Mechanisms of opsonized HIV entry in normal B lymphocytes

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Abstract Using our in vitro model of normal B cell infection that functions with low doses of HIV but requires virus opsonization by seropositive patient serum, and complement, we analyzed what receptors allowed virus entry. Here, we show that HIV infection of B cells occurs through 2 major receptors: the CD4 antigen and the CR1/CR2 complex. These 2 pathways work independently since a complete inhibition of virus entry requires both CD4 and CD21/CD35 blockade on CD4^{dim} tonsillar B cells whereas only the latter is critical on CD4-negative B cells.

Key words: B lymphocyte; CD4; Complement receptor; CD21; CD35; HIV

1. Introduction

Although in vivo infection of B lymphocytes remains to establish, B lymphocyte phenotype and functions are strongly impaired in human immunodeficiency virus (HIV)-infected patients. This impairment, appearing early in the course of the disease, alters B cells from the periphery as well as those located in secondary lymphoid organs [1,2]. In particular, Casareale et al. (1984) obtained HIV- and EBV-coinfected B cell lines from peripheral blood lymphocyte cultures of seropositive patients, evidencing HIV infection of EBV-positive B cells and subsequent ex vivo immortalization [3]. In 1991, a HIV$^+$ B cell line was obtained after in vitro HIV infection of normal B lymphocytes from EBV-seropositive normal donors [4]. In addition, the expression of HIV provirus in lymphoma cells of seropositive patients [5,6] has been reported and in situ hybridization has allowed the detection, by Prevot et al., of HIV mRNA in polymorphic B cell proliferation of 2 seropositive patients and in numerous B cell lymphomas [7].

In 1993, we have shown, for the first time, that normal B lymphocytes are indeed susceptible to in vitro HIV infection and produce infectious virus in culture [8]. This productive infection requires opsonization of HIV, with antibodies from asymptomatic patient and human complement, and virus entry, thus, involves complement receptors (CRs) type 1 (CR1/CD35)

and 2 (CR2/CD21). CD35 binds 3 complement fragments (C3b, C4b and iC3b) and is able to cleave C3 into iC3b and C3dg, which are ligands for CD21, showing a functional link between these 2 CRs [9,10]. In contrast, Zhang and Henderson demonstrated that tonsil B cells do express CD4 mRNA and, although CD4 molecule remains undetectable by flow cytometry, it allowed infection by antibody-free virion. Indeed, the infection was strongly decreased by CD4-blocking, suggesting that CD4 is a critical mediator for unopsonized HIV-1 infection of surface CD4-negative B cells [11]. Recently, infection of CD40 plus IL4-activated normal B cells by high dose of HIV (multiplicity of infection = 1) have been demontrated in the absence of detectable CD4 expression [12].

HIV infection results in a strong activation, by HIV and HIV-circulating-immune-complexes [13,14], of both classical and alternative complement pathways [15,16]. In spite of this activation and of virus opsonization, with anti-HIV antibodies and complement or complement only, HIV seems to be protected from human complement lytic action [17,10]. Non-lysed opsonized HIV may, thus, bind to complement receptors and infect CRs-bearing cells, as described on T cell lines [18], monocytic cell lines [19–21], B cell lines [22–24], thymocytes [25], primary monocytes [21], peripheral and tonsil B lymphocytes [8].

The presence of anti-HIV antibodies and complement covering virions leads to an enhanced infection (C'-ADE infection) of targeted cells, characterized by a more rapid apparition of infection stigmas (giant cells, *syncytia*, cell death) [26,27], and is CRs-dependent and/or CD4-dependent, according to the cellular model. Indeed, OKB7 monoclonal antibody (anti-CD21) blocks C'-ADE both in CR2$^+$ CD4$^+$ MT2 cells [18] and in CR2$^+$ CD4$^-$ IC-1 cells [23]. Blocking of CR3 in CR3$^+$ CD4$^+$ U937 cell line also allows inhibiton of C'-ADE infection [19]. If these studies clearly showed that CRs are involved in enhanced cell infection, the role of CD4 remains unclear in this infection process. Some studies performed on different CD4$^+$ cells reported a clearcut inhibition of infection by CD4-blocking [18,23], thus suggesting that CD4 is also required for C'-ADE cell infection, whereas other studies, performed with low doses of complement opsonized virus, described an enhancement of infection of MT2 [28] and U937 cells [19,20], in a CD4-independent manner. Taken as a whole, these data suggest that the respective roles of CD4 and CRs in HIV infection are variable according to experimental model.

While normal B lymphocyte infection has been demonstrated in vitro, little is known about mechanisms of HIV entry into these cells. Here, we present data on the respective role of CRs type 1 and 2, and CD4 molecule, in opsonized HIV entry in normal B lymphocytes.

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2. Materials and methods

2.1. B cell source and isolation

B lymphocytes were isolated from tonsils of HIV-seronegative children with chronic tonsillitis. After tonsil dilaceration in isotonic NaCl solution, cell suspensions contain approximately 70% of T cells and 30% of B cells. B cells were depleted from T cells by rosetting with aminoethyl-isothiouronium bromehydrate-treated sheep erythrocytes as previously described in [8]. After the first cycle of rosetting, non-T cell fraction was extensively washed (1700 and 750 × g, 5 min, 4°C) and centrifuged on a 40% Percoll layer (Pharmacia Bioprocess, Sweden) to discard monocytes-macrophages and follicular dendritic cells (25 min at 1200 × g and 4°C). Pellets, containing B cells, were then submitted to a second cycle of rosetting and highly purified B cells were frozen and stored in liquid nitrogen until use.

2.2. Cultures

All cultures were performed in RPMI 1640 medium containing 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, 1 mM sodium pyruvate, 10 mM HEPES buffer and 10% heat-inactivated fetal calf serum (FCS) (complete medium; Gibco BRL). In some experiments, purified B lymphocytes were stimulated by phorbol 12-myristate 13-acetate (PMA) or by phorbol 12,13-dibutyrate (PDB) at a concentration of 5 ng/ml in complete medium for 2 days before HIV infection (see infection protocol). Cells were cultured in 96-well flat-bottomed plates (Costar, Cambridge, MA) at 10^6 cells/ml in complete medium supplemented with IL2 (50 U/ml) and IL4 (25 ng/ml). Unstimulated infected B cells were cultured in the presence of 1 ng/ml PMA in IL2-and IL4-supplemented medium.

2.3. Infection protocol

In all experiments, the source of virus was the Lai strain of HIV-1 (provided by Dr. F. Barré-Sinoussi, Paris, France) which always grew in primary lymphocyte cultures. Infections were performed with 50 tissue culture infective doses 50% ($TCID_{50}$)/million cells, as determined by a limiting dilution microtiter assay on MT2 cells [8]. Before cell infection, virus was opsonized for 40 min at 4°C with HIV-positive heat-inactivated serum (1/16) and normal non-heated serum as a source of complement (1/12), in a final volume of 100 μl. For infection, cells (5 × 10^6 cells/ml) were incubated with opsonized virus suspension at 37°C for 40 min and, after 3 washes, cells were cultured in triplicates as described above.

2.4. Reagents and blocking experiments

Four monoclonal antibodies (mAbs) to the CD4 molecule were used to block CD4-HIV-binding: OKT4a (IgG2a, Ortho Diagnostic System), Q4120 (IgG1, generously provided by Dr. Q. Sattentau through the MRC AIDS directed program), NuTh/i (IgG1, obtained through our participation to the 5th International Workshop on Human Leukocyte Differentiation Antigens, Boston 1993; 5th IWHLDA) and anti-leu3a (IgG1, Becton Dickinson). Three mAbs directed to CR1 (CD35), J3D3, J3B11 and J8B10 (all IgG1) were a kind gift of Dr. E. Fischer and Professor M.D. Kazatchkine (Paris, France) and were pooled to obtain a complete blockade of CR1 function. The OKB7 mAb (IgG1; Ortho Diagnostic System) was used to block CR2 (CD21) function. F97-6B3 mAb (kind gift of Dr. P. Poncelet, Marseille, France), another anti-CD21 mAb which is not directed to the C3dg-binding site, was also used. Two mAbs directed to CD5, which have no implication in opsonized HIV infection of B lymphocytes [8], were obtained through the 5th IWHLDA and used as controls: ICO-80 (IgG1) and A50 (IgG2a). Recombinant CD4 coupled to HSA (CD4-HSA) was provided by Rhône Poulenc Rorer.

For blocking experiments, 6.5 × 10^5 B lymphocytes were incubated in complete medium with saturating concentrations of specific mAbs for CD4, CD21, CD35 or CD5, alone or in combination, for 1 h at 4°C. Infection was then performed as described above. After 3 washes, cells were resuspended in culture medium and each treatment was performed in triplicates in 96-well flat-bottomed plates as indicated above (see section 2.2.). Recombinant CD4 coupled to HSA was also used to inhibit HIV entry in B lymphocytes. To block infection, CD4-HSA was mixed with virus during opsonization for 40 min at 4°C.

2.5. Detection of p24 by antigen capture assay

Major viral core protein p24 production was assessed in culture supernatants using an ELISA kit (DuPont de Nemours, Les Ulis, France) as specified by the manufacturer. In all experiments, infection with heat-inactivated opsonized virus was performed, as negative control (mock infection), to quantify residual antigen amounts. Each p24 quantitation then included the subtraction of mock-infected control p24 results (between 0–10 pg/ml according to experiments).

2.6. Cell Immunostaining and flow cytometry

Mabs used to stain B cells were directed to CD3 (anti-Leu4a, IgG1, Becton Dickinson), CD4 (anti-Leu3a, IgG1, Becton Dickinson), CD14 (RMO52, IgG1, Immunotech), CD19 (J4–119, IgG1, Immunotech), CD20 (BB6, IgM, Immunotech), CD21 (BL13, IgG1, Immunotech) and CD35 (J3D3, IgG1, Immunotech). We also used the 12B1 antibody which recognizes dendritic reticulum cells in the germinal center (GC) of lymph nodes, spleen and tonsils (IgG2a, Immunotech).

We used mouse IgG1, IgG2a or IgM monoclonal antibodies (ICN, Costa Mesa, CA) as controls.

To avoid non-specific binding, B cells (5 × 10^5/test) were incubated for 1 h at 4°C in PBS containing 1% bovine serum albumin (Fraction V, Sigma), 0.1% NaN_3 (incubation medium) and 10% normal human heat inactivated AB^+ serum. Cells were then washed and incubated for 30 min at 4°C in 50 μl incubation medium containing a saturating concentration of non-fluorescent mouse monoclonal antibody directed to a specific molecule. After 2 washes with incubation medium, staining of cells was revealed by a second incubation (30 min at 4°C) with FITC-conjugated goat anti-mouse Ig F(ab')₂ fragments (1/30) (Immunotech, Marseille, France). After washes, cells were fixed in PBS containing 1% paraformaldehyde. Immunostaining analysis was performed on gated viable B lymphocytes, using a FACScan flow cytometer (Becton Dickinson, San José, CA).

To determine the number of a specific molecule expressed on cell surface, 2 batches of beads were utilized: (1) low expressions were determined with a QIFI-Low kit (Biocytex, Marseille, France); and (2) high expressions with a QIFI-Kit (DAKO, France). QIFI-Low kit was especially designed by Biocytex for the quantitation of weakly expressed surface antigens, by using murine monoclonal antibodies, and consists in blank beads and beads coated with 180 to 23,000 antigenic sites. QIFI-Kit allows to quantitate surface antigens within a 5,500 to 640,000 sites/cell density range.

3. Results

3.1. Purity of B cell suspensions

Each B cell suspension was tested for the expression of CD19, CD3, CD4, 12B1 and CD14. In all cases, the percentage of $CD19^+$ B cells was higher than 97% and that of $CD3^+$ cells and $CD14^+$ cells were lower than 1 and 0.5%, respectively. As expected, no $12B1^+$ cells were recovered in our B cell suspensions (data not shown). When CD4 expression was assessed, less than 0.5% of total cells exhibited a high CD4 density, characteristic of $CD4^+$ T cells. In contrast, a weak CD4 expression ($CD4^{dim}$) was observed on a variable fraction of tonsillar B cells as shown in Fig. 1.

3.2. Quantitative CD4 and complement receptor expression in B cell suspensions

5 × 10^5 B cells were stained with antibodies to CD4, CD21 and CD35 to quantify their surface expression by flow cytometry. As shown in Table 1, tonsillar B cells expressed high number of CD21 and CD35 antigens. Nevertheless, a lower expression of these molecules was observed on 2-day PMA- or PDB-stimulated B lymphocytes (mean 7,000 and 27,000 sites/cell for CD21 and CD35, respectively) than on freshly thawed B cells molecules (mean 28,000 and 60,000 sites/cell for CD21 and CD35, respectively). As indicated before, a weak CD4 surface expression was detectable on both unstimulated and 2-day stimulated B lymphocytes isolated from 2 of 3 tonsils. On the two $CD4^+$ tonsillar suspensions after thawing, CD4 expres-
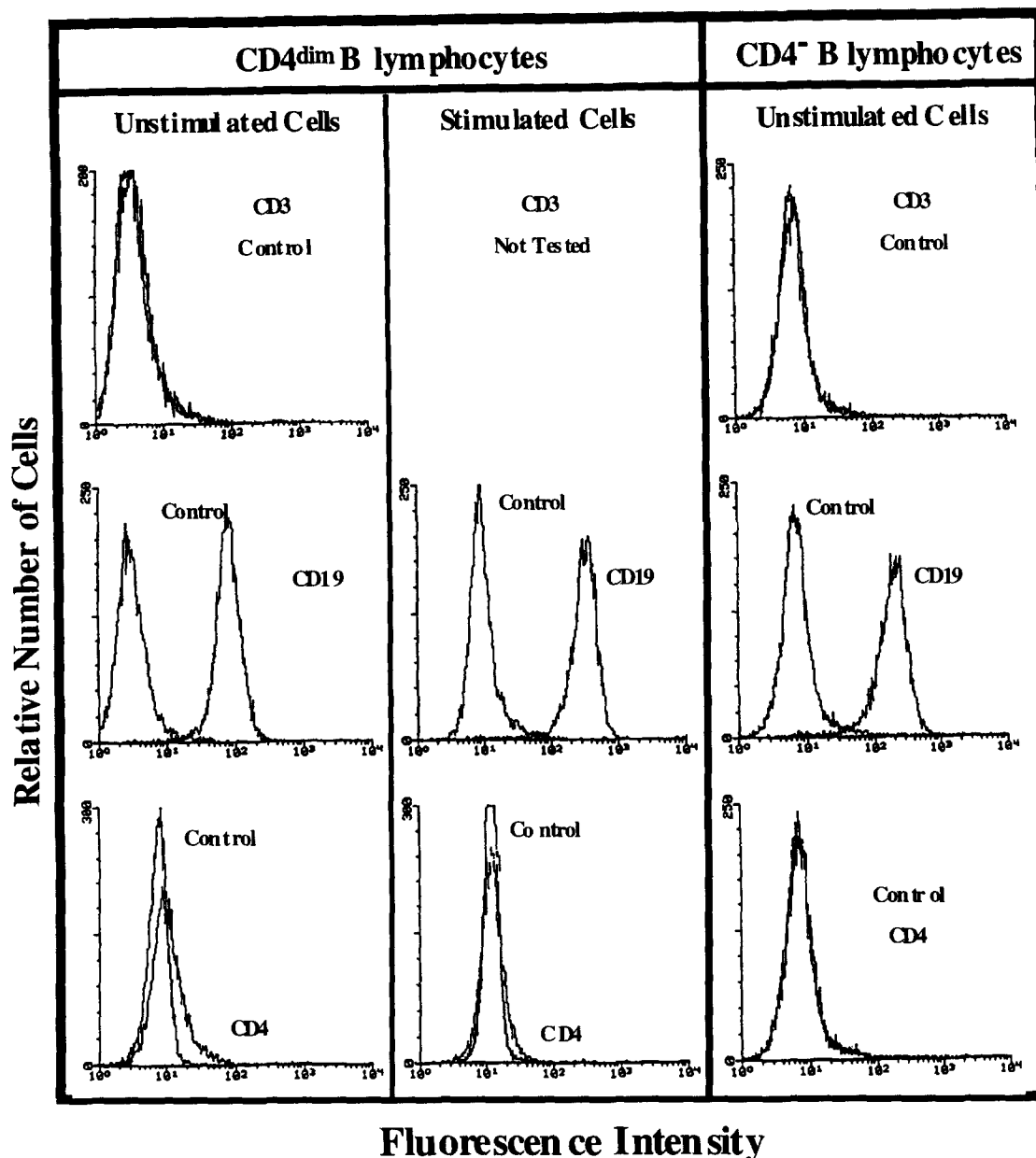
Fig. 1. **B lymphocytes immunostainings** Purity of B cell suspension was evaluated by immunostainings with monoclonal antibodies directed to CD3 and CD19 and CD4 molecules. B cell suspensions was pure higher than 97% and contained less than 1% of CD3$^+$ cells whatever state of activation. CD4 expression, variable following tonsils, was decreased with cell stimulation to all normal tonsil B lymphocytes used. No CD4 cell surface expression could be detected by flow cytometry with 1 tonsil only.

sion was decreased by about 60% by phorbol ester stimulation. In all cases, CD19 expression was upregulated by about 25% upon stimulation.

3.3. CD4–HSA and CD4 antibodies differently inhibit HIV infection of CD4^{dim} and CD4$^-$ tonsillar B cells

B cells were infected according to the C′-ADE protocol and production of virions was assessed by measurement of p24 antigen in culture supernatants harvested at day 5 postinfection. In preliminary experiments, we have determined that maximal inhibition of viral replication was reached with 5 μg/ml monoclonal antibodies directed against CD4, whereas 220

μg/ml was required for optimal CD4–HSA inhibition (data not shown). The percentage of inhibition was calculated by comparison with p24 amounts in isotype-matched positive controls, except for CD4–HSA-treated cultures (Fig. 2). On these various experiments, p24 values ranged from 127 to 230 for unstimulated B cells and from 174 to 268 pg/ml for phorbol ester-stimulated B cells cultured in the absence of blocking antibodies. In the presence of control antibodies, these values varied less than 10%.

On CD4^{dim} tonsillar B lymphocytes (BT254, BT286), we obtained an efficient while partial inhibition of viral replication with all CD4-blocking reagents. Inhibition was more pro-
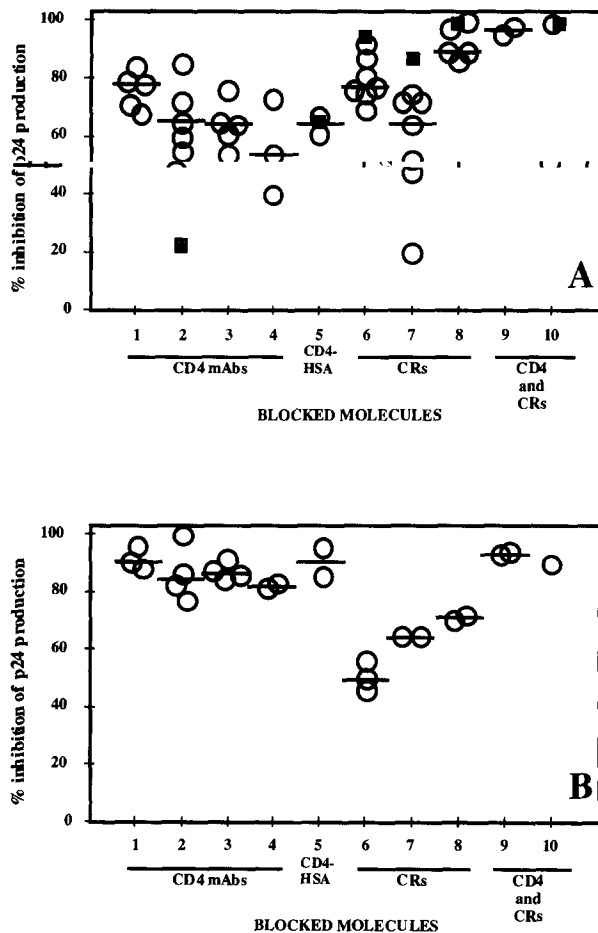
Fig. 2. **Blocking experiments** HIV replication inhibition in non-stimulated (A) and 2-day stimulated (B) tonsil B lymphocytes by monoclonal antibodies and reagents used alone [OKT4a(1), Q4120 (2), NuTh/i (3), anti-Leu3a (4), HSA–CD4 (5), OKB7 (6), pool of anti-CD35 (7)] or in combination [OKB7 + pool of anti-CD35 (8), OKB7 + pool of anti-CD35 + OKT4a (9), OKB7 + pool of anti-CD35 + Q4120 (10)]. B cells were incubated for 1 h at 4°C with 5 µg/ml of mAbs, infected with opsonized virus for 40 min at 37°C, and cultured. CD4–HSA was mixed to virus during opsonization step at the concentration of 220 µg/ml. p24 level were measured by ELISA at day 5 p.i. Percentage of inhibition is calculated by comparison with isotype-matched positive controls. Each plot is 1 experiment. CD4-negative (▨) and CD4^{dim} (o) tonsil B lymphocytes are represented. Median of independent results is indicated by a black line.

nounced on 2-day stimulated B lymphocytes than on freshly thawed B cells (Fig. 2, B and A, respectively). In stimulated B lymphocytes, blocking of CD4 with OKT4a, Q4120, NuTh/i and anti-Leu3a, and CD4–HSA reached 90, 83.5, 86.5, 82 and 90% of inhibition, respectively (median of 3, 4, 4, 2 and 2 experiments), whereas in unstimulated B lymphocytes, these CD4-blocking Ab induced 78, 65, 64, 54 and 64% of inhibition, respectively (median of 5, 5, 5, 3 and 2 experiments).

HIV infection of freshly thawed CD4$^-$ tonsil B lymphocytes (BT291) was weakly sensitive to CD4 mAb Q4120 effect (22% inhibition). Nevertheless, CD4–HSA treatment of virions during opsonization led to an inhibition similar to that obtained with CD4^{dim} tonsil B lymphocytes.

3.4. Complement receptors control a second pathway of HIV infection different from CD4

Dose-dependent addition of CD35 and CD21 Ab have previously established that maximal inhibition of tonsillar B cells HIV infection was reached in the presence of 5 µg/ml Ab. When CD35 and CD21 were independently blocked, viral production was decreased by 64% (median of 7 experiments) and 77% (median of 7 experiments) in freshly thawed B lymphocytes, and by 64 and 49% in 2-day stimulated normal B lymphocytes (median of 2 and 3 experiments, respectively). The simultaneous addition of CD35 and CD21 Ab increased inhibition up to 89% on unstimulated B cells (median of 5 experiments) and to 71% on 2-day stimulated B cells (median of 2 experiments).

CD21- and CD35-independent blocking performed with unstimulated CD4$^-$ B lymphocytes (BT291) allowed a higher inhibition than that obtained with unstimulated CD4^{dim} B lymphocytes (94 vs. 77% for CD21-blocking, and 87 vs. 64% for CD35-blocking). Moreover, when both CD21 and CD35 molecules were blocked, inhibition of viral replication reached 98.5% instead of 89% reached in CD4^{dim} tonsillar B cells. Interestingly, no additive effect of CD4 monoclonal antibody was then observed.

On the other hand, the combination of CD4, CD35 and CD21 Ab allowed a powerful inhibition on freshly thawed as well as on stimulated CD4^{dim} B lymphocytes, whatever the CD4 antibody used (Q4120 or OKT4a). With OKT4a antibody this inhibition reached 92.5% (median of 2 experiments) and 96.5% (median of 2 experiments) with stimulated and unstimulated B cells, respectively. With Q4120 antibody, viral replication was inhibited by 89% with stimulated B cells and by 99% in unstimulated ones.

4. Discussion

Taken together, our data show that opsonized HIV-1/Lai entry in normal B lymphocytes involves 2 major pathways, the CD4 and the CR pathways. Whatever the activation state of B lymphocytes, blockage of CD4 induced a consistent inhibition of viral replication. In unstimulated B cells, this inhibition occurred independently of the CD4^{dim} (BT254, BT286) or CD4$^-$ (BT291) phenotype of tonsil B cells but with a very low efficiency in the latter case (64–78 vs. 22%). This observation, that fits well with data from Zhang and Henderson showing inhibition of free-virus entry by CD4 Ab on CD4-negative B cells [11], suggests that, while undetectable by flow cytometry, CD4 is indeed present on such B cells.

Table 1
Cytofluorometric quantitation of surface receptors on unstimulated and 2-day stimulated normal tonsil B lymphocytes

Tonsil code	CD4	CD21	CD35
Unstimulated B lymphocytes			
BT254	516	31935	73902
BT286	180	29054	64416
BT291	0	24280	40527
2-day stimulated B lymphocytes			
BT254	206	7345	31039
BT286	65	6122	23055

Results are expressed as the number of each specified molecule/cell, as assessed with QIFI-Low (CD4 antigenic density) and QIFI-Kit (CD21 and CD35 antigenic density) beads.

In contrast to the low efficiency of CD4 Ab to inhibit viral replication on BT291 CD4⁻ cells, CD4–HSA-mediated inhibition is as efficient on CD4⁻ as on CD4^{dim} B lymphocytes. A major difference between CD4 Ab and CD4–HSA treatment was that CD4–HSA was directly incubated with virus during the opsonization step and would dissociate uncovalently linked gp120-gp41 complexes [29]. Such a dissociation would, thus, lead to a gp120-depleted virus, less efficient in infecting normal B cells in our experimental conditions, keeping in mind that gp120 binds C3b fragments [30]. Taken together, these results clearly establish that CD4 molecules contribute to viral entry in normal B lymphocytes whether HIV is opsonized or not. Since total inhibition of viral replication was never reached on B cells with CD4-specific blocking reagents, one can speculate that other receptor molecules exist.

Extending our previous data [8], we showed that complement receptors type 1 (CD35) and 2 (CD21) constitute a second infection pathway. Indeed, viral replication was decreased by up to 77 and 64% by the independent blockade of these CRs with unstimulated and 2-day stimulated cells, respectively, and inhibition reached up to 89 and 71% in the presence of CD35 and CD21 Ab with unstimulated and 2-day stimulated cells, respectively. Of note, F97–6B3 (SB2) mAb, another anti-CD21 antibody, which is directed against the short consensus repeats (SCR) 5 to 8, different from the C3dg-binding site (J.-P. Aubry, pers. commun.) inhibited by 53% HIV replication in unstimulated normal B lymphocytes. When F97–6B3 and OKB7 were added simultaneously, inhibition reached 95% (data not shown) instead of 53 and 77%, respectively, suggesting that both SCR1–2 and SCR 5–8 are involved in HIV–CD21 interactions, or that binding of mAb to SCR 5–8 inhibits the link between CD35 and CD21. On B cells, CD21 does not work alone, it is associated either with CD19 or with CD35 molecules [31]. As our previous data have shown that addition of CD19 Ab neither inhibited viral entry, when added alone, nor increased CD21-mediated inhibition [8], it seems likely that HIV interacts with CD21/CD35-associated molecules. In addition, the observation that F97–6B3 mAb inhibited virus entry as well as OKB7 Ab suggests that other sites than SRC 1 and 2 are involved in the interactions between HIV and CRs. Inhibition of opsonized HIV infection by CD21 reagents have also been described in T cell lines [18,28], B cell lines [22–24] and thymocytes [25]. On B cell lines, Tremblay et al. [22] have obtained a complete inhibition of the C'-ADE HIV infection with OKB7 whereas this antibody only induced a partial one in our normal B cells. In these cells as well as in thymocytes [32], CD35 synergize with CD21 for the blocking of HIV entry. As CD35 may function as a cofactor for the cleavage of iC3b into C3dg, a ligand for CD21 [9], inhibition of HIV infection would result from a deficit in C3 cleavage. From data on HIV infection of HPB-ALL T cells, it seems more likely that CD35 functions as a receptor for opsonized HIV [32]. This hypothesis is strengthened by data on monocytes showing that blockade of CD35 on CD35^{high} primary monocytes allowed a total inhibition of opsonized virus entry [21] and suggesting that the role of CD35 as HIV receptor would be particularly important in the absence of CD21.

With CD4-negative B lymphocytes, independent CD21- or CD35-blocking is more efficient than with CD4^{dim} B lymphocytes (Fig. 2A). In this case, contribution of CD4 molecule is minimal since no additive effect of CD4 mAb was observed,

demonstrating that CD21 and CD35 are indeed an alternate pathway for normal B lymphocyte infection by opsonized HIV. Nevertheless, as with CD4-blocking, no total inhibition was reached by their simultaneous blocking. We, thus, performed blockade of both CRs and CD4 pathways. Results showed that inhibition was extremely important but not total, particularly in 2-day stimulated B lymphocytes (see Fig. 2B), suggesting another putative CD4- and CRs-independent process for HIV entry. Curtis et al. have showed that a C-type lectin which normally binds mannose is able to bind the glycosylated envelope glycoprotein gp120 with high affinity [33]. Once bound to the lectin, gp120 is rapidly internalized, suggesting that C-type lectin might mediate cell infection by a CD4-independent mechanism. Since no monoclonal antibodies to the mannose-binding site of this lectin were available, we used 2 concentrations of mannose (10 and 100 μg/ml) to compete with the possible binding of HIV. Whatever the mannose concentration, no significant inhibition of infection was obtained (data not shown), suggesting that either C-type lectin is absent from the B cell surface or that lectin plays a marginal role in HIV entry in these cells.

In the present study, we showed that CD4 and CR1/CR2 are the main pathways for HIV entry in normal B cells exposed to opsonized virus. As these results have been obtained with the HIV-1/Lai strain, it would be interesting to test whether these 2 pathways are similarly used by clinical isolates of the virus, opsonized by autologous patient's antibodies. Nevertheless, our HIV-1/Lai stock has always been grown in primary human cells, and was then never selected for cell line infection capacity. Our results are, consequently, not subjected to this possible bias. It is worth noting that, while opsonized, the virus entry in normal B cells used both CD4 and CRs. This suggests that, in lymphoid organs which constitute a major site of intense viral replication [34] and where the virus is mainly present as immune complexes (virus opsonized by both anti-HIV antibodies and complement) at the surface of follicular dendritic cells, HIV would enter in B cells through similar mechanisms. As follicular dendritic cells are located in germinal centers of lymphoid organs, one can speculate that GC B cells will be particularly sensitive to HIV infection. Although CD21 and CD35 expression decrease with B cell activation, GC B cells still express important levels of both antigens, whereas in vivo infection of lymphoid organ B cells is infrequently detected. This discrepancy would suggest that, once infected, B lymphocytes are unable to normally interact with their microenvironment, and die. We feel that our in vitro infection model will help us to evaluate this hypothesis.

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