Differential Effects of CD40 Ligand/Trimer Stimulation on the Ability of Dendritic Cells to Replicate and Transmit HIV Infection: Evidence for CC-Chemokine-Dependent and -Independent Mechanisms

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Differential Effects of CD40 Ligand/Trimer Stimulation on the Ability of Dendritic Cells to Replicate and Transmit HIV Infection: Evidence for CC-Chemokine-Dependent and -Independent Mechanisms

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The role of exogenous stimulation of CD40 by CD40 ligand (CD40L) in dendritic cell (DC) maturation, CC-chemokine production, and CCR5 receptor expression was examined using a soluble trimeric CD40L agonist protein (CD40LT). Stimulation of monocyte-derived DCs with CD40LT enhanced the production of the CC-chemokines macrophage inflammatory protein (MIP)-1α, MIP-1β, and RANTES and diminished surface expression of CCR5. Based on these findings, the functional role of CD40LT stimulation on the ability of DCs to replicate and transmit HIV viral infection was studied. The addition of CD40LT to cocultures of naive CD4+ T cells and autologous DCs (T/DC) infected with the macrophage-tropic isolate, HIVBaL, caused a striking reduction in reverse transcriptase (RT) activity after 10 and 14 days of culture. The addition of a mixture of Abs against CC-chemokines abrogated the decrease in RT activity, demonstrating that the inhibitory effect mediated by CD40LT was CC-chemokine-dependent. In contrast, the presence of CD40LT in T/DC cocultures infected with the T cell-tropic isolate, HIVIIIB, caused an increase in RT activity that was CC-chemokine-independent. Of note, CD40LT stimulation also inhibited RT activity in cultures containing macrophage-tropic virus (HIVBaL)-infected DC only. However, in contrast to the results seen in the T/DC cocultures, CD40LT stimulation inhibited RT activity in cultures of DCs alone in a CC-chemokine-independent manner. Together, these results show that CD40LT stimulation of DCs suppresses HIV replication and transmission to CD4+ T cells by two potentially different mechanisms. *The Journal of Immunology, 1999, 162: 3711–3717.*

CD40/CD40L ligand (CD40L)3 costimulation plays a major role in regulating both humoral and cellular immune responses (1). The interaction between CD40L with its counter-receptor CD40 on APCs is critical for Ag-specific T cell responses (2). The ability of CD40/CD40L stimulation to regulate T cell-dependent responses is mediated by at least two mechanisms. First is the ability of CD40/CD40L stimulation to enhance the expression of accessory molecules on APCs, including CD54, CD80, CD86, and MHC class II Ags (3, 4). In addition, CD40/CD40L stimulation induces and/or enhances production of inflammatory cytokines, such as IL-12, TNF-α, IL-1α, IL-1β, IL-6, and IL-8, from macrophages and dendritic cells (DCs) (5–7). Furthermore, in one report, CD40 stimulation also resulted in production of the CC-chemokine macrophage inflammatory protein (MIP)-1α by monocytes and cord blood-derived DCs (3). This raised the question of whether other CC-chemokines might be induced from APCs through CD40/CD40L stimulation.

In assessing the effect of CD40/CD40L stimulation on CC-chemokine production, Kornbluth et al. (8) recently demonstrated that macrophages are a potent source of CC-chemokines following stimulation by cells transfected with CD40L. In addition, supernatants from these CD40L-stimulated monocytes diminished the ability of HIVSF162, a strain of HIV-1 that uses CCR5 as a coreceptor, to infect CD4+ T cells. These data clearly supported a CC-chemokine-dependent pathway by which CD40L-stimulated monocytes could limit HIV transmission to CD4+ T cells. These data are in contrast to earlier work in which CD40/CD40L stimulation enhanced HIV-1 replication in DC cocultures with CD4+ T cells. It should be noted, however, that these studies were done using the HIVLAI strain, which was subsequently shown to use CXCR4 as a coreceptor (9). While these discrepancies are likely explained by the coreceptor usage of the particular strain of HIV used, we were interested in determining the net biologic effects that stimulation of CD40 by exogenous CD40L exerted on both macrophage (M)-tropic and T cell (T)-tropic HIV-1 replication in vitro. To this end, we used a soluble trimeric CD40L agonist (CD40LT) protein to examine whether such exogenous CD40L/CD40L stimulation enhanced production of CC-chemokines from CD14+ monocyte-derived DCs and whether this had an impact on the replication of HIV in cocultures with CD4+ T cells. In an attempt to mimic events that occur in vivo, DCs were infected with HIV-1 and then cultured with or without purified autologous CD4+ T cells in the presence or absence of CD40LT. Cells were
cultured for a period of 2 wk, during which time HIV replication was monitored by reverse transcriptase (RT) activity. We demonstrate that stimulation with CD40LT diminished RT activity from infected DCs alone and in cocultures with T cells plus autologous DC (T/DC) in a CC-chemokine-independent and -dependent manner, respectively. The potential physiologic role of CD40/CD40L stimulation in regulating the ability of DCs to serve as reservoirs of HIV, as well as to transmit virus to CD4+ T cells, is discussed.

Materials and Methods
Reagents
Complete medium consisting of RPMI 1640 supplemented with 10% heat-inactivated human AB sera (Sigma, St. Louis, MO), penicillin (100 U/ml), streptomycin (100 U/ml), and t-glutamine (2 mM) were used in all cultures. Lymphocyte separation medium was purchased from Organon Teknika (Durham, NC).

Subjects
Monocytes were obtained via countercurrent elutriation from apheresed subjects from the National Institutes of Health normal donor pool. Autologous, highly enriched T cells were also obtained from the same donors and cryopreserved.

Recombinant cytokines
Human rIL-4 was purchased from Genzyme (Cambridge, MA). Human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) and CD40LT were gifts from Immunex (Seattle, WA).

Antibodies
Goat anti-human Abs to MIP-1α, MIP-1β, RANTES, and control Abs were purchased from R&D Systems (Minneapolis, MN).

Cell preparation
Fresh elutriated human monocytes (90% CD14+ cells) were cultured in 6-well dishes (Costar, Cambridge, MA) at a concentration of 10⁶ cells/ml. Cells were cultured in AIM-V (Gibco, Grand Island, NY) medium supplemented with 5% FCS with exogenous IL-4 (100 ng/ml) and GM-CSF (50 ng/ml) added every 2–3 days for 7 days. Highly purified autologous CD4+ T cells (>95%) were obtained by positive selection using CD4 magnetic beads and Detach-a-Bead (Dynal, Lake Success, NY) according to the manufacturer’s instructions.

Induction of chemokine production
DCs were harvested after 7 days’ culture in GM-CSF plus IL-4 and washed three times in HBSS. DCs (5 × 10⁵ cells/ml) were added to 24-well plates (Costar) in the presence or absence of CD40LT (2 μg/ml). Supernatants were collected at 48 h and stored at −70°C until used. It should be noted that endotoxin content in the CD40LT preparations was <5 endotoxin U/ml. In addition, to rule out an effect of LPS in the cultures, we demonstrated that CC-chemokine production was not diminished by addition of endotoxin. Moreover, it has been previously reported that activated fresh human monocytes stimulated with CD40L-transfected cells were induced to secrete CC-chemokines MIP-1α, MIP-1β, and RANTES (8). We sought to extend these findings by determining whether monocyte-derived DCs were also a potent source of CC-chemokines using CD40LT. To this end, fresh elutriated monocytes were stimulated for 7 days with GM-CSF plus IL-4, and cells were evaluated by surface staining to determine their phenotype. The starting population of monocytes (day 0) were CD14+ expression was abrogated in the presence of neutralizing Abs to CD40LT on DC maturation and expression of CCR5, elutriated monocytes were induced to constitutively express MIP-1α and MIP-1β but not RANTES when cultured in medium alone. Addition of CD40LT to cultures resulted in a 4-fold increase in production of MIP-1α (Fig. 1A) and a 2-fold increase in MIP-1β (Fig. 1B). Moreover, CD40LT stimulation induced substantial production of RANTES (Fig. 1C). Thus, CD40LT stimulation strikingly enhanced production of CC-chemokines from DCs.

CD40/CD40L stimulation down-regulates expression of CCR5 on DCs
In addition to the aforementioned effects of CD40LT stimulation on CC-chemokine production by DCs, CD40/CD40L stimulation has also been shown to induce DC maturation (3, 4). Moreover, it has been reported recently that down-regulation of CCR5 expression is coincident with phenotypic maturation of DCs after culturing in the presence of monocyte-conditioned medium (11). To determine the effect of stimulation with CD40LT on DC maturation and expression of CCR5, elutriated monocytes were first cultured for 7 days with GM-CSF plus IL-4 to generate DCs. Cells were then washed and cultured in the presence or absence of CD40LT for 48 h, at which time CCR5 and CD83 expression was determined by FACScan analysis. As shown in Fig. 2A, fresh monocytes constitutively express CCR5, which is diminished after 7 days of culture in the presence of GM-CSF plus IL-4. Addition of CD40LT to cultures resulted in a significant further down-regulation of CCR5 (Fig. 2A) while simultaneously enhancing the expression of CD83. Furthermore, the CD40LT-induced down-regulation of CCR5 expression was abrogated in the presence of neutralizing Abs to the CC-chemokines (Fig. 2B). It should be noted, however, that
neutralization of the endogenous CC-chemokines did not affect CD40LT enhancement of CD83 expression. Together, these data support a role for CD40LT stimulation in the differentiation of DCs (as assessed by CD83 expression) and show that its ability to induce CC-chemokines has a biologic effect in down-regulating expression of CCR5.

**FIGURE 1.** CD40LT enhances production of CC-chemokines from DCs. Elutriated monocytes were stimulated for 7 days with GM-CSF and IL-4, washed extensively, and placed in 24-well plates at a concentration of $5 \times 10^5$ cells/ml in the presence or absence of CD40LT (1 $\mu$g/ml). Supernatants were assayed 48 h later for CC-chemokines MIP-1$\alpha$ (A), MIP-1$\beta$ (B), and RANTES (C) by ELISA (limit of detection, 32 pg/ml). Results are represented as the mean $\pm$ SE of six separate experiments. Starred values indicate statistical significance compared with cells in medium alone.

**FIGURE 2.** CD40LT down-regulates CCR5 expression in DCs. A, Fresh elutriated monocytes were cultured in GM-CSF and IL-4 for 7 days, washed, and stimulated in the presence or absence of CD40LT ($1 \mu$g/ml) for an additional 48 h. B, In a separate experiment, CD40LT was added in the presence or absence of anti-CC-chemokine neutralizing Abs (anti-MIP-1$\alpha$, anti-MIP-1$\beta$, anti-RANTES; 50 $\mu$g/ml), and DCs were assessed for expression of CCR5 and CD83. Surface expression for CCR5 and CD83 was assessed by FACS analysis. Results are representative of six separate experiments. Values represent the percentage of positively staining cells.

The Journal of Immunology
CD40LT suppresses HIV replication in T cell/DC cocultures infected with M-tropic HIV but increases replication in cocultures infected with T-tropic virus

Because CD40LT stimulation resulted in a significant effect on CC-chemokine induction from DCs, we determined the functional role that CD40LT had in regulating HIV viral replication using an in vitro model. DCs were exposed to either HIV_{BaL} (M-tropic) or HIV_{IIIB} (T-tropic) strains, washed extensively, and placed in culture with or without autologous CD4^+ T cells (T/DC) in the presence (T/DC plus CD40LT) or absence of CD40LT. HIV-1 replication was assessed 7, 10, and 14 days later by RT activity. It should be noted that these conditions are sufficient to induce HIV replication in the absence of mitogen stimulation or addition of exogenous IL-2 (12). Cultures containing T/DC in which DCs were infected with HIV_{BaL} demonstrated a progressive increase in RT activity over the 14-day culture period (Fig. 3, A and B). Addition of exogenous CD40LT (T/DC plus CD40LT) reduced RT activity at all time points tested (Fig. 3, A and B). This suppression was abrogated by addition of a mixture of anti-CC-chemokine Abs. Furthermore, the addition of exogenous CC-chemokines to T/DC cultures (T/DC plus anti-CC-chemokine) that did not contain CD40LT reduced RT activity in a manner similar to that observed by stimulation with CD40LT (Fig. 3A). As a control, the addition of anti-CC-chemokines to T/DC cultures (T/DC plus anti-CC-chemokine) resulted in similar RT activity to that of T/DC alone (Fig. 3B). Together, these data strongly suggest that CD40LT induction of CC-chemokines from DCs was able to inhibit replication of HIV_{BaL} in CD4^+ T cells stimulated with infected DCs. Over seven independent experiments, the median inhibition in RT activity observed was 83% (range, 66–97%). To verify that CC-chemokines were induced in the T/DC cocultures, we measured the production of MIP-1\alpha, MIP-1\beta, and RANTES on days 7, 10, and 14. As shown in Table I, production of MIP-1\alpha and MIP-1\beta but not of RANTES was detected in the supernatants from the T/DC cocultures. Addition of exogenous CD40LT enhanced production of all three CC-chemokines, consistent with the data presented in Fig. 1.

In striking contrast to the aforementioned results, cocultures of T/DCs exposed to HIV_{IIIB} and stimulated with CD40LT (T/DC plus CD40LT) had an increased RT activity at all time points tested vs T/DC alone (Fig. 3C). Over seven independent experiments, the mean enhancement of RT activity was 2.5-fold (range, 1.5- to 4.0-fold). Of note, addition of anti-CC-chemokines to cultures containing CD40LT (T/DC plus CD40LT plus anti-CC-chemokines) did not abrogate the enhancement in RT activity. These data suggest that for HIV_{IIIB}, CD40LT can enhance replication.

### Table I. CC-chemokine induction in CD4^+ T/DC cocultures infected with HIV_{BaL}

<table>
<thead>
<tr>
<th>Exp. 1</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaL (CD4^+ T/DC)</td>
<td>2,200</td>
<td>1,960</td>
<td>1,000</td>
</tr>
<tr>
<td>MIP-1\alpha</td>
<td>3,780</td>
<td>4,200</td>
<td>2,400</td>
</tr>
<tr>
<td>MIP-1\beta</td>
<td>7,800</td>
<td>15,320</td>
<td>ND</td>
</tr>
<tr>
<td>RANTES</td>
<td>700</td>
<td>880</td>
<td>780</td>
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</table>

<table>
<thead>
<tr>
<th>Exp. 2</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaL (CD4^+ T/DC)</td>
<td>1,500</td>
<td>1,840</td>
<td>ND</td>
</tr>
<tr>
<td>MIP-1\alpha</td>
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<td>8,260</td>
<td>ND</td>
</tr>
<tr>
<td>MIP-1\beta</td>
<td>17,800</td>
<td>15,320</td>
<td>ND</td>
</tr>
<tr>
<td>RANTES</td>
<td>5,660</td>
<td>5,060</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Supernatants from T/DC cocultures (outlined in Fig. 3) were assayed for production of MIP-1\alpha, MIP-1\beta, or RANTES by ELISA on days 7, 10, and 14. Values shown are reported in pg/ml. The sensitivity of the assay was 32 pg/ml. Data are representative of five separate experiments.
independent of the CC-chemokines MIP-1α, MIP-1β, and RANTES. It should also be noted that the addition of endogenous CC-chemokines (T/DC plus CC-chemokines) also resulted in a modest increase in RT activity compared with T/DC alone. This observation was reproducible in all experiments. These latter data are consistent with recent reports showing that CC-chemokines can enhance T-tropic replication (13, 14). Together, these data suggest that CD40LT leads to the differential regulation of HIV-1 replication by reducing M-tropic but increasing T-tropic replication in a T/DC coculture system.

CD40LT diminishes M-tropic HIV replication in DC in a CC-chemokine-independent fashion

As a control in Fig. 3A, it was noted that RT activity from infected DC alone was suppressed by the addition of CD40LT to the cultures. Because DCs may serve as a reservoir for HIV, we further examined whether CD40LT alters HIV replication in DCs. Similar to the experiment illustrated in Fig. 3A, DCs were exposed to HIVBaL for 2 h, extensively washed, and then cultured in the presence or absence of CD40LT. As shown in Fig. 4A, the addition of CD40LT (DC plus CD40LT) markedly diminished RT activity at days 10 and 14 compared with DC-only cultures. Over six independent experiments, the median inhibition of HIVBaL RT activity was 84% (range, 70–93%). Moreover, addition of anti-CC-chemokine Abs did not significantly change RT activity in HIVBaL-infected DC cultures in any experiments, whether in the presence or absence of CD40LT (Fig. 4A). After extensive washing, DCs (10^7/200 μl) were cultured in 96-well plates in the presence or absence of CD40LT (1 μg/ml). B. Anti-CC-chemokine neutralizing Abs (anti-CC-chemokines; 50 μg/ml) were added to DC cultures in the presence or absence of CD40LT. RT activity was assessed on days 7, 10, and 14. The results in B are from the same normal donor/experiment as those shown in Fig. 3B. Data are representative of six independent experiments.

Discussion

The present study demonstrates that stimulation of DCs with CD40LT enhances CC-chemokine production, reduces expression of CCR5, and inhibits HIV replication in cocultures of stimulated DCs and CD4^+ T cells acutely infected with M-tropic HIV (HIVBaL). Of note is the fact that this inhibition of HIV replication occurred only with M-tropic HIV, while replication of T-tropic HIV (HIVinm) was actually enhanced under similar conditions. The reduced replication of M-tropic HIV in T/DC cocultures was dependent on the induction of CC-chemokines in DCs by stimulation with CD40LT. In contrast, the replication of M-tropic HIV in DCs themselves was inhibited by CD40LT in a CC-chemokine-independent manner.

CD40LT stimulation enhances production of CC-chemokines but diminishes CCR5 expression on DCs

In addition to its effects on enhancing production of CC-chemokines, this study shows that CD40LT stimulation causes a decrease in the expression of CCR5 on DCs. With regard to the effect of cytokines on CCR5 expression, a recent report by DiMarzio et al. (20) showed that GM-CSF caused a rapid decrease in CCR5 mRNA in monocytes that correlated with decreased viral entry. Furthermore, a recent report by Wang et al. (21) showed that fresh monocytes cultured with GM-CSF and IL-4 also had diminished CCR5 expression. It should be noted, however, that in this latter study (21), cells cultured in GM-CSF alone actually had an increase in CCR5 expression. Our study demonstrates that DCs generated from monocytes cultured in GM-CSF and IL-4 for 7 days had a marked reduction in CCR5 expression. Furthermore, stimulation with CD40LT caused a further down-regulation of CCR5 expression that was abrogated by the addition of anti-CC chemokine Abs to cultures. These data are consistent with a previous report showing rapid and extensive down-regulation and internalization of CCR5 by RANTES (22). Finally, it should be noted that while CD40LT stimulation diminished expression of CCR5, it also increased expression of CD83, a marker often used to characterize “mature” or more fully differentiated DCs (23). These data are consistent with recent work by Delgado et al. (11), who showed that CCR5 expression was strikingly reduced when immature DCs...
were further differentiated in monocyte-conditioned medium. Together, these studies suggest that CCR5 expression may vary in the course of DC differentiation.

The role of CD40/CD40L stimulation in the regulation of HIV infection by CD4+ T cells and DCs

In earlier studies examining the role of CD40/CD40L stimulation in HIV replication, Pichuk et al. (9) showed that cross-linking mAbs against CD40 increased DC transmission of HIV-1 to CD4+ T cells. This augmentation in HIV transmission was blocked by anti-CD80 Abs or a soluble fusion protein of the CD80 ligand, CTLA4Ig. It should be noted, however, that these studies used the T-tropic strain, HIV_LAI. Subsequently, Weissman et al. (24), using another T-tropic strain, HIV_RL, demonstrated that mature CD80- /CD83+ DCs were substantially more proficient at passing HIV-1 infection in vitro to CD4+ T cells as compared with immature DCs not expressing high levels of CD80/CD83 or with blood monocytes. Indeed, our results using exogenous CD40LT to stimulate DCs via CD40 support the notion that stimulation of DCs in this manner increases viral replication in T/DC cocultures infected with T-tropic HIV-1. Furthermore, the CD40LT-mediated enhancement of T-tropic replication appeared to be independent of CC-chemokines (Fig. 3C). This latter observation may be due to the fact that CD40LT enhances maturation of DCs and up-regulates expression of B7 costimulatory molecules (data not shown), thereby leading to enhanced CD4+ T cell activation. These observations underscore the important role of enhanced immune activation (25) in HIV-1 transmission, particularly in the setting of T-tropic infection, and suggest that the propagation of T-tropic strains may be more sensitive to higher levels of activation based on the variable regulation of CXCR4 vs CCR5 (26). In contrast to the results using T-tropic HIV-1, the ability of CD40LT to enhance production of CC-chemokine from DCs was shown to be functionally important using M-tropic HIV-1. In this regard, the presence of CD40LT in T/DC cocultures caused a striking reduction in RT activity that was abrogated by anti-CC-chemokine Abs. These data clearly demonstrate that for the M-tropic strain of HIV-1 (HIV_RL), the ability of CD40LT to inhibit viral replication is CC-chemokine dependent.

To conclude, in our T/DC coculture system, there is a dichotomy in the mechanism by which CD40LT stimulation differentially regulates HIV replication depending on the tropism of the virus. Thus, CD40LT stimulation causes an increase in T-tropic replication and a decrease in M-tropic replication. Moreover, the inhibition of M-tropic replication is CC-chemokine-dependent, while the enhancement of T-tropic replication is CC-chemokine-independent. With regard to this latter point, however, two recent reports have demonstrated that the presence of CC-chemokines enhanced T-tropic replication in T cells (13, 14). Thus, in the experiments reported here, while the presence of CD40LT in T/DC cocultures appeared to increase T-tropic replication in a CC-chemokine-independent manner (Fig. 3C), it is possible that under other experimental conditions, CD40LT-mediated enhancement of CC-chemokine production could also effect T-tropic replication.

CD40/CD40L stimulation enhances maturation of DCs and inhibits their ability to replicate HIV

In addition to the role of DCs in transmitting HIV-1 infection to CD4+ T cells, HIV-infected blood and skin DCs may represent an important viral reservoir in disease pathogenesis. There are several conflicting reports in the literature with regard to HIV-1 replication in DCs. Several earlier studies suggested that HIV-1 can replicate actively in DCs (27–29); by contrast, others have shown a relatively low level of infection in DCs (30). Moreover, this low level of infection occurred despite the efficient entry M-tropic and T-tropic strains (31). Some of these discrepancies, however, may be accounted for by differences in cell populations as well as by the methods of cell culture and ultimate level of maturation of the DCs examined. There is recent evidence that immature DCs derived from monocytes selectively replicate M-tropic but not T-tropic HIV-1 strains, whereas mature DCs do not support replication of either (15). Our results support a lack of efficient viral replication by T-tropic strains (HIV_RL) in immature or mature DCs in contrast to the M-tropic strain (HIV_RL), which readily replicates in immature DCs. The treatment of DC cultures with CD40LT after HIV-1 pulse infection markedly reduced the level of HIV_RL replication compared with immature DCs. Of interest, addition of anti-CC-chemokine Abs to DC cultures treated with CD40LT had no effect on viral replication. This was in marked contrast to the reversal of suppression seen in T/DC-infected cocultures within the same experiment. These data support a CC-chemokine-independent pathway whereby CD40LT stimulation reduces M-tropic HIV-1 viral replication in DCs.

With regard to the CC-chemokine-independent mechanism by which CD40LT inhibited viral replication in DC cultures, there remain several possibilities. First, CD40LT could limit viral replication in DCs through a decrease in viral entry. This is consistent with data shown in Fig. 2 in which CCR5 expression was markedly diminished on DCs cultured with CD40LT. However, in the experiments shown in Figs. 3 and 4, DCs were exposed to HIV before the addition of CD40LT. A second possibility is that the addition of CD40LT to DC cultures after infection could limit cell-to-cell spread by decreasing CCR5 expression. Because CD40LT stimulation diminished CCR5 expression through enhancement of CC-chemokines (Fig. 2), the data in Fig. 4 showing that the addition of anti-CC-chemokines to DC cultures stimulated with CD40LT did not affect viral replication makes this latter mechanism less likely.

CD40LT also induced high levels of expression of CD83, MHC class II, CD80, CD86, and ICAM-1 and a notable morphologic change in dendrite formation (data not shown), suggesting that CD40LT enhances DC maturation. Consequently, these mature DCs do not support a high level of HIV-1 infection following CD40 triggering, in contrast to their immature precursors. Thus, CD40 stimulation may be an important mechanism whereby M-tropic HIV-1 spread is limited within the DC reservoir. It should be noted that while CD40LT substantially limits HIV replication to a very low level within mature DCs themselves, these cells are still able to pass virus to CD4+ T cells. These data are consistent with several reports demonstrating that mature DCs, despite a very low level of endogenous infection, still retain the ability to effectively spread HIV-1 infection to CD4+ T cells (30, 32–35). Thus, the capacity of DCs to initiate and propagate HIV-1 infection in neighboring CD4+ T cells appears to be distinct from HIV-1 replication within the DC reservoir itself (35–37).

Therapeutic potential of CD40LT in HIV infection

CD40LT stimulation has differential effects on HIV-1 replication that vary according to viral tropism. CD40LT reduces M-tropic HIV-1 replication in CD4+ T cells but enhances T-tropic HIV-1 replication. While the protective effects from M-tropic HIV-1 on CD4+ T cells are mediated primarily through the induction of CC-chemokines from DCs, the reduction in M-tropic HIV-1 replication in DCs themselves appears to be a direct effect of CD40LT acting to induce DC maturation. This “antiviral” role of CD40LT stimulation, combined with recent studies showing an “immune enhancing” role of CD40/CD40L stimulation in restoring CTL activity in the absence of CD4+ T cell help (38–40), provides a
rationale for considering CD40LT as a therapeutic modality for HIV infection. Furthermore, CD40LT enhancement of DC function may increase CD8+ T cell activity, which could limit HIV replication through both CC-chemokine-dependent and -independent mechanisms (41, 42). One caveat, however, is that CD40LT therapy could alter or enhance HIV-1 viral selection toward a T-tropic predominance. The potential enhancement in CTL activity, however, combined with the availability of potent antiviral chemotherapy, may obviate this concern.

Acknowledgments

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References