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The effects and comparative differences of neutrophil specific chemokines on neutrophil chemotaxis of the neonate☆

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Abstract

Neutrophil specific chemokines are potent chemoattractants for neutrophils. IL-8/CXCL8 is the most extensively studied member of this group, and its concentrations increase during inflammatory conditions of the newborn infant including sepsis and chronic lung disease. A significant amount of information exists on the effects of IL-8/CXCL8 on neutrophil chemotaxis of neonates, but little is known about the other neutrophil specific chemokines. The aim of this study was to determine the relative potency of the neutrophil specific chemokines on chemotaxis of neonatal neutrophils and to compare this effect with the effect on adult neutrophils. Neutrophils were isolated from cord blood or healthy adult donors and incubated in a Neuroprobe chemotaxis chamber. Chemokine concentrations ranging from 1–1000 ng/mL were used. Differences in chemotactic potency existed among the seven neutrophil specific chemokines. Specifically, at 100 ng/mL, the order was IL-8/CXCL8 > GRO-α/CXCL1 > GCP-2/CXCL6 > NAP-2/CXCL7 > ENA-78/CXCL5 > GRO-γ/CXCL2 > GRO-β/CXCL3. This pattern was observed for adult and neonatal neutrophils. We conclude that (1) neutrophils from cord blood exhibit the same pattern of potency for each ELR chemokine as neutrophils from adults, and (2) migration of neonatal neutrophils is significantly less than that of adults at every concentration examined except the lowest (1 ng/mL).

Keywords: Chemokine; Chemotaxis; Migration; Neonate; Neutrophil

1. Introduction

Chemokines are 8–10 kDa proteins responsible for the movement of leukocytes [1]. Two subfamilies of chemokines exist and are distinguished according to the arrangement of the first two cysteines which can be either adjacent (C–C) or separated by one amino acid (C–X–C). CXC chemokines act mainly on neutrophils, and possess the amino-terminal sequence Glu-Leu-Arg (ELR+), which is necessary for receptor recognition and signaling [2]. Seven neutrophil specific chemokines have been identified (Table 1). All ELR+-CXC chemokines are activators of CXCR-2, but only IL-8/CXCL8 and GCP-2/CXCL6 are potent agonists for CXCR-1 [2].

Abbreviations: CXCL, cysteine "X" cysteine ligand; CXCR, cysteine "X" cysteine receptor; ENA-78/CXCL5, epithelial derived neutrophil chemoattractant; GCP-2/CXCL6, granulocyte chemotactic peptide two; GRO-α/CXCL1, growth related oncoprotein alpha; GRO-β/CXCL2, growth related oncoprotein beta; GRO-γ/CXCL3, growth related oncoprotein gamma; IL-8/CXCL8, interleukin 8; LTB_4, leukotriene B_4; NAP-2/CXCL7, neutrophil activating peptide two; PAF, platelet activating factor; PMN, polymorphonuclear.

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Table 1
Neutrophil specific chemokines

Chemokine	Classification	Associated receptor (s)
ENA-78	CXCL5	CXCR-2
GCP-2	CXCL6	CXCR-1;CXCR-2
GRO-α	CXCL1	CXCR-2
GRO-β	CXCL2	CXCR-2
GRO-γ	CXCL3	CXCR-2
IL-8	CXCL8	CXCR-1;CXCR-2
NAP-2	CXCL7	CXCR-2

The relative chemotactic potency of neutrophil specific chemokines for neutrophils in the newborn infant has only been studied for IL-8/CXCL8. Thus the focus of the present study was, (1) to quantify the effect of the remaining six neutrophil specific chemokines on chemotaxis of neutrophils from neonates and (2) to compare the response of neonatal neutrophils with the chemotactic response of adult neutrophils exposed to the same chemokines.

2. Methods

2.1. Materials

Stock solutions (1×10^{-5} M) of each neutrophil specific chemokine (Table 1) were prepared by dissolving in phosphate buffered saline (PBS) containing 0.1% human serum albumin (endotoxin free, Sigma Diagnostics, St. Louis, MO) and held at −80 °C until utilized. The chemokines were further diluted (1−1000 ng/mL) in PBS with 0.1% human serum albumin immediately prior to each chemotaxis assay.

2.2. Blood samples

Cord blood (40 mL) or adult peripheral blood (40 mL) was collected immediately prior to the performance of the chemotaxis assay. Cord blood samples were obtained from the fetal side of placentas within 5 min after elective cesarean section of term pregnancies without evidence of intra-amniotic infection. We chose to use cord blood neutrophils from term infants after elective cesarean section, since previous studies have shown that the mode of delivery does not affect the chemotaxis of cord blood neutrophils [3]. Samples of adult blood were obtained from healthy volunteer donors. All blood samples were drawn into syringes containing preservative-free sodium heparin (50 U/mL). The Institutional Review Board at the University of South Florida approved the collection of cord blood and adult blood samples.

2.3. Neutrophil isolation

Using sterile technique, whole blood (16 mL) was diluted 1:1 with 1× Dulbecco's Phosphate Buffered Salt Solution (DPBS) (Cellgro, Herndon, VA) in a 50 mL conical centrifuge tube. Histopaque 1119 (Sigma Diagnostics) 16 mL was placed in another 50 mL conical centrifuge tube. Histopaque 1077 (16 mL) was then layered onto the layer of Histopaque 1119 followed with a layer of the diluted blood. The tube was then centrifuged at 700 × *g* for 30 min at room temperature (RT). The neutrophil layer was removed (to within <0.5 cm of the RBC layer) and placed into a separate 15 mL tube. The cells were washed by the addition of 10 mL 1× DPBS, gently resuspended, and centrifuged at 300 × *g* for 10 min at RT. The supernatant was then aspirated and discarded. The wash was repeated an additional time, and the cells were resuspended in DPBS and counted.

2.4. Hypotonic lysis of RBCs

Sterile water (5 mL) was added to the cell suspension for 20 s immediately followed by 5 mL of 1.8% filtered NaCl solution. The cells were washed and the step was repeated up to three times.

2.5. Cell viability assay

Viability of neutrophils was determined by the trypan blue dye exclusion assay [4]. Freshly isolated neutrophils were incubated with trypan blue solution (5 mg/mL in saline; Sigma Diagnostics) for 3−5 min before being counted using a hemacytometer. The viability of neutrophils was determined by the following formula: (number of unstained cells/total number of cells) × 100%.

2.6. Staining of neutrophils by Calcein AM

Calcein AM (1:1000; Molecular Probes, Eugene, OR) was added to the suspension of cells. The cells were incubated for 30 min at 37 °C, and then washed up to four times with DPBS before being resuspended in 2 mL DMEM without calcium or magnesium. The cells were counted and brought to a final concentration of approximately 2000 cells/μL [5]. Under these conditions, activation of PMN has not been demonstrated when using Calcein AM-labeled PMN [5].

2.7. Chemotaxis assay

Twenty-nine microliters of chemokine/chemoattractant or PBS (negative control), were placed in the bottom of the well of a microchemotaxis plate (Neuroprobe, Gaithersburg, MD). As a positive control, 23 μL of labeled cells were placed in a bottom well. A framed filter (5 μm) was then placed over the microplate and 23 μL of the prepared cells were placed on the top of the filter. The plate was then incubated at 37° C in humidified air with 5% CO_2 for 30 min. After incubation, a clean smooth