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CXCR1 and CXCR2 Are Rapidly Down-Modulated by Bacterial Endotoxin Through a Unique Agonist-Independent, Tyrosine Kinase-Dependent Mechanism

Masud H. Khandaker,* Luoling Xu,† Rahbar Rahimpour,‡ Gordon Mitchell,§ Mark E. DeVries,** J. Geoffrey Pickering,‡ Sharwan K. Singhal,* Ross D. Feldman,† and David J. Kelvin2*‡

The expression of the seven-transmembrane domain chemokine receptors CXCR1 and CXCR2 modulates neutrophil responsiveness to the chemoattractant IL-8 and a number of closely related CXC chemokines. In the present study, we investigated the mechanism by which bacterial LPS induces the down-modulation of IL-8 responsiveness and CXCR1 and CXCR2 expression on human neutrophils. Treating neutrophils with LPS reduced IL-8R expression to 55 ± 5% of the control within 30 min and to 23 ± 2% within 1 h of stimulation. Furthermore, this down-modulation could not be attributed to increased concentrations of IL-8, TNF-α, or IL-1β, since ELISA studies indicated that LPS-stimulated neutrophils did not release detectable amounts of these proteins before 2 h poststimulation. The tyrosine kinase (TK) inhibitors genistein and herbimycin A attenuated the LPS-mediated down-modulation of CXCR1 and CXCR2, indicating that the activation of a TK is required for LPS to mediate its effect. The effect of LPS on receptor expression paralleled the hyperphosphorylation of the protein TK p72syk. Although IL-8 induced a comparable down-modulation of CXCR1 and CXCR2, TK inhibitors did not attenuate this effect. These studies provide the first evidence of an agonist-independent, TK-dependent pathway of chemokine receptor regulation by endotoxin.

Materials and Methods

Reagents

Escherichia coli LPS (055:B5) was purchased from Difco Laboratories (Detroit, MI). IL-8 and TNF-α were purchased from PeproTech (Rocky Hill, NJ). Genistein, staurosporine, and pertussis toxin (PT) were purchased from Sigma (St. Louis, MO). Herbimycin A and calphostin C were purchased from Calbiochem (La Jolla, CA). FITC-conjugated anti-CXCR1 and phyceroerythrin (PE)-conjugated anti-CXCR2 Abs were purchased from PharMingen (San Diego, CA). Agarose-conjugated phosphotyrosine mAb PY20 and anti-p72syk polyclonal Ab were purchased from Santa Cruz Biotechnologies (Santa Cruz, CA).

Isolation of leukocytes

Peripheral blood leukocytes that were enriched for mononuclear cells or granulocytes were obtained from healthy donors. Granulocytes were purified by dextran sedimentation, followed by Ficoll gradient centrifugation and hypotonic lysis of RBCs. PMNs were collected, washed in PBS, and resuspended at 5 x 10⁶/ml in RPMI 1640 supplemented with 10% FCS. The purity of the PMN preparations was judged to be >95% by morphologic criteria; the remaining cells were typically lymphocytes.

Measurement of CXCR1 and CXCR2 surface expression

Isolated neutrophils were preincubated in RPMI 1640 (10% FCS) at 37°C for 30 min followed by LPS, IL-8, or TNF-α stimulation. Cells were washed twice with PBS and then incubated with optimal concentrations of FITC-conjugated anti-CXCR1 or PE-conjugated anti-CXCR2 Abs for 1 h at 4°C. Subsequently, cells were washed with PBS and resuspended at 5 x 10⁶/ml for analysis on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Lysis software (Becton Dickinson) was used to acquire samples. CellQuest software (Becton Dickinson) was used to analyze electronically gated cell populations.

Measurement of IL-8, TNF-α, and IL-1β protein by ELISA

Isolated neutrophils cultured in RPMI 1640 (10% FCS) at 37°C were stimulated with LPS for 0, 0.5, 1, and 2 h at 37°C. Cells were centrifuged at 750 x g for 5 s, and the supernatant was removed. An additional centrifugation step removed any remaining cells. The isolated neutrophil supernatant was tested for IL-8, TNF-α, and IL-1β protein amounts according to the methods described in the Quantikine ELISA kit (R&D Systems, Minneapolis, MN).

Measurement of [Ca²⁺]ᵢ

The [Ca²⁺]ᵢ, in Indo-1-AM-loaded cells was monitored using a dual wavelength fluorometer (model RF-M2004, Photon Technology International, Indianapolis, IN). Human PMNs were incubated at a density of 1 x 10⁶/ml for 2.5 h at 37°C in medium containing 5 μM Indo-1-AM (Molecular Probes, Eugene, OR). The cells were then washed once with RPMI 1640 and resuspended in HBSS containing Ca²⁺ (1 mM). The [Ca²⁺]ᵢ, in Indo-1-loaded cells was measured with an excitation wavelength at 340 nm and an emission wavelength at 405 and 485 nm to detect bound and free Indo-1, respectively.

Western blotting analysis

Purified peripheral blood PMNs were stimulated with increasing doses of LPS for 10 min at 37°C. Cells were then lysed in buffer containing 1% Nonidet P-40 and protease inhibitors. After immunoprecipitation with agarose-conjugated phosphotyrosine mAb PY20 (Santa Cruz Biotechnologies), samples were reduced and loaded onto 10% SDS-polyacrylamide gels, separated, and transferred electrophoretically to polyvinylidene difluoride membranes. Membranes were probed with phosphotyrosine mAb 4G10 (Upstate Biotechnology, Lake Placid, NY), and autoradiography was performed using enhanced chemiluminescence reagents (Amersham, Cleveland, OH). After stripping the same membrane of 4G10, the membrane was reblotted with Ab to p72syk protein (Santa Cruz Biotechnologies). Autoradiography was performed as described above.

Results

LPS stimulation rapidly down-regulates CXCR1 and CXCR2 on human neutrophils

Previously, we have demonstrated that stimulating human neutrophils with LPS resulted in a decrease in 125I IL-8 cell surface binding (25). To determine whether this change in binding was accompanied by a decrease in cell surface receptor expression, we explored whether LPS stimulation results in a decrease in the immunofluorescent staining of cell surface CXCR1 and CXCR2 on human neutrophils. Figure 1 illustrates the rate at which LPS treatment mediates CXCR1 and CXCR2 down-modulation as assessed by staining with FITC- and PE-labeled Abs to CXCR1 and CXCR2. Stimulating neutrophils with LPS (100 ng/ml) for 30 min resulted in a 45 ± 5.1% reduction in fluorescence intensity for CXCR1 staining. LPS treatment for 1 h resulted in a maximal loss of CXCR1 staining, as the mean fluorescence intensity (MFI) dropped to 23 ± 2.2% of untreated control neutrophils. No recovery of CXCR1 staining was seen for up to 3 h following LPS stimulation. Over a similar time course, LPS mediated a decrease in immunofluorescent staining for CXCR2 that was comparable with that found for CXCR1 (Fig. 1B). Thus, these initial studies confirmed that the previously observed LPS-induced down-modulation of 125I IL-8 binding is due to the loss of cell surface CXCR1 and CXCR2.

LPS-stimulated neutrophils secreted little or no detectable levels of IL-8, TNF-α, or IL-1β protein before 2 h poststimulation

Next, we investigated whether the down-modulation of CXCR1 and CXCR2 was due to an autocrine mechanism. The basis for these experiments is that previous reports have shown that LPS can induce the expression of a variety of cytokines, including IL-8 (26); the increased expression of IL-8 could account for the down-modulation of CXCR1 and CXCR2 through rapid receptor internalization (22, 23). To determine whether this autocrine mechanism could account for CXCR down-modulation, we examined the time course for the LPS-stimulated production of IL-8, TNF-α, and IL-1β (Fig. 2, A–C). No alterations in the basal levels of IL-8, TNF-α, and IL-1β were observed.
TK inhibitors prevent LPS-mediated down-modulation of CXCR1 and CXCR2 expression

In an attempt to identify the molecular basis for the CXCR1- and CXCR2-induced down-modulation, we subsequently explored the signaling pathways involved in this process using a variety of inhibitors. Tables I and II summarize the data on the effect of tyrosine and serine/threonine kinase inhibitors and PT on the LPS-induced down-modulation of CXCR1 and CXCR2. As indicated, genistein and herbimycin A were effective in attenuating the LPS-induced down-modulation of CXCR1 and CXCR2. As indicated, genistein and herbimycin A were effective in attenuating the LPS-induced down-modulation of CXCR1 and CXCR2, while staurosporine attenuated only CXCR1 down-modulation. Calphostin C, an inhibitor of protein kinase C, was unable to block the LPS-induced down-modulation of CXCR1 and CXCR2, while staurosporine attenuated only CXCR1 down-modulation. Calphostin C, an inhibitor of protein kinase C, was unable to block the LPS-induced down-modulation of CXCR1 and CXCR2.

The TK inhibitor genistein prevents TNF-α- but not IL-8-stimulated down-modulation of CXCR1 and CXCR2

To demonstrate the extent of the generalization of the TK-dependent mechanism of CXCR down-modulation, we compared the effect of LPS with that of IL-8 and TNF-α, which mediated a comparable down-modulation of CXCR1 and CXCR2 expression. IL-8 (500 ng/ml) induced a rapid decrease in the staining of CXCR1 and CXCR2 (Fig. 4A). However, unlike LPS, the IL-8-induced down-modulation of CXCR1 and CXCR2 was not attenuated by the TK inhibitor genistein (Fig. 4C). These results indicate that LPS and IL-8 stimulate down-modulation through different mechanisms. TNF-α induces CXCR1 and CXCR2 down-modulation over a time course that is comparable with that induced by LPS (Fig. 4B). In addition, the TNF-α-induced down-modulation of CXCR1 and CXCR2 was blocked by genistein, which was similar to LPS but in contrast to IL-8 stimulation (Fig. 4D). These results indicate that like LPS, the TNF-α-mediated down-regulation of IL-8Rs requires the activity of TKs; however, the IL-8-mediated down-regulation of IL-8Rs is independent of TK activity.

LPS stimulates hyperphosphorylation of tyrosine residues on a 70- to 75-kDa protein identified as p72syk

Based on the results of the TK inhibitor experiments on CXCR1 and CXCR2 expression, we subsequently investigated which specific tyrosine phosphorylation events might be triggered by LPS. Neutrophils were stimulated for 10 min with increasing doses of
LPS or genistein plus LPS; next, cells were lysed, and tyrosine-phosphorylated proteins were immunoprecipitated with PY20 Ab and resolved by SDS-PAGE (Fig. 5A). Following transfer to an immobilon membrane, tyrosine-phosphorylated proteins were detected by immunoblotting with antiphosphotyrosine mAb (4G10). Tyrosine phosphorylation of several proteins was induced by LPS at concentrations of 100 and 500 ng/ml, with the most striking increase in tyrosine phosphorylation observed for a protein with an apparent molecular mass of 70 to 75 kDa. Anti-p72syk was used to reprobe the membrane after it had been stripped of 4G10. A comparison of the 4G10 and anti-p72syk immunoblots demonstrated that LPS stimulation of phosphorylated p72syk (Fig. 5B) overlapped perfectly with the hyperphosphorylated protein that was detected just above the 68-kDa marker (Fig. 5A). In addition, genistein was able to inhibit the LPS-induced tyrosine phosphorylation of several substrates, including p72syk.

**TK inhibitors preserve G protein signaling in LPS-treated neutrophils**

Previously, we have demonstrated that endotoxin treatment of neutrophils resulted in an inhibition of IL-8-induced neutrophil chemotaxis (25), suggesting that CXCR1R and CXCR2R down-modulation was the molecular cause of IL-8 hyporesponsiveness. To determine whether blocking LPS-induced CXCR1R and CXCR2R down-modulation with TK inhibitors could also preserve IL-8-induced signaling, we examined the effect of TK inhibitors on the levels of [Ca^{2+}]_{i} in IL-8-stimulated neutrophils that had been treated with LPS. Following treatment, neutrophils were stimulated with 250, 125, or 50 ng/ml of IL-8, and the rise in [Ca^{2+}]_{i} was detected. Treating neutrophils with 100 ng/ml of LPS resulted in an IL-8 hyporesponsive state, wherein LPS-treated cells showed minimal or no rise in [Ca^{2+}]_{i}, which was detected. Neutrophils with 100 ng/ml of LPS showed minimal or no rise in [Ca^{2+}]_{i} when stimulated with increasing doses of IL-8 (Fig. 6). However, pretreating neutrophils with genistein or herbimycin A blocked the LPS-mediated IL-8 hyporesponsiveness, although genistein was more effective than herbimycin A (Fig. 6, A and B vs C). These results indicate that the cell surface signals transduced by CXCR1 and CXCR2Rs are functionally preserved in LPS-stimulated cells that have been treated with TK inhibitors.

**Discussion**

Recent studies have suggested that the chemokine receptors CXCR1 and CXCR2 are regulated by agonist-dependent mechanisms. IL-8 binding rapidly down-modulates CXCR1 and CXCR2 due to the internalization of the ligand-receptor complex (22, 23), and continuous stimulation leads to receptor desensitization. There is evidence that the carboxyl terminal domain of CXCR1 and CXCR2 is involved in IL-8-mediated receptor desensitization, signaling, and internalization. We demonstrated previously (27) that truncation of the C terminus of CXCR2 in transfected 293 cells resulted in the loss of IL-8-dependent migration. In addition, Mueller et al. (28) mutated multiple serine residues in the C terminus of CXCR2 and demonstrated their involvement in the regulation of ligand-induced desensitization. Studies by Prado et al. (29) showed the importance of the carboxyl1 terminus of both CXCR1 and CXCR2 in receptor internalization.

In the present work, we conclude that bacterial endotoxin rapidly down-modulates the intact cell surface expression of CXCR1 and CXCR2 by a mechanism that is both independent and distinct from agonist-mediated internalization. This conclusion is based on three observations. First, the LPS-induced loss of CXCR1 and CXCR2 expression is rapid and maximal by 1 h (Fig. 1); however, this time course is not as rapid as the ligand-induced loss of receptor expression, which can be detected by 5 min and is maximal by 15 min (Fig. 4A). Moreover, the histogram profiles of surface receptor fluorescence intensity qualitatively suggest a difference in LPS-induced (Fig. 3) vs IL-8-induced (Fig. 4C) down-modulation; IL-8 consistently appears to cause a uniform attenuation of CXCR expression among the total neutrophil population, which is in contrast to the nonuniform-mediated down-modulation that was observed for LPS. Second, although activated neutrophils produce IL-8 and the proinflammatory cytokines TNF-α and IL-1β, this production cannot account for the loss of CXCR1 and CXCR2 expression induced by LPS. Our conclusion is derived from three main observations: 1) LPS stimulated neutrophils did not secrete detectable levels of IL-8, TNF-α, or IL-1β (Fig. 2) before 2 h of stimulation; 2) Prior studies have shown that IL-8, TNF-α, and IL-1β mRNA levels are not detectable until 1 h following LPS stimulation and are maximal at 2 to 4 h poststimulation (30, 31); however, we have observed a 45 ± 5% decrease in CXCR1 and CXCR2 cell surface expression by 30 min after LPS stimulation (Fig. 1). 3) Cycloheximide pretreatment did not inhibit the LPS-mediated down-modulation of CXCR1 and CXCR2 (data not shown), indicating that de novo protein synthesis is not required for receptor down-modulation by LPS. Taken together, these three observations preclude the possibility of agonist-stimulated CXCR1 and CXCR2 chemokine receptor down-modulation by LPS. Finally, TK inhibitors can attenuate the LPS-stimulated (Fig. 3) but not the IL-8-stimulated (Fig. 4C) down-modulation of CXCR1 and CXCR2 chemokine receptors.

The molecular mechanism of the endotoxin-mediated down-modulation of chemokine receptors is poorly understood. Previously, we have shown that LPS treatment regulated the steady-state mRNA levels of IL-8Rs by rapidly degrading IL-8R mRNA.

**Table II. **Effect of various kinase inhibitors on the LPS-induced down-modulation of CXCR2 expression on human neutrophils

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Unstained MFI</th>
<th>Neutrophils Only MFI</th>
<th>+ LPS MFI</th>
<th>+ Inhibitor MFI</th>
<th>+ Inhibitor + LPS MFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genistein</td>
<td>5.46 ± 0.28^a</td>
<td>58.53 ± 15.09</td>
<td>25.25 ± 6.37</td>
<td>49.81 ± 19.07</td>
<td>42.52 ± 11.74^c</td>
</tr>
<tr>
<td>Herbimycin A</td>
<td>5.39 ± 0.15</td>
<td>41.60 ± 2.57</td>
<td>16.19 ± 1.78</td>
<td>43.18 ± 5.35</td>
<td>25.26 ± 5.66^e</td>
</tr>
<tr>
<td>Stauorosporine</td>
<td>5.49 ± 0.63</td>
<td>43.18 ± 14.69</td>
<td>21.23 ± 3.49</td>
<td>39.31 ± 17.44</td>
<td>26.06 ± 7.84</td>
</tr>
<tr>
<td>Calphostin C</td>
<td>5.13 ± 0.04</td>
<td>67.57 ± 21.47</td>
<td>21.49 ± 3.71</td>
<td>41.00 ± 24.65</td>
<td>17.51 ± 8.22</td>
</tr>
<tr>
<td>PT</td>
<td>5.28 ± 0.08</td>
<td>54.13 ± 20.23</td>
<td>19.72 ± 3.11</td>
<td>51.10 ± 15.04</td>
<td>19.82 ± 5.33</td>
</tr>
</tbody>
</table>

^a MFI values were acquired using FACSscan and calculated using Lysis software.

^b Neutrophils were pretreated for 30 min with inhibitors at the following concentrations: genistein, 10 μM; herbimycin, 5 μM; stauorosporine, 50 μM; calphostin C, 300 nM; or PT, 1000 ng/ml.

^c Average of MFI values for n = 3 experiments ± SEM.

^d Statistical significance using one-way ANOVA for inhibitor + LPS vs LPS alone: p < 0.05.
and inhibiting transcription (25). Similar studies on monocytes by us (32) and Sica et al. (33) have demonstrated that LPS causes a reduction in CCR2 and, to a lesser extent, CCR1 and CCR5 mRNA levels. In these studies, chemokine receptor mRNA levels did not change significantly during the first hour of LPS stimulation; levels then degraded rapidly over the following 2 to 4 h. However, we have observed that even at 2 h following cycloheximide treatment alone, CXCR1 and CXCR2 cell surface expression was only slightly reduced (data not shown), suggesting a relatively stable cell surface expression of IL-8Rs. Thus, even though

**FIGURE 3.** Effect of various inhibitors on the LPS-induced down-modulation of CXCR1 and CXCR2. Purified peripheral blood PMNs were preincubated with genistein (10 μM) (A), herbimycin A (5 μM) (B), staurosporine (Stauro) (50 μM) (C), or PT (1000 ng/ml) (D) for 30 min in media (RPMI 1640/10% FCS) followed by the addition of LPS (100 ng/ml) for 2 h at 37°C. The x-axis indicates the fluorescence intensity as measured on a log10 scale, and the y-axis indicates event counts per channel on a linear scale. MFI values for individual histograms are indicated above each histogram.
FIGURE 4. Effect of IL-8 and TNF-α stimulation on CXCR1 and CXCR2 expression. Purified peripheral blood PMNs were incubated in media (RPMI 1640/10% FCS) for 0 to 3 h in the presence or absence of IL-8 (500 ng/ml) (A) or TNF-α (50 ng/ml) (B). PMNs were preincubated with genistein (10 μM) for 30 min, and IL-8 (500 ng/ml) (C) or TNF-α (50 ng/ml) (D) was added. Cells were then incubated for 1 h at 37°C, and CXCR1 and CXCR2 expression was measured cytofluorometrically. MFI was measured on a log_{10} scale.
the loss of IL-8R mRNA may ultimately lead to an inhibition of the de novo synthesis of CXCR1 and CXCR2 at timepoints beyond 2 h, it is unlikely that this is the mechanism responsible for the rapid down-modulation of CXCR1 and CXCR2 by LPS that is observed by 0.5 h.

In the studies reported here, we examined the role of kinases in the endotoxin-mediated regulation of CXC chemokine receptors. CXCR1- and CXCR2-induced down-modulation by LPS could be substantially reversed by the TK inhibitors genistein and herbimycin A, while CXCR1 down-modulation was significantly inhibited by staurosporine. In contrast, genistein did not attenuate the IL-8-mediated down-modulation of CXCR1 or CXCR2. The protein kinase C inhibitor, calphostin C, and the Gi signaling inhibitor, PT, had no effect on the LPS-mediated down-modulation of CXCR1 or CXCR2. These data suggest that TK-dependent and -independent pathways regulate CXCR1 and CXCR2 cell surface expression.

The stimulation of various cell types, including monocytes and neutrophils, by LPS induces hyperphosphorylation and the activation of several TKs. Specifically, the Src-related TKs p59hck, p53–56lyn, and p58fgr have all been shown to be activated by stimulation with LPS (34–36). However, Meng et al. (34) have shown that hck, fgr, and lyn are not essential for LPS-induced macrophage activation and signal transduction by generating null mutations of all three kinases. We found that LPS could stimulate the phosphorylation of a 70 to 75 kDa protein that was identified as the nonreceptor protein TK p72syk. Syk is activated by several classes of receptors, including the B cell receptor (37–39), the IgER (40–42), the FcγRII (43), as well as receptors for IL-2 (44) and integrins (45, 46). Syk is also activated by G protein-coupled receptors (47, 48) and has been shown to be involved in nonclathrin-mediated pathways of endocytosis (49). Endotoxin stimulation has also been observed to be associated with the tyrosine phosphorylation of syk in monocytes (50). It is clear that the mechanism of CXCR1 and CXCR2 down-modulation by LPS stimulation involves the activation of TKs, and p72syk may represent one of the potential candidate TKs that regulates chemokine receptor expression in neutrophils.

The binding of intact, functional IL-8Rs by IL-8 triggers G protein signaling, Ca2+ mobilization, chemotaxis, granule exocytosis, and respiratory burst (51–54). We have demonstrated, as have others (13), that the stimulation of neutrophils with IL-8 induces an immediate release of Ca2+ from internal stores. In contrast, LPS-stimulated neutrophils abrogate IL-8-stimulated Ca2+ release (Fig. 6). These results indicate the loss of functional CXCR1 and

![Image](http://www.jimmunol.org/)

**FIGURE 5.** LPS stimulation augments the tyrosine phosphorylation of a 70- to 75-kDa protein identified as the protein TK p72syk. Purified peripheral blood PMNs were stimulated with increasing doses of LPS (left panel) or with 100 ng/ml of LPS in the presence or absence of 10 μM genistein (Gen) (right panel) for 10 min at 37°C. Subsequently, cells were lysed in buffer containing 1% Nonidet P-40, and protease inhibitors and phosphotyrosine proteins were immunoprecipitated with mAb PY20. A indicates membranes blotted with phosphotyrosine mAb 4G10. B indicates membranes reblotted with Ab to p72syk.

![Image](http://www.jimmunol.org/)

**FIGURE 6.** TK inhibitors preserve LPS-treated neutrophil responsiveness to IL-8. Purified peripheral blood PMNs that had been suspended in Indo-1-AM medium were preincubated with genistein (10 μM) or herbimycin A (5 μM) for 30 min at 37°C followed by the addition of LPS (100 ng/ml). The cells were then incubated for an additional 2 h at 37°C. Increasing doses of IL-8 were added to cells, and the Ca2+ efflux was then measured.
CXCR2 signaling. Both genistein and herbimycin A could restore levels of intracellular Ca\(^{2+}\) mobilization and G protein signaling upon IL-8 stimulation in neutrophils that had been treated with LPS. These data again indicate that TK inhibitors can prevent the LPS-induced down-modulation of functional IL-8Rs from the cell surface.

TNF-\(\alpha\) stimulation also down-modulated CXCR1 and CXCR2 expression in a manner that was apparently similar to the LPS-induced down-modulation. Interestingly, the time course and histogram profiles of the LPS- and TNF-\(\alpha\)-mediated down-modulation of both CXCR1 and CXCR2 show remarkably similar patterns. LPS and TNF-\(\alpha\) both induce maximal IL-8R down-modulation by 1 h poststimulation. As with LPS, the TK inhibitor genistein also blocked the TNF-\(\alpha\)-mediated down-modulation of CXCR1 and CXCR2. Thus, it is likely that LPS and TNF-\(\alpha\) regulate CXCR1 and CXCR2 chemokine receptor expression through similar or identical pathways.

Our findings identify the existence of two separate pathways for CXC chemokine receptor regulation. It is possible that the IL-8 and endothelin-induced down-modulation of CXCR1 and CXCR2 occur through different pathways of receptor internalization. Entry into the cell is mediated by various mechanisms, including clathrin-coated pits, caveolae, and phagocytic vesicles (55). Different pathways for the internalization of G protein-coupled receptors have been observed. The adrenergic (56, 57) and thrombin (58) receptors are internalized by the classical endocytic pathway through clathrin-coated pits. Other G protein-coupled receptors, such as angiotensin II type 1AR, are internalized through nonclathrin-coated vesicles (59). Glycosylphosphatidylinositol-anchored proteins (60) and TKs (61) have been found to be associated with caveolae or plasmalemmal vesicles, which are large detergent-insoluble membrane complexes. At this time, we have not determined whether internalization is important for the endothelin-induced down-modulation of CXC chemokine receptors.

Another possible mechanism of LPS- and TNF-\(\alpha\)-induced down-modulation is through the activation of a protease. Recently, metalloproteases have been found to be involved in the down-modulation and cleavage of several receptors, including CD16 (62), CD43, CD44 (63), L-selectin (64), and TNF-\(\alpha\) (65, 66). In addition, it has been suggested that a Ca\(^{2+}\)-dependent aminopeptidase is involved in the LPS-mediated inhibition of IL-8 binding (67). Preliminary work in our laboratory suggests that the LPS-induced down-modulation of CXCR1 and CXCR2 is dependent upon metalloprotease degradation of the receptors. However, we have not yet determined whether this event occurs at the cell surface or within an internalized compartment. Hence, further studies are required to elucidate the precise mechanism of chemokine receptor regulation by LPS.

The identification of a novel regulatory pathway that can markedly and rapidly alter surface chemokine receptor expression raises interesting issues related to the ability of microbial organisms to modulate the immune system. A pathway involving agonist-independent, TK-dependent regulation of CXCR1 and CXCR2 by bacterial endotoxin may represent a common strategy entailing the regulation of various chemokine receptors; such a pathway provides new insight with regard to the involvement of chemokines and chemokine receptors in viral and bacterial pathogenesis.

Acknowledgments

We thank Joseph Andrews, Dr. Longsi Ran, and Anne Leaist for their technical assistance and Dr. Bruce Gill for his critical review of this manuscript.

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