

gp120- and TNF- α -induced modulation of human B cell function: Proliferation, cyclic AMP generation, Ig production, and B-cell receptor expression

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Background: It is well known that HIV-1 infection induces profound alterations in the immune system, including hyperactivation of B cells. TNF- α induces HIV-1 replication and immunodysregulation, including polyclonal B-cell activation. **Objective:** We sought to determine the effects of surface-binding HIV-1 envelope glycoprotein (gp120) and TNF- α on human B-cell function.

Methods: HIV-1 seronegative peripheral blood human B cells were purified and activated by CD40 mAb and IL-4. In vitro studies of B-cell proliferation, cyclic AMP (cAMP) generation, receptor expression, and Ig production were performed.

Results: gp120, an Ig superantigen, stimulated HIV-1 seronegative and HIV-1 seropositive human B-cell cAMP generation, proliferation, and Ig production. These gp120-induced B-cell responses were demonstrated to be specific as evidenced by the abrogation of the stimulatory response in the presence of anti-gp120 mAb, blocking of CD4 resulting in no change on gp120-induced B-cell responses, and the binding of gp120 in these B cells. TNF- α also stimulated cAMP generation, proliferation, and Ig production in B cells, and the binding of gp120 to these B cells stimulated by TNF- α further enhanced cell proliferation, cAMP generation, and Ig production. Antigenic expression of the B-cell receptor CD79b was down-regulated by gp120 but was not altered by the addition of TNF- α .

Conclusion: gp120 modulation of TNF- α -induced B-cell receptor- and cAMP-mediated signal transduction events may be involved in the B-cell abnormalities observed in HIV-1 infection. (*J Allergy Clin Immunol* 2000;105:975-82.)

Key words: gp120, TNF- α , B cells, cyclic AMP, B-cell receptor, CD79b, HIV-1 long terminal repeat

Abnormalities of the humoral response occurs on HIV-1 infection, including polyclonal hypergammaglobulinemia, B-cell hyperplasia, circulating immune complexes, spontaneous Ig secretion, and proliferation along with elevated autoantibodies.¹⁻³

Abbreviations used

BCR:	B-cell receptor
cAMP:	Cyclic AMP
CRE:	Cyclic AMP responsive element
HIV-1 LTR:	HIV-1 long terminal repeat
NF- κ B:	Nuclear factor- κ B
PE:	Phycoerythrin
SPA:	Staphylococcal protein A

Viral glycoproteins that are expressed on the virion envelope and membranes of the viral-infected cells are potent inducers of humoral and cellular immune responses and thus are prime candidates for the B-cell responses observed in HIV-1-infected individuals. Berberian et al⁴ presented evidence of binding by gp120 to the V_H3 subpopulation of normal B cells by membrane Ig. gp120 has now been identified as a member of the new family of Ig superantigens.⁵

TNF- α , secreted by B lymphocytes in HIV-1 infection, is a known progression factor for B cells that contributes to the support of B-cell proliferation and differentiation in an autocrine manner.⁶⁻¹⁰ One of the earliest events after B-cell stimulation is the transcription of the TNF- α gene.¹¹ TNF- α is involved in the immunodysregulation related to HIV-1 infection, including polyclonal B-cell activation.¹² TNF- α regulates HIV-1 replication^{13,14} and binding of nuclear factor- κ B (NF- κ B) to HIV-1 long terminal repeat (HIV-1 LTR).¹⁵ In macrophages TNF- α is required for the gp120-induced down-regulation of CD4 expression.¹⁶ In B cells gp120 has been demonstrated to augment TNF- α secretion.¹⁷

The antigen-dependent activation of B cells is mediated by surface Ig and the associated molecules of the B-cell receptor (BCR). CD79b, the invariant component of the antigen receptor in B lymphocytes, is involved in antibody responses and B-cell differentiation.¹⁸ The BCR, which normally activates downstream signal transduction responsible for linkage to nuclear activation, is decreased in HIV-1-seropositive B lymphocytes.^{19,20}

TNF- α and cyclic AMP (cAMP) regulate cellular events in HIV-1 infection by HIV-1 LTR induction.²¹ cAMP activates protein kinase A induction of HIV-1 LTR²² with resultant increased levels of cAMP.²³ Aandahl et al²⁴ show that T cells from HIV-1-seropositive individuals exhibit significantly higher levels of cAMP than do T cells of HIV-1-seronegative individuals. In

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monocytes TNF- α in the presence of a cAMP activator act synergistically to enhance HIV-1 replication at the transcriptional level²⁵ and to augment the induction of the TNF receptor.²⁶

In this study we examined the effects of TNF- α on HIV-1-seronegative activated human B-lymphocyte signal transduction, proliferation, and Ig production. In addition, we examined the ability of gp120 to modulate these TNF- α -induced B-cell events and the specificity of these interactions.

MATERIAL AND METHODS

Reagents

The following reagents (and their sources) were used: recombinant gp120 (HIV-1, IIIB, produced in the Baculovirus Expression System, Intracel, Cambridge, Mass), gp120 mAb and gp120-FITC mAb (Intracel, Cambridge, Mass), recombinant human TNF- α , recombinant human IL-4 (Genzyme, Cambridge, Mass), CD4 mAb (Becton-Dickinson, Mountain View, Calif) and CD40 mAb (Immunotech, Westbrook, Me), *Staphylococcus aureus* Cowan I protein (Sigma, St Louis, Mo), and recombinant staphylococcal protein A (SPA) (Calbiochem, La Jolla, Calif).

B-cell purification

B lymphocytes were positively selected from platelet-depleted HIV-1-seronegative buffy coats according to the technique of Funderud et al.²⁷ Briefly, CD19-coated magnetic beads (Dynabeads 450, Dynal, Oslo, Norway) were added to the blood in a target-to-bead ratio of 1:5. The mixture was incubated at 4°C. The bead-cell complexes were collected and washed in media. The cells were then separated from the magnetic beads according to the protocol of Rasmussen et al.²⁸ Analysis by flow cytometry (Epics, Coulter, Hialeah, Fla) revealed that our population of B cells was comprised of >97% CD20-positive B cells with approximately 2% T cells and <1% monocytes as determined by staining with CD2 and CD14 (Becton-Dickinson).²⁹ The monocyte depletion of this population was also confirmed by peroxidase staining.

Human studies issues

Informed consent for HIV-1-negative donor blood was obtained and donor selection was made according to the Guidelines of the Gulf Coast Regional Blood Bank, Houston, Tex. Blood from HIV-1-positive individuals was obtained in a manner approved by the Institutional Review board at Baylor College of Medicine.

Culture conditions

B cells were cultured for an initial incubation of 48 hours in the presence of a combination of CD40 mAb (10 ng/mL) and IL-4 (50 U/mL) in RPMI 1640 medium supplemented with penicillin, streptomycin, glutamine, and heat-inactivated FCS (GIBCO, Grand Island, NY) at 37°C in a humidified atmosphere of 5% carbon dioxide. These conditions are acknowledged to be an equivalent of T-cell activation of B cells. The cells were then washed in RPMI 1640 medium, resuspended in fresh medium, and aliquoted.

Measurement of B-lymphocyte proliferation

B cells (1×10^5) were cultured in 96-well plates with 200 μ L of RPMI 1640 medium supplemented with FCS in the presence or absence of various stimulators for 2 days at 37°C in 5% carbon dioxide. The cells were pulsed with 1 μ Ci per well of tritiated thymidine (New England Nuclear, Wilmington, Del) during the last 18 hours of culture and were harvested. Radioactivity was determined on a Packard beta counter.

Measurement of Ig synthesis

B cells (5×10^5) were cultured in RPMI 1640 medium with FCS with the designated test agents or controls for 7 days at 37°C in 5% carbon dioxide. The supernatants were harvested and frozen at -20°C until assayed. Supernatant Ig levels were measured by ELISA.⁹

cAMP determination

Cells were diluted to a final concentration of 4×10^6 cells per milliliter in RPMI 1640 medium plus FCS. The cell cultures were preincubated with 1 mmol/L IBMX, a phosphodiesterase inhibitor (prevents hydrolysis of cAMP), after which time the designated test agents were added. The reaction was terminated by centrifugation, and cAMP was extracted from the cells by the addition of 95% acidic ethanol before homogenization. After washing, the supernatants were evaporated and the cAMP content was determined by use of a competitive protein binding assay (Diagnostic Products, Los Angeles, Calif).³⁰

Flow cytometric analysis

Phenotypic analysis was performed on cells incubated with phycoerythrin (PE)-conjugated CD79b FITC-conjugated CD20 mAb or CD20-PE (Becton-Dickinson) or gp120-FITC (Intracel). The cells were washed and fixed in 1% formaldehyde. Surface staining was analyzed with an EPICS cytometer (Coulter). Nonviable cells were excluded from analysis on the basis of their forward-sideward scatter signal. With the use of appropriate isotype-matched control mAbs (IgG₁ and IgG_{2a}), the percentage positive cells were calculated.

Statistical analysis of the data

All statistical comparisons were made with the Student *t* distribution for unpaired data. Variation among similar experiments was assessed by ANOVA testing.

RESULTS

Augmentation of TNF- α -induced proliferation by gp120 in human B cells

TNF- α alone stimulated DNA synthesis in CD40 mAb plus IL-4-activated HIV-1-seronegative normal human peripheral blood B lymphocytes in a dose-dependent manner with a plateau of a 75% increase at 100 ng/mL ($P < .01$) (Fig 1). As indicated, gp120 (100 ng/mL) alone also caused significant stimulation of the B-cell proliferative response ($P < .01$) (Fig 1). gp120 exerted its effects on these B-cell functions in a dose-dependent manner (data not shown). The addition of gp120 to TNF- α -induced B cells augmented the stimulatory proliferative response in a dose-responsive manner. There was a peak enhanced proliferative response of 65% and 62% at TNF- α concentrations of 10 and 50 ng/mL over that of gp120 levels, respectively ($P < .01$) (Fig 1). Proliferative studies on HIV-1-seropositive human B cells demonstrated results similar to those seen with HIV-1-seronegative human B cells ($P < .01$) (Table I).

Potentiation of TNF- α -stimulated B-cell Ig synthesis by gp120

TNF- α alone stimulated IgM secretion in a dose-dependent fashion with a 3-fold increase observed at 50 ng/mL ($P < .001$) (Fig 2, A). TNF- α alone also stimulat-

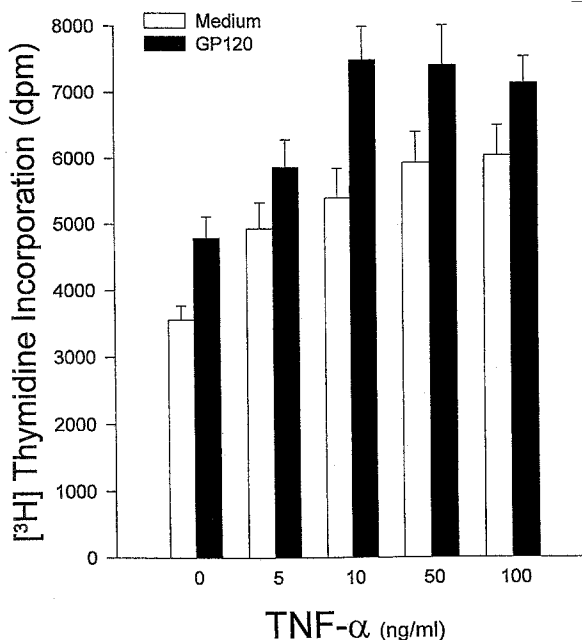


FIG 1. TNF- α -induced proliferative response and enhanced lymphoproliferation by gp120. As noted in Methods before subsequent treatments indicated in legends to Figs 1 to 5, freshly isolated HIV-1-seronegative B cells were activated with CD40 mAb plus IL-4. These activated cells were treated for 1 hour with gp120 (100 ng/mL) before addition of TNF- α (5-100 ng/mL). Activated B cells cultured in medium or gp120 alone are represented at 0 point. Tritiated thymidine incorporation was measured on day 2. Data represent mean $\pm$ SE of at least 4 experiments in quadruplicate.

TABLE I. Effects of gp120 and TNF- α on HIV-seropositive human B cells*

B cells cultured in presence of	Proliferation (dpm)†	IgM secretion (ng/ml)†
Medium	1223 $\pm$ 82	50 $\pm$ 3.8
gp120	2882 $\pm$ 176‡	81 $\pm$ 4.2‡
TNF- α	2412 $\pm$ 182‡	70 $\pm$ 4.7‡
gp120 + TNF- α	2915 $\pm$ 220‡	131 $\pm$ 10.8‡

*B cells were stimulated with CD40 mAb (10 ng/mL) plus IL-4 (50 U/mL), followed by gp120 (100 ng/mL) and TNF- α (50 ng/mL).

†The data are representative of the mean $\pm$ SE of 2 experiments in duplicate.

‡ $P < .01$, over medium.

ed IgG secretion over a concentration-dependent range ($P < .01$) (Fig 2, B). gp120 (100 ng/mL) alone stimulated both IgM and IgG production as well ($P < .01$) (Fig 2). The addition of gp120 to the TNF- α -induced B-cell culture system resulted in the augmentation of IgM production, 91% over that of gp120-enhanced IgM and a 5-fold increase over baseline at TNF- α (10 and 50 ng/mL) ($P < .001$) (Fig 2, A). A similar trend was observed in IgG production but at a reduced level of response ($P < .01$) (Fig 2, B). IgM production in HIV-1-seropositive B cells was enhanced by gp120 alone and the combination of gp120 plus TNF- α (Table I).

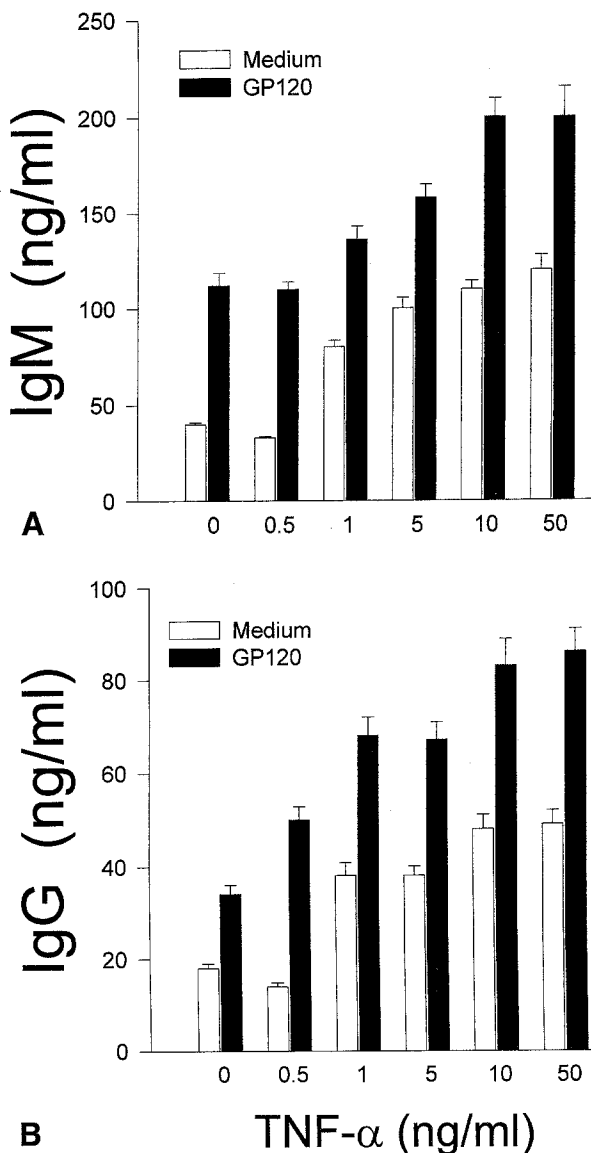


FIG 2. Dose-response curve of TNF- α on Ig production of activated HIV-1-seronegative human B lymphocytes with potentiation by gp120. For TNF- α dose-response, B cells were recultured with increasing doses of TNF- α alone (0.5-50 ng/mL). Activated B cells cultured in medium or gp120 (100 ng/mL) alone are represented at 0 point. Supernatants were harvested and quantitated by ELISA on (A) day 7 for IgM and (B) day 10 for IgG production. Data represent mean $\pm$ SE of 4 experiments in triplicate.

Specificity of gp120-induced B-cell responses

The specificity of these gp120-induced B-cell responses was established in several different ways. First, to determine whether the gp120-induced responses were direct effects of the HIV-1 envelope glycoprotein, a gp120-specific mAb (100 ng/mL) was added to the B-cell culture system 2 hours before the addition of gp120 (100 ng/mL) (Table IIA). The stimulatory effect of gp120 was abrogated in the presence of the anti-gp120 mAb in both gp120-induced B-cell proliferation and IgM production, indicative of the specificity of gp120. Second, to

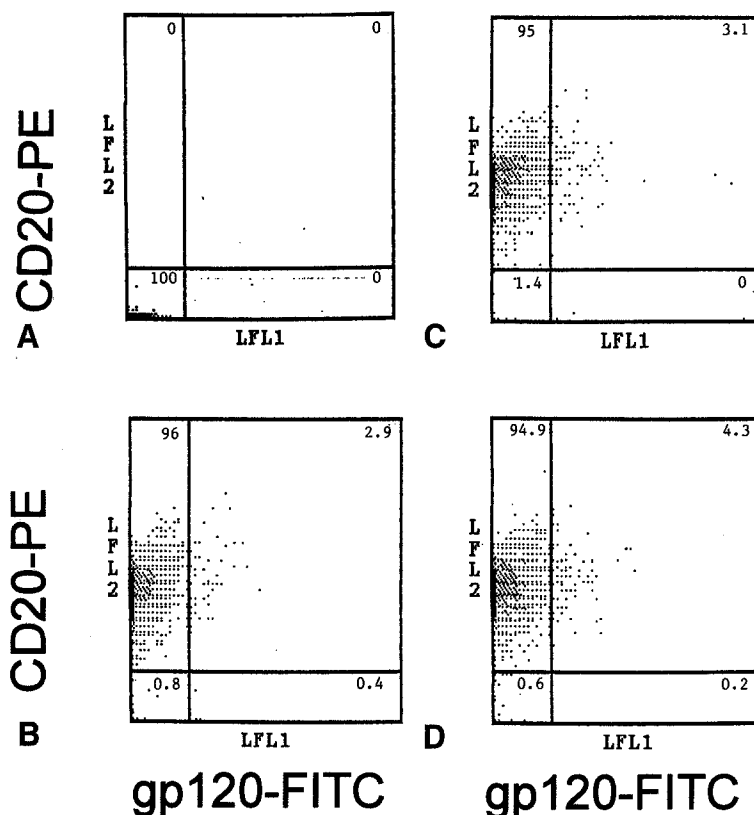


FIG 3. Binding of gp120 by activated HIV-1-seronegative human B lymphocytes. These cells were cultured in the presence of (B) medium, (C) *S aureus* Cowan I protein plus IL-2, or (D) CD40 and IL-4 for 48 hours at 37°C. Cells were double stained with CD20-PE and gp120-FITC. Two-color immunostaining profiles of lymphocytes were gated by forward and 90-degree light scatters. Background levels are shown in A. For each quadrant the percentage of total cells is indicated. The upper right quadrant contains B cells bound by gp120. The results are a representation of 3 independent experiments.

substantiate that a CD4-receptor mechanism was not involved in the modulation of these gp120-induced B-cell functions, an anti-CD4 mAb (3 ng/mL) was added to the culture system. The addition of anti-CD4 mAb exerted no effect on either gp120-induced B-cell proliferation or IgM production (Table IIA). Third, substitution of gp120 with a B-cell superantigen such as SPA did not augment TNF- α -induced proliferation (Table IIB).

Finally, gp120-binding properties were assessed by flow cytometric analysis (Fig 3). B cells that were unstimulated (Fig 3, B), *S aureus* Cowan I protein (0.01%) plus IL-2 (50 U/mL) activated (Fig 3, C), or CD40 mAb (10 ng/mL) plus IL-4 (50 U/mL) activated (Fig 3, D) were incubated with gp120 from HIV-1_{IIIB}, double-stained with antisera to HIV-1_{IIIB} gp120, and CD20 mAb analyzed by flow cytometry. Background levels are shown in Fig 3, A. Under these conditions 2.9% to 4.3% of the B-cell population (CD20+) bound gp120 (Fig 3, B, C, and D). The net result of these findings demonstrates the degree of specific binding of gp120 to human B cells.

Modulation of TNF- α -induced B-cell cAMP generation by gp120

CD40 mAb plus IL-4-activated HIV-1-seronegative human B cells were cultured with increasing concentrations of TNF- α (5-100 ng/mL) for 2 hours at 37°C. gp120 (100 ng/mL) alone stimulated cAMP generation in this B-cell culture system ($P < .01$) (Fig 4). The addition of gp120 with TNF- α for a 2-hour incubation at 37°C resulted in the enhancement of the TNF- α -stimulated B-cell cAMP generation in a dose-dependent manner ($P < .01$) (Fig 4). All cell cultures were treated with the phosphodiesterase inhibitor IBMX (1 mmol/L), suggesting that the gp120 effect was cAMP dependent. IBMX was required to obtain measurable baseline values (data not shown).

Influence of gp120 on BCR cell surface molecules on human B lymphocytes

Flow cytometric analysis of cells in the presence of gp120 (100 ng/mL) for 24 hours (Fig 5, B) demonstrated a down-regulation of dual-positive CD79b/CD20 cells by

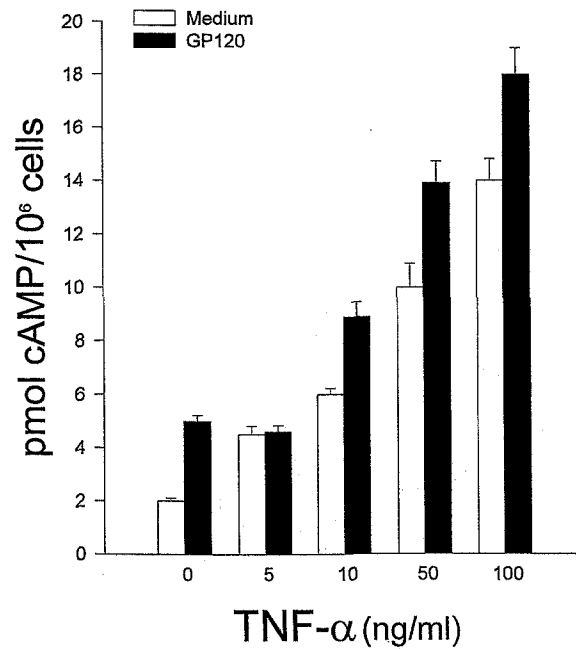


FIG 4. Modulation of TNF- α -induced B-cell cAMP generation by gp120. Activated HIV-1-seronegative human B cells were cultured for 2 hours at 37°C with medium alone, various concentrations of TNF- α (5-100 ng/mL), gp120 (100 ng/mL), or TNF- α plus gp120. The values are represented as means $\pm$ SE of 3 independent experiments performed in triplicate.

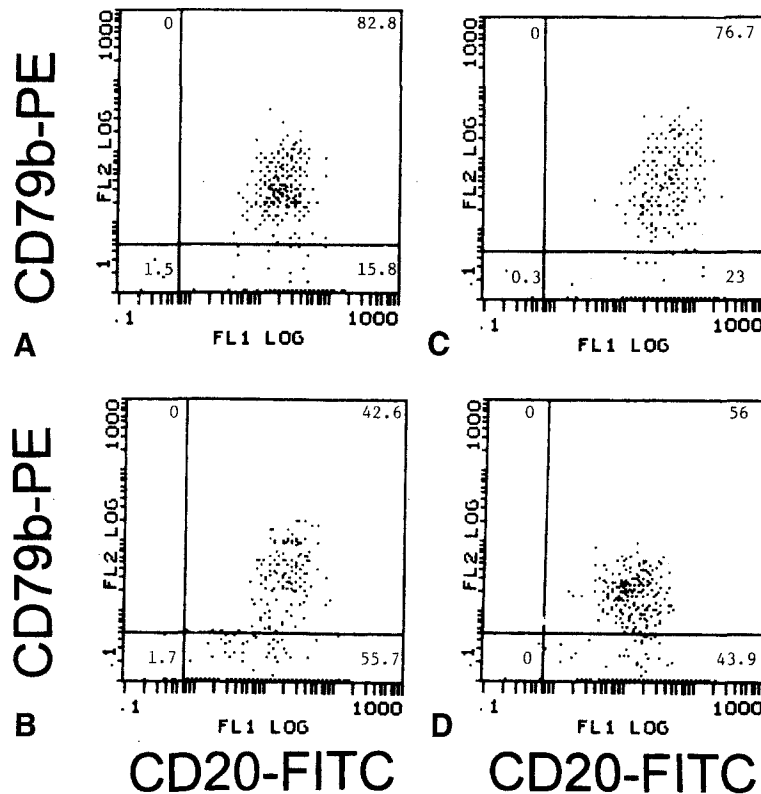


FIG 5. Two-color immunofluorescence flow cytometric analysis of CD79b expression in B cells. Activated HIV-1-seronegative human B cells were treated for 24 hours with (A) medium, (B) gp120 (100 ng/mL), (C) TNF- α (100 ng/mL), or (D) TNF- α and gp120. Cells were double stained with FITC-labeled CD20 mAb and PE-labeled CD79b mAb. Two-color immunostaining profiles of lymphocytes were gated by forward-side scatter. For each quadrant the percentage of total cells is indicated. The results shown are a representation of 3 independent experiments.

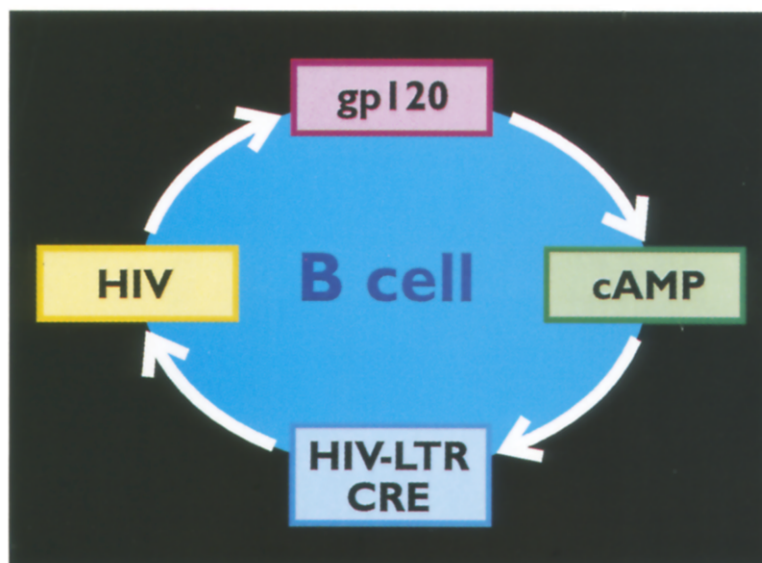


FIG 6. Hypothesis of gp120 modulation of B-cell signal transduction. The cAMP pathway activated by gp120 with augmentation by TNF- α impinges on the HIV-1 LTR CRE, resulting in a reservoir system for HIV-1 virus. Individually, cAMP and TNF- α induce NF- κ B binding to HIV-1 LTR, resulting in HIV-1 viral expression with an enhanced response resulting on combination of both.

TABLE IIA. Specificity of gp120 on HIV-seronegative B-cell proliferation and IgM production on human B cells*

B cells cultured in the presence of		Proliferation (dpm) [†]		IgM production (ng/mL) [‡]	
gp120 mAb	CD4 mAb	Control	gp120	Control	gp120
–	–	1085 $\pm$ 89	1750 $\pm$ 135§	29 $\pm$ 1.1	69 $\pm$ 2.8§
+	–	960 $\pm$ 74	1155 $\pm$ 98	24 $\pm$ 0.8	32 $\pm$ 1.2
–	+	899 $\pm$ 75	1692 $\pm$ 149§	30 $\pm$ 0.6	59 $\pm$ 1.8§

*B cells were stimulated with CD40 mAb (10 ng/mL) plus IL-4 (50 U/mL); gp120 (100 ng/mL) was added after a 2-hour incubation with gp120 mAb (100 ng/mL) and/or CD4 mAb (3 mg/mL).

[†]The data represent the mean $\pm$ SE of 3 experiments in quadruplicate.

[‡]The data represent the mean $\pm$ SE of 3 experiments in triplicate.

§ $P < .01$, gp120-induced responses over control.

TABLE IIB. Specificity of gp120 augmentation on TNF- α -stimulated HIV-seronegative B-cell proliferation and production of IgM

B cells cultured in the presence of		Proliferation (dpm)*		IgM production (ng/mL) [†]	
gp120 (100 ng/mL)	SPA (10 ng/mL)	Control	TNF- α (50 ng/mL)	Control	TNF- α (50 ng/mL)
–	–	3504 $\pm$ 198	5875 $\pm$ 329‡	42 $\pm$ 2.1	112 $\pm$ 7.9‡
+	–	4772 $\pm$ 291‡	6921 $\pm$ 370‡,§	111 $\pm$ 7.2‡	189 $\pm$ 14.6‡,§
–	+	4602 $\pm$ 401‡	5725 $\pm$ 301‡	79 $\pm$ 5.8‡	85 $\pm$ 5.1‡

*The data represent the mean $\pm$ SE of 3 experiments in quadruplicate.

[†]The data represent the mean $\pm$ SE of 3 experiments in triplicate.

‡ $P < .01$, over control.

§ $P < .01$, over TNF- α .

52% ($P < .01$) from that of baseline CD79b⁺/CD20⁺ cells (Fig 5, A). Treatment of these B cells with TNF- α (50 ng/mL) (Fig 5, C) exerted no change in baseline of CD79b surface expression. Addition of TNF- α to gp120 did not enhance the down-modulation of CD79b antigen expression over that caused by gp120 alone (Fig 5, D). Cytometric analysis of other B-cell surface markers, in particular CD22, CD23, and CD71, demonstrated no significant modulation by either TNF- α or gp120 or their combination (data not shown).

DISCUSSION

The current study supports the premise that the HIV-1 envelope protein gp120 exerts an effect on human B cells that is further modulated by TNF- α . gp120 stimulated B-cell proliferation, Ig secretion, and cAMP generation in HIV-1-seronegative, CD40 mAb, and IL-4-activated human peripheral blood B lymphocytes. This enhanced activation and differentiation was shown to be gp120 specific because anti-HIV-1-gp120 antibodies blocked these

B-cell responses. Flow cytometric analysis established binding of gp120 to a small proportion of the CD20⁺ B lymphocyte population. This study demonstrated that TNF- α alone induced B-cell proliferation, Ig synthesis, and cAMP generation paralleling that observed with gp120. The addition of gp120 to TNF- α -induced B-cell cultures augmented B-cell proliferation and Ig production. Modulation of signal transduction was observed with resultant gp120-induced down-regulation of the BCR with no abrogation of this inhibitory effect by TNF- α . TNF- α -induced cAMP generation was enhanced by gp120.

With use of B cells from HIV-1-infected individuals, Rieckmann et al¹⁷ presented evidence to support the stimulatory effect of gp120 on IgG and IgM synthesis. This correlates with our HIV-1 seropositive data. Yet in their study the addition of gp120 to unstimulated seronegative donor B cells produced no effect on either IgM or IgG production. Their results may be due to the resting state of the seronegative B cells in their study. Incubation of gp120 with resting B cells in our system likewise exerted no response (data not shown). Activation appears to be required for a gp120 response.

Evidence has shown that a direct effect of HIV-1 on B cells is mediated through gp120, a member of Ig superantigens.^{31,32} CD40 mAb and IL-4, which mimic T-cell help, allow gp120-stimulated B cells from HIV-1-negative donors to secrete V_H3 Ig.⁴ The activation signal delivered by gp120 is not strictly linked to infection, as evidenced by these studies and others. The level of gp120 binding in our B-cell population (3%-4%) is consistent with those reported by other investigators.^{9,14,33}

The response of B cells to pathogens is mediated, in part, by the cell surface B-cell receptor for antigen. Conge et al²⁰ have shown that HIV-1-seropositive B cells have a defect in early signal transduction by BCR/CD40 pathways. We show a down-regulation of the BCR by gp120, which may be an explanation of the defect in the early stages of HIV-1 B-cell activation. Although this early defect in HIV-1 B-cell signaling may be responsible for rendering them sensitive to activation-induced cellular death, this may initially result in the gradual loss of B-cell function during the progression of HIV-1 infection. The longitudinal study of 805 children born to HIV-1-infected women, for example, demonstrated by univariate analysis that lower B-cell numbers ($P = .004$) and lower IgG serum levels ($P = .003$) were associated with early death (Shearer et al, in preparation).

cAMP modulates cell activation, which is required for HIV-1 gene expression. Here we show that cAMP generation in B lymphocytes is induced by gp120 and augmented by TNF- α . Aandahl et al²⁴ observed an increase in cAMP levels in T cells from HIV-1-infected patients above that seen in HIV-1-seronegative individuals, demonstrating the importance of cAMP signaling in HIV-1 infection.

As a regulator of HIV-1 replication and transcription, cAMP transduces a signal that results in the alteration of cellular and viral gene expression. Polyclonal B-cell activation seen in HIV-1 infection is mediated by a non-antigen-specific MHC-unrestricted membrane TNF- α recep-

tor-mediated pathway.^{6,13,34} Anti-TNF- α drugs (vesnarionone), already in clinical trials, have shown inhibition of HIV-1 replication and apoptosis in HIV-1-infected individuals.^{35,36} We have presented data to show that TNF- α stimulates B-cell cAMP generation and that the TNF- α -induced cAMP generation is further augmented by modulation of gp120.

Lapointe et al²² presented evidence in B cells of cAMP regulation of HIV-1 LTR and NF- κ B activity. cAMP has been shown to enhance NF- κ B binding to DNA and the induction of the TNF- α -receptor with subsequent induction of viral expression in HIV-1-positive cells. This resulted in enhanced binding of NF- κ B to the HIV-1 LTR.²⁵ In monocytes enhanced HIV-1 expression was induced in the presence of TNF- α , cAMP activator, or both.^{21,25} It appears that the cAMP pathway is involved in signal transduction of HIV-1 LTR activity.

Hypothesis

Modulation of signal transduction pathways by gp120 in B cells may indicate a role of cAMP as a trigger of latent HIV-1 infection because B cells may well be a reservoir of HIV-1 virus (Fig 6).³⁷ We propose that, because TNF- α (by NF- κ B binding) and cAMP (by cAMP responsive element [CRE]) regulate HIV-1 expression through the HIV-1 LTR, the sustained cAMP levels may interfere with the cell's ability to respond to external stimuli. For example, the gp120 modulated down-regulation of the BCR may interrupt downstream signal transduction. If validated, this model may be important in the design of a B-cell vaccine and immunomodulatory therapy of HIV-1 infection.

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REFERENCES

1. Yarchoan R, Redfield R, Broder S. Mechanisms of B cell activation in patients with acquired immunodeficiency syndrome and related disorders. *J Clin Invest* 1986;78:439-47.
2. Lane HC, Fauci AS. Immunologic abnormalities in the acquired immunodeficiency syndrome. *Annu Rev Immunol* 1985;3:477-500.
3. Shirai A, Cosentino M, Leitman-Klinman SF, Klinman DM. Human immunodeficiency virus infection induces both polyclonal and virus-specific B cell activation. *J Clin Invest* 1992;89:561-6.
4. Berberian L, Goodlick L, Kipps TJ, Braun J. Immunoglobulin VH3 gene products: natural ligands for HIV gp120. *Science* 1993;261:1588-91.
5. Goodlick L, Zevit N, Neshat MS, Braun J. Mapping the Ig superantigen-binding site of HIV-1 gp120. *J Immunol* 1995;155:5151-9.
6. Gras G, Legendre C, Krzysiek R, Mormont D, Galanaud P, Richard Y. CD40/CD40L interaction and cytokines regulate HIV replication in B cells in vitro. *Virology* 1996;220:309-19.
7. Dalglish A. The immune responses to HIV: potential for immunotherapy. *Immunol Today* 1995;16:356-8.
8. Kehrl JH, Miller A, Fauci AS. Effect of tumor necrosis factor on mitogen-activated human B cells. *J Exp Med* 1987;166:786-91.
9. Rodriguez C, Roldan E, Navas G, Brieve JA. Essential role of tumor necrosis factor- α in the differentiation of human tonsil in vivo induced B cells capable of spontaneous and high-rate immunoglobulin secretion. *Eur J Immunol* 1993;23:1160-4.
10. Jelinek DF, Lipski PE. Enhancement of human B cell proliferation and differentiation by tumor necrosis factor- α and interleukin 1. *J Immunol* 1987;139:2970-6.
11. Boussiotis VA, Nadler LM, Strominger JL, Goldfeld AE. Tumor necrosis factor- α is an autocrine growth factor for normal human B cells. *Proc Natl Acad Sci U S A* 1994;91:7007-11.

12. Macchia D, Almerigogna F, Parronchi P, Ravina A, Maggi E, Romagnani S. Membrane tumour necrosis factor- α is involved in the polyclonal B-cell activation induced by HIV-infected human T cells. *Nature* 1993;363:464-6.
13. Rosenberg Z, Fauci AS. Immunopathogenic mechanisms of HIV infection: cytokine induction of HIV expression. *Immunol Today* 1990;11:176-80.
14. Poli G, Kinter A, Justement J, Kehrl J, Bressler P, Stanley S, et al. Tumor necrosis factor- α functions in an autocrine manner in the induction of human immunodeficiency virus expression. *Proc Natl Acad Sci U S A* 1990;87:782-5.
15. Osborn L, Kunkel S, Nabel G. Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. *Proc Natl Acad Sci U S A* 1989;86:2336-40.
16. Karsten V, Gordon S, Kim A, Herbein G. HIV-1 envelope glycoprotein gp120 down-regulates CD4 expression in primary human macrophages through induction of endogenous tumour necrosis factor- α . *Immunology* 1996;88:55-60.
17. Rieckmann P, Poli G, Fox CH, Kehrl JH, Fauci AS. Recombinant gp120 specifically enhances tumor necrosis factor- α production and Ig secretion in B lymphocytes from HIV-infected individuals but not from seronegative donors. *J Immunol* 1991;147:2922-7.
18. Nakamura T, Koyama M, Koike Y, Miyazaki K, Yoneyama A, Higashihara M, et al. Suppression of humoral immunity by monoclonal antibody to CD79b, an invariant component of antigen receptors on B lymphocytes. *Int J Hematol* 1996;64:39-46.
19. Van Kooten C, Galibert L, Seon B, Garrone P, Liu Y, Banchereau J. Cross-linking of antigen receptor via Ig- β (B29, CD79b) can induce both positive and negative signals in CD40-activated human B cells. *Clin Exp Immunol* 1997;110:509-15.
20. Conge A, Tarte K, Jacques R, Segondy M, Gerfaux J, Zembala M, et al. Impairment of B-lymphocyte differentiation induced by dual triggering of the B-cell antigen receptor and CD40 in advance HIV-1 disease. *AIDS* 1998;12:1437-49.
21. Rabbi M, Al-Harhi L, Roebuck K. TNF α cooperates with the protein kinase A pathway to synergistically increase HIV-1 LTR transcription via downstream TRE-like cAMP response element. *Virology* 1997;237:422-9.
22. Lapointe R, Lemieux R, Olivier M, Darveau A. Tyrosine kinase and cAMP-dependent protein kinase activities in CD40-activated human B lymphocytes. *Eur J Immunol* 1996;26:2376-82.
23. Hofmann B, Nishanian P, Nguyen P, Insizengmay T, Fahey J. Human immunodeficiency virus proteins induce the inhibitory cAMP/protein kinase A pathway in normal lymphocytes. *Proc Natl Acad Sci U S A* 1993;90:6676-80.
24. Aandahl E, Aukrust P, Skalleberg B, Muller F, Froland S, Hansson V, et al. Protein kinase A type I antagonist restores immune responses of T cells from HIV-infected patients. *FASEB J* 1998;12:855-62.
25. Chowdhury IH, Koyanagi Y, Horiuchi S, Hazeki O, Ui M, Kitano K, et al. cAMP stimulates human immunodeficiency virus (HIV-1) from latently infected cells of monocyte-macrophage lineage: synergism with TNF. *Virology* 1993;194:345-9.
26. Scheurich P, Kobrich G, Pfizenmaier K. Antagonistic control of tumor necrosis factor receptors by protein kinases A and C: enhancement of TNF receptor synthesis by protein kinase A and transmodulation of receptors by protein kinase C. *J Exp Med* 1989;170:947-58.
27. Funderud S, Erikstein B, Asheim H, Nustad K, Stokke T, Blomhoff H, et al. Functional properties of CD19+ B lymphocytes positively selected from buffy coats by immunomagnetic separation. *Eur J Immunol* 1990;20:201-6.
28. Rasmussen AM, Smeland EB, Erikstein BK, Caignault L, Funderud S. A new method for detachment of dynabeads from positively selected B lymphocytes. *J Immunol Meth* 1992;146:195-202.
29. Patke CL, Green CG, Shearer WT. Differential effects of interleukin-2 and interleukin-4 on immunomodulatory role of platelet-activating factor in human B cells. *Clin Diagn Lab Immunol* 1994;4:424-32.
30. Yokota S, Geppert T, Lipsky P. Enhancement of antigen on mitogen-induced human T lymphocyte proliferation by tumor necrosis factor- α . *J Immunol* 1988;140:531-6.
31. Berberian L, Shukla J, Jefferis R, Braun J. Effects of HIV infection on V β 3 (D12 idiotope) B cells in vivo. *J Acquired Immune Defic Syndr* 1994;7:641-6.
32. Patella V, Florio G, Petraroli A, Marone G. HIV-1 glycoprotein 120 induces IL-4 and IL-13 release from human Fc ϵ RI+ cells through interaction with the V β 3 region of IgE. *J Immunology* 2000;164:589-95.
33. Juompan L, Lambin P, Moncef Z. Selective deficit in antibodies specific for the superantigen binding site of gp120 in HIV infection. *FASEB J* 1998;12:1473-80.
34. Macchia D, Almerigogna F, Parronchi P, Ravina A, Maggi E, Romagnani S. Membrane tumour necrosis factor- α is involved in the polyclonal B-cell activation induced by HIV-infected human T cells. *Nature* 1993;363:464-6.
35. Maruyama I, Maruyama Y, Nakajima T, Kitajima I, Osame M, Zhao J, et al. Vesnarinone inhibits production of HIV-1 in cultured cells. *Biochem Biophys Res Commun* 1993;195:1264-71.
36. Oyaizu N, McCloskey T, Than S, Pahwa S. Inhibition of CD4 cross-linking-induced lymphocytes apoptosis by vesnarinone a novel immunomodulating agent: vesnarinone inhibits Fas expression and apoptosis by blocking cytokine secretion. *Blood* 1996;87:2361-8.
37. Fritsch L, Marechal V, Schneider V, Barthet C, Rozenbaum W, Moisan-Coppey M, et al. Production of HIV-1 by human B cells infected in vitro: characterization of an EBV genome-negative B cell line chronically synthesizing a low level of HIV-1 after infection. *Virology* 1998;244:542-51.