Mucosal Plasma Cell Repertoire During HIV-1 Infection


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Mucosal Plasma Cell Repertoire During HIV-1 Infection


Impaired development of local Ab responses may predispose HIV-1-infected patients to an increased rate, severity, and duration of mucosal infections. We characterized the repertoire of Ig-producing cells in the intestinal effector compartment (the lamina propria) of HIV-1-infected (n = 29) and seronegative control (n = 27) subjects. The density of Ig-producing cells per area was similar in both groups. However, the proportions of IgA-producing cells were lower in both the duodenum and colon from HIV-1-infected patients compared with those of control subjects (p < 0.05), with compensatory increases in IgG-producing cells in the colon and IgM-producing cells in the duodenum. Similarly, among Abs in the lumen the proportions of IgA were also decreased and the proportions of IgG were increased among HIV-1-infected patients. On a molecular level, V_H gene repertoire analyses by RT-PCR revealed comparable proportions of the V_H3 family among duodenal IgA transcripts (50–53%) from both groups. V_H3 expression was decreased only for IgM among patients with advanced HIV-1 disease (n = 6) compared with that of control subjects (n = 8) (48 ± 8 vs 62 ± 13%; p < 0.01). Moreover, the frequencies of individual IgM and IgA V_H3 genes were comparable in each group, including rates of putative HIV-1 gp120-binding V_H3 genes (V3-23, V3-30, V3-30/30-30.5). We conclude that, despite a decrement in local IgA producing cells, the density and molecular V_H repertoire of mucosal plasma cells are relatively intact among patients with HIV-1 infection. These data suggest that HIV-1-infected patients use functional regulatory mechanisms to provide sufficient V_H diversity and effective induction and differentiation of mucosal B cells. The Journal of Immunology, 2002, 169: 4008–4016.

Patients infected with HIV-1 experience extremely high rates of gastrointestinal (GI) symptoms and secondary mucosal infections (1–4). Underlying these high rates may be the impaired Ab responses to luminal Ags, which complicate HIV-1 infection (5–10). The mechanisms of this impairment of B cell function may derive from T cell (11, 12) or intrinsic B cell defects (13–16). HIV-1 has been proposed to induce intrinsic defects in B cells through selective depletion of cells expressing Ig H chains of the third variable region family (V_H3) (17–21), which comprise about half of the expressed circulating V_H repertoire (22–26). HIV-1 surface envelope gp120 may bind in a superantigen-like fashion to conserved V_H3 framework regions (27, 28) and activate (29) and subsequently deplete these cells (reviewed in Ref. 30).

We have shown that V_H3 gene use in the blood of HIV-1-infected patients is normal among resting, naïve IgM+IgD+ B cells (21), but decreased in serum IgM Abs and in previously activated IgG memory B cells (21). Thus, selective deletions of V_H3 family B cells may occur preferentially in activated and/or differentiated B cells. Moreover, earlier work suggested a decrement in mucosal IgA-producing cells (31, 32) and recent data from our laboratory suggest that rates of somatic hypermutation may be decreased in mucosal plasma cells from HIV-1-infected patients (5). Both class switch recombination (CSR) and somatic hypermutation are typically dependent on engagement of CD40 by its ligand on activated T cells (33, 34), and compromised CD4+ T cell number and activity are hallmarks of HIV-1 disease (35). Therefore, we determined whether the dramatic effects of HIV-1 on intestinal T cells (36–39) are associated with perturbations in mucosal plasma cell number, isotype, and V_H repertoire, including the pattern of V_H3 gene use in the GI tract.

Materials and Methods

Patient samples

HIV-1-infected patients (n = 29; 22 (76%) males) and control subjects with low-risk for HIV-1 exposure (n = 27; 16 (59%) males) were enrolled after written informed consent with protocols approved by Institutional Review Boards at the Minneapolis Veterans Affairs Medical Center and the University of Minnesota. Subjects with a history of chronic liver or GI disease, diarrhea, febrile illness within 5 days of entry, recent use of antimotility agents, diabetes, immunosuppressive therapy, or cancer were excluded from the study. The HIV-1-infected group had a higher mean age (40.4 vs 30.9 years) and was more racially diverse (28 vs 4% African-American, respectively) than control subjects. The HIV-1-infected patients represented all clinical stages of disease (Centers for Disease Control stages: A1–3, 12 patients; B1–3, 3 patients; C1–3, 14 patients) (40) (Table I). Their peripheral blood CD4+ T cell counts per microliter were ≤200 in 12 patients, 200–500 in 12 patients, and ≥500 in 5 patients. Plasma HIV-1 RNA levels (Amplicor HIV-1 Monitor version 1.0; Roche Molecular Systems, Pleasanton, CA) ranged from <400 to 106 virions/mL (median, 11.237; mean, 139,949). Six patients were receiving no antiretroviral therapy and another six received multidrug combinations with protease inhibitors. Colon samples were obtained from 15 HIV-1-infected patients and 11 control subjects and duodenal samples from 23 HIV-1-infected patients.
and 18 control subjects, including eight HIV-1-infected patients and two control subjects who underwent both procedures.

After an overnight fast, study participants drank a nonabsorbable osmo- 
lar agent (Colyte; Reed and Carnrick, Piscataway, NJ) at the rate of 250 ml 
every 15 min until the onset of watery stools. Clear liquid specimens were 
collected, filtered, centrifuged twice, and treated with protease inhibitors as 
previously described (41). Clarified supernatants were stored at −70°C until total IgGs were measured by ELISA as previously described (41). After 
lavage, either upper endoscopy or colonoscopy was performed to obtain 
biopsies from control subjects and HIV-1-infected patients. Three 
biopsies were fixed in 10% formalin and paraffin-embedded for immuno-
histochemistry, and four from discrete sites were rinsed and immediately 
formalinized in TRIzol (Life Technologies, Rockville, MD) and stored at 
−70°C.

**Immunohistochemistry**

**Morphological analysis.** Standard 5-μM-thick, formalin-fixed, paraffin-
embedded tissue sections were mounted on glass slides, stained with H&E, 
and examined by light microscopy. The samples were evaluated grossly at 
histology, and examined by light microscopy. The samples were evaluated grossly at 

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* Eight patients (with two CD4⁺ T cell and plasma HIV-1 RNA values) were examined on two separate occasions (4–17 mo apart)—once for the colonoscopy and once for the upper endoscopy procedures.

* Clinical HIV-1 disease stage. Centers for Disease Control (40).

* PI, protease inhibitors; NRTI, nucleoside analog reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors.

* Patients selected for cloning and sequencing of VH3 IgM and IgA RT-PCR products.

* ND, Not done.

**Plasma cell analysis.** Tissue sections (5 μM) were cut onto slides coated with VECTABOND (Vector Laboratories, Burlingame, CA), and heated at 56°C overnight. Slides were deparaffinized by heating to 60°C for 30 min followed by incubations in xylene. Sections were rehydrated by sequential passage through graded alcohols into distilled water. To unmask Abs, sections were heated in 0.01 M sodium citrate (pH 6) in capped Coplin jars in an 800 W microwave for 1 min on high and 5 min on medium. Sections were blocked in PBS containing 7% goat serum for 30 min. For simultaneous three-color staining and quantitation of mucosal plasma cells in a single section, directly labeled fluorescent polyclonal goat anti-human IgGs (Jackson Immunochemicals, Birmingham, AL; IgA (7-amino-4-methyl-coumarin-3-acetic acid; blue, 1/40), IgM (FITC; green, 1/40), and IgG (Cy3; red, 1/500)) were used. The same Abs with different fluorochromes were used to obtain confocal images of triple-stained mucosal plasma cells (IgA, Cy3, 1/75; IgM, Cy5, blue, 1/40; and IgG, FITC, 1/75). Sections were incubated at 37°C in a humidified chamber for 60 min with the goat anti-human IgG Ab alone, washed in PBS, and then similarly incubated with the anti-IgA and anti-IgM Abs. Fluorochrome-labeled normal goat IgG diluted to equivalent concentrations as the specific antiserum was used as a negative control. Plasma cells expressing VH3 IgGs were identified in selected colon and duodenal tissue sections by dual labeling with anti-IgA-
Cy3 or anti-IgM-Cy3 (as above) and a VH3-spe cific biotinylated chicken single-chain Fv (scFv; LJ-26, 15 μg/ml) (47) visualized with FITC-labeled streptavidin (Zymed Laboratories, South San Francisco, CA). As a negative control, a biotinylated chicken scFv to an irrelevant Ag was used at an equivalent concentration to LJ-26. After mounting with VECTASHIELD aqueous medium (Vector Laboratories), digital images of fluorescently stained sections were acquired on an Olympus BX60 microscope (Olympus America, Melville, NY) equipped with a digital spot camera using METAMORPH software (Universal Imaging, Downingtown, PA). Positive cells for each Ig isotype were quantitated by both image threshold (48) and 18 control subjects, including eight HIV-1-infected patients and two control subjects who underwent both procedures.

After an overnight fast, study participants drank a nonabsorbable osmo-
and direct counting \((r = 0.97)\) on the basis of total number per square area in at least eight randomly selected lamina propria fields \((\geq200\text{cells/section})\). Epithelium and muscularis, as well as organized lymphoid nodules, were outlined by hand and excluded in each field. To acquire confocal images, immunoreactive cells were scanned using a confocal laser scanning microscope (model 1024; Bio-Rad, Hercules, CA) with a fluorescence microscope (Nikon, Melville, NY). Images were acquired using Comos software (version 6.05.8; Bio-Rad) and further processed using NIH Image (version 1.62; http://rsb.info.nih.gov/nih-image) and Adobe Photoshop (version 6.0; Adobe Systems, San Jose, CA).

**RT-PCR amplification of V\(_H\) genes**

V\(_H\) family-specific RT-PCR was performed with duodenal RNA, extracted with TRIzol (Life Technologies), using a set of V\(_H\) family-specific leader primers as described previously (21). Briefly, DNase I-treated (Life Technologies) RNA was reverse transcribed to first-strand cDNA with Moloney murine leukemia virus reverse transcriptase (Life Technologies), oligo(dT) (Life Technologies), and random hexamers (Promega, Madison, WI) (21). For each cDNA sample, a set of six V\(_H\) family-specific (V\(_H\)1–6) PCR primers with [\(\alpha-\mathrm{P}\)]dCTP were separately assembled with IgM and IgA constant region primers as described (21). The constant region primers (IgM, 5'-CGGGAAGATCTCAGGAC-3'; IgA, 5'-GAGGCTCAGCGGGAAGACC-3') were from Kabat et al. (49). Amplification of IgA V\(_H\) sequences was performed at an annealing temperature of 59°C in reactions containing 2 mM MgCl\(_2\). The relative radioactivity in the V\(_H\) family-specific PCR bands was measured with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). All samples were assayed in duplicate and results are expressed for each V\(_H\) family as a percentage of the total signal obtained for all six V\(_H\) families. Data for expression of V\(_H\)2 and V\(_H\)6 families, which consistently accounted for <5% of the total signal in all subjects, are not shown.

The number of cycles required for linear amplification of IgA (22 cycles) and IgM (26 cycles) V\(_H\) sequences were determined with cycle curves (18–34 cycles) based on representative control and HIV-1-infected samples as described (21).

**Cloning and sequencing of V\(_H\) genes**

Biopsies from three HIV-1-infected patients with advanced disease and three seronegative control subjects were selected for VH 3 gene cloning (Table I). Nonradioactive V\(_H\)3 IgM and IgA PCR products were cloned into Escherichia coli and plasmid inserts were sequenced in both directions, as described previously (21). Determination of the V\(_H\)3 gene of cloned sequences was performed by alignment with germline V\(_H\)3 sequences (Vbase; http://www.mrc-cpe.cam.ac.uk/imt-doc) using DNAplot accessed at this site.

**Statistical analyses**

Mean values for two clinical groups were tested for differences using an unpaired \(t\) test. ANOVA was performed for analyses comparing three or more groups. Means for significant main effects were tested by Fisher’s probable least-square difference test with the Bonferroni correction. A \(\chi^2\) analysis was used to analyze the frequencies of specific V\(_H\)3 gene usage in HIV-1-infected patients compared with control subjects and in IgA vs IgM plasma cells.

**Results**

**GI morphology**

To determine whether HIV-1 infection in these patients was associated with gross anatomical changes in the intestinal mucosa, we characterized the morphology of H&E-stained colonic and duodenal tissue sections. Sections of small intestine from 18 HIV-1-infected patients (six of whom had <200 CD4\(^+\) T cells/ml) showed normal villous architecture and normal numbers of mononuclear cells in the lamina propria and epithelial layers compared with those of 12 control subjects. Moreover, polymorphonuclear cells were not detected in either group, confirming the absence of significant acute inflammation in the duodenum of this cohort. Similarly, in colon tissue sections from 12 HIV-1-infected patients (four of whom had <200 CD4\(^+\) T cells/ml), the crypt architecture was normal and no polymorphonuclear cells were detected. Most HIV-1-infected patients had normal numbers of intraepithelial lymphocytes in colon sections compared with those of control subjects. Two HIV-1-infected patients showed increases in intraepithelial lymphocytes—one focally at the surface and the other in the crypts. In addition, two other HIV-1-infected patients had increased numbers of macrophages in the colonic lamina propria. However, in general, morphological analysis suggested that histologic features of colon and duodenum were relatively normal in our cohort of HIV-1-infected patients.

**Isotype distribution of intestinal lavage Ig and plasma cells**

Although IgA comprised the majority of Ig in intestinal lavage fluids in both groups, IgA accounted for a lower proportion of total Ig in fluids from HIV-1-infected patients \((n = 27)\) compared with that of control subjects \((n = 26)\) \((69.1 \pm 16.4\% vs 78.2 \pm 14.6\%\), respectively \((\text{mean} \pm \text{SD})\); \(p < 0.05\) (Fig. 1). In contrast, the proportion of IgG \((15.5 \pm 11.1\% vs 8.5 \pm 8.2\%\); \(p < 0.05\) was higher in those with HIV-1 disease (Fig. 1), whereas values for IgM were comparable in both groups. These differences were not directly related to CD4\(^+\) T cell numbers in peripheral blood \((<200, 200–500, \text{and} >500\text{cells/μl})\) or plasma HIV-1 RNA levels \((<10^3, 10^3–10^5, \text{and} >10^5\text{viriions/ml})\).

We next determined whether the altered distribution of Igs in lavage fluids correlated with perturbations in the isotype distribution of intestinal plasma cells in the duodenum and colon. Consistent with results in lavage fluids, the proportions of IgA-producing cells by simultaneous three-color immunofluorescence were significantly decreased in HIV-1-infected patients compared with those of control subjects in both duodenum and colon (Figs. 2 and 3). The proportion of IgM-producing cells was increased in duodenum, as was the proportion of IgG-secreting plasma cells in the colon. Within the duodenum, the alterations in proportions of IgA- and IgM-producing plasma cells were restricted to those patients with early HIV-1 disease (high CD4\(^+\) T cell levels and low plasma HIV-1 RNA levels) (Fig. 3, lower panel, inset).

Despite changes in the proportions of isotype-specific plasma cells in duodenum and colon, the overall density of total Ig-producing plasma cells per area in the duodental and colonic lamina propria did not differ between HIV-1-infected patients and control subjects (duodenum, 1952 ± 109 vs 2160 ± 94 cells/mm\(^2\); colon, 1370 ± 173 vs 1422 ± 129 cells/mm\(^2\), respectively). However, the density of duodenal IgA plasma cells was lower among HIV-1-infected patients compared with that in control subjects (1433 ± 82 vs 1692 ± 65 cells/mm\(^2\), respectively; \(p < 0.05\)). The density of IgA plasma cells exhibited no consistent pattern when patients...
were stratified by CD4+ T cell or plasma HIV-1 RNA group. Moreover, the density of IgM plasma cells was increased 60% among HIV-1-infected patients with CD4+ T cell counts $>500/\mu l$ compared with that of control subjects (617 ± 78 vs 385 ± 34 cells/mm², respectively; $p < 0.005$). Taken together, these data indicate that the distributions of colonic and duodenal isotype-specific plasma cells are altered in HIV-1-infected patients. However, the magnitude of these changes appears to be relatively small and most prevalent in those with less severe disease (high CD4+ T cells and low plasma HIV-1 RNA).

*V*_H* family expression in duodenum of HIV-1-infected patients*

We next determined whether changes in the patterns of differentiation of intestinal plasma cells among HIV-1-infected patients, as reflected by their isotype distribution, was accompanied by alterations in the patterns of *V*_H* gene selection and use. We compared the relative expression of the six *V*_H* gene families for IgM and IgA in duodenal biopsies to explore whether specific gene families, particularly *V*_H*3, were selectively deleted, as has been described in blood cells from HIV-1-infected patients (17–21). Among IgM mRNA transcripts, the *V*_H*3 family tended to be underrepresented in 14 HIV-1-infected patients compared with those of nine control subjects (53.4 ± 2.9 vs 62.2 ± 4.2%, respectively; $p < 0.06$) (Fig. 4, *upper panel*). These differences were most prevalent in HIV-1-infected patients with <200 CD4+ T cells/μl ($n = 6$), among whom the relative expression of *V*_H*3 IgM mRNA (47.7 ± 3.2%) was 24% lower than that of control subjects ($p < 0.01$) (Fig. 4, *upper panel*). Compensatory increases in the relative expression of IgM mRNA of the *V*_H*1 family were present in patients with advanced HIV-1 disease vs control subjects (9.1 ± 1.8 vs 4.8 ± 1.5%; $p < 0.01$) (Fig. 4). Finally, the relative expression of *V*_H*4 IgM mRNA was increased in the whole HIV-1-infected group (Fig. 4, *upper panel*, $p < 0.05$) as well as among patients with low viral loads (<10,000 virions/ml) compared with that of control subjects (37.2 ± 4.5 vs 26.6 ± 1.7%, respectively; $p < 0.005$).

In contrast to IgM, we found that the expression of each IgA *V*_H* family gene was comparable among control subjects and HIV-1-infected patients (Fig. 4, *lower panel*), including in each CD4+ T cell and plasma HIV-1 RNA stratum. The decrement in the frequencies and proportions of IgA-secreting cells described above in tissues was not related to preferential perturbations of any *V*_H* family. To further support that HIV-1-infected patients had relatively normal proportions of *V*_H*3 expression among their intestinal IgA and IgM plasma cells, we used immunofluorescence to dual-label colon tissue sections with an Ig isotype-specific Ab and a biotinylated *V*_H*3-specific scFv reagent (LJ-26) raised in chickens (47). In two representative HIV-1-infected patients and two control subjects, the percentage of LJ-26+ IgA and IgM cells in colon tissue was similar (IgA, 37–52%; IgM, 26–44%).

However, because HIV-1-associated abnormalities in IgM intestinal plasma cells involved both their relative frequencies and *V*_H*3 family expression, we next determined whether the decrement in IgM *V*_H*3 was associated with preferential changes in individual *V*_H*3 gene products, including selective deletion of those proposed to interact with HIV-1 gp120 (29, 30, 50, 51).

*V*_H*3 gene-specific frequencies in duodenal IgM- and IgA-producing plasma cells among HIV-1-infected patients

The distribution of individual IgM *V*_H*3 genes was broad; 18 of 22 *V*_H*3 genes were expressed in the intestinal mucosa (Fig. 5, *upper panel*). As in naive IgM+ IgD+ peripheral blood B cell populations (21, 52, 53), V3-23 was the most commonly used gene in the mucosa (~25% of all *V*_H*3 genes) from three advanced HIV-1-infected patients and two control subjects. Similarly, as in blood, V3-30/3-30.54 and V3-74 were also prominent (10–16 and 9–11%, respectively). In contrast, V3-07 was overrepresented in the intestine compared with the naive circulating B cell repertoire (21). Among cloned IgM transcripts, we observed no dramatic differences in the frequency of *V*_H*3 gene use between HIV-1-infected patients and control subjects (Fig. 5, *upper panel*). Indeed, even among the *V*_H*3 genes proposed to react directly with HIV-1 gp120 (V3-23, V3-30, V3-30/30.5, and to a lesser extent V3-73) (54), we identified no selective decrement in gene frequency (38 vs 40%, HIV-1 vs control, respectively) among HIV-1-infected patients (Fig. 5, *upper panel*, genes in shaded box).

Regarding IgA plasma cells in the duodenum, the comparable proportions of *V*_H*3 family mRNA described above from both groups did not exclude the possibility that selected *V*_H*3 genes might be underrepresented in samples from patients with HIV-1 disease. However, the distribution of individual *V*_H*3 genes expressed by IgA plasma cells was very similar to frequencies of IgM clones (21 of 22 *V*_H*3 genes expressed) and was also very similar among HIV-1-infected patients and control subjects (Fig. 5, *lower panel*). Indeed, the putative gp120-reactive gene products (V3-23 and V3-30/3-30.5)4 comprised comparable proportions of

4 Includes sequences that align to *V*_H*3 genes located at two distinct loci (V3-30 and V3-30.5).
VH3 genes (37.1 vs 37.8%, respectively) in the two groups. Moreover, these frequencies were remarkably similar to those reported previously for naive peripheral blood IgD⁺/H11001 cells (21) (Fig. 6).

Independent of HIV-1 status, V3-07 was less common among intestinal IgA compared with IgM plasma cells (χ², p < 0.03), whereas V3-09 was more prevalent (χ², p < 0.02). In addition, V3-09 and V3-15 tended to be overrepresented in both isotypes in HIV-1-infected patients compared with control subjects (for IgA cells, χ², p < 0.01 and p < 0.04, respectively). These individual changes may be due to the immunologic sequela of HIV-1 infection, differences in the magnitude of mucosal antigenic exposure, or the genetic differences in the VH repertoire of the patients tested.

Discussion
Abs serve an important protective role in controlling many mucosal pathogens (e.g., Salmonella spp., Campylobacter jejuni, Shigella spp., Giardia lamblia, Clostridium difficile, and Cryptosporidium) that complicate HIV-1 infections, particularly in resource-poor nations (1, 4, 55, 56). Impaired humoral responses to mucosal Ags during HIV-1 disease may increase the incidence and prolong the prevalence of these infections (5–9). However, despite the extensive analyses of HIV-1-associated B cell defects in the systemic compartment (13–21, 27–29, 50, 51, 57–59) and innovative studies on early SIV infections in macaques (36, 39, 60), few data are available on the basic structural and molecular elements of the human mucosal humoral immune system in this population. We characterized the integrity of the effector arm of humoral mucosal defense in the intestinal lamina propria of HIV-1-infected patients. Although we found significant perturbations in the isotype distribution and VH repertoire of mucosal B cells from HIV-1-infected patients, by and large plasma cells appear to be relatively spared, even in the patients tested with advanced disease.

Nevertheless, consistent with previous reports (31, 61, 62), we identified lower proportions of intestinal IgA Abs in HIV-1-infected patients compared with control subjects. This decreased proportion of IgA in intestinal lavage samples of HIV-1-infected patients may arise in part due to transudation of serum IgG across a compromised mucosal barrier (31, 62). However, despite similar overall densities of plasma cells in the two groups, we also found lower proportions of IgA-producing plasma cells in both duodenal...
and colonic tissues, suggesting local B cell imbalances. Furthermore, confirming parallel studies in HIV-1-infected humans (32) and SIV-infected macaques (39, 63), we found a significant increase in the density of duodenal IgM plasma cells for HIV-1-infected patients that was most prevalent in those with CD4 cells >500/μl. Among the potential mechanisms underling such an increase in IgM-producing plasma cells and a decrease in IgA-producing plasma cells in the lamina propria is a decrease in CSR from IgM to IgA during the course of HIV-1/SIV infection.

Mucosal B cells undergo CSR in germinal centers (GC) in organized lymphoid tissue (e.g., lymphoid nodules and Peyers patches in the ileal and appendix regions of the intestine). Intestinal lamina propria plasma cells are likely derived from B cells activated and selected in these organized lymphoid tissues (64). Impaired GC formation, structure, and function in the systemic compartment are well described in HIV-1-infected patients (59, 65, 66) and may also relate to changes in the isotype distribution of mucosal B cells during HIV-1 infection. Prominent data from HIV-1-infected humans and SIV-infected macaques suggest that widespread depletion of CD4 T cells in the effector lamina propria occurs early after infection, while these cells remain more numerous in mucosal inductive sites (12, 39, 67). Although these GC T cells could supply the signaling that B cells require to initiate CSR (see below), several studies have also shown that these cells can harbor HIV-1 or SIV (36, 39, 68). Further characterization of the anatomic and functional integrity of mucosal inductive sites in HIV-1-infected patients is needed to address these issues.

In general, CSR is dependent on CD4 T cell help in the form of cytokines and engagement of the CD40/CD40 ligand (CD40L) system. Stimulation of a human monoclonal B cell line, CL-01, with CD40L, IL-4, and IL-10 induced CSR to all seven downstream H chain isotypes (33). Complete loss of CD40L, as occurs in patients with X-linked hyper-IgM syndrome (69–71), completely blocks CSR. Attenuated activation of intestinal T cells with decreased expression of CD40L and, potentially, of cytokines may contribute to the apparent reduced levels of CSR within mucosal plasma cells of HIV-1-infected patients. Impaired CD40L expression has been documented in systemic CD4 T cells from HIV-1-infected patients (58, 72), but whether these results are recapitulated among CD4 T cells in mucosal inductive sites is not yet known. In related studies,5 we have recently shown that targeting of

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replacement somatic mutations to the complementarity-determining regions of mucosal V_{H}3 IgM and IgA sequences, another process dependent on CD40L (73), is normal in HIV-1-infected patients. These results suggest that CD40L may be adequately expressed by CD4^+ T cells at mucosal inductive sites of HIV-1-infected patients.

In addition to CD4^+ T cells, some studies suggest that epithelial cells or APCs (e.g., dendritic cells) supply cytokines that regulate CSR (e.g., TGF-B1 for IgA production) (74, 75). Decreased expression of such stromal cell-derived cytokines during HIV-1 infection could also adversely affect CSR in these patients. Finally, in the murine system, T cell-dependent class switching of IgM-producing B cells to IgA under the influence of lamina propria stromal cells has been described (76). The authors hypothesized that these were B1 B cells that develop outside of GCs and may preferentially target commensal bacteria in the intestine (77). However, the role of B-1 (CD5^+) B cells in the lamina propria of humans is controversial (78, 79).

Reduced numbers of IgA plasma cells in the intestinal lamina propria of HIV-1-infected patients may also result from defects in homing of B cells as they traffic from the inductive Peyer’s patches to the effector lamina propria (80, 81). Such defects could involve altered expression of the receptor-ligand pairs responsible for initial adhesion or recruitment of lymphocytes to the mucosa (82). In this regard, cells expressing a_{B}B_{2} integrin were dramatically reduced in rectal lymphoid nodules and the lamina propria of SIV-infected macaques (39). However, expression of the ligand for a_{B}B_{2}, mucosal cell adhesion molecule-1 on mucosal endothelial venules, was elevated in the duodenum of HIV-1-infected patients before highly active antiretroviral therapy (83). After initiation of highly active antiretroviral therapy, mucosal cell adhesion molecule-1 expression returned to normal levels in most patients but remained elevated in those with intestinal infections, although the infections had resolved (83). In addition, a recently described chemokine (C-C chemokine ligand 25/thymus-expressed chemokine) strongly attracts IgA-secreting plasma cells in mice and is highly expressed by small intestinal epithelial cells (84). Epithelial cell dysfunction among HIV-1-infected patients may alter expression of this important chemotactic factor.

We investigated another potential mechanism for decreased numbers of IgA-expressing plasma cells in the intestinal lamina propria of HIV-1-infected patients, selective loss of V_{H}3-expressing mucosal B cells. HIV-1 surface envelope gp120 has been proposed to bind in a superantigen-like fashion to conserved V_{H}3 framework regions (27, 28) and to activate (29) and subsequently deplete these cells. This scenario is unlikely to occur directly with lamina propria plasma cells because these cells are terminally differentiated, do not express surface Ig, have highly mutated Ig genes (85, 86), and likely no longer bind HIV-1 gp120 (54). However, HIV-1 could exert such effects on naive B cells at immune inductive sites in the intestine (36, 39, 68), resulting in reduced numbers of effector lamina propria plasma cells producing V_{H}3 Ig. However, we identified no signs of V_{H}3 deletion by both V_{H} family and V_{H}3 gene-specific analyses among IgA-producing B cells from HIV-1-infected patients. Thus, we conclude that specific deletion of V_{H}3 IgA plasma cells does not contribute to decreased numbers of intestinal IgA plasma cells or IgA Ab in HIV-1-infected patients.

In contrast to IgA, duodenal V_{H}3 family expression for IgM was reduced by 24% among HIV-1-infected patients with CD4^+ T cell counts <200/μl compared with that of control subjects. However, this apparent decrease in V_{H}3 IgM expression was not correlated with reduced expression of gp120-reactive V_{H}3 genes, V3-23, V3-30, and V3-30/3-30.5. Indeed, the proportion of plasma cells expressing IgM was elevated in the duodenal lamina propria of HIV-1-infected patients. One might speculate that an influx of B-1 B cells, purported to be elevated in the blood of HIV-1-infected patients (87), into the intestinal lamina propria could account for these differences in IgM-producing plasma cells. In this context, the preferential usage of V_{H}4 genes by B-1 B cells (88) could then account for both the decreased V_{H}3 expression and the increased V_{H}4 expression that we measured in lamina propria IgM-producing plasma cells. However, as noted above, convincing evidence supporting a prominent role for B-1 B cells in the human intestinal mucosa is quite limited to date (78, 79).

The results of our characterization of V_{H} family and V_{H}3 gene analyses are consistent with those in the literature. The relative proportions of each V_{H} family that we found (V_{H}3 > V_{H}4 > V_{H}1 > V_{H}5) for our samples are comparable to those reported by McCabe et al. (89). Moreover, while others have examined V_{H}4, V_{H}5, and V_{H}6 genes from microdissected B cells in ileal lamina propria and Peyer’s patches (64, 90, 91), and bulk V_{H} genes from single intestinal IgA and IgM plasma cells (85), our data set of 422 unique clones is the largest to specifically characterize V_{H}3 genes expressed by intestinal plasma cells. In the latter study (85), >80% of the V_{H} genes detected were V_{H}3 family members and, of these, ~34% were putative gp120-reactive genes (V3-23 and V3-30/3-30.5), similar to the percentages we report (37-38%). In addition, we now show that the normal distribution of these selected V_{H}3 genes extends from the resting naive IgD^+ blood B cell repertoire of HIV-1-infected patients (21) to terminally differentiated IgA- and IgM-producing cells in the lamina propria of the duodenum (see Fig. 6).

In summary, distinct and statistically significant abnormalities are present in the isotype and V_{H} family distribution of plasma cells in GI mucosa of HIV-1-infected patients. However, the magnitude of the changes seems relatively limited and the functional potential of mucosal plasma cells appears overall intact. The ability of HIV-1-infected patients, including those with very advanced disease, to adequately populate their upper and lower intestinal mucosa with Ig-producing plasma cells with relatively intact isotype distribution, V_{H}4 gene use, and appropriate levels of somatic hypermutation in Ag-binding complementarity-determining regions supports this conclusion. These processes are regulated, in large part, by initial Ag-responsive activation and selection in Peyer’s patches and solitary lymphoid aggregates. Limited data to date suggest that, in contrast to peripheral lymph nodes, Peyer’s patch structure and T cell integrity may be, to a large extent, spared during HIV-1 infection. Such preservation of inductive sites, as suggested by the integrity of the effector sites, provides hope that these anatomic resources can be harnessed to initiate protective immune responses against the onslaught of mucosal pathogens in this high-risk population.

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