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Engagement of CD28 Modulates CXC Chemokine Receptor 4 Surface Expression in Both Resting and CD3-Stimulated CD4+ T Cells

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Optimal CD4+ T cell activation requires the cooperation of multiple signaling pathways coupled to the TCR-CD3 complex and to the CD28 costimulatory molecule. In this study, we have investigated the expression of surface CXC chemokine receptor 4 (CXCR4) in enriched populations of CD4+ T PBL, stimulated with anti-CD3 and anti-CD28 mAbs, immobilized on plastic. Anti-CD3 alone induced a progressive down-regulation of surface CXCR4, accompanied by a significant decline in the entry of the HXB2 T cell line-tropic (X4-tropic) HIV-1 clone in CD4+ T cells. Of note, this effect was strictly dependent on the presence in culture of CD4+ monocytes. On the other hand, anti-CD28 alone induced a small but reproducible increase in the expression of surface CXCR4 as well as in the entry of HXB2 HIV-1 clone in resting CD4+ T cells. When the two mAbs were used in combination, anti-CD28 potently synergized with anti-CD3 in inducing the expression of CD69 activation marker and stimulating the proliferation of CD4+ T cells. On the other hand, anti-CD28 counteracted the CXCR4 down-modulation induced by anti-CD3. The latter effect was particularly evident when anti-CD28 was associated to suboptimal concentrations of anti-CD3. Because CXCR4 is the major coreceptor for the highly cytopathic X4-tropic HIV-1 strains, which preferentially replicate in proliferating CD4+ T cells, the ability of anti-CD28 to up-regulate the surface expression of CXCR4 in both resting and activated CD4+ T cells provides one relevant mechanism for the progression of HIV-1 disease. The Journal of Immunology, 2000, 164: 4018–4024.

The 44-kDa homodimer CD28 is present on the surface of most CD4+ and some CD8+ T cells (reviewed in Refs. 1 and 2). Functional studies by numerous laboratories have shown that binding of CD28 by its counterreceptors (CD80/B7.1, CD86/B7.2/B70, B7.3), expressed on the surface of APC, such as monocytes, dendritic cells, and activated B cells (2), determines the fate of the T cell response (1–2). In fact, optimal CD4+ T cell activation requires a non-Ag-driven cosignal provided by CD28 in addition to engagement of the TCR-CD3 complex with CD4 (2). CD28 plays a critical role in CD4+ T cell costimulation leading to the production of cytokines (in particular IL-2) (3), and expression of cytokine receptors, including the high affinity α-chain of the IL-2R (4). Both events are required for the progression of T cell response. It has also been shown that CD28 engagement induces various biochemical events associated with signal transduction (2, 5), including activation of c-Jun N-terminal kinase (JNK) (6–8), and phosphatidylinositol 3-kinase (PI 3-K) (9–11).

Chemokines are a group of proteins that mediate directed leukocyte migration (reviewed in Ref. 12). Based on the locations of four cysteine residues that form disulfide bonds, these small (6–14 kDa) basic substances have been classified into two main families, CC or CXC chemokines. Both CC and CXC chemokines bind to seven-transmembrane G protein-coupled receptors, which transduce signals through heterotrimeric G proteins (reviewed in Ref. 13). A number of studies demonstrated that the CCR5 (R5) and CXCR4 (X4) chemokine receptors act as major coreceptors for the entry of macrophage-tropic and T cell line-tropic HIV-1 strains, respectively, into target CD4+ cells (reviewed in Ref. 14).

Most resting T lymphocytes show a functional expression of surface CXCR4, which is down-regulated by exposure to its high affinity ligand, stromal derived factor-1α, as well as by phorbol esters (15–18). Although earlier studies reported an up-regulation of the CXCR4 promotor and mRNA in the presence of mitogenic stimulation (19–21), subsequent studies have shown that mitogenic agonists induce the internalization of surface CXCR4 molecule (22–25). This issue is particularly relevant because it has been clearly demonstrated that HIV-1 preferentially replicates in activated/proliferating CD4+ T cells (26). Thus, the aim of this study was to analyze the expression of surface CXCR4 in resting CD4+ T cells as well as in cells treated with anti-CD3 mAb alone or in combination with anti-CD28 mAb, which mimics the physiological activation of CD4+ T cells (1–2).

Abbreviations used in this paper: JNK, c-Jun N-terminal kinase; CXCR, CXC chemokine receptor; LTR, long terminal repeat; MFI, mean fluorescence intensity; PI 3-K, phosphatidylinositol 3-kinase.
Materials and Methods

Cells

PBMC were isolated by Ficoll-Hypaque density-gradient centrifugation (Pharmacia, Uppsala, Sweden) of heparinized leukocyte units obtained from 14 healthy adult donors, who gave their informed consent to this research according to the Helsinki declaration of 1975. Enriched populations of resting CD4+ T cells were isolated by immunomagnetic negative selection with Dynabeads M450 (Dynal, Polyscience, PA). For this purpose, we used a cocktail of mAbs against CD19 and CD20, present on B cells; CD16 on granulocytes and NK; CD56 and CD57, on NK, CD14 on monocytes; and CD8 on T cells (all mAb were from Immunotech-Coulter, Marseilles, France). The final cultures of CD4+ T cells thus obtained were always >80% pure, as determined by staining performed with a combination of FITC-conjugated anti-CD3 plus PE-conjugated anti-CD4 mAb (Becton Dickinson, San Jose, CA), followed by flow cytometry analysis. The major contaminating cell population was represented by CD14+ monocytes, whose number was between 5 and 10% in the different cell preparations, as evaluated by forward-side scatter analysis and staining with PE-conjugated anti-CD14 (Becton Dickinson), followed by flow cytometry.

In some experiments, PBMC from the same donors were divided in two aliquots and subjected to the immunomagnetic negative selection procedure either in the absence or presence of anti-CD14 mAb. When anti-CD14 mAb was not included in the cocktail of mAbs, the number of CD14+ monocytes recovered in the final cell population ranged between 20 and 30%. When anti-CD14 mAb was included in the cocktail of mAbs, the number of CD14+ monocytes was comprised between 5 and 10%. After the immunomagnetic negative selection containing anti-CD14 mAb, one-half of the cell preparation was seeded in tissue flasks for 3 days at 37°C to eliminate residual monocytes by plastic adherence. After this step, the number of CD14+ monocytes was lowered to <1%. At the end of purification, cells were resuspended in AIM-V serum-free medium (Life Technologies, Grand Island, NY) at 1.8 × 10^6 cells/ml and seeded (0.6 ml/well) in 48-well flat-bottom plates, coated as described below.

Adherence of Abs and proteins to microtiter plates

Anti-CD3 (IgG2a-clone × 35; Immunotech-Coulter), anti-CD28 (IgG1; Becton Dickinson), and isotype-matched irrelevant mAbs (Becton Dickinson) as well as full-length synthetic HIV-1 Tat protein (86 aa, derived from the HIV-1 T cell line-tropic BH10 strain; Technogen, Caserta, Italy) (27) were resuspended in PBS containing 0.1% BSA (Sigma, St. Louis, MO). Forty-eight-well flat-bottom polystyrene plates (Costar, Cambridge, MA) were coated overnight at 4°C with anti-CD3, anti-CD28, isotype-matched irrelevant mAb (IgG1 and IgG2a; Immunotech-Coulter), Tat, BSA, used alone or in combination, at the concentrations indicated in the text. Plates were then rinsed with AIM-V serum-free medium (Life Technologies) to remove nonadherent proteins, and medium was immediately added to the plates after the final wash.

FACS evaluation of surface markers

The surface expression of CXCR4, CCR5, and CD69 was evaluated by direct staining with the PE-conjugated anti-CCR5 (PharMingen, San Diego, CA; clone 12G5), FITC-conjugated anti-CCR5 (R&D Systems, Minneapolis, MN; clone 45502.111), and PC5-conjugated anti-CD69 (Immunotech-Coulter) mAbs. Briefly, aliquots of 3 × 10^5 cells were stained with 5 μl of each mAb in 200 μl of PBS containing 2% FCS, at 4°C for 30 min. Nonspecific fluorescence was analyzed by using isotype-matched controls. After staining procedures, samples were analyzed using FACS Calibur (Becton Dickinson). In the experiments described, with increasing concentrations of monocytes, CD4+ T lymphocytes and CD14+ monocytes were identified by forward-side scatter analysis and by double staining with PE-conjugated anti-CXCR4 + FITC-conjugated anti-CD4 (Becton Dickinson for CD4+ T lymphocytes) or PE-conjugated anti-CXCR4 + FITC-conjugated anti-CD14 (for monocytes). Data collected from 10,000 cells are presented as either percentage of positive cells or mean fluorescence intensity (MFI) values.

[^3]HThymidine incorporation assay

Cell proliferation was evaluated by [^3]Hthymidine incorporation assay, performed as described previously (28). CD4+ T cells were first seeded in 48-well flat-bottom plates coated with BSA, anti-CD3 (1 μg/ml), anti-CD28 (1 μg/ml), anti-CD3 + anti-CD28 (1 μg each/well) mAbs at the concentration of 1 × 10^5 cells/ml (600 μl/well), as described above. Thirty-six hours postseeding, aliquots of 0.15 ml were harvested and seeded in 96-well tissue culture plates (Costar), supplemented with 1 μCi [%^3]Hthymidine (6.7 Ci/mmol; DuPont, Boston, MA) for additional 12 h.

Radioactivity incorporated into DNA was measured using an automated liquid scintillation counter. Results were expressed as arithmetic mean cpm of triplicate cultures.

HIV-1 infection assay

At 48 h post-seeding, cells were infected with HIV-1 (HXB2 clone; Advanced Biotechnologies, Columbia, MD; multiplicity of infection of 0.01) for 3 h and then washed three times with PBS. Viral stocks were treated before use with RNAse-free DNase I (Boehringer Mannheim, Indianapolis, IN) to remove contaminating DNA. Fourteen hours postinfection, CD4+ T cells were collected, resuspended in proteinase K-lysis buffer, and allowed to incubate at 56°C for 60 min, then at 98°C for 20 min. Serial dilutions of cell lysates were subjected to HIV-1 DNA PCR by using the following primers designed based on previously published sequences (29): 5’ primer, 5’-TCTCTCTGCTTAGGACAGACTGTCAGAGATGAAGTGTTTGTCG; 3’ primer, 5’-ACTCTCA GAGATTTCACACCTG. These primers, which amplify a 180-bp fragment in theLTR R/U5 region, were designed to detect early steps in reverse transcriptase. (30). Samples were subjected to 40 cycles of amplification (95°C for 1 min, 50°C for 1 min, and 72°C for 1 min). Negative controls were represented by samples containing buffer only or uninfected cells. The PCR products were separated on 2% agarose gel, transferred to a nylon membrane, hybridized with an [%^3]P-labeled oligonucleotide probe (5’-CTCAATAAAAGCTTGGTTCGACATCGT; GTTGTGTCG) against an internal sequence of the HIV PCR product, and analyzed after exposure to x-ray film. To normalize for the quantity of DNA in each sample, β-globin PCR was conducted (β-globin primers; Stratagene, La Jolla, CA). β-Globin PCR products were visualized under UV light after staining of agarose gels with ethidium bromide. Each sample was amplified in duplicate or triplicate.

Statistical analysis

The results were expressed as means ± SDs of three or more experiments performed in duplicate. Statistical analysis was performed using the two-tailed Student’s t test.

Results

Stimulation with either anti-CD3 mAb or anti-CD28 mAb alone shows striking differential effects on surface CXCR4 in enriched CD4+ T cells

The surface CXCR4 levels were initially evaluated in enriched populations of CD4+ T PBL, before and after seeding in serum-free medium on wells coated with BSA, anti-CD3, anti-CD28, or isotype-matched irrelevant mAbs. The dose of proteins or Abs reported in all experiments is that added overnight to the plates to coat the wells and does not represent the amount of proteins bound to each well. This has been estimated to be 1–4% of the dose added (28, 30).

Surface CXCR4 was restricted to 20–40% of freshly isolated CD4+ T cells. Upon culture at 37°C in BSA-treated control wells, CXCR4 expression increased rapidly, with the percentage of CXCR4+ cells raising up to 90% after 4–24 h in culture, persisting to high levels for up to 72 h (Fig. 1A). Interestingly, when cells were seeded on plates coated with anti-CD3 mAb alone (1 μg/ml), a significant (p < 0.01) decrease in the number of CXCR4+ cells was noticed from 24 h onward (Fig. 1A). Maximal levels of CXCR4 inhibition were observed at 48 h of culture (Fig. 1A), as also shown by the analysis of CXCR4 MFI (Fig. 1B). On the other hand, the percentage of CXCR4+ cells did not significantly (p > 0.1) differ between plates coated with BSA or anti-CD28 mAb. However, with respect to control cells treated with BSA, anti-CD28 mAb induced a small but reproducible (p < 0.05) increase of CXCR4 surface expression, evaluated as MFI, between 24 and 48 h of culture (Fig. 1C).

In parallel, we have analyzed the surface expression of CD69 early activation marker (31), which was confined to 10–30% of freshly isolated CD4+ T cells. Surface CD69 expression was transiently up-regulated by seeding the cells in serum-free BSA-treated cultures at 37°C, reached maximal expression (50–90% of
CD691 cells at 4 h, and rapidly declined thereafter (Fig. 2). Anti-CD3 induced a persistent and significant ($p < 0.01$) increase in the number of CD691 cells with respect to BSA from 24 h onward. In turn, anti-CD28 alone induced only a transient up-regulation of CD69 at 24 h (Fig. 2). No significant differences in CXCR4 and CD69 surface expression were noticed between cells seeded on plates coated with BSA or irrelevant mAb. Therefore, in next experiments, we only used BSA as a negative control.

**CD3- and CD28-induced modulation of surface CXCR4 correlates with entry of the HXB2 X4-tropic strain**

In next experiments, primary CD4+ T cells were cultured for 48 h in BSA- or anti-CD3- or anti-CD28-coated plates, and then incubated with HXB2 inoculum for 3 h. After additional 14 h of culture in fresh medium, samples were analyzed by PCR for the presence/amount of viral DNA as a measurement of viral entry. Semiquantitative PCR of strong-stop DNA (with LTR R/U5 primers), an early product of reverse transcription, revealed significant lower levels of proviral DNA in cells seeded on CD3-coated plates with respect to those seeded on control (BSA-coated) plates (Fig. 3).
the other hand, cells seeded on CD28-coated plates showed levels of proviral DNA reproducibly higher than those found in both CD3- and BSA-coated plates. These findings are in agreement with previous data from our group (30) and other groups (22–24), who have demonstrated that the susceptibility of CD4\(^+\) T cells to highly cytopathic (X4-tropic) HIV-1 strains is directly related to the levels of CXCR4 exposed on the cell surface.

CD28 counteracts the CD3-mediated down-regulation of surface CXCR4 expression in enriched CD4\(^+\) T cells

In the following experiments, cells were seeded on plates coated with high concentrations (1 \(\mu\)g/well) of anti-CD3 plus anti-CD28 mAbs, a treatment mimicking the in vivo activation of CD4\(^+\) T cells by APC (1, 2). Of note, in cells costimulated by anti-CD3 +
anti-CD28 mAbs, surface CXCR4 expression showed a progressive decline reaching the lowest values at 48 h after the beginning of the treatment (Fig. 4A). However, at this time point (48 h), the number of CXCR4+ cells in well coated with anti-CD3 plus anti-CD28 was significantly \((p < 0.01)\) higher than that observed in well coated with anti-CD3 alone (Fig. 4A). On the other hand, anti-CD3 + anti-CD28 synergized in inducing maximal stimulation of CD69 activation marker. In fact, the levels of CD69 MFI were significantly \((p < 0.05)\) higher in CD4+ T cells plated on anti-CD3 + anti-CD28 than those observed in cells seeded on BSA, anti-CD3, or anti-CD28 (Fig. 4B).

In parallel, the effect of anti-CD3 and anti-CD28, used alone or in combination, was evaluated on CD4+ T cell proliferation. As shown in Fig. 4C, most primary CD4+ T cells seeded for 36 h on BSA or anti-CD28 mAb remained quiescent. Anti-CD3 mAb alone induced a...

FIGURE 6. CXCR4 surface expression in CD4+ T cells cultured with increasing concentrations of CD14+ monocytes. Cells were seeded on plates coated with BSA, anti-CD3 (1 μg/well), anti-CD3 plus anti-CD28 (1 μg/well, each), or anti-CD3 (1 μg/well) plus HIV-1 Tat protein (0.4 μg/well). CD4+ T lymphocytes were distinct from CD14+ monocytes on the basis of their forward-side scatter features and bright expression of surface CD4 (CD4+ T cells) vs dim expression of surface CD4 and bright expression of surface CD14 (monocytes). Data are expressed as means ± SD of three to five independent experiments, in which the levels of surface CXCR4 were analyzed by FACS at 48 h and expressed as percentage of positive cells.
significant ($p < 0.01$) increase of [methyl-3H]thymidine uptake, with respect to cells seeded on BSA or anti-CD28. The combination of immobilized anti-CD3 + anti-CD28 mAbs potently stimulated the proliferation of CD4$^+$ T cells to levels significantly greater ($p < 0.01$) than those observed in cells seeded on anti-CD3 alone (Fig. 4C).

The up-regulation of CXCR4 mediated by CD28 mAb was reminiscent of a similar effect observed in the presence of extracellular HIV-1 Tat protein immobilized on plastic (30). To further investigate these similarities, anti-CD3 mAb was titrated down to 0.1 ng/well in the absence or presence of predetermined optimal concentrations of anti-CD28 mAb (1 μg/well) or Tat protein (0.4 μg/well) (Fig. 5A). The CD3 mAb-induced CXCR4 down-regulation was dose dependent, with significant ($p < 0.01$) decrease of surface CXCR4 being observed for concentrations of anti-CD3 mAb ranging between 1000 and 10 ng/well at 48 h. Anti-CD28 mAb and extracellular Tat were able to efficiently ($p < 0.05$) counteract the suppression of surface CXCR4 induced by 10–1000 ng/well (for anti-CD28) and 100–1000 ng/well (for Tat) of anti-CD3 mAb (Fig. 5A). This effect was particularly evident when anti-CD28 mAb (Fig. 5, A and B) or extracellular HIV-1 Tat (Fig. 5, A and C) was combined to 100 ng/well of anti-CD3 mAb.

**The CD3-mediated down-modulation of surface CXCR4 in CD4$^+$ T cells requires the presence in culture of CD14$^+$ monocytes**

Previous studies investigating the role of mitogenic stimulation on CXCR4 surface expression were performed using either PBMC or partially purified CD4$^+$ T cells (19, 21–24). Similarly, the data illustrated above were obtained using enriched (>80% pure) CD4$^+$ T cells, which contain a fraction of residual CD14$^+$ monocytes (<10% in the different cell preparations). To ascertain the potential role of these accessory cells, the effect of anti-CD3, used alone or in combination with anti-CD28 or HIV-1 Tat protein, was next evaluated in CD4$^+$ T cell preparations containing increasing concentrations of CD14$^+$ monocytes. When the percentage of CD14$^+$ monocytes was lowered to <1% by immunomagnetic negative selection and plastic adherence, anti-CD3 mAb (1 μg/well) was unable to down-regulate ($p > 0.1$) surface CXCR4 in CD4$^+$ T cells (Fig. 6). The possibility that the unresponsiveness of CD4$^+$ T cells was due to the adherence procedure itself rather than to the monocyte depletion was ruled out, analyzing CD4$^+$ T cell preparations from the same donors progressively enriched in monocytes. The enrichment in CD14$^+$ cells (reaching 20–30% of the total cell population) significantly ($p < 0.05$) increased the ability of anti-CD3 to down-regulate surface CXCR4 expression in CD4$^+$ T cells (Fig. 6).

**Anti-CD28 mAb and extracellular Tat do not significantly affect the CCR5 surface expression in enriched CD4$^+$ T cells**

In the last group of experiments, the effect of anti-CD3 mAb, anti-CD28 mAb, and Tat, used alone or in combination, was evaluated on the surface expression of CCR5. In agreement with previous studies (32–34), we found that surface CCR5 was expressed by a minority of freshly purified resting CD4$^+$ T cells and it was substantially unaffected by any of the combinations used to stimulate the cells for up to 72 h of culture (Fig. 7).

**Discussion**

In contrast to most chemokine systems, which recruit cells to sites of inflammation, the stromal derived factor-1/CXCR4 system is believed to localize cells to hemopoietic and lymphoid organs (15, 35, 36). In fact, transgenic mice overexpressing high levels of human CXCR4 on the membrane of CD4$^+$ T cells present a disturbed lymphocyte trafficking, mainly characterized by a severe loss of CD4$^+$ T lymphocytes from peripheral blood and homing of these cells in bone marrow (37). Consistently, we and other authors (22–24) have shown that freshly isolated PBL CD4$^+$ T cells express low levels of surface CXCR4 and that the receptor is rapidly expressed on the cell surface upon culture at 37°C.

In this study, we have dissected for the first time the individual role of CD3 and CD28 in modulating CXCR4 expression. Coligation of CD3 and CD28 is required for optimal stimulation of CD4$^+$ T cells (1, 2), as also documented in this study by their ability to up-regulate CD69 activation marker and to induce a significant increase of [methyl-3H]thymidine uptake with respect to anti-CD3 alone. However, the effects of anti-CD3 and anti-CD28 on surface CXCR4 were divergent. In fact, anti-CD3 mAb induced an abrupt down-regulation of surface CXCR4 in CD4$^+$ T cells that required the presence in culture of at least 5–10% CD14$^+$ monocytes. On the other hand, anti-CD28 mAb alone induced a small but reproducible up-regulation of CXCR4 surface expression in resting CD4$^+$ T cells. When cells were maximally stimulated by the combination of anti-CD3 plus anti-CD28 mAbs, they showed intermediate levels of surface CXCR4, with respect to cells treated with single mAb. Of note, the changes in surface expression levels of CXCR4 showed a clear correlation with the ability of BH2 X4-tropic HIV-1 strain to infect CD4$^+$ T cells, as also described in previous studies (30, 36, 37). However, we have not ruled out the possibility that both CD3 and CD28 activation may interfere with HIV-1 replication also at a postentry step, an issue that deserves further investigation. Finally, a striking parallelism was observed between anti-CD28 mAb and a synthetic peptide corresponding to Tat protein of the BH10 X4-tropic HIV-1 strain, as both molecules were able to up-regulate surface CXCR4.

The cell surface expression of CXCR4 appears to be regulated both by gene expression (19, 20), and by continual recirculation of the receptor between the cell surface and endosomal compartments (18, 23, 25, 38). It has been shown that anti-CD3 down-regulates surface CXCR4 through a protein kinase C-dependent pathway (22, 23, 25). Although we have not addressed the molecular mechanisms underlying the ability of CD28 to counteract the CD3-mediated CXCR4 down-regulation, it is well established that, upon interaction with its counterreceptors predominantly expressed on APC (2), CD28 triggers two main intracellular signals involving JNK (6–8) and PI 3-K (9–11). Interestingly, extracellular Tat is also able to activate both JNK (39) and PI 3-K (40) in lymphoid CD4$^+$ T cells. It is, therefore, possible that both anti-CD28 and extracellular Tat activate common intracellular pathways to eliciting the up-regulation of surface CXCR4 in CD4$^+$ T cells.

Although it has been shown that in vitro HIV-1 can use several chemokine receptors to enter CD4$^+$ cells, CCR5 and CXCR4 are the most important coreceptors for HIV-1 infection in vivo (14). In the course of HIV-1 infection, R5 viral strains predominate during the early phase of infection, while dual-tropic and X4 viral strains predominate during disease progression to AIDS (reviewed in Ref. 14). Moreover, evidence showing that deletion in the CCR5 gene does not protect against infection by X4-tropic strains (41), and the fact that patients heterozygous for the D32 CCR5-harboring virus with an X4 phenotype have a poor prognosis (42, 43), strengthen the role of CXCR4 as a main HIV-1 coreceptor. In agreement with previous data demonstrating that CXCR4 and CCR5 are differentially expressed in T lymphocytes (32–34), we found that the CD28- and Tat-mediated up-regulation of CXCR4 surface expression was not accompanied by significant modifications of surface CCR5 chemokine receptor in CD4$^+$ T cells. Thus, CD3/CD28 costimulation of CD4$^+$ T cells may exert selective pressure in favor of X4-tropic isolate production through a combination of mechanisms, including production of high levels of β-chemokines (21), lack of induction, or even down-regulation
(44) of β-chemokine (CC) receptors and sustained expression of CXCR4.

The entry of X4-tropic HIV-1 into CD4+ T cells is strictly related to the expression levels of surface CXCR4 (23–25, 29, this study), while critical steps in the HIV-1 cycle, such as integration and initiation of transcription, depend on cell activation (26, 29). Even if X4-tropic strains are able to infect resting lymphocytes expressing CXCR4, proviral integration and subsequent HIV-1 replication will not occur in the absence of cell activation. The existence of an inverse correlation between the CD3-mediated activation of T lymphocytes and the surface expression of CXCR4 suggests that the activation of CD4+ T cells by TCR/CD3 would not be an advantage to the propagation of X4-tropic strains in CD4 lymphocytes because of CXCR4 down-regulation. However, our data have demonstrated for the first time that both CD28 ligands, not be an advantage to the propagation of X4-tropic strains in CD4+ T cells, and 2) by sustaining the surface expression of CXCR4. Due to the primary role of CD28 as costimulatory molecule (2), its ability to synergize with CD3 in stimulating the proliferation of surface CXCR4 most likely has a relevant functional significance for the progression toward AIDS, facilitating the spreading of X4-tropic strains.

References


CD28 UP-REGULATES CXCR4 SURFACE EXPRESSION

The entry of X4-tropic HIV-1 into CD4+ T cells is strictly related to the expression levels of surface CXCR4 (23–25, 29, this study), while critical steps in the HIV-1 cycle, such as integration and initiation of transcription, depend on cell activation (26, 29). Even if X4-tropic strains are able to infect resting lymphocytes expressing CXCR4, proviral integration and subsequent HIV-1 replication will not occur in the absence of cell activation. The existence of an inverse correlation between the CD3-mediated activation of T lymphocytes and the surface expression of CXCR4 suggests that the activation of CD4+ T cells by TCR/CD3 would not be an advantage to the propagation of X4-tropic strains in CD4 lymphocytes because of CXCR4 down-regulation. However, our data have demonstrated for the first time that both CD28 ligands, not be an advantage to the propagation of X4-tropic strains in CD4+ T cells, and 2) by sustaining the surface expression of CXCR4. Due to the primary role of CD28 as costimulatory molecule (2), its ability to synergize with CD3 in stimulating the proliferation of surface CXCR4 most likely has a relevant functional significance for the progression toward AIDS, facilitating the spreading of X4-tropic strains.

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