent Ab responses could be further impaired by chronic activation of B cells by gp120 and gp120-induced BAFF. These T cell-independent stimuli would cause terminal differentiation and functional exhaustion, thereby lowering the number of B cell precursors available for the initiation of protective T cell-dependent Ab responses. In addition, nonspecific and low-affinity B cells polyclonally expanded by T cell-independent stimuli might compete with high-affinity B cells emerging from the T cell-dependent Ab pathway for survival factors and favorable anatomical niches. This competition would be particularly disadvantageous for B cells producing neutralizing Abs due to their physiologically low precursor frequency. Consistent with this point, early polyclonal hypergammaglobulinemia is inversely correlated with the initiation of virus-neutralizing Ab responses (78). In light of these considerations, administration of BAFF-blocking agents to individuals chronically infected by HIV-1 might not only attenuate the clinical manifestations of B cell hyperactivation disorders, but also improve the quality of Ab responses to HIV-1 infection, opportunistic pathogens, and vaccines.

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Disclosures

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