HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals

Susan Moir*†‡, Angela Malaspina*†, Kisani M. Ogwaro*, Eileen T. Donoghue*, Claire W. Hallahan*, Linda A. Ehler*, Shuying Liu*, Joseph Adelsberger§, Réjean Lapointe¶, Patrick Hwu¶, Michael Baseler§, Jan M. Orenstein‖, Tae-Wook Chun*, Jo Ann M. Mican*, and Anthony S. Fauci*

*Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, and ¶Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; §Science Applications International Corporation/National Cancer Institute–Frederick Cancer and Development Center, Frederick, MD 21702; and ‖Department of Pathology, George Washington University, Washington, DC 20037

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A number of perturbations of B cells has been described in the setting of HIV infection; however, most remain poorly understood. To directly address the effect of HIV replication on B cell function, we investigated the capacity of B cells isolated from HIV-infected patients to respond to a variety of stimuli before and after reduction of viremia by effective antiretroviral therapy. B cells taken from patients with high levels of plasma viremia were defective in their proliferative responses to various stimuli. Viremia was also associated with the appearance of a subpopulation of B cells that expressed reduced levels of CD21. After fractionation into $CD21^{high}$- and $CD21^{low}$-expressing B cells, the $CD21^{low}$ fraction showed dramatically reduced proliferation in response to B cell stimuli and enhanced secretion of immunoglobulins when compared with the $CD21^{high}$ fraction. Electron microscopic analysis of each fraction revealed cells with plasmacytoid features in the $CD21^{low}$ B cell population but not in the $CD21^{high}$ fraction. These results indicate that HIV viremia induces the appearance of a subset of B cells whose function is impaired and which may be responsible for the hypergammaglobulinemia associated with HIV disease.

B cells of HIV-infected individuals show numerous signs of aberrant hyperactivity, including hypergammaglobulinemia (1, 2), spontaneous secretion of immunoglobulins in culture (3), increased risk of neoplastic transformation (4), and increased expression of activation markers (5, 6). B cell abnormalities during HIV infection have been shown to reflect both HIV-specific and nonspecific responses as evidenced by high frequencies of Ab-secreting cells directed against HIV and nonviral antigens (7). Paradoxically, HIV-infected patients respond poorly to immunizations that elicit humoral responses (8–10), and their B cells respond abnormally when stimulated *ex vivo* (1, 2, 11).

In vitro studies on cells isolated from normal donors and exposed to HIV or HIV-related factors have described several potential sources of B cell perturbations. These include direct effects of the virus on B cells (12), indirect effects of HIV-impaired T cell help on B cells (13), and dysregulation of B cells by cytokines that are associated with HIV infection (14, 15). Few studies have addressed the issue of B cell abnormalities relative to viral replication *in vivo*. However, a recent study has demonstrated that levels of serum immunoglobulins and frequency of Ab-secreting cells are normalized after effective antiretroviral therapy, thereby strongly suggesting that viremia drives B cell hyperactivity *in vivo* (16). Furthermore, a cross-sectional study examining the capacity of B cells to differentiate in response to CD40 and B cell receptor (BCR) triggers suggested that loss of reactivity was associated with plasma viral load and disease progression (17). In the present study, we evaluated the direct effect of *in vivo* viral load on phenotypic and functional attributes of B cells by studying patients before and after reduction of viral load by antiretroviral therapy. We show that high viremia is associated with generalized B cell dysfunction and the appearance of a phenotypically distinct subpopulation of B cells that fail to proliferate in response to B cell stimuli yet secrete high levels of immunoglobulins.

Materials and Methods

Study Patients. Study subjects included patients chronically infected with HIV and normal donors. Six of the patients chronically infected with HIV were studied before and after receiving effective antiretroviral regimens. Lymphopheresis and standard blood draws were conducted in accordance with protocols approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases.

Cell Preparation and Culture Conditions. Peripheral blood mononuclear cell-derived B cells were isolated from lymphopheresis products by using a column-based purification technique (StemCell Technologies, Vancouver), as described (18). The purity of B cell suspensions was generally greater than 95%. Fractionation of B cells into CD21-enriched and CD21-depleted populations was performed by cell sorting, using an EPICS ELITE cell sorter (Beckman Coulter) as described (18). Alternatively, fractionation was performed by immunomagnetic selection using anti-CD21 mAb BL13 (Beckman Coulter) and rat anti-mouse IgG Abs coupled to magnetic beads through a cleavable DNA linker (Dynal, Lake Success, NY). Cultures of 1.5×10^5 cells per well in 96-well plates were established in RPMI medium 1640 supplemented with 10% (vol/vol) FCS and one of the following B cell stimulatory conditions: 10 ng/ml phorbol 12-myristate 13-acetate (PMA) and 1 μg/ml ionomycin (Sigma–Aldrich); 1/4,000 fixed and killed *Staphylococcus aureus* protein-A-positive cells (SAC; Roche Molecular Biochemicals); 500 ng/ml CD40 ligand (kindly provided by Immunex) and 100 ng/ml IL-4 (PeproTech, Rocky Hill, NJ); or 20 μg/ml goat anti-human IgM (Jackson ImmunoResearch) with or without 20 ng/ml IL-4. Cells were cultured for 72 h, after which proliferation was measured by [^{3}H]thymidine uptake during an additional 16-h incubation. In some experiments, culture supernatant was removed at 72 h and assayed by an ELISA (Cygnus Technologies, Plainville, MA) for IgG secretion.

Abbreviations: BCR, B cell receptor; Q-FACS, quantitative flow cytometry (fluorescence-activated cell sorting); SAC, *S. aureus* protein-A-positive cells; PMA, phorbol 12-myristate 13-acetate.

†S.M. and A.M. contributed equally to this work.

‡To whom reprint requests should be addressed at: Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 10, Room 6A02, Bethesda, MD 20892. E-mail: smoir@niaid.nih.gov.

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Table 1. Profiles of patients chronically infected with HIV-1

Study groups	Patient*	Therapy status	CD4 count per μl	Plasma HIV RNA copies per ml[†]	Months of suppressed viremia[§]	$CD21^-$ B cells, %[¶]	Serum IgG levels, mg/ml
High viral load	1A	Nonadherence	18	56,600		13	17.1
vs. low viral	1B	HAART	123	53	4	4.5	7.9
load	2A	Nonadherence	252	39,125		18	21
	2B	HAART	438	<50	5	6.3	14.5
	3A	Nonadherence	227	176,481		37	14.1
	3B	HAART	273	<50	3	14	9.7
	4A	Resistance	241	119,410		50	19.8
	4B	HAART	288	701	1.5	43	16.9
	5A	Resistance	102	43,463		56	41.2
	5B	HAART	216	<50	2	38	16.2
	6A	Naïve	6	427,675		15	19.8
	6B	HAART	145	2,680	1	18	24.1
$CD21^{high}$ vs.	7	Nonadherence	577	422[‡]	NA	27	26.7
$CD21^{low}$	8	Naïve	287	124,053	NA	32	17.2
	9	Nonadherence	233	316,418	NA	41	13.8
	10	Resistance	163	47,195	NA	25	22.6
	11	HAART	764	<50[‡]	NA	35	11.6
	12	Nonadherence	969	74,301	NA	37	7.9

NA, not applicable; HAART, highly active antiretroviral therapy.
*''A'' refers to time point prior to effective HAART treatment, and ''B'' refers to the time point after HAART.
[†]Plasma HIV RNA was measured by using the bDNA (Chiron) assay with a detection limit of 50 copies ml^{-1} plasma.
[‡]Patient plasma viral loads had been above 10,000 on previous recent samplings.
[§]Calculated as the time the plasma HIV RNA levels were within 1/2 a log of the level at time of study.
[¶]Percentage of $CD19^+$ cells that are $CD21^-$.

Quantitative Flow Cytometry (Q-FACS). The number of CD21 receptors per B cell was measured by Q-FACS (fluorescence-activated cell sorting), using Quantum Simply Cellular microbeads (Sigma–Aldrich), according to the manufacturer's specifications. Briefly, the number of Ab-binding sites per cell was determined from a calibration curve generated by incubating a mixture of Quantum Simply Cellular microbeads possessing incremental capacities to bind mouse immunoglobulins with a saturating quantity of phycoerythrin (PE)-conjugated anti-CD21 mAb (BD PharMingen, San Diego), in parallel with the B cells. The B cells were also stained with FITC-conjugated CD19 mAb (BD PharMingen) to establish the number of anti-CD21 binding sites per CD19-positive cell. Labeled cells and microbeads were analyzed on a FACSCalibur (Becton Dickinson) by using CELLQUEST software (Becton Dickinson).

Reverse Transcription–PCR. Total RNA was isolated from 1–8 $\times$ 10^6 B cells by using the TRIzol reagent according to manufacturer specifications (Invitrogen). After preheating to 80°C for 5 min, 0.1 μg RNA was annealed with random hexamers by slow cooling and was reverse transcribed into cDNA with avian myeloblastosis virus (AMV) reverse transcriptase (Life Sciences, St. Petersburg, FL). cDNA representing 0.01 μg of total RNA was then used as the template for real-time PCR in an iCycler (Bio-Rad). The amplification reaction included 0.5 μM primers, 0.2 μM fluorescent probe, 0.8 mM dNTPs, 5 mM $MgCl_2$, and 2.5 units of Platinum *Taq* polymerase (Invitrogen) in 50 μl of total volume. The following primers were used: 5′-GAG GCT CTA CAC CCT ACA GAC ATG-3′ (5′ primer) and 5′-GTC ACA GAC AAT CCT GGA GCA ATG-3′ (3′ primer) for CD21; and 5′-CTT CCT GAG CCC TCA TGG GTC AGC-3′ (5′ primer) and 5′-GCC ACC TGA GGA TCA CCT GGT GC-3′ (3′ primer) for CD19. The fluorescent probes consisted of 5′-6FAM-ACA CAG GTT GGT AGT CGT GTC GGC-TAMRA-3′ for CD21 and 5′-6FAM-CCC AAT CAT GAG GAA GAT GCA GAC TC-TAMRA-3′ for CD19. PCR conditions consisted of a denaturation step at 95°C for 3 min followed by 45 cycles of 15 sec at 95°C and 1 min at 60°C. Copy numbers of CD21 and CD19 mRNA were determined from a standard curve that was generated with serial dilutions of *in vitro*-transcribed CD21 and CD19 mRNA (MEGAscript *In Vitro* Transcription kit; Ambion, Austin, TX) by using cDNAs cloned into pSP64 poly(A) vector (Promega) as starting material.

Transmission Electron Microscopy. Freshly isolated B cells were fixed in 2.5% (vol/vol) glutaraldehyde, gelled into warm agar, postfixed in OsO4, dehydrated in graded ethanol and propylene oxide, and embedded in Spurr's epoxy for sectioning. Thin sections were stained with uranyl acetate and lead citrate and were analyzed by using a Zeiss EM10 electron microscope at 60 kV (LEO Electron Microscopy).

Statistical Analysis. Group comparisons for number of CD21 receptors and transcripts per B cell were made by using an ANOVA with Tukey's multiple comparison test for means. The Q-FACS data on percentage of $CD21^-$ B cells were not normally distributed; therefore, the median percentage of $CD21^-$ B cells in each group were compared by using the Kruskal–Wallis test with the Wilcoxon two-sample test. *P* values for comparisons of medians were adjusted for multiple testing by the Bonferroni method. The association of the median fold enhancement of proliferation with CD4 count at high plasma viremia was determined by the Spearman rank correlation.

Results

Comparison of Proliferative Capacity of B Cells Before and After Control of Plasma Viremia. We isolated B cells from peripheral blood mononuclear cells of six patients chronically infected with HIV-1 at 2 time points—once before and once after reduction of viral burden with effective antiretroviral therapy. Patient profiles shown in Table 1 indicate that between the 2 time points, plasma HIV RNA levels dropped by at least 2 logs for all

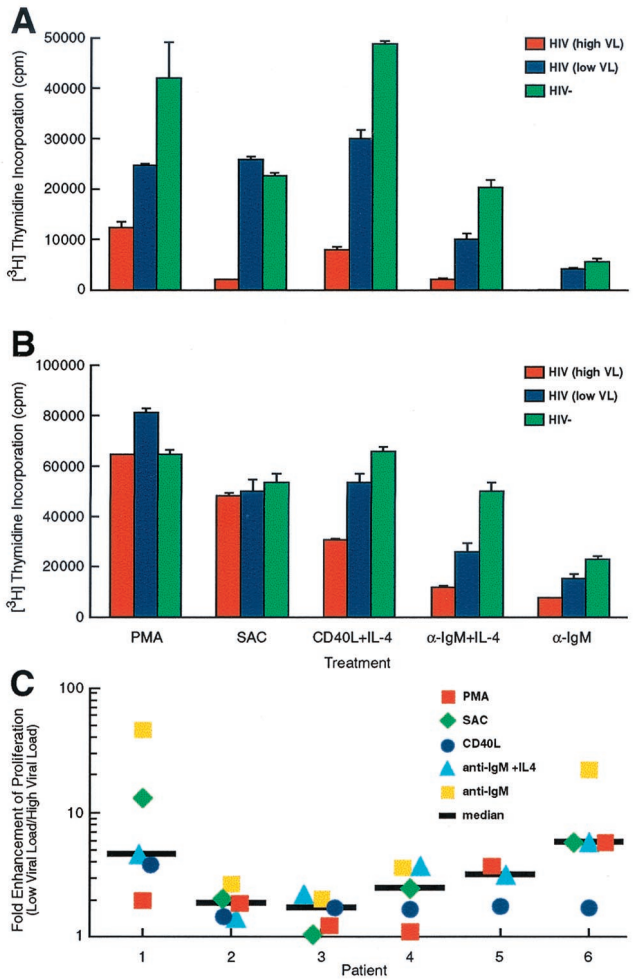

Fig. 1. Proliferation of B cells relative to plasma viremia. Peripheral blood mononuclear cell-derived B cells were isolated from patients at high viral load, and compared with B cells isolated at low or undetectable viral load and to B cells isolated from a normal HIV-negative donor. Absolute levels of proliferation of B cells isolated from Patient 1 (*A*) and Patient 3 (*B*) are indicated, as well as compiled data from all 6 patients expressed as fold enhancement of B cell proliferation at low viral load (VL) over B cell proliferation at high viral load (*C*). In *A* and *B*, data are mean ± SD of triplicates, and in *C*, data include median fold enhancement for each patient.

patients, and $CD4^+$ T cell counts increased in all patients, albeit to various degrees. Furthermore, in agreement with previous findings (16), reduction of viral load was associated with a reduction in serum levels of IgG in five of the six patients studied (Table 1).

To minimize assay to assay variations, we measured the capacities of B cells to proliferate in response to various stimuli simultaneously by using cryopreserved peripheral blood mononuclear cells from both time points. Cells were cultured with a broad range of B cell stimuli, including PMA and ionomycin for T cell-independent stimulation, CD40 ligand plus IL-4 for T cell-dependent stimulation, and SAC or anti-IgM or anti-IgM plus IL-4 for various levels of BCR stimulation. For B cells of Patient 1, who initially had a very low $CD4^+$ T cell count, proliferation to all stimuli tested was dramatically enhanced after the reduction of viral load by effective highly active antiretroviral therapy (Fig. 1*A*). At low plasma viremia, B cell responses were restored to 50% or more of levels measured for the normal donor, compared with below 30% before reduction of viremia. For Patient 3, whose $CD4^+$ T cell count was higher than that of Patient 1 (Table 1), enhancement of B cell proliferation after reduction of plasma viral load was not as profound as that observed in Patient 1, especially in response to the respectively strong and BCR-multimerizing stimuli PMA/ionomycin and SAC (Fig. 1*B*). When data for all five treatments were combined for each patient and reported as fold enhancement of proliferation at low viral load over proliferation at high viral load (Fig. 1*C*), median fold enhancement of proliferation tended to decrease with increasing $CD4^+$ T cell count (correlation = 0.94, $P = 0.005$; Table 1). Patients 1 and 6, who had the lowest initial $CD4^+$ T cell counts, had median fold enhancements of proliferation of 4.6 and 5.8, respectively, whereas Patient 5, with an intermediate $CD4^+$ T cell count, had an intermediate median fold enhancement of proliferation of 3.2. Patients 2, 3, and 4, whose initial $CD4^+$ T cell counts were similarly the highest of the group, also had the lowest median fold enhancements of proliferation of 1.9, 1.7, and 2.5, respectively, suggesting clinical status reflected by $CD4^+$ T cell count influences the capacity of B cells to proliferate. On the other hand, plasma viral load was also an important determining factor for B cell dysfunction, especially with the more subtle physiologic form of BCR triggering represented by anti-IgM with or without IL-4. Of note, Patients 3 and 4, whose $CD4^+$ T cell counts increased marginally between high and low viral load, B cells nonetheless showed fold enhancements of proliferation of between 2.1 and 3.7 in response to stimulation with anti-IgM. Taken together, these data suggest that viral load is an important predictor of the extent of B cell dysfunction in HIV-infected individuals.

Association Between Viral Load and CD21 Expression. In a previous study, we demonstrated that HIV binds to B cells *in vivo* through interactions between CD21 expressed on the surface of B cells and its ligands, i.e., complement breakdown products of C3 attached to the surface of HIV immune complexes (18). In agreement with other studies (19, 20), we also reported that HIV-infected chronically viremic patients manifested a subpopulation of B cells in peripheral blood that expressed reduced levels of CD21. In the present study, we found that a decrease in plasma viral load was associated with a rapid increase in the expression of CD21 on B cells, which paralleled the reduction of serum IgG levels in five of six patients (Table 1). In the patient whose percent CD21 expression did not increase as viral load decreased (Patient 6 of Table 1), the mean fluorescence of intensity was measured at 7.3 before and 15.6 after reduction of viral load, respectively, suggesting that a normalization of CD21 expression had occurred in this patient as well.

To evaluate more accurately and succinctly the effect of HIV replication on CD21 expression, we used Q-FACS (21) to measure the number of CD21 molecules per B cell. This approach, which combines Ab-specific standardization and the use of saturating conditions, can detect low levels of expression that would have been reported as negative by standard flow cytometry (21). Accordingly, we used Q-FACS to compare the number of CD21 molecules per B cell among a group of HIV-infected chronically viremic patients (plasma HIV RNA above 15,000 copies per milliliter), a group of normal donors, and a group of chronically HIV-infected patients whose plasma HIV RNA levels have been stable at below 5,000 copies per milliliter for over 6 months (referred to as low plasma viral load group). B cells of HIV-infected patients with high plasma viral load expressed a significantly lower mean number of CD21 receptors per B cell (7,395 ± 784) compared with that of normal donors (23,610 ± 2,211) and HIV-infected patients with low plasma viral load (21,502 ± 2,538; $P < 0.001$; Fig. 2*A*). By Q-FACS, a median of 4.9% (0–28.2) of B cells from the viremic group were truly negative for CD21, compared with an undetectable median for both normal donors (undetectable for all) and HIV-infected patients with low plasma viral load (0–2.2;

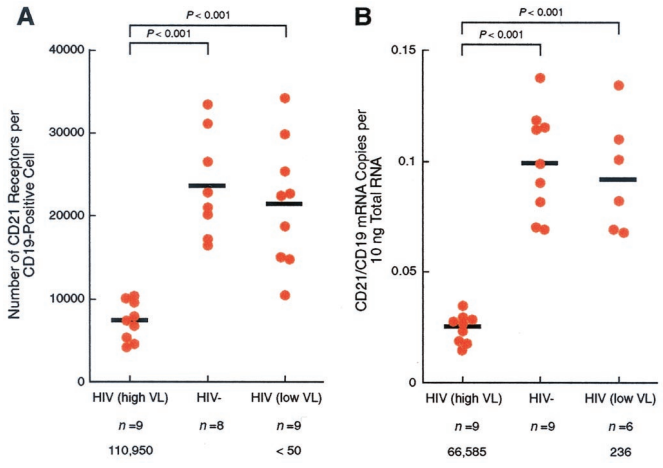

Fig. 2. Expression of CD21 in B cells. The number of CD21 receptors per $CD19^+$ cell (*A*) and the copy number of CD21 mRNA relative to CD19 mRNA (*B*) were measured on a group of HIV-infected patients at high plasma viral load (VL), compared with a group of HIV-infected patients at low plasma viral load and a group of normal donors. Means of these data are reported for each group. Median HIV RNA levels in copies per milliliter for each group are shown below the number of patients studied in the respective group. Because of restrictions on the number of cells available, 2 of 9 samples in the high viral load group and 4 of 9 samples in the low viral load group were not subjected to both CD21 mRNA and surface protein measurements.

data not shown). To determine whether the decreased number of CD21 surface receptors was paralleled by a reduction in the number of CD21 transcripts, we performed quantitative real-time reverse transcription–PCR to measure CD21 mRNA copy number relative to CD19 mRNA copy number. The reduced levels of CD21 surface protein were also accompanied by a significant reduction in the mean number of CD21 transcripts (0.025 ± 0.002) when comparing B cells of HIV-infected patients with high plasma viral load to those of B cells of normal donors (0.100 ± 0.008) and HIV-infected patients with low plasma viral load (0.094 ± 0.011; $P < 0.001$; Fig. 2*B*). For both measurements tabulated on CD21 expression, no significant difference was found between the group of normal donors and the group of chronically infected patients at low plasma viral load (Fig. 2). Taken together, these data indicate that HIV viremia is associated with reduced B cell surface levels of CD21 that is also observed at the transcriptional level.

Functional Correlates of Reduced CD21 Expression. We then sought to determine whether the above phenotype of reduced CD21 expression on B cells of HIV-infected patients with high plasma viremia had a functional correlate. Accordingly, B cells of six viremic patients were separated by cell-sorting or immunomagnetic bead treatment into CD21-depleted and CD21-enriched fractions. Patient profiles shown in Table 1 (Patients 7–12) indicate that by qualitative flow cytometry, 25–41% of the B cells studied expressed reduced levels of CD21. A representative flow cytometry profile of the sorted cell populations obtained is shown in Fig. 3*A* for Patient 11, indicating that the CD21-enriched and CD21-depleted populations showed a B cell purity, as measured by CD19 staining, above 95%. The fractionated cells then were stimulated with the various B cell stimuli described previously. As shown by reporting as fold enhancement of proliferation of $CD21^{high}$ over $CD21^{low}$ B cells (Fig. 3*B*), the CD21-depleted B cell fractions of all five patients studied demonstrated profoundly reduced proliferative capacities to all stimuli when compared with their counterpart CD21-enriched B cell fractions. Furthermore, whereas the strong stimulus PMA/ionomycin resulted in a small median fold enhancement of 1.7,

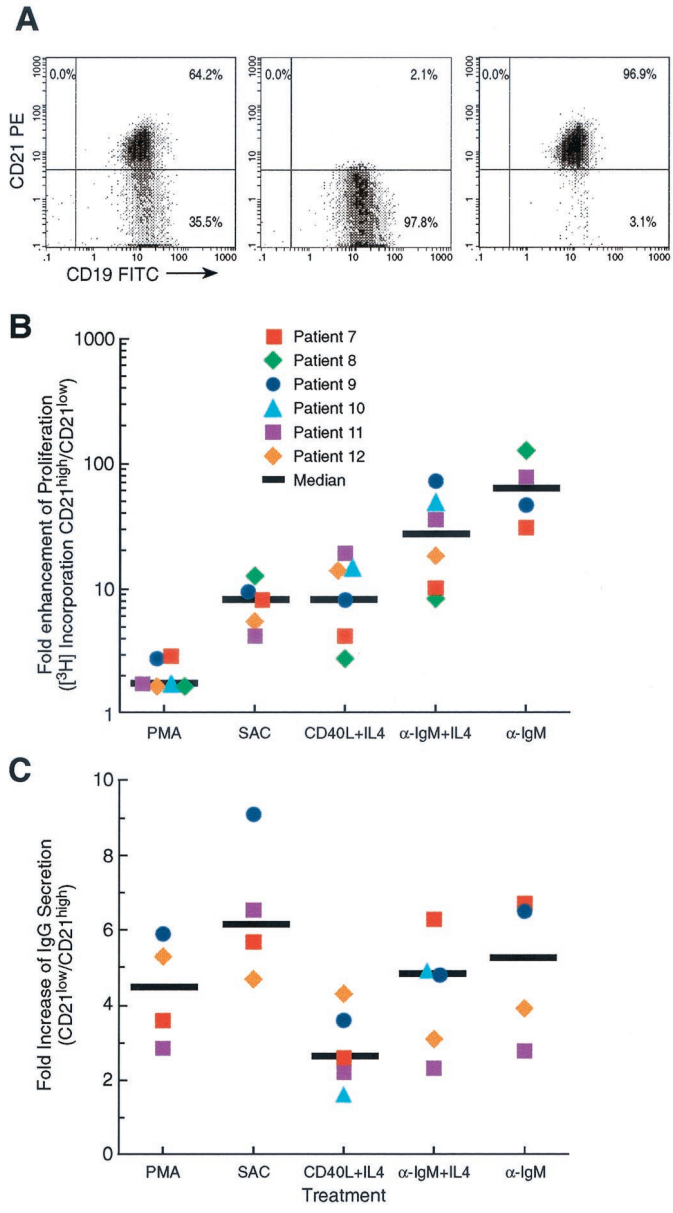

Fig. 3. Properties of CD21-depleted and CD21-enriched B cells of HIV viremic patients. (*A*) Representative B cells of a chronically viremic patient (*Left*) were sorted into $CD21^{low}$ (*Center*) and $CD21^{high}$ (*Right*) fractions. (*B*) Proliferation of fractionated cells of six patients expressed as fold enhancement of $CD21^{high}$ B cells over $CD21^{low}$ B cells, and (*C*) IgG secretion in 3-day cultures expressed as fold enhancement of $CD21^{low}$ B cells over $CD21^{high}$ B cells. Medians of data are reported for *B* and *C*.

the more subtle physiologic forms of stimulation resulted in much higher levels of proliferation in the $CD21^{high}$ fractions, with median fold enhancements of proliferation of 8.2 for CD40-triggering and 61.6 for BCR-triggering with anti-IgM. Finally, BCR-triggering with the stronger stimuli SAC and anti-IgM plus IL-4 resulted in median fold enhancements of proliferation of 8.1 and 26.9, respectively. Taken together, these data demonstrate a profoundly impaired capacity of CD21-depleted B cells to proliferate in response to B cell stimuli, particularly when the triggering signals used were subtler.

Down-regulation of CD21 and loss of proliferative response to B cell stimuli are hallmarks of B cells that have differentiated into plasma cells (22). To test the hypothesis that the B cells of

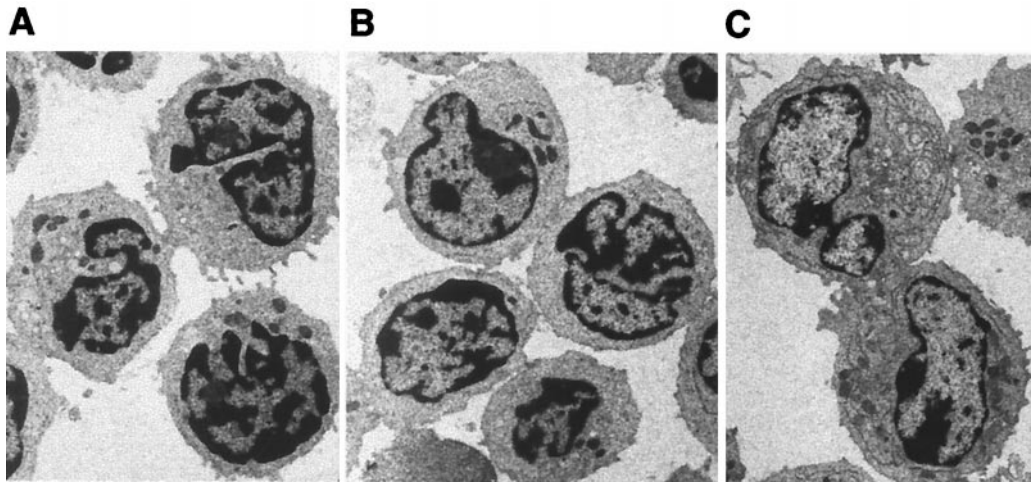

Fig. 4. Electron micrographs showing morphology of normal B cells (*A*), and B cells of a chronically viremic patient sorted into $CD21^{high}$ (*B*) and $CD21^{low}$ (*C*) fractions. Original magnifications were ×9,200, ×7,200, and ×5,800 for *A*, *B*, and *C*, respectively.

viremic patients expressing reduced levels of CD21 had progressed toward terminal differentiation, we measured levels of IgG in the supernatant of the cultured $CD21^{high}$ and $CD21^{low}$ B cell fractions. Substantially higher amounts of IgG were secreted from the $CD21^{low}$ fractions than the $CD21^{high}$ fractions for all stimuli studied (Fig. 3*C*). Median fold enhancements of IgG secretion in the $CD21^{low}$ cultures over $CD21^{high}$ cultures varied from 2.6 for CD40 ligand to 6.1 for SAC. The other B cell stimuli, PMA/ionomycin, anti-IgM, and anti-IgM plus IL-4, induced intermediate enhancements of IgG secretion in the $CD21^{low}$ cultures, with median fold enhancements of IgG secretion of 4.4, 5.2, and 4.8, respectively. To determine whether the enhanced secretion of immunoglobulins could be explained by the presence of plasma cell-like morphology in the CD21-depleted B cell fractions, electron microscopy was performed on freshly isolated bulk B cells of viremic patients and normal donors, as well as on their CD21-depleted and CD21-enriched fractions. A significant enrichment of cells with typical plasmacytoid features, namely irregularly shaped nuclei and extensive endoplasmic reticulum and Golgi apparatus formations, were observed in the CD21-depleted B cell fractions (Fig. 4*C*). These features were almost completely absent from the CD21-enriched B cell fractions (Fig. 4*B*) and normal donor B cell preparations (Fig. 4*A*).

Discussion

In the present study we have demonstrated that the defect in proliferation *in vitro* in response to various stimuli of B cells of HIV-infected patients is directly related to the level of ongoing viral replication *in vivo*. B cells of patients with high plasma viremia showed consistent functional impairment when compared with B cells of the same patients assayed at times of low plasma viremia. Furthermore, we have demonstrated that viremia induces the appearance of a subpopulation of B cells that express reduced levels of CD21. In several respects, this subpopulation of B cells resembles plasma cells, exhibiting typical morphological features of plasma cells and enhanced levels of Ig secretion when compared with their CD21-enriched counterparts. However, certain features of normal plasma cells, such as reduced expression of CD20 and surface Ig (23), did not segregate with CD21 expression (data not shown), indicating that the $CD21^{low}$ B cells of HIV viremic patients were engaged only partially in their differentiation pathway or that the cells were following an abnormal pathway. Nonetheless, considering that the frequency of Ab-secreting cells has been shown to be elevated during HIV viremia and decreases with suppression of viral load (16), we suggest that the CD21-depleted subpopulation described here may represent these Ab-secreting cells and may account at least in part for the hypergammaglobulinemia that is often described in HIV disease.

Previous studies on patients infected with HIV (19, 20), and other reports on inflammatory diseases both in humans (24) and in other species (25, 26), have shown that disease-induced B cell hyperactivity is associated with a reduction of CD21 expression. However, this is the first study, to our knowledge, to demonstrate a direct link between decreased CD21 expression and ongoing HIV replication and a correlation with plasma cell differentiation. It is unclear whether decreased CD21 expression on B cells is driven by the same mechanisms in HIV and other inflammatory diseases; however, there are indications that each disease is characterized by unique features. In the autoimmune disease systemic lupus erythematosus (SLE), the presence of abnormal B cells was associated with enhanced expression of CD27 and the typical phenotypic features of plasma cell phenotypes (27). In contrast, HIV disease has been associated with reduced expression of CD27 on B cells (28, 29), and we failed to observe enhanced CD27 expression on the plasma cells that we described (data not shown). In the case of rheumatoid arthritis, decreased CD21 expression has been observed in B cells isolated from synovial fluids of inflamed tissues (30), but expression was increased on peripheral blood B cells (31). In the case of African swine fever, B cell hyperactivity that followed infection was associated with a decrease in CD21 expression; however, the B cells also showed enhanced capacity to proliferate in response to CD40 ligand and IL-4 (26), a response that was opposite to our findings. Taken together, these observations indicate that HIV replication seems to induce a form of B cell hyperactivity that has unique effects on CD21 expression.

Several mechanisms of action could be responsible for the decreased expression of CD21 on B cells of HIV viremic patients, including ligand-mediated down-regulation of the receptor, defects in capacity to express CD21, or terminal differentiation. Although ligands of CD21 such as C3-derived immune complexes, soluble CD23, and Epstein–Barr virus (EBV) have been associated with chronic HIV disease (4, 18, 32), our findings indicate that at least part of the explanation for the CD21-depleted B cells recovered from viremic patients lies in terminal differentiation toward plasma cells. Of note, the recent finding that EBV primarily infects resting IgD-negative memory B cells in immunosuppressed individuals (33), combined with

our observation that $CD21^{low}$ and $CD21^{high}$ B cells express similar levels of IgD (data not shown), would suggest that EBV is unlikely to be involved in the down-modulation of CD21 described here. On the other hand, the decreased CD21 transcriptional activities that we observed in parallel with the decreased CD21 surface receptor would also be compatible with terminal differentiation (34). Whether terminal differentiation is being induced by abnormal activation and/or aberrations in B cell trafficking between lymphoid tissues, where plasma B cells normally reside, and peripheral blood, where plasma cells are normally excluded, remains to be determined. Both HIV-induced lymphoid tissue hyperplasia, which may contribute to the release of plasma cells into the blood stream, and B cell hyperactivity have been shown to decrease with decreasing viral replication (16). Hence, our finding that loss of CD21 expression on B cells is reversed with decreasing viremia is consistent with the overall concept that viral replication is responsible for numerous B cell abnormalities.

Finally, there are potential functional consequences of reduced levels of CD21 on B cells. $CD21^{low}$ B cells that are engaged in the terminal differentiation pathway will ultimately die without further contribution to immune surveillance, whereas $CD21^{low}$ B cells that arise from other mechanisms will also be deficient responders as CD21 has been shown to be an essential component of T-dependent Ab responses (35, 36). In this regard, the effects of HIV viremia on CD21 expression on B cells are likely to be deleterious to immune function and add to the compromise of host defenses in HIV-infected individuals.

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