Cutting Edge: HIV-1 Tat Protein Differentially Modulates the B Cell Response of Naive, Memory, and Germinal Center B Cells

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Cutting Edge: HIV-1 Tat Protein Differentially Modulates the B Cell Response of Naive, Memory, and Germinal Center B Cells

Eric A. Lefevre,* Roman Krzysiek,† Erwann P. Loret,‡ Pierre Galanaud,* and Yolande Richard²*

Critical steps of B cell differentiation occur within lymphoid organs that are also major sites of HIV-1 replication. Because Tat can be released by infected cells, we investigated whether extracellular HIV-1 Tat modulates cell proliferation of B cells at critical stages of their differentiation. Here we show that extracellular Tat inhibited the proliferation of B cell receptor-triggered naive and memory B cells by >80% but had no effect on their CD40 mAb and IL-4-mediated proliferation. In striking contrast, Tat doubled the germinal center B cell proliferation induced by CD40 mAb and IL-4. These effects were dose dependent and required the addition of Tat at the initiation of the culture, suggesting that Tat acts on early stages of cell cycle progression. By its effects on B cell subsets, Tat might directly affect the normal B cell differentiation process in HIV-positive patients and favor the occurrence of AIDS-associated B cell lymphomas. The Journal of Immunology, 1999, 163: 1119–1122.

Human immunodeficiency virus-1 infection is associated with a strong polyclonal B cell activation, leading to an increased percentage of B cells expressing an activated and/or immature phenotype (1–4). Although spontaneously secreting Ig (1), B cells from HIV-positive patients are nevertheless unable to mount a T cell-dependent B cell response (4, 5). In secondary lymphoid organs of HIV-positive patients, B cell activation leads to a strong and sustained follicular hyperplasia during the asymptomatic phase of the disease (6, 7). This hyperplasia is associated with the loss of germinal center (GC) polarization, leading to a random distribution of centroblasts, centrocytes, and T cells, whereas the mantle zone, essentially composed of naive B cells, is thinner than in noninfected subjects and has numerous disruptions (8). During the symptomatic phase, the HIV-1-specific B cell response decreases in the periphery, and a progressive involution of GC occurs in lymphoid organs (7). Antiretroviral therapies decrease HIV-1-driven B cell hyperactivity and polyclonal B cell activation in patients (9), strongly suggesting that HIV-1, by its sustained replication, alters the B cell differentiation process within lymphoid organs.

Soluble Tat, present as a biologically active extracellular protein released by infected cells in HIV-positive patients, is readily taken up by uninfected cells and targeted to the nucleus (10, 11). Extracellular Tat stimulates the growth of Kaposi’s sarcoma cells, potentiates anergy and apoptosis of uninfected T cells, and promotes chemotaxis and invasive behavior by monocytes (12). In B cell lines, Tat also modulates the production of cytokines and the expression of their receptors (13, 14). It also increases CD95 expression on peripheral B cells during short term cocultures with T cells and monocytes (15).

It is thus possible that Tat locally produced in lymphoid organs in HIV-positive patients might act directly on primary B cells and participate in the B cell abnormalities observed in vivo. Here, we demonstrate that Tat exerts a direct effect on all primary B cell subsets but differentially modulates the anti-IgM Ab- and CD40 mAb-induced proliferation of naive, memory, and GC B cell subsets isolated from lymphoid organs of HIV-negative donors.

Materials and Methods

Reagents

Recombinant Tat HIV-1IIIB (aa 1–86) from Dr. J. Raina was obtained from the National Institute of Biological Standards and Control Centralised Facility for AIDS Reagents (Potters Bar, U.K.). The stock solution was diluted in saline-citrate buffer as recommended, and aliquots were stored at −80°C until use. The concentration of endotoxin was below 0.01 endotoxin unit (EU)/mg of protein.

B cell preparation and stimulation

Human mononuclear cells were obtained from palatine tonsils removed from children with chronic tonsillitis. Total B cells, obtained by one cycle of rosette formation and depletion of residual T cells with CD2 magnetic beads (Dynabeads M-450, Dynal, Oslo, Norway), were 93 ± 4% CD19⁺.

Abbreviations used in this paper: GC, germinal center; MIP, macrophage inflammatory protein.
RESULTS AND DISCUSSION

The addition of extracellular Tat decreased the proliferation of tonsillar B cells stimulated with anti-IgM Ab and IL-4 or anti-IgM and CD40 Ab, in a dose-dependent manner (Fig. 1). At 0.5 μg/ml Tat, B cell proliferation induced by anti-IgM Ab and IL-4 was inhibited by 83 ± 4% (n = 6, p < 0.05) whereas that induced by anti-IgM and CD40 Ab was decreased by only 68 ± 15% (n = 10, p = 0.005). At 1 μg/ml Tat, the former inhibition was 94 ± 1%, whereas the latter was 86 ± 3%. This inhibition was not due to B cell apoptosis, because Tat increased the percentage of apoptotic cells by only 10 to 20% as assessed by staining with FITC-conjugated annexin V and propidium iodide (data not shown). However, the addition of Tat to the anti-IgM Ab- and IL-4-treated culture led to a 2-fold increase in the percentage of CD95+ B cells.

59 ± 6% IgD+, 81 ± 16% CD44+, 21 ± 6% CD38high and <1% CD14+, CD3+, and DR/C1+ (n = 10). Total B cells were separated into IgD- (naive B cells) and IgD+ populations using anti-IgD mAb (TA4-1) and goat anti-mouse IgG magnetic beads (Dynalt) as previously described (16). IgD- B cells were further separated into CD44+ (memory) and CD44- (GC) B cells by a similar protocol with CD44 mAb (BF24, Diaclone, Besançon, France). All purification procedures were conducted at 4°C to prevent spontaneous apoptosis. As assessed by flow cytometry, naive B cells were 96 ± 4% CD19+, 83 ± 4% IgD+, 97 ± 2% CD44+, and 7 ± 3% CD38high; memory B cells were 92 ± 6% CD19+, 20 ± 6% IgD+, 73 ± 17% CD44+, and 10 ± 10% CD38high; and GC B cells were 94 ± 3% CD19+, 11 ± 4% IgD+, 11 ± 10% CD44+, and 90 ± 2% CD38high (n = 5).

B cells were cultured in RPMI 1640 (Life Technologies, Paisley, Scotland) containing 10 mM HEPES, 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate, and 10% heat-inactivated FCS (complete medium). B cells (1 × 10^6 cells/well) were activated by incubation in complete medium for 2 days with one or more of the following: polyclonal anti-IgM Ab coupled to beads (Irvine Scientific, Santa Ana, CA, 5 μg/ml), CD40 mAb (G28.5, 1 μg/ml), IL-4 (Schering Plough, Kenilworth, NJ, 20 ng/ml), IL-2 (Chiron, Amsterdam, The Netherlands, 50 U/ml), and IL-10 (Schering Plough, 50 ng/ml). Tat was added at the initiation of the culture, unless otherwise indicated.

Proliferation assays

Proliferation was measured by supplying the cultures with a pulse of 0.5 μCi per well [methyl-3H]thymidine (Amersham, Les Ulis, France) for the last 12 h of the third day of incubation. Cells were collected by filtration through a glass fiber filter, and [3 H]thymidine incorporation was measured by a β-scintillation counter (Betaplate 1205, EGG Wallac, Turku, Finland). Results are expressed in cpm (mean of triplicates ± SD).

Ig quantification

Total or naive B cells (1 × 10^7/well) were stimulated by IL-2 (100 U/ml) and IL-10 (50 ng/ml) with or without CD40 mAb, in the presence or absence of Tat. IgM, IgG, and IgA concentrations in cell-free supernatants harvested on day 10 were determined by specific ELISA.

Statistical analysis

Statistical significance was determined using Wilcoxon’s nonparametric test. p values <0.05 were regarded as being significant.

RESULTS AND DISCUSSION

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FIGURE 1. Extracellular Tat modulates B cell proliferation. Total B cells (1 × 10^5/well) were stimulated by anti-IgM Ab and IL-4 (A, □), anti-IgM and CD40 Ab (B, □), or CD40 mAb and IL-4 (C, □) in the presence or absence of one of a series of concentrations of Tat. Results of [3 H]thymidine incorporation are expressed as mean cpm ± SD of triplicate determinations. Values are representative of 6 (A, C) or 10 (B) independent experiments.
It is therefore possible that B cells are more prone to CD95 ligand-mediated apoptosis after Tat exposure. In striking contrast to its effect on anti-IgM Ab-induced proliferation, Tat (0.5 μg/ml) increased the B cell proliferation induced by CD40 mAb and IL-4 (Fig. 1) by 2- to 6-fold (range, 1.3- to 2.8-fold, n = 6, p < 0.05). The effects of Tat on B cell proliferation were totally inhibited by its preincubation with heparin (5 μg/ml) (data not shown). The ID₅₀ (1 μg/ml heparin) was similar to that reported for blocking Tat-induced HIV-1 trans-activation (17).

The inhibitory effect of 0.5 μg/ml Tat on anti-IgM Ab- and IL-4-mediated cell proliferation, still maximal when Tat was added 8 h after the beginning of the culture, was much smaller if the addition was delayed by 24 h (19% vs 81% inhibition) (Fig. 2A). Similar results were obtained in anti-IgM and CD40 Ab-stimulated cultures (data not shown). When B cells were stimulated by CD40 mAb and IL-4, Tat induced a 2.8-fold increase of cell proliferation (85,501 ± 787 cpm with vs 30,625 ± 953 cpm without Tat) only if it was present at the beginning of the culture (Fig. 2B). These results suggest that Tat acts on early stages of B cell activation, probably before the G₁ to S phase transition. This view was supported by two additional observations: Tat modulated [³H]thymidine and [³H]uridine incorporation to the same extent; and Tat inhibited the anti-IgM Ab-induced proliferation without changing the percentage of CD69⁺ or CD71⁺ cells (data not shown). In agreement with these data, Kundu et al. (18) recently reported that Tat elongates the G₁ phase of the cell cycle in glial cell lines.

Naive and memory B cells proliferate strongly after B cell receptor or CD40 triggering in the presence of IL-4, whereas only CD40 mAb and IL-4 induce GC B cell proliferation. We thus compared the effect of Tat on the proliferation of B cell subsets. Tat inhibited the anti-IgM Ab-mediated proliferation (in the presence of IL-4 or CD40 mAb) of the naive and memory B cells by 88–93% (Fig. 3). Tat had no significant effect on the CD40 mAb and IL-4-mediated proliferation of naive (1.2- ± 0.5-fold increase, n = 5, p = 0.46) or memory B cells (1.1 ± 0.4, n = 5, p = 0.5), but specifically increased that of GC B cells by 2- ± 0.8-fold (n = 5, p < 0.05). Whereas CD40 mAb and IL-2 plus IL-10 induced a strong B cell proliferation and Ig production, only Ig production

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>HIV-1 Tat</th>
<th>Total B Cells</th>
<th>Naive B Cells</th>
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<tr>
<td></td>
<td></td>
<td>IgM</td>
<td>IgG</td>
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<tr>
<td>IL-2 + IL-10</td>
<td>−</td>
<td>235 ± 34</td>
<td>284 ± 8</td>
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<td></td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD40 mAb and IL-2 + IL-10</td>
<td>−</td>
<td>1326 ± 264</td>
<td>2115 ± 401</td>
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<tr>
<td></td>
<td>+</td>
<td>430 ± 16</td>
<td>996 ± 169</td>
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* Results are expressed as mean ng/ml ± SD of triplicate determinations. Values are representative of two independent experiments.

b Below the detection limit of the ELISA kit.
was inhibited by Tat. In total B cells, Tat decreased IgM, IgG, and IgA production by 67, 47, and 46%, respectively (Table I). IgG production by naive B cells is strictly dependent on CD40 ligation and was not impaired by Tat addition, whereas IgM production was decreased by 42%. This suggests that Tat acts on terminal B cell differentiation rather than on isotype switching. These results showed that the efficiency of Tat modulation of the B cell response depends not only on the differentiation state of the B cell target but also on the pathway of stimulation. In all experiments, the maximal effect of Tat was obtained at concentrations of 0.5 to 1 μg/ml (50 to 100 nM), values that are higher than the concentrations of circulating Tat reported in vivo (10 PM to 24 nM). However, it seems likely that the concentrations of Tat are locally higher in the lymphoid organs of HIV-positive patients, as suggested by Westendorp et al. (10).

Because Tat exerts some of its biological activity by interfering with cytokine production (12, 19), we tested its effect on the production of several B cell-derived cytokines. No significant change in IL-8, IL-10, or TGF production was observed after Tat addition in our experimental conditions. Addition of Tat weakly modified the production of macrophage inflammatory protein (MIP)-1α, MIP-1β, TNF-α, and IL-6 in CD40 mAb and IL-4-stimulated cultures and strongly decreased their production in anti-IgM Ab-stimulated cultures. It seems unlikely that Tat controls B cell proliferation via the modulation of cytokine production because: 1) Tat increased CD40 mAb- and IL-4-induced cell proliferation but not cytokine production; and 2) addition of recombinant TNF-α, MIP-1α, MIP-1β, or IL-6 did not reverse the Tat-induced inhibition of anti-IgM Ab-dependent B cell proliferation (data not shown). However, the decreased production of B cell-derived MIP-1β and MIP-1α may impair the recruitment of CD4+CD45RO+ helper T cells (16) and interfere with T/B interactions leading to abnormal T cell-dependent maturation of the B cell response.

The mechanisms by which Tat acts on B cell response are still unknown but do not involve the cysteine-rich region of Tat. Indeed, two synthetic Tat variants (Tat Oyi and cmC Tat Bru), bearing modified cysteines and devoid of the HIV-1 long terminal repeat-trans-activating activity (20), modulated the B cell proliferation to the same extent as did the wild-type synthetic Tat proteins, Tat Mal and Tat Eli (data not shown). In addition, Tat does not exert its inhibitory effect on the anti-IgM Ab-induced B cell response by blocking 1-type calcium channels as previously reported (21) because the addition of Bay K8644 did not prevent or reverse its effects in human B cells (data not shown). Based on previous data (18), Tat might exert its inhibitory effects on B cell proliferation by modulating cyclin-dependent kinase activity. Alternatively, Tat might favor the survival of GC B cells by enhancing the DNA-binding activity of NF-κB (22). Experiments are in progress to evaluate these hypotheses.

Here, we have shown that exogenous Tat acts directly on B cells and differentially modulates the B cell response of naive/memory and GC B cells. Its ability to enhance GC B cell proliferation might thus play an important role in promoting early HIV-associated centrofollicular hyperplasia and favor the occurrence of autoimmune disorders and B cell malignancies in lymphoid organs of HIV-positive patients.

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References