Reduced Expression of Interleukin-8 Receptors A and B on Polymorphonuclear Neutrophils from Persons with Human Immunodeficiency Virus Type 1 Disease and Pulmonary Tuberculosis

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The expression of the two human interleukin (IL)-8 receptors, designated IL-8RA (CXCR-1) and IL-8RB (CXCR-2), on the surface of whole blood polymorphonuclear leukocytes (PMNL) was determined by use of receptor-specific monoclonal antibodies and flow cytometry. Sixteen subjects each were included in 4 study groups: healthy blood donors (ND), patients with pulmonary tuberculosis (TB), human immunodeficiency virus type 1–seropositive patients (HIV), and HIV-1–seropositive subjects with pulmonary tuberculosis (HIV/TB). A significant reduction in the percentage of PMNL expressing IL-8RA and IL-8RB and in their respective fluorescence intensities was found in TB, HIV, and HIV/TB groups compared with that obtained for the ND group. The greatest down-regulation of both receptors occurred in the HIV/TB group. Furthermore, associated with this reduced expression of IL-8 receptors was impairment of both intracellular calcium flux and migration of PMNL in response to IL-8 in a group of HIV/TB patients compared with that in healthy persons.

The chemokines are a family of secreted proteins that mediate a variety of inflammatory processes, including activation and chemotaxis of neutrophils, monocytes, and lymphocytes to sites of infection [1]. Members of this family share a highly conserved structural motif of four cysteine residues, the arrangement of which forms the basis of dividing the family into two subfamilies. In the first subfamily, the first two cysteine residues are adjacent (C-C), whereas in the second subfamily, the first cysteine pair is separated by an intervening amino acid (C-X-C). A third subfamily, subsequently described, is known as the C-chemokine subfamily and has only one member, lymphotactin, described to date [2].

The C-X-C chemokines, which act predominantly on neutrophils, can be further subdivided into ELR+ and ELR- groups, on the basis of the presence or absence of a Glu-Leu-Arg (ELR) sequence motif in the adjacent position N-terminal to the C-X-C group [3]. Interleukin-8 (IL-8), a member of the ELR+ group and the best-characterized of the C-X-C chemokines, is an 8-kDa polypeptide produced by a number of cell types [4], although the most prominent source is monocytes/macrophages [5]. Biologic actions of IL-8 on neutrophils include the induction of respiratory burst and of chemotaxis [6], promoting release of lysosomal enzymes, and enhanced killing of various microorganisms, including Mycobacterium tuberculosis [4].

Studies have identified two types of IL-8 receptors, namely IL-8RA (CXCR-1) [7] and IL-8RB (CXCR-2) [8], which share 77% amino acid identity and have a 29%–34% sequence homology with the C5a and FMLP receptors [7]. Both receptors are members of the superfamily of seven transmembrane G protein–coupled receptors [9]. IL-8 has been shown to bind with high affinity (0.5–3 nM) to both receptors, whereas all of the other C-X-C chemokines have a high affinity for IL-8RB only [10, 11]. Furthermore, the ELR+ C-X-C chemokines growth-regulated oncogene (GRO)α, GROβ, GROγ, neutrophil-activating peptide-2 (NAP-2), and epithelial cell–derived neutrophil-activating peptide-78 (ENA-78) are potent agonists for IL-8RB but not IL-8RA [12].

Chuntharapai et al. [13] found that the receptor for IL-8 is present on all polymorphonuclear leukocytes (PMNL) and monocytes and 5%–25% of total lymphocytes. The highest levels of IL-8RA and IL-8RB are found on PMNL, with IL-8RA and IL-8RB expression occurring at an approximately equal ratio. On monocytes and IL-8 receptor–positive lymphocytes, higher levels of IL-8RB than of IL-8RA are expressed. Within the IL-8 receptor–positive lymphocyte population, 7%–42% of these cells were CD8 T cells and 39%–76% were CD56 NK cells.

Studies with monoclonal antibodies (MAbs) raised against each of the IL-8 receptors of human neutrophils have indicated that IL-8RA and IL-8RB are functionally different [14]. Neutrophil responses, such as the release of granule enzymes and changes in cytosolic free Ca2+ [14] and chemotaxis [15], are mediated by both IL-8RA and IL-8RB. The activation of phospholipase D and respiratory burst via NADPH oxidase are, however, triggered only through IL-8RA but not IL-8RB [14].
Infection with human immunodeficiency virus (HIV)-1 has been shown to be the largest known risk factor for the development of tuberculosis [16], which is typically an early complication of HIV infection, occurring before AIDS-defining illness in 50%–67% of HIV-infected patients [17]. In addition, infection with M. tuberculosis promotes the progression of HIV-1 disease [18, 19]. Studies have shown that in active tuberculosis, patients who are coinfected with HIV-1 have a reduced survival rate compared with that of patients without HIV infection [20, 21], and dually infected persons are at increased risk of acquiring new secondary infections compared with patients infected with HIV-1 alone [22].

Monitoring of IL-8 levels has been shown to be of clinical prognostic significance in patients with tuberculosis [23]. Levels of this chemokine are also known to be raised in the serum of HIV-1–infected persons [24], suggestive of a role in HIV-1 disease pathogenesis. Insight into what role IL-8 may play in pathogenesis of either disease state can, in part, be gleaned from evaluating the distribution and expression of IL-8–specific receptors on immune cells. PMNL, which are the major cell type involved in host defense against primary or secondary bacterial or fungal infection, have a substantial dependence on IL-8 for their directed migration to sites of inflammation and, on arrival, for a wide range of their effector antimicrobial functions. The purpose of this study was therefore to determine the effect of HIV-1 infection, pulmonary tuberculosis, and coinfection of HIV-1 and M. tuberculosis on the expression of the C-X-C chemokine receptors IL-8RA and IL-8RB on human PMNL. We then analyzed cellular events following the binding of IL-8 to IL-8 receptors on PMNL isolated from coinfected patients.

Materials and Methods

Subjects. Four subject groups were recruited for IL-8 receptor studies and included 16 healthy volunteers as controls (normal donors [ND]), 16 patients with pulmonary tuberculosis (TB), 16 HIV-1–seropositive patients (HIV), and 16 persons infected with HIV-1 and M. tuberculosis (HIV/TB). The immunologic characteristics of these subjects are shown in table 1. A further 11 ND and 25 HIV/TB subjects were recruited for studies on PMNL function. All TB and HIV/TB patients were receiving standard four-drug antituberculosis therapy: rifampin, isoniazid, pyrazinamide, and ethambutol. At the time of sampling, patients were receiving no other medication. Blood was collected by venipuncture into EDTA Vacutainers (Becton Dickinson, San Jose, CA). The blood was processed immediately for assays of PMNL function and analyzed by flow cytometry within 6 h of collection.

Reagents. Mouse MAbs to IL-8RA (9H1) and IL-8RB (10H2) were supplied by Genentech (San Francisco) [13]. Mouse isotype antibodies IgG1 and IgG2a from Serotec (Oxford, UK) were used as controls for IL-8RA and IL-8RB, respectively. Secondary antibody was fluorescein isothiocyanate–conjugated goat anti-mouse (GAM-FITC) obtained from Dako (Copenhagen). FACS lysing solution (10× concentration) was from Becton Dickinson. The cell fixative was 1.5% (vol/vol) formaldehyde (Merck, Darmstadt, Germany) with 2% (wt/vol) bovine serum albumin (Sigma, St. Louis). Fura-2–acetoxyethyl ester (Fura-2/AM) and IL-8 were from Boehringer Mannheim (Mannheim, Germany).

Flow cytometry. A flow cytometer (FACSort; Becton Dickinson) with a 488-nm argon laser was used for all analyses. Forward (FSC) and side light scatter (SSC) characteristics were used in gating the granulocyte population. The data were analyzed by use of Cellquest version 1.0 software (Becton Dickinson) and expressed as the percentage of cells expressing IL-8RA or IL-8RB and their respective fluorescence intensities or median channel concentrations of IL-8 receptor antibodies minus the median channel number of the corresponding isotype antibody control sample). For comparison, lymphocytes were gated using FSC and SSC on the same samples, and the proportion of these cells expressing either IL-8 receptor was determined.

Isolation of PMNL. PMNL were isolated from anticoagulated peripheral blood by centrifugation on a double-layered Histopaque-Ficoll (Sigma, Oxford, UK) gradient. Residual erythrocytes were lysed with a solution of 0.15 M NH4Cl, 10 mM KHCO3, and 1 mM sodium-EDTA, pH 7.2, and PMNL were washed twice with PBS. Purified PMNL suspensions were >98% viable as determined by trypan blue exclusion.

Calcium mobilization. PMNL were resuspended at 107 cells/mL in Ca2+- and Mg2+-free Hanks’ buffered saline solution (HBSS), pH 7.4, containing 2.5 μM Fura-2/AM, and incubated for 30 min at 37°C in the dark. The cells were washed twice and then resuspended in HBSS at 2 × 106 cells/mL. PMNL suspension (2 mL) was placed in a cuvette in a luminescence spectrometer (LS50; Perkin-Elmer Cetus, Norwalk, CT) and fluorescence (F) was monitored at excitation wavelengths of 340 nm and 380 nm and an emission wavelength of 510 nm (Fluorescence Data Manager software; Perkin-Elmer Cetus). After the determination of the basal [Ca2+]i, expressed as a ratio (Rb = F340 nm/F380 nm), IL-8 was then added to the cuvette to give a final concentration of 100 ng/mL, and the fluorescence shift was recorded (RIL-8). As controls for each sample, the maximum (Rmax) and minimum (Rmin) amounts of intracellular calcium available were determined by lysing PMNL with Triton X-100 (0.1% vol/vol final) and adding EGTA (1 mM final), respectively. Data are expressed as stimulation indices: the fluorescence ratio due to IL-8 (RIL-8) divided by that for resting unstimulated PMNL (Rb).
**Chemotaxis assay.** PMNL chemotaxis through polycarbonate filters (not treated for tissue culture) with a diameter of 6.5 mm and a pore size of 3 μm was monitored by use of 24-well plates (Transwell; Corning Costar, Badhoevedorp, Netherlands). IL-8 in 500 μL (100 ng/mL) of RPMI 1640 medium containing 10% fetal calf serum (FCS) was placed in the lower chamber (below the filter). Controls wells received medium without IL-8. Purified PMNL, resuspended at 5 × 10⁶/mL in RPMI medium containing 10% FCS, were added to the top chambers in 100-μL volumes. Control and test experiments were done in duplicate for each subject. The plates were incubated at 37°C in 5% CO₂ for 60 min in a humidified chamber. After incubation, PMNL migrating randomly (control) or directly in response to IL-8 were counted by trypan blue exclusion. PMNL viability remained unchanged over the duration of the assay.

**Statistical analysis.** Statgraphics software (STSC, Rockville, MD) was used in all of the statistical analyses. Comparison of IL-8RA and IL-8RB percentages, relative fluorescence intensities, calcium stimulation indices, chemotactic indices, and various immunologic parameters between the groups was done by use of the Mann-Whitney U test. Correlation coefficients comparing data within groups were determined by Spearman rank correlations.

**Results**

**Immunologic status of study groups.** Significant differences were observed between the total white blood cell counts of the TB group and those of the ND, HIV, and HIV/TB groups (P < .001) (table 1). Significant differences in absolute PMNL counts were found between ND and TB (P < .001), TB and HIV (P < .001), and TB and HIV/TB (P < .05) groups. For the granulocyte percentages, significant differences were found only between the ND and TB groups (P < .05) and the TB and HIV groups (P < .05). Highly significant differences (P < .001) were observed when the CD4 cell counts of the ND group were compared with those of the HIV and HIV/TB groups and when those of the TB group were compared with those of the HIV and HIV/TB groups. CD4 cell counts did not differ between the HIV and HIV/TB groups (P > .05). The immunologic characteristics of the 25 HIV/TB patients recruited for evaluations of their PMNL function were similar to those for the 16 HIV/TB patients described in table 1 (data not shown).

**IL-8RA and IL-8RB expression on peripheral blood PMNL.**

The effect of HIV-1 disease and pulmonary tuberculosis on the expression of IL-8 receptors on PMNL was evaluated with whole venous blood from subjects in the 4 groups by indirectly staining cells with MAbs that specifically bind each of the two IL-8 receptors [13]. Figure 1A shows the proportion of PMNL expressing IL-8RA for the different groups. For healthy subjects, a median of 97.7% of PMNL expressed IL-8RA (range, 94.2%–99.0%). The same was true for comparing data within group (P < .05). The immunologic characteristics of the 25 HIV/TB patients recruited for evaluations of their PMNL function were similar to those for the 16 HIV/TB patients described in table 1 (data not shown).

**Table 1. Immunologic characteristics of study groups in investigation of IL-8 receptors.**

<table>
<thead>
<tr>
<th>Study group</th>
<th>ND</th>
<th>TB</th>
<th>HIV</th>
<th>HIV/TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.4 ± 1.9</td>
<td>36.9 ± 4.9</td>
<td>34.4 ± 1.7</td>
<td>33.9 ± 1.7</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>6/10</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
</tr>
<tr>
<td>Race (black/white)</td>
<td>6/10</td>
<td>16/0</td>
<td>12/4</td>
<td>16/0</td>
</tr>
<tr>
<td>White blood cell count (×10³/μL)</td>
<td>5.4 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>5.0 ± 0.3</td>
<td>5.7 ± 0.5</td>
</tr>
<tr>
<td>Granulocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count (×10³/μL)</td>
<td>2.8 ± 0.2</td>
<td>6.7 ± 1.0</td>
<td>2.4 ± 0.2</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>%</td>
<td>50.6 ± 2.0</td>
<td>62.4 ± 3.6</td>
<td>47.8 ± 2.6</td>
<td>55.9 ± 3.4</td>
</tr>
<tr>
<td>CD4 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count (cells/μL)</td>
<td>982 ± 46</td>
<td>1030 ± 113</td>
<td>337 ± 50</td>
<td>296 ± 68</td>
</tr>
<tr>
<td>%</td>
<td>44.8 ± 1.7</td>
<td>45.3 ± 2.4</td>
<td>14.2 ± 2.0</td>
<td>15.3 ± 2.1</td>
</tr>
<tr>
<td>CD8 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count (cells/μL)</td>
<td>580 ± 36</td>
<td>804 ± 93</td>
<td>1577 ± 227</td>
<td>1130 ± 207</td>
</tr>
<tr>
<td>%</td>
<td>27.8 ± 1.7</td>
<td>30.7 ± 2.1</td>
<td>61.6 ± 3.2</td>
<td>60.9 ± 2.7</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>1.7 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.3 ± 0.05</td>
<td>0.3 ± 0.05</td>
</tr>
</tbody>
</table>

NOTE. Results are expressed as means ± SEs. Study groups: ND, normal donors; TB, tuberculosis patients; HIV, HIV-infected patients without tuberculosis; HIV/TB, coinfected patients (n = 16 for each group).
with a greater reduction observed in HIV-1-seropositive subjects with concurrent pulmonary tuberculosis.

Relationship between IL-8 receptor expression and duration of antituberculosis treatment. As a much greater degree of suppression of IL-8RB occurred on PMNL of subjects in the 2 TB groups than on PMNL of HIV-only patients, a situation unlike that found for the IL-8RA, we thought it important to consider the possible role of antituberculosis drug therapy in this phenomenon.

The mean duration of antituberculosis treatment did not differ significantly between the TB and HIV/TB groups ($P > .05$), and the times ranged from 1 week to a maximum of 26 weeks. When the 2 groups were combined, there was no correlation between the expression of either IL-8 receptor on PMNL and the duration of their antituberculosis drug therapy ($P > .05$). Interestingly, the TB group on its own showed a weak negative correlation between the fluorescence intensity of IL-8RA and the duration of antituberculosis treatment ($r = - .63, P < .05$). When patients were stratified into 2 groups on the basis of duration of treatment, about half had received treatment for $< 2$ months and the rest for $> 2$ months. There was no significant difference between the expression of IL-
Figure 2. IL-8RB staining of granulocytes from normal donors (ND), patients with tuberculosis (TB), patients with HIV infection (HIV), and coinfected patients (HIV/TB). Granulocytes were gated, and % of positive cells (A) and relative fluorescence intensity (B) were determined. Data are individual values ( ■ ), medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th percentiles (bars). Significant differences between groups are indicated.

Relationship between IL-8 receptor expression on PMNL and lymphocytes. The percentages of lymphocytes expressing IL-8RA and IL-8RB, determined only on the basis of their FSC and SSC characteristics, are shown in table 2. The purpose of monitoring each patient’s lymphocytes was to provide an indication of whether there might be a relationship between down-regulation of IL-8 receptors on PMNL and that on lymphocytes. The range of percentages of IL8R-positive lymphocytes obtained in the ND group was in agreement with previously described values [13]. The lymphocyte compartment was largely unaltered in the TB group with respect to IL-8RA and IL-8RB expression compared with that of the ND group. Significant reductions in expression were mainly observed in the 2 HIV-1–infected groups for IL-8RA and only in the HIV/TB group for IL-8RB. There was no correlation between expression of either receptor on PMNL (percentage or fluores-
Table 2. Gross determination of percentage of lymphocytes showing positivity for IL-8RA and IL-8RB in 4 study groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-8RA</th>
<th>P*</th>
<th>IL-8RB</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>16.01 (4.77–26.59)</td>
<td>—</td>
<td>17.68 (4.33–30.27)</td>
<td>—</td>
</tr>
<tr>
<td>TB</td>
<td>11.64 (5.01–22.86)</td>
<td>—</td>
<td>14.68 (4.24–31.02)</td>
<td>—</td>
</tr>
<tr>
<td>HIV</td>
<td>7.62 (0.28–16.61)</td>
<td>.003</td>
<td>13.07 (6.47–29.13)</td>
<td>—</td>
</tr>
<tr>
<td>HIV/TB</td>
<td>5.99 (2.66–11.33)</td>
<td>&lt;.001</td>
<td>7.23 (0.88–23.14)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

NOTE. Data are median (range). Study groups: ND, normal donors; TB, tuberculosis patients; HIV, HIV-infected patients without tuberculosis; HIV/TB, coinfected patients (n = 16 for each).
* Significance for each infection group compared with ND group. Other significant differences: TB and HIV (P = .04) and TB and HIV/TB (P = .003) for IL-8RA; TB and HIV/TB (P < .001) and HIV and HIV/TB (P < .001) for IL-8RB.

Gross measurement of fluorescence intensity and proportions of lymphocytes expressing the particular IL-8 receptor (P > .05).

IL-8–induced calcium mobilization in PMNL. To determine if PMNL functions in response to IL-8 were impaired because of infection, we recruited a further 11 ND as controls and 25 HIV/TB patients, as this latter group showed the greatest overall reduction in IL-8 receptor expression on their PMNL (figures 1, 2). Within these groups, IL-8 receptor expression, calcium mobilization, and chemotaxis were evaluated; the numbers of subjects tested for each varied depending on whether sufficient PMNL were isolated. The capacity of PMNL to mount a response to IL-8 can be demonstrated by a transient increase in cytosolic free Ca^{2+} that is mobilized from intracellular stores in the absence of extracellular calcium. We used the highly sensitive indicator of intracellular free Ca^{2+}, Fura-2 [25], to measure the increase in calcium fluorescence on IL-8 receptor–ligand interaction. Basal [Ca^{2+}], and that induced by IL-8 was determined as the relative ratio of fluorescence obtained at 340 nm and 380 nm. Stimulation indices were determined as this ratio calculated for IL-8 divided by that calculated for the basal response in the absence of any stimuli. The results obtained for PMNL from 10 of the ND group and 21 of the HIV/TB patients are shown in figure 3 and demonstrate that the addition of IL-8 to PMNL from infected subjects produced a significantly reduced calcium flux compared with that in controls (P < .001). There were no significant correlations between calcium stimulation index and immunologic characteristics of these patients.

IL-8–induced chemotaxis. Directed migration in response to IL-8 was measured by use of a Transwell chemotaxis assay. Figure 4 shows the chemotactic indices determined for 11 of the ND and 22 of the HIV/TB patients. When further stratified on the basis of the magnitude of the chemotactic response, indices obtained for the HIV/TB patients could be subgrouped as either low (chemotactic index, <4.0), intermediate (chemotactic index, 4.0–6.0), or high (chemotactic index, >6.0). These all independently were significantly different from the ND group (P < .05). There were no significant correlations between chemotaxis and immunologic characteristics of these patients.
also significantly less in the HIV/TB patients with intermediate or high chemotactic indices than in the normal control group (\( P < .05 \)), whereas the IL-8RA percentages were not different (\( P > .05 \)). The fluorescence intensities of both receptors in all of the HIV/TB chemotactic index subgroups were significantly reduced relative to the normal control group (\( P < .05 \)). The proportion of IL-8 receptor–positive PMNL was a stronger predictor for reduced function in the low chemotactic index subgroup than was receptor density. A difference as small as 2% resulted in a notable difference in chemotaxis in response to IL-8. In the intermediate and high chemotactic index subgroups, the reduced function from normal was the result of a combination of a lower proportion of IL-8RB–fluorescing PMNL and reduced intensity of fluorescence of either IL-8RA or IL-8RB, various combinations in different subjects giving rise to the range of chemotactic capacities seen in these groups. Although cytosolic calcium increases (stimulation indices) due to IL-8 did not differ significantly from each other within the different chemotactic index subgroups despite trends to this effect, all were significantly less than that for the ND group (\( P < .05 \)).

**Discussion**

This study has shown that PMNL from patients with HIV-1, pulmonary tuberculosis, or dual infection have significantly diminished expression of both IL-8 receptors compared with expression of those receptors in uninfected persons. It was further apparent that these defects, common to both HIV-1 disease and tuberculosis, were present irrespective of the patient’s immunologic status. One difference noted in expression of the two receptors was the pronounced decrease in IL-8RB on PMNL from persons with tuberculosis, this being further aggravated by concurrent HIV-1 infection. As all tuberculosis patients were receiving antituberculosis drug therapy, the possibility that antituberculosis drugs may play a role in reduced IL-8RB expression cannot be excluded and requires further evaluation. What was clear, however, was that the duration of antituberculosis treatment had no apparent effect on IL-8RB expression, whereas fluorescence intensity of IL-8RA was higher in tuberculosis patients receiving treatment for <2 months than it was in those treated for >2 months. Future longitudinal studies of tuberculosis patients are required to clarify the possible role of antituberculosis drug(s) in diminished IL-8 receptor expression on PMNL.

We also questioned if the down-regulation of IL-8 receptors on PMNL was a result of an overall down-regulation of these receptors on all leukocytes or whether there may be some cell type–specific regulation. The presence of tuberculosis had no significant effect on the proportion of IL-8RA– or IL-8RB–positive lymphocytes, whereas HIV-1 markedly affected the number of cells expressing IL-8RA and IL-8RB, this being most reduced in the presence of both HIV-1 disease and pulmonary tuberculosis. As there was no correlation between the

![Figure 4. IL-8-induced chemotaxis in granulocytes from normal donors (ND) and coinfected patients (HIV/TB). Chemotaxis in response to IL-8 (100 ng/mL) was monitored by Transwell assay. Results are expressed as mean chemotactic index from duplicate determinations: ratio of number of cells migrating in response to IL-8 divided by number of randomly migrating cells in control wells. ■, individual values; error bars, 10th and 90th percentiles; boxes, values between 25th and 75th percentiles; median is indicated.](image-url)
Table 3. Comparison of IL-8 receptor expression, calcium mobilization, and capacity to migrate in response to IL-8 of polymorphonuclear leukocytes from normal donors (ND) and coinfected patients (HIV/TB).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIV/TB</th>
<th>ND (CI, 7.0–12.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (CI, &lt;4.0)</td>
<td>Intermediate (CI, 4.0–6.0)</td>
</tr>
<tr>
<td>IL-8 receptors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8RA</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>%</td>
<td>95.14 (94.12–96.84)</td>
<td>97.95 (95.45–98.77)</td>
</tr>
<tr>
<td>MCS</td>
<td>345.80 (263.88–509.18)</td>
<td>345.97 (268.48–422.22)</td>
</tr>
<tr>
<td>IL-8RB</td>
<td>94.90 (94.30–97.54)</td>
<td>97.66 (96.85–98.37)</td>
</tr>
<tr>
<td>%</td>
<td>213.83 (127.86–400.32)</td>
<td>123.76 (64.1–165.72)</td>
</tr>
<tr>
<td>Calcium mobilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>SI</td>
<td>1.02 (1.01–1.11)</td>
<td>1.06 (1.05–1.08)</td>
</tr>
</tbody>
</table>

NOTE. Data are median (range). CI, chemotactic index; SI, stimulation index; MCS, median channel shifts.

degree of expression of either IL-8 receptor on PMNL and on lymphocytes, it seems likely that their regulation in vivo is cell type–specific and may be altered differentially depending on the disease state.

A number of mechanisms may be responsible for down-regulation of IL-8 receptors due to disease. Levels of IL-8 have been reported to be elevated in the serum of HIV-1–infected patients [24] and in the plasma of some tuberculosis patients [23, 26]. On human neutrophils, IL-8 has been shown to dynamically regulate its own receptor expression. IL-8 very rapidly down-regulates its receptor expression, but the down-regulated receptor can be rapidly recycled to the surface of neutrophils [27]. The down-regulation of IL-8 receptors seen here may therefore be modulated by IL-8 itself or by other cytokines whose levels may be altered. Alternatively, cells bearing IL-8 receptors may be defective in their ability to recycle these receptors, perhaps through some intracellular interference mechanism. Besides altered recycling of these receptors through extracellular or intracellular means, another related factor may be that peripheral neutrophils, at least in HIV-1–infected persons, are in an activated state. This is supported by a number of findings, including enhanced phagocytosis by neutrophils from HIV-1–infected persons [28, 29], increased CD11b expression [30], reduced FcyRIII (CD16) expression [31], and enhanced apoptosis on neutrophil isolation [32].

Our results further suggest that functions dependent on sufficient IL-8 receptor expression on neutrophils may be impaired in persons with HIV-1 disease and tuberculosis, and we have demonstrated a deficiency in calcium mobilization and cell migration in response to IL-8 of PMNL from patients with HIV and tuberculosis, the group with the greatest decrease in receptor expression. The impaired PMNL response was largely due to a reduction in both IL-8 receptors in cells with a poor chemotactic response to IL-8. Those with intermediate and high chemotactic responses within the HIV/TB group had proportions of IL-8RA–expressing PMNL essentially similar to those of the ND group but had reduced proportions of PMNL expressing IL-8RB and reduced densities of both receptors on their PMNL relative to normal controls. In vivo, one might therefore expect, depending on the extent of decreased IL-8RA and IL-8RB expression, impairment of IL-8–dependent functions that are triggered through high-affinity IL-8RA, which specifically binds only IL-8, and functions triggered through IL-8RB, which include interactions with a wider spectrum of ligands belonging to the C-X-C chemokine subfamily (GROα, GROβ, GROγ, NAP-2, and ENA-78) in addition to IL-8. Neutrophils are among the first cells to arrive at the site of M. tuberculosis infection [33], and killing of this organism occurs via nonoxidative means [34], a process shown to be enhanced by IL-8 [35]. Effective killing by neutrophils on exposure to microbes is therefore reliant on a number of essential processes ranging from adhesion of peripheral blood neutrophils to endothelial cells as a first step, followed by transendothelial migration and entry into the site of infection. This migration of neutrophils is governed by a chemokine concentration gradient established by the release of high concentrations of IL-8 mainly by monocytes/macrophages, either due to direct infection or phagocytosis or indirectly through other cytokines, such as tumor necrosis factor-α. For cells in the periphery to be responsive to IL-8, they would require expression of specific IL-8 receptors on the surface of naive cells or require preactivation, with the subsequent acquisition of the IL-8 receptor, prior to a cell migration response. The combined effect of multiple chemokines is assumed to be responsible for the cellular composition at inflammatory sites, and therefore, any alteration of chemokine composition or concentration would likely result in the influx of cell populations different from normal.

In addition to effects on cellular trafficking, neutrophil functions dependent on ligand–IL-8 receptor interactions important
to microbial killing may be impaired. These include oxidative killing via activation of NADPH oxidase (respiratory burst) or nonoxidative killing through release of potent antimicrobial polypeptides (degranulation). Neutrophils of HIV-1–infected persons have an inability to kill Candida organisms; this occurs despite enhanced phagocytosis and unimpaired oxidative burst [29]. This suggests defective microbial killing by neutrophils via nonoxidative means. Reduced expression of both IL-8RA and IL-8RB on PMNL would support a deficient degranulation response in HIV–1–infected persons, as both receptors mediate agonist-induced granule release [14]. Furthermore, the reduction in IL-8RA expression on PMNL from HIV–1–infected persons obtained in our study may not be sufficient to alter functions such as oxidative burst found to be triggered only through this receptor.

These data suggest that an altered IL-8 receptor repertoire on neutrophils, with resultant dysregulation of the corresponding ligands as a consequence, may be an important feature of HIV–1 and tuberculosis pathogenesis and further may have profound effects not only on normal cell function but also on cellular trafficking in response to infection. In addition, cellular responses dependent on specific receptor engagement and the subsequent translation of signal transducing events that lead to phagocyte effector functions may be impaired in IL-8R–deficient phagocytes. An understanding of the effect of diseases such as HIV–1 infection and pulmonary tuberculosis on regulation of G-coupled protein receptors will not only contribute toward the elucidation of chemokine networks operational in vivo but also help in the quest to unravel their ever-increasing role in disease pathogenesis.

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References


26. Friedland JS, Hartley JC, Hartley CGC, Shattock RJ, Griffin GE. Cytokine secretion in vivo and ex vivo following chemotheraphy of Myco-