



Case Report/Relato de Caso

Abnormal spontaneous interleukin 8 receptor expression: a brief report of two cases

Expressão espontânea anômala do receptor de interleucina 8: um breve relato de dois casos

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ABSTRACT

Interleukin 8 (CXCL8) is an autocrine chemokine specific for the chemoattraction and activation of granulocytes, NKT cells and T lymphocytes. Patients with tuberculosis and latent *Mycobacterium tuberculosis* infection were assessed for the spontaneous expression of CXCR1 (CD128) and CXCR2 on lymphocytes and monocytes. Compared with *ex vivo* profiles, increased spontaneous CXCR2 expression and normal CXCR1 expression were found on lymphocytes in two out of 59 individuals. Monocytes showed normal *ex vivo* profiles for both receptors. After stimulation with purified protein derivative, the *in vitro* levels of CXCL8 were below the median levels of all patients with prior tuberculosis. Spontaneous CXCR2 modulation did not cause notable variation in the *in vitro* levels of CXCL8.

Keywords: Interleukin 8 receptor. Lymphocytes. Cytokines.

RESUMO

Interleucina 8 (CXCL8) é quimiocina autócrina específica para atração e ativação de granulócitos, assim como NKT e linfócitos T. Pacientes com infecção por *Mycobacterium tuberculosis* e latentes foram recrutados para comparar expressão espontânea dos receptores CXCR1 (CD128) e CXCR2 nos mononucleares. Comparado com perfis *ex vivo* dos linfócitos, observou-se aumento em CXCR2; porém, expressão normal de CXCR1 em dois dos 59 indivíduos. Monócitos mostraram perfis *ex vivo* normais; após estimulação específica *in vitro* das citocinas estudadas com extrato bruto total, não se encontrou correspondência na anomalia observada *ex vivo*. Modulação espontânea de CXCR2 não causou grande variação *in vitro* nos níveis de CXCL8.

Palavras-chaves: Receptor de interleucina 8. Linfócitos. Citocinas.

INTRODUCTION

Interleukin 8 (CXCL8), an important autocrine chemokine, belongs to the glutamic acid-leucine-arginine-CXC chemokine family that specifically chemoattracts and activates human neutrophilic granulocytes (as previously reviewed¹). To date, mice do not appear to produce or respond to this putative chemokine. Two CXCL8 receptor subtypes have been identified on human leukocytes: receptor A, or CXCR1 (later designated CD128), and receptor B,

or CXCR2². Both receptors share 77% amino acid homology but differ in their binding properties. CXCR1 is specific for CXCL6 and CXCL8 with high binding affinity, whereas CXCR2 binds to CXCL8 and other CXC chemokine family members with equally high affinity. Binding to CXCR2 usually promotes angiogenesis and endothelial cell chemotaxis. Normal granulocytes not only produce a large amount of CXCL8 but also promptly respond to CXCL8 stimulation via these two receptors (as previously reviewed¹). In a pioneering study, staining of T cells for induced CXCR1 and CXCR2 expression showed strongly elevated levels of expression exclusively in NKT cells and CD8⁺ T cells³. In addition, CD8⁺ T lymphocytes, but not B cells or CD4⁺ T cells, expressed CXCL8 receptors. Lymphocytes also expressed higher levels of CXCR2 than CXCR1³.

In this study, the specific and spontaneous *ex vivo* CXCR1 and CXCR2 expression levels were compared by flow cytometry. In parallel, cytokine levels were also measured in stimulated primary cell cultures. We compared 2 cohorts of individuals and determined the presence of any abnormality in either CXCL8 receptors or spontaneous expression.

CASE REPORT

Twenty-nine prior tuberculosis (TB) patients and 30 control patients with latent *Mycobacterium tuberculosis* infection were identified through the Baltimore City Health Department Eastern Chest Clinic and the Nashville Metropolitan Health Department Tuberculosis Clinic (USA). The eligibility and exclusion criteria have been described elsewhere⁴. This study was approved by the institutional review boards (IRB) of both sites. All study participants provided written informed consent. Peripheral blood mononuclear cells (PBMC) were purified (to > 92% purity) within 24h of obtaining the specimens from study participants using a previously described protocol⁴, and the flow cytometric staining was assayed and analyzed as described before with slight modifications⁵. Cytokine levels were measured by a multiplex bead array, and CXCL8 was detected at 96h via enzyme-linked immunosorbent assay (ELISA) after stimulation with purified protein derivative (PPD). Anti-HLA monoclonal antibodies were purchased from Pharmingen Inc. (USA). IgG2a-isotype control antibodies and anti-CXCR1 and anti-CXCR2 monoclonal antibodies were obtained from Becton & Dickinson Biosciences Co. (USA). PPD was regularly purchased from Statens Serum Institut (Denmark). *Escherichia coli*-derived lipopolysaccharide (LPS) was obtained from Sigma-Aldrich (USA). Recombinant human IFN- γ was obtained from Genentech (USA), and a commercial CXCL8 ELISA kit was obtained from Pierce (USA).

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The levels of *ex vivo* CXCR1 and CXCR2 expression at the screening and the single-cell levels were compared among persons who had had been previously treated for TB and among control patients with latent *M. tuberculosis* infections. **Table 1** depicts the spontaneous levels found for each patient group. Due to the homogeneous pattern, all data were further combined for receptor expression (below). As an internal control, negative responses to isotype control antibodies were detected in all individuals tested (**Figure 1**). All individuals assayed demonstrated standard staining for CXCR1, showing means (\pm SEM) values of 3.7% (\pm 0.3) and 63.6% (\pm 2.1) in the lymphocyte and monocyte (R1) gated populations, respectively (**Figures 1A and B**).

Likewise, 57 examined individuals showed a similar, regular pattern for CXCR2 expression on both lymphocytes (**Figure 1C**) and monocytes (2.2% \pm 0.1 and 65.2% \pm 2.3, respectively). In contrast, two clinically characterized prior extrapulmonary TB patients showed spontaneously high CXCR2 expression *ex vivo*. The first patient was a 47-year-old male (#ID: 125) who was culture-positive for *M. tuberculosis* with a PPD reaction of 18 mm, who responded well to the standard treatment and who had had pleural effusion plus extensive infection (**Table 1**). The second subject was a 52-year-old male (#ID: 162) who was culture-positive for *M. tuberculosis* with no reaction to PPD, who responded well to standard treatment and who exhibited bone/joint lesions (**Table 1; Figure 1C**). During

TABLE 1 - Specific staining for CXCR1 and CXCR2 expression (%) and the 96h purified protein derivative-stimulated CXCL8 levels (pg/ml) on both lymphocyte and monocyte populations (10^6 cells/ml) performed in prior tuberculosis (TB) patients and controls with latent *M. tuberculosis* infection (LTBI). Likewise, the 2 discrepant individuals for CXCR2 staining (please see text) are also shown for comparison.

	Parameters				CXCL8**
	CXCR1*		CXCR2*		
	lymphocytes	monocytes	lymphocytes	monocytes	
TB	5.7 (\pm 0.5)	69.7 (\pm 2.2)	2.4 (\pm 0.2)	67.4 (\pm 2.6)	374.9 (106.6-1,010.8)
LTBI	2.6 (\pm 0.3)	59.2 (\pm 3.5)	2.1 (\pm 0.3)	60.2 (\pm 3.6)	344.3 (157.0-1,296.8)
ID #125	1.0	50.6	53.1	84.8	242.6
ID #162	5.0	47.7	42.9	98.3	316.1

*mean (\pm SEM), **median (min-max), PPD at 10 μ g/ml.

TB: tuberculosis, LTBI: latent tuberculosis infection, ID: identification.

sample handling, the latter patient showed an increased number of erythrocytes when the PBMCs were purified. However, a normal blood pattern was exhibited when the monocytes of those cases were also compared, indicating that the abnormality did not extend to the other mononuclear populations, making it unlikely that an artifact was observed during the cell purification and staining procedures (**Figure 1D**). In addition, the remote possibility of cross-staining of the granulocyte population was ruled out because all studied patients showed similar light scattering profiles (**Figure 2**). Remarkably, the cytokine profile did not change significantly (data not shown), even though the CXCL8 level was slightly decreased at 96h when PBMCs (10^6 cells/ml) were cultured after PPD

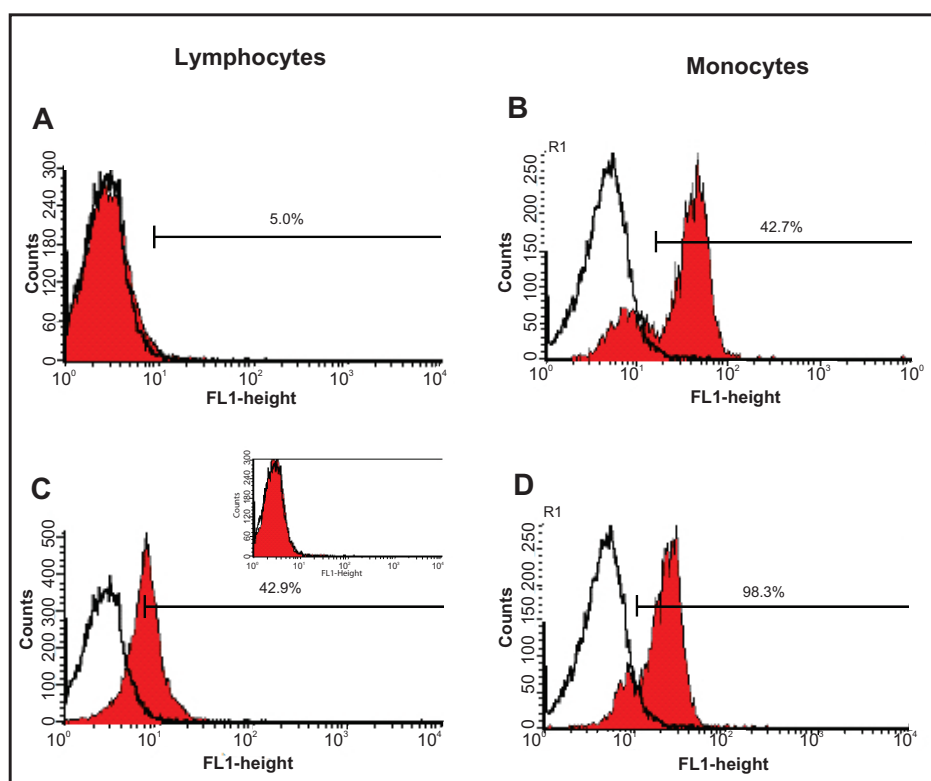


FIGURE 1 - Two *ex vivo* profiles of CXCR1 (A and B) and CXCR2 (C and D) expression on lymphocytes (A and C) and monocytes (B and D) obtained from peripheral blood mononuclear cells of a prior extrapulmonary tuberculosis patient.

Panel A depicts typical CXCR1 expression, but **Panel C** depicts abnormal spontaneous CXCR2 expression on lymphocytes (Insert: typical CXCR2 expression in controls with latent *M. tuberculosis* infection). One out of 3 representative and independent experiments is shown. The thick lines show the control isotype. The percentages of positive cells for the respective histograms are indicated in each graphic.

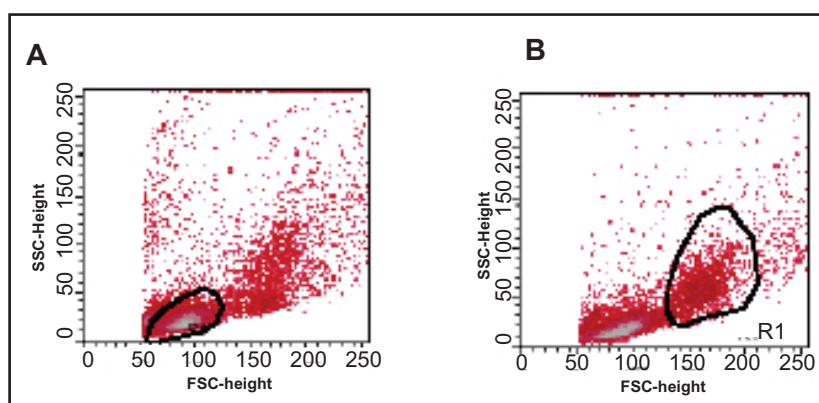


FIGURE 2 - Two *ex vivo* profiles of lymphocytes (A) and monocytes (B) from peripheral blood mononuclear cells derived from a prior extrapulmonary tuberculosis patient.

As denoted in the dot plots, the two distinct populations, lymphocytes (A) and monocytes (B - R1), were gated on the basis of their light scattering properties.

(10mg/ml) stimulation (**Table 1**). To better explore this abnormality, staining for HLA was also performed in parallel for one patient (#ID: 162). As expected, monocytes exhibited a positive modulation for HLA when LPS (200ng/ml) and IFN γ (1,000U/ml) were used alone or in combination: 19.0-fold, 92.6-fold and 67.4-fold for LPS, IFN γ and LPS+IFN γ , respectively. This result ruled out possible non-responsiveness during the cell culture procedures. Unfortunately, because of IRB conflicts at the time that this study was performed, no additional analysis could be carried out.

DISCUSSION

It is hypothesized that increased serum CXCL8 regulates its own receptors on granulocytes by inducing receptor internalization and proteolytic cleavage (as previously reviewed¹). The same hypothesis could be extended to other cell populations, such as T lymphocytes. In addition, blocking experiments with neutralizing anti-CXCL8 antibodies and pretreatment with bacterial LPS, which rapidly downmodulates CXCL8 receptor levels, showed that the inducing activity was due to the presence of CXCL8 in the conditioned medium⁶. Nevertheless, the almost consistent *in vitro* CXCL8 levels that were found in the present study cannot explain the disparities in *ex vivo* CXCR2 expression. A recent study showed that CXCR2 expression is affected by medications (as previously reviewed¹). In another setting, increased gene expression of CXCL8 and the corresponding receptors was detected in PBMCs, mainly during chronic heart failure, resulting in the selective recruitment of leukocyte subpopulations into inflamed tissues⁷. However, marked abnormalities in genes from those patients were significantly modulated in a normal direction during specific therapy. Three single-nucleotide polymorphisms (SNP) have been reported in the CXCR2 gene. The +1208 T/C SNP, located in the non-coding region of the CXCR2 gene, might provide valuable information for the pathogenesis of and susceptibility to chronic inflammatory disease⁸. In addition, a highly significant association has been found between the homozygous +1208 TT genotype and an aggressive phenotype of breast carcinoma⁸. Furthermore, the +1440 G/A SNP and some haplotypes are associated with periodontitis in Brazilian individuals. In contrast, the +785 C/T SNP, located in exon 11 and resulting in a silent codon change (no amino-acid substitution), may be important in protecting against pulmonary inflammation, despite exhibiting no association with Kawasaki disease. Thus, CXCR2 is

an important and interesting new candidate gene that is potentially involved in regulating the inflammatory process in the airways⁹. It has been postulated previously that the CXCR2 +785 T allele may be associated with protection against decreased expiratory flow rates and anomalous gas exchange in chronic obstructive pulmonary disease (COPD) and with a number of quantitative spirometric abnormalities, such as reduced lung function and respiratory symptoms¹⁰. Conversely, this SNP is associated with angiogenesis in systemic sclerosis in individuals homozygous for the CXCR2 +785 C allele⁸. Although differential expression in both PBMC populations ruled out a primary deficiency, any association of the CXCR2 gene SNPs and the 2 individuals described here deserve further investigation, as does the analysis of the intrinsic regulation of monocytes and lymphocytes by CXCR2 because chemokine receptors are selectively and differentially expressed on different leukocyte subsets in individuals with arthritis.

In the current study, 2 patients showed stimulated-CXCL8 production that fell within the range of all patients tested and the normal range of CXCR1 expression, but those 2 patients showed abnormal CXCR2 expression when compared with the other analyzed individuals. A probable pharmacological explanation seems unlikely because those 2 individuals were medication-free (except for TB treatment) for more than two years. Unfortunately, for logistical reasons, neither patient could be followed after the completion of this study. Detailed clinical histories were not available. However, previous complete blood count analyses did not show any discrepancies between the 2 cases and the rest of the cohort. Unlike CXCR1, CXCR2 can bind other CXC chemokines with high affinity. Accordingly, CXCR2 plays more a pleiotropic role in inflammation than does CXCR1 (as previously reviewed¹). This difference may be detected in the 2 patients found here. It is worth noting that the regular cytokine levels found in the 2 patients do not seem to explain the disparities found in this study. In addition, the CXCR2 observed on granulocytes in patients suffering from active systemic lupus erythematosus is more important than CXCR1 in CXCL8-mediated inflammatory responses. The real mechanism of the increased spontaneous CXCR2 expression on lymphocytes from the 2 patients remains to be elucidated.

In conclusion, two original results were found: I) increased *ex vivo* spontaneous expression of the CXCL8 receptor CXCR2, but not CXCR1, was observed in two individuals, whereas II) the secretion of CXCL8 and other cytokines was not substantially impaired. This abnormal modulation of CXCR2 in these atypical cases warrants further investigation.

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