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Stimulation through TLR represents a new therapeutic approach for enhancing Ab responses to vaccination. Considering that Ab responses are decreased in HIV disease and that B cells express TLR9 and respond to TLR9 agonists, we investigated the responsiveness of B cell subpopulations from HIV-infected and uninfected individuals to the TLR9 agonist CpG oligonucleotide type B (CpG-B) in the presence and absence of BCR ligation and T cell help (CD40L). CpG-B was equally effective in stimulating the proliferation of naive B cells of HIV-infected individuals and HIV-negative individuals, and, when combined with BCR and CD40 ligation, cytokine secretion by naive B cells was also comparable in HIV-infected and uninfected individuals. In contrast, CD27+ memory/activated B cells of HIV-infected individuals with active disease were less responsive to CpG-B in terms of proliferation and cytokine secretion when compared with CD27+ B cells of HIV-negative and HIV-infected individuals whose viremia was controlled by antiretroviral therapy. These findings suggest that despite abnormalities in memory B cells of HIV-infected individuals with active disease, naive B cells of HIV-infected individuals, irrespective of disease status, can respond to TLR9 agonists and that the incorporation of such agents in vaccine formulations may enhance their Ab responses to vaccination. The Journal of Immunology, 2008, 181: 1199–1206.

The B cells express a number of innate immune receptors of the TLR family that enable them to recognize and respond to unique molecular patterns present in pathogens (1). Among these is unmethylated CpG-containing DNA, which is more frequent in the genomes of bacteria and viruses than in those of mammals, and was first recognized for its ability to activate murine B cells (2). TLR9 was identified as the primary receptor for CpG DNA and synthetic CpG-containing oligonucleotides (ODN) (3), and its expression was later described in human B cells and plasmacytoid dendritic cells (pDC) (4). Three distinct classes of CpG ODN motifs have been identified. CpG-A induces pDC to secrete high levels of IFN-α; however, it does not recognize B cells. CpG-B is a potent B cell stimulator and induces pDC to secrete low levels of IFN-α, and CpG-C has effects that are intermediate to CpG-A and CpG-B (5–7).

CpG-B has been shown to provide survival and proliferation signals to human B cells, especially memory B cells, and to enhance their ability to secrete cytokines and Ig (8). Terminal differentiation of memory B cells by polyclonal stimulation with CpG-B has been proposed as a mechanism for maintaining long-term B cell memory in the absence of Ag (9). The effect of CpG-B on naive B cells is less clear, with some studies indicating that multiple signals are required for stimulation of naive B cells (10), whereas more recent studies indicate that CpG-B alone is sufficient to induce effector responses from naive B cells and to enhance their survival (11, 12). These effector responses include increased potential of CpG-B-activated naive B cells to present Ags to T cells, which could have implications in the enhancement of humoral and cell-mediated immunity (12).

CpG-B is a very effective costimulator of B cells, especially of memory/activated B cells of HIV-infected individuals with active disease (25). In one study performed on a small cohort of HIV-infected individuals (27), the addition of a TLR9 agonist to a vaccine against the hepatitis B virus (HBV) was shown to enhance titers of HBV-specific Abs, suggesting that this approach could improve B cell responses to immunization. However, little is known regarding the responsiveness of B cells per se to TLR9 agonists in the setting of HIV disease.

In the present study, we evaluated the proliferative, survival and effector responses to CpG-B of B cells isolated from HIV-infected individuals whose viremia was controlled or not by antiretroviral therapy and from HIV-negative individuals. Our findings indicate that CpG-B is a very effective costimulator of B cells, especially of naive B cells, in HIV-infected individuals, and thus suggest a

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3 Abbreviations used in this paper: ODN, oligodeoxynucleotides; pDC, plasmacytoid dendritic cell; LT, lymphotoxin.

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potentially beneficial effect of including TLR9 agonists in vaccine preparations.

Materials and Methods

Study participants

Three groups of individuals were investigated: 12 HIV-negative individuals; 12 HIV chronically infected individuals with a median HIV plasma viremia of 21,851 RNA copies/ml (1,897–924) and a median CD4+ T cell count of 445 cells/µl (225–924), referred to as the HIV-viremic group; and 12 HIV chronically infected individuals with a median HIV plasma viremia below the limit of detection of 50 RNA copies/ml and a median CD4+ T cell count of 641 cells/µl (383–1526), referred to as the HIV-aviremic group. Of the 12 HIV-viremic individuals 11 were antiretroviral therapy naïve and 1 was not fully compliant with his antiretroviral regimen, whereas the HIV-aviremic individuals were receiving effective antiretroviral therapy. CD10+/CD27 by using a biotin-based microbead enrichment system (Miltenyi Biotec) as well as functionally possible inclusions, was verified as described previously (28).

Cell preparation

PBMC were isolated from leukapheresis or blood draw products by Ficoll-Hypaque density gradient centrifugation. CD10+ B cells were isolated from PBMCs by negative selection using an immunomagnetic column-based technique and a B cell mixture to which was added anti-CD10 tetrameric mAbs (both from Stem Cell Technologies). The purity of the CD10+ B cells, typically >95%, was verified as described previously (28). The CD10+ B cells were then separated into CD27+ and CD27− fractions by using a biotin-based microbead enrichment system (Miltenyi Biotec) as described previously (29). Purity of each B cell fraction ranged from 80 to 95%. The CD27+ B cell fractions of HIV-viremic individuals were further separated into CD21high and CD21low fractions by using a FITC-based microbead enrichment system (Miltenyi Biotec), resulting in purities ranging from 80 to 95%. Five-color staining was performed with mAbs against CD10/CD21/CD14/CD27/TLR9 and with the following fluorochromes listed in the same order as the markers: PE-Cy7 tandem (PE-Cy7)/fluorescein (FITC)/allophycocyanin/allophycocyanin-Alexa 750/PE. The anti-CD19 and -CD10 mAbs were obtained from BD Biosciences; the anti-CD21 mAb was obtained from Beckman Coulter; the anti-CD27 mAb was obtained from Invitrogen; and the anti-CD27 mAb was obtained from Imgenex. For intracellular analysis, cells were first stained for cell surface markers, fixed (FACS Lysing Buffer; BD Biosciences), permeabilized (Permeabilizing Solution 2; BD Biosciences), and then stained for intracellular markers. Data were acquired on a FACSCanto flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star).

Cell culture

B cells (2×10⁵ cells/well) were cultured in a 96-well round-bottom plate in the presence or absence of 2.5 µg/ml CpG-B (ODN-2006; Operon), and in the presence or absence of 10 µg/ml P(ah)₅ goat anti-human IgA/IgM/IgG, referred to as anti-Ig (catalog no. 109-006-064; Jackson Immunoresearch Laboratories). After overnight incubation, the cells were washed to remove the anti-Ig, which interferes with the measurement of Ig in culture supernatants, and recultured at 1×10⁵ cells/well in a 96-well flat-bottom plate in the presence or absence of CpG-B and in the presence or absence of 50 ng/ml CD40L (30). 72 h in culture, supernatant was collected to measure levels of cytokine and Ig secretion and cells were pulsed for 16 h with [³H]thymidine to measure proliferation. Proliferation was also evaluated by CFSE labeling. Briefly, B cells were labeled with 0.5 µM CFSE (Molecular Probes) in PBS for 8 min at room temperature, followed by addition of RPMI 1640-10% FBS and extensive washing. Cells (2×10⁵/well) were then cultured for 5 days in a 96-well round-bottom plate with the above stimuli. The effect of CpG-B on B cell survival was evaluated by annexin V staining as previously described (28), followed by CFSE staining at 37°C in the presence or absence of 2.5 µg/ml CpG-B. Culture supernatants were assayed for the secretion of cytokines IL-2, IL-4, IL-6, IL-10, IL-12p70, IFN-γ, lymphotixin (LT) α, and TNF-α and of immunoglobulins IgM, IgA, and IgG using a multiplex bead array system (CBA Flex Sets) and accompanying FACSArray instrumentation and software (BD Biosciences).

Statistical analysis

Comparisons were made by Kruskal-Wallis test with Dunn’s posttest or by paired t test where appropriate.

Results

Characteristics of the B cell subpopulations generated for functional analyses

To evaluate the responsiveness of naive and memory B cells of HIV-viremic and HIV-aviremic individuals to CpG-B and to compare these responses to those of corresponding B cell subpopulations in HIV-negative individuals, we sought to generate comparable B cell fractions from these groups of subjects. Given that CD10-expressing immature/transitional B cells are
overrepresented in HIV-viremic individuals (29) and that including them as a separate fraction would be technically challenging, we decided to exclude this subpopulation from all analyses by generating CD10-depleted B cells from the PBMCs of all study participants. The CD10\(^{+/−}\) B cells were then fractionated into CD27\(^{+/−}\) and CD27\(^{+/−}\) fractions, which in HIV-aviremic and HIV-negative individuals largely represent resting/memory and naive B cells, respectively. In HIV-viremic individuals, CD27\(^{+/−}\) B cells are comprised of mature/activated B cells and plasma cells in addition to resting/memory B cells (31). Furthermore, the CD27\(^{+/−}\) B cell fraction of HIV-viremic individuals also contains CD21\(^{low}\) cells that have not yet been fully characterized but are thought to be distinct from naive B cells (32, 48). Thus, the CD27\(^{+/−}\) B cell fraction of HIV-viremic individuals was further fractionated by CD21 to generate a truly naive CD21\(^{high}\)CD27\(^{+/−}\) B cell population that was phenotypically comparable to the CD27\(^{+/−}\) B cells of HIV-aviremic and HIV-negative individuals and thus appropriate for functional comparison. To verify that the CD21 fractionation itself did not affect B cell function, CD27\(^{+/−}\) B cells of HIV-negative individuals were also fractionated by CD21 and the resulting CD21\(^{high}\)CD27\(^{+/−}\) B cells were found to be identical to the single-fractionated CD27\(^{+/−}\) B cells both in terms of proliferation and secretion of cytokines and Ig (data not shown).

**Effect of CpG-B on proliferation of B cell fractions**

The effect of CpG-B on the proliferation of B cell fractions isolated from HIV-viremic, HIV-aviremic, and HIV-negative individuals was evaluated in the presence or absence of other B cell stimuli. The proliferation of B cell fractions was very low in the absence of any stimulation or in the presence of BCR stimulation alone (data not shown). Consistent with previous reports (9), CpG-B alone induced the proliferation of CD27\(^{+/−}\) B cells (Fig. 2A) and naive B cells (Fig. 2B) of HIV-negative (HN), 12 HIV-viremic (V), and 12 HIV-aviremic (AV) individuals. Culture supernatants were collected at day 3 and assayed for LT-α, TNF-α, IL-6, and IL-10. Black horizontal lines indicate median counts.

**Cytokine Secretion (pg/ml)**

- LT-α: HN > AV > V
- TNF-α: HN > AV > V
- IL-6: HN > AV > V
- IL-10: HN > AV > V

**Immunoglobulin Secretion (ng/ml)**

- IgM: HN > AV > V
- IgA: HN > AV > V
- IgG: HN > AV > V

**FIGURE 2.** CpG-B enhances secretion of cytokines from both CD27\(^{+/−}\) and naive B cells. CD27\(^{+/−}\) (A) and naive (B) B cells of 12 HIV-negative (HN), 12 HIV-viremic (V), and 12 HIV-aviremic (AV) individuals were cultured with CpG-B (2.5 μg/ml), anti-Ig (10 μg/ml), and CD40L (500 ng/ml). Culture supernatants were collected at day 3 and assayed for LT-α, TNF-α, IL-6, and IL-10. Black horizontal lines indicate median counts.

**FIGURE 3.** CpG-B alone induces secretion of Ig from CD27\(^{+/−}\) B cells. CD27\(^{+/−}\) B cells of 12 HIV-negative (HN), 12 HIV-viremic (V), and 12 HIV-aviremic (AV) individuals were cultured without stimulation (A) or with CpG-B (2.5 μg/ml) (B). Culture supernatants were collected at day 3 and assayed for total IgM, IgA, and IgG. Black horizontal lines indicate median counts.
cells in all three groups of individuals, although the CD27+ B cells of HIV-viremic individuals proliferated at a significantly lower level compared with CD27+ B cells of HIV-aviremic and HIV-negative individuals (Fig. 1A). However, when CpG-B was combined with either BCR stimulation alone (anti-Ig) or in the added presence of CD40 ligation, the differences in proliferation between HIV-viremic individuals and the other two groups of individuals were not significant (Fig. 1A). In contrast, the response of CD27+ B cells of HIV-viremic individuals to BCR and CD40 ligation in the absence of CpG-B was significantly lower than that of CD27+ B cells of HIV-aviremic and HIV-negative individuals. These data suggest that CpG-B has a normalizing effect on CD27+ B cell proliferation in HIV-viremic individuals.

Consistent with a recent report (11), CpG-B alone induced the proliferation of naive B cells in all three groups of individuals and levels of proliferation were comparable among the three groups (Fig. 1B). When naive B cells were stimulated via BCR and CpG-B in the presence or absence of CD40 ligation, there was no significant difference in proliferation among the three groups of individuals. However, naive B cells of HIV-viremic individuals proliferated significantly less than those of the other two groups upon stimulation via BCR and CD40 alone, suggesting a less effective response under certain suboptimal conditions for HIV-viremic individuals. These data indicate that naive B cells of HIV-infected individuals respond robustly to CpG-mediated signals irrespective of disease status.

Effect of CpG-B on cytokine and Ig secretion

The effector properties of B cells following CpG-B stimulation were evaluated in terms of cytokine and Ig secretion. CpG-B alone or any two of the three stimuli shown in Fig. 1 had relatively marginal effects on cytokine secretion (data not shown). However, when CpG-B was combined with BCR and CD40 ligation, several cytokines were detected at moderate to high levels in the culture supernatant of both CD27+ B cells (Fig. 2A) and naive B cells (Fig. 2B). CD27+ B cells from all three groups secreted high levels of IL-6, although levels were significantly lower for HIV-viremic compared with HIV-negative individuals. CD27+ B cells from both HIV-aviremic and HIV-negative individuals secreted moderate levels of LT-α and TNF-α, and these levels were significantly higher compared with CD27+ B cells from HIV-viremic individuals. The secretion of IL-10 by CD27+ B cells was equally low in all three groups of individuals. In contrast, secretion of IL-10 by naive B cells was equally high in all three groups of individuals (Fig. 2B). In addition, naive B cells from all three groups of individuals secreted high levels of IL-6 and modest levels of TNF-α and LT-α, although levels of LT-α secreted by naive B cells were lower for HIV-viremic compared with HIV-aviremic and HIV-negative individuals.

CpG-B alone induced CD27+ B cells from all three groups of individuals to secrete moderate to high levels of IgM, IgA, and IgG (Fig. 3B), whereas lower levels were induced with other conditions or in the absence of stimulation. In the case of naive B cells, relatively negligible levels of Ig were secreted under all conditions (data not shown). Levels of spontaneous Ig secretion in the absence of any stimulation are shown in Fig. 3A for CD27+ B cells of the three groups of individuals. Levels of spontaneous IgG secretion from CD27+ B cells of HIV-1-viremic individuals were significantly higher compared with CD27+ B cells of HIV-aviremic and HIV-negative individuals, whereas spontaneous secretion of IgM and IgA was significantly higher for CD27+ B cells from HIV-viremic compared with HIV-negative individuals. Levels of IgG secreted by CD27+ B cells of HIV-viremic individuals in response to CpG-B alone were significantly higher when compared with CD27+ B cells of HIV-negative individuals (p < 0.001), but not HIV-aviremic individuals (Fig. 3B). Levels of IgM and IgA secreted by CD27+ B cells varied considerably within each group, although there was no significant difference between groups (Fig. 3B).
Taken together, these data indicate that the addition of CpG-B to BCR and CD40 ligation increased the effector function of both naive and CD27+B cells in terms of cytokine secretion, whereas CpG-B alone was a strong inducer of Ig secretion in CD27+B cells. Of note, the observation that CD27+B cells of HIV-viremic individuals were more effective at secreting Ig, especially IgG, whether spontaneously or upon CpG-B stimulation, and less effective at secreting cytokines, is most likely a reflection of the more activated and differentiated nature of these CD27+B cells compared with the more resting state of the CD27+B cells of HIV-aviremic and HIV-negative individuals.

To determine whether differences in the levels of expression of TLR9 could explain the differences in responses of B cells to CpG-B among the three groups of individuals studied, we performed intracellular TLR9 staining on PBMCs that were gated for total B cells (CD19+) and for CD10−CD27+ and naive (CD10−CD27highCD27−) B cell subpopulations (Fig. 4). Consistent with previous studies (33), we detected a significantly higher expression of TLR9 in CD27+B compared with naive B cells for all groups of individuals that could explain the lower proliferative capacity of naive compared with CD27+B cells in response to CpG-B (cf Fig 1, A to B). However, levels of TLR9 expression in

FIGURE 6. Proliferation and differentiation of CD27+B cells in the presence of CpG-B. Representative flow cytometric dot plots of CFSE-labeled (A) CD27+B cells from a HIV-negative (HN), a HIV-viremic (V), and a HIV-aviremic (AV) individual and naive B cells (B) from a HIV-negative (HN), a HIV-viremic (V), and a HIV-aviremic (AV) individual stimulated with CpG-B alone (CpG), CpG-B plus anti-Ig, anti-Ig plus CD40L, or a combination of CpG-B, anti-Ig, and CD40L.
CD27⁺ B cells were not significantly different among the three groups of individuals and thus could not explain the lower proliferative capacity to CpG for CD27⁺ B cells of HIV-viremic compared with HIV-aviremic and HIV-negative individuals shown in Fig. 1A.

Effect of CpG-B on survival
Considering that CpG-B has been shown to provide survival signals to B cells and pDC (8), we tested the survival effect of CpG-B on CD27⁺ and naive B cells isolated from HIV-viremic, HIV-aviremic, and HIV-negative individuals. For all three groups of individuals, the presence of CpG-B had a significant survival effect on the CD27⁺ B cells (Fig. 5A), although the effect was more moderate on the CD27⁺ B cells of the HIV-viremic individuals. In contrast, CpG-B had a significant and comparable survival effect on the naive B cells from all three groups of individuals (Fig. 5B).

Effect of CpG-B on population dynamics
Finally, we sought to gain a better understanding of the overall proliferative and differentiation effects of CpG-B on CD27⁺ and naive B cells by labeling the two B cell fractions with CFSE and staining them for the expression of CD27 as a marker of activation after 5 days in culture. When CFSE-labeled CD27⁺ B cells that had been stimulated with CpG-B alone or in combination with BCR ligation were costained with CD27 at day 5, levels of CD27 expression were increased from baseline levels on cells that had undergone several division cycles (Fig. 6A), consistent with the ability of CpG-B to induce terminal differentiation. This was especially true for CD27⁺ B cells of HIV-viremic individuals, where a very distinct population of B cells expressing high levels of CD27 and decreased levels of CD20 (data not shown), two hallmark features of terminal differentiation, could be observed (Fig. 6A). Of note, whereas the addition of CD40L to CpG-B and BCR ligation had the effect of inducing the highest percentage of CD27⁺ B cells to undergo cell division, consistent with the proliferation data in Fig. 1A, CD40 ligation also had a dampening effect on the induction of high CD27-expressing cells (Fig. 6A). This effect was most evident when CD27⁺ B cells were stimulated with CD40 and BCR ligation in the absence of CpG-B (Fig. 6A). These data are consistent with studies demonstrating that CD40 ligation inhibits terminal differentiation (34).

The effect of CpG-B on cell division and differentiation of naive B cells was less distinctive and more uniform across the three groups of individuals than its effect on CD27⁺ B cells. As shown in Fig. 6B, fewer naive B cells underwent cell division when stimulated with CpG-B alone than when CD40 and/or BCR ligation was included, consistent with the proliferation data in Fig. 1B. Furthermore, none of these conditions induced the naive B cells to differentiate into CD27-expressing memory or plasma cells (Fig. 6B), although as others have reported (12), the presence of CpG-B did increase the expression of activation markers (data not shown). Although these data may appear to be partially inconsistent with reports of CpG-induced B cell differentiation from naive B cells, it should be noted that B cell cultures described herein were not maintained for the extended periods described by others (10–12). Taken together, these data support a role for CpG-B in promoting proliferation of naive B cells and a role in promoting proliferation and differentiation of CD27⁺ B cells.

Discussion
The potent stimulatory effect of CpG ODN on cells of the immune system has led to their consideration as adjuvants to vaccine formulations in a number of clinical trials (18). The inclusion of CpG-B in a vaccine formulation for HBV was shown to induce a more rapid Ab response, a higher and more sustained Ab titer, and an increased HBV-specific Th response in HIV-negative individuals when compared with vaccine formulations that did not include CpG-B (35–38). Similar, albeit less extensive, findings were observed in a trial conducted on HIV-infected individuals (27). However, while the enhanced immune response in HIV-infected individuals to a HBV vaccine resulting from the inclusion of a TLR9 agonist is promising, little is known regarding the responsiveness of TLR9-expressing cells to CpG-B or other TLR9 agonists in HIV-infected individuals. Given that B cells and pDC are the principal cells of the immune system to express TLR9 (4) and that CpG-B ODN are more effective at stimulating B cells than pDC (8), it is reasonable to assume that B cells represent the major targets of CpG-B-based adjuvants. Accordingly, the aim of the present study was to investigate B cell responses to CpG-B in HIV-infected individuals and to compare these responses to those of healthy HIV-negative individuals. Our findings indicate that regardless of disease status, B cells of HIV-infected individuals have the capacity to respond to TLR9 agonists and that this is especially true for naive B cells. Thus, the addition of TLR9 agonists to vaccine preparations may be most effective at compensating for the paucity of CD4⁺ T cell help during the initiation of an Ab response, particularly a primary response, in immunocompromised individuals.

CpG-B has been shown to have distinct effects on the two main B cell subpopulations circulating in the peripheral blood, namely, naive and memory B cells (10). Given that B cells of HIV-infected individuals, and especially HIV-viremic individuals, are comprised of several B cell subpopulations, some of which are abnormally represented in peripheral blood (29, 31), it was important to devise a strategy that would consider comparable B cell subpopulations in the three groups of individuals. As such, we excluded CD10⁻/-immature/transitional B cells and CD21⁺/CD27⁻ activated B cells, both of which are overrepresented in HIV-viremic individuals (31). Thus, we were able to focus on truly naive B cells, defined as CD10⁺ CD21⁺/CD27⁻ B cells, and show that they were highly responsive to CpG-B in all three groups of individuals studied (HIV-negative, HIV-infected aviremic, and HIV-infected viremic individuals), especially when combined with BCR and CD40 costimulation. The beneficial effects of CpG-B on naive B cell responses in both HIV-infected and HIV-negative individuals included enhanced proliferation and enhanced secretion of cytokines IL-6, TNF-α, and LT-α. To our knowledge, this is the first report that CpG-B enhances the secretion of LT-α. The importance of this observation is underscored by the fact that B cells are thought to be the main source of LT-α and that in the absence of LT-α, germinal centers do not form and Ab responses are defective (39).

The effect of CpG-B on CD27⁺ B cells (predominantly memory cells) was more heterogeneous across the three groups of individuals when compared with its effect on naive B cells. Although the response of CD27⁺ B cells of HIV-aviremic individuals to CpG-B was similar to that of HIV-negative individuals, the response of CD27⁺ B cells of HIV-viremic individuals was clearly deficient with regard to proliferation and cytokine secretion. This was not surprising considering that
CD27⁺ B cells of HIV-viremic individuals are more activated and terminally differentiated compared with B cells of HIV-aviremic and HIV-negative individuals (28, 31). These cells are thus more likely to secrete Ig, either spontaneously or following stimulation, and less likely to further proliferate and secrete cytokines upon stimulation when compared with the more resting CD27⁺ B cells of HIV-aviremic and HIV-negative individuals. The higher responsiveness of CD27⁺ B cells of HIV-aviremic compared with HIV-viremic individuals is consistent with observations of improved B cell responses to various immunogens following successful reduction in plasma viremia by antiretroviral therapy (40–42). However, none of these studies included a TLR agonist.

In the present study, when CpG-B was combined with BCR and CD40 ligation, the proliferation of CD27⁺ B cells of HIV-viremic individuals was indistinguishable from that of the other two groups of individuals. Thus, CpG-B may be a key factor in overcoming proliferative deficiencies in CD27⁺ B cells of HIV-viremic individuals. It should be noted that CpG-B may exacerbate hypergammaglobulinemia, a hallmark of active HIV disease (19). However, such an effect would likely be transient and may be outweighed by the benefits of an enhanced Ag-specific immune response. Of note on a related issue, the predictions that CpG-based immunotherapies would induce or exacerbate autoimmune responses have thus far not been observed in clinical trials where such responses were investigated (18).

We also demonstrated that CpG-B had a strong survival effect on naive B cells and CD27⁺ B cells of HIV-infected and HIV-uninfected individuals, although the effect was less pronounced on CD27⁺ B cells of HIV-viremic individuals. These data are consistent with other studies and may underscore yet another advantage of including CpG in vaccine formulations given the high susceptibility of B cells to death by apoptosis in HIV-infected individuals (28).

We (31) and others (43) have recently reported on the increase in resting/memory CD27⁺CD21low B cells following effective antiretroviral therapy. Despite this increase, the percentage of resting/memory B cells remained below the average 40% reported in the peripheral blood of HIV-negative healthy individuals (44). This underrepresentation of memory B cells, irrespective of treatment status, is consistent with previous studies on HIV-infected individuals (45, 46). These data are also consistent with our and other studies indicating that Ag-specific memory B cell responses remain low even in patients successfully treated with antiretroviral therapy (25, 47). Taken together, these observations are a further indication of the potential benefits of using CpGs as a means of enhancing Ag-specific Ab responses in immune-compromised individuals such as HIV-infected individuals.

In summary, we have demonstrated that B cells of HIV-infected individuals, regardless of disease status, respond robustly to CpG-mediated signals and that this is especially true for naive B cells. These findings provide a further scientific basis for the consideration of performing clinical trials using CpG ODN as vaccine adjuvants to enhance Ab responses in HIV-infected and other immunocompromised individuals.

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