Cryptococcal Glucuronoxylomannan Induces Interleukin (IL)-8 Production by Human Microglia but Inhibits Neutrophil Migration toward IL-8

Myriam M. Lipovsky, Genya Gekker, Shuxian Hu, Laura C. Ehrlich, Andy I. M. Hoepelman, and Phillip K. Peterson

On the basis of the clinical observation that the cerebrospinal fluid (CSF) of patients with cryptococcal meningitis contains high levels of the chemokine interleukin (IL)-8 but few polymorphonuclear leukocytes (PMNL), the production of IL-8 by cultured brain glial cells after stimulation with two serotypes of cryptococcal capsular polysaccharide glucuronoxylomannan (GXM) was studied, followed by an assessment of the effect of GXM on PMNL migration toward IL-8. GXM serotype A but not D was capable of inducing IL-8 production in human fetal microglial cell but not in astrocyte cultures. When added directly to the PMNL, GXM (both serotypes) potently blocked PMNL migration toward IL-8. The mechanism of GXM's inhibitory effect appeared to involve cross-desensitization. These findings suggest that GXM can induce IL-8 production in the brain but that GXM in the systemic circulation inhibits migration of PMNL toward IL-8.

The encapsulated yeastlike fungus Cryptococcus neoformans is the leading mycologic cause of central nervous system infection in the immunocompromised host. Cell-mediated immunity plays an important role in host defense against this opportunistic. However, the pathogenesis of cryptococcosis is still incompletely understood [1].

Recently, Chaka et al. (unpublished data) analyzed cerebrospinal fluid (CSF) samples from human immunodeficiency virus—positive patients with cryptococcal meningitis and found high levels of interleukin (IL)-8 (nanogram per milliliter range), much higher than IL-8 detected in parallel serum samples. IL-8 is a potent chemoattractant for polymorphonuclear leukocytes (PMNL), and in an animal model in which IL-8 was delivered intracerebrally, PMNL rapidly invaded the blood-brain barrier (BBB) [2]. However, the CSF of patients with cryptococcal meningitis typically shows a paucity of leukocytes, predominantly mononuclear cells and virtually no PMNL. Taken together, these observations suggest that in cryptococcal meningitis, IL-8 is produced within the brain but that PMNL do not cross the BBB in response to IL-8.

In patients with cryptococcal meningitis, high levels of cryptococcal capsular polysaccharide antigen are detectable in the CSF and serum of most patients. As early as 1951, Drouhet and Segretain [3] described that cryptococcal polysaccharide could inhibit chemotaxis of leukocytes toward FMLP. Moreover, the major polysaccharide component, glucuronoxylomannan (GXM), which consists of an α-1,3–linked polymannose backbone with O-acetyl substituents and β-linked monomeric branches of xylose and glucuronic acid, has been shown to induce the production of proinflammatory cytokines [4] and chemokines [5] by human leukocytes. Therefore, in the present study, two hypotheses were tested: that cryptococcal GXM can induce IL-8 production by brain cells but that GXM blocks the migration of PMNL toward IL-8.

Materials and Methods

Reagents. IL-8, IL-1β, and anti–IL-8 antibodies were provided by R&D Systems (Minneapolis). Anti-goat horseradish peroxidase (HRP) conjugate was obtained from Jackson Immune Research (West Grove, PA). Lipopolysaccharide (LPS) and polymyxin B were acquired from Sigma-Aldrich (St. Louis).

Cryptococcal capsular polysaccharide. Purified GXM, obtained as previously described [6] from C. neoformans serotype A (ATCC 62066) and serotype D (B3501), was a gift from R. Cherniak (Georgia State University, Atlanta). Briefly, C. neoformans was autoclaved after growth in a chemically defined medium for 5 days. Polysaccharide was precipitated with calcium acetate and ethanol. After being dissolved in NaCl and undergoing brief ultrasonic irradiation, the polysaccharide was precipitated by differential complexation with hexadecyltrimethylammonium bromide. Purified GXM was precipitated by ethanol, dissolved, ultrasonically irradiated for 2 h, centrifuged, dialyzed, and finally recovered by lyophilization. Analysis of the samples through various methods revealed that none of the purified polysaccharides contained constituents other than those known to occur in GXM [6]. GXM was dissolved in 50 mg/mL Hanks’ balanced salt solution (Sigma) and stored at −20°C.
Glial cell cultures. Human fetal microglia and astrocytes were obtained from brain tissues of 16- to 22-week aborted fetuses, using previously described techniques [7, 8]. Greater than 99% of cells in the microglial cell preparation stained positively with anti-CD68, a marker for human macrophages, and <1% stained with antibodies to glial fibrillary acid protein (GFAP, an astrocyte marker; Dako, Carpinteria, CA). Greater than 99% of cells in the astrocyte cultures stained positively with antibodies to GFAP. Cell viability, assessed by trypan dye exclusion, was >98%.

ELISA for IL-8 detection. IL-8 levels in glial cell cultures were quantified by an ELISA. Briefly, purified mouse anti-human IL-8 antibodies were coated overnight at 4°C onto 96-well microtiter plates at 1 μg/mL. The wells were blocked and washed with 1% bovine serum albumin (BSA) in PBS for 1 h at 37°C, followed by the addition of samples and a series of dilutions of standard IL-8 in triplicate for 2 h at 37°C. The wells were then washed with PBS–Tween 20 (0.5%) and incubated with goat anti-human IL-8 antibody (2 μg/mL) for 1.5 h, followed by washing and incubation with donkey anti-goat HRP conjugate (120,000 in PBS) for an additional 1 h at 37°C. After intensive washing, substrate buffer of K-blue (ELISA Technology, Lexington, KY) was used for color development, and the reaction was stopped after 30 min by addition of 1 M H2SO4 and read by 450 nm. The detection limit for this assay was 10 pg/mL IL-8.

Experimental design for chemokine induction. Human microglia or astrocytes were seeded onto 96-well plates (5 × 10³ cells/well in 100 μL of Dulbecco’s MEM [DMEM; Sigma] plus 10% BSA). After 24 h (microglia) or 72 h (astrocytes), medium was replaced with DMEM containing 1% BSA, and GXM serotype A or D (0.1–3 mg/mL) in DMEM was added. After 24 h of incubation at 37°C in a humidified 5% CO2 incubator, supernatants were harvested and assayed for IL-8. All experiments were performed in triplicate and repeated with glial cells from at least 3 different brain specimens.

Chemotaxis assay. Human PMNL were obtained by a method described previously [5, 9]. PMNL migratory activity was assessed by using a 48-well modified Boyden chamber (Neuro Probe, Bethesda, MD), as described previously [9] with slight modifications. A polyvinylpyrrolidone-free polycarbonate membrane of 3-μm pore size (Poretics Products, Livermore, CA) was used. Materials being assessed for chemoattractive activity (IL-8 and GXM) and medium control (Gey’s balanced salt solution; Sigma), were placed in the lower wells of the chambers. PMNL suspension, with or without added GXM (20 min of preincubation), was added to the upper chamber wells. Each potential chemoattractant was examined in triplicate. After 45 min of incubation at 37°C, the membrane separating both chambers was removed, and nonmigrating cells were gently scraped from the upper side of the filter. Cells on the lower side of the membrane were stained (Diff-Quick; Baxter, McGaw Park, IL) and examined with light microscopy. Five microscopic fields per well were examined under high power (×1000), and the average number of PMNL per field that had migrated to the other side of the membrane was determined.

Statistical analysis. Data are expressed as mean ± SE of triplicate values from a representative experiment or as mean ± SE of three separate experiments (performed in triplicate). Differences between the means of two groups were analyzed by Student’s t test (Statview II; Abacus Concepts, Berkeley, CA).

Results

IL-8 production. To test whether GXM is capable of inducing IL-8 production by glial cells, human fetal microglia and astrocytes were incubated with various concentrations of GXM serotype A and D. GXM serotype A but not serotype D induced IL-8 production by microglial cells in a dose-dependent manner (figure 1A). To exclude LPS contamination as an explanation for the observed results, we showed that polymyxin B (20 μg/mL) had no effect on GXM A−induced IL-8 production (GXM A [1 mg/mL]: 22.2 ± 2.1 ng/mL; GXM A plus polymyxin B: 23.1 ± 1.2 ng/mL), while polymyxin B inhibited 10 ng/mL LPS−induced IL-8 production by >85% (LPS [10 ng/mL]: 106.0 ± 10.6 ng/mL).

Figure 1. Production of IL-8 by (A) human microglia and (B) astrocytes, stimulated with cryptococcal glucuronoxylomannan (GXM), serotypes A and D, at indicated concentrations. GXM was added to highly enriched preparations of each glial cell type, and after 24 h of incubation, culture supernatants were harvested for IL-8 assessment. As negative control, cells were incubated in medium alone; as positive control for astrocytes, cells were stimulated with IL-1β (10 ng/mL). Data represent mean ± SE of triplicate values and are representative of 3 independent experiments with cells from different brain tissue specimens.
Figure 2. Effects of (A) glucuronoxylomannan (GXM), serotypes A and D or (B) IL-8 on polymorphonuclear leukocyte (PMNL) migration toward chemoattractants IL-8 (50 ng/mL) and/or GXM (both serotypes, 500 μg/mL). Chemoattractants or medium, as indicated at top of diagram, were added to lower chamber of Boyden chamber chemotaxis device. PMNL, in absence (medium) or presence of (A) GXM A (500 μg/mL), GXM D (500 μg/mL), or (B) IL-8 (50 ng/mL), were added to upper chamber and migration of cells toward lower chamber was quantified after 45 min by counting 5 high-power fields (×1000) per triplicate. Data represent mean ± SE of (A) three independent experiments or (B) replicates of representative experiment of 2 independent experiments, using cells from different donors.

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mL; LPS plus polymyxin B: 12.9 ± 0.9 ng/mL). For the highest GXM dose tested, neither GXM serotype was capable of inducing IL-8 production by astrocytes (figure 1B). The failure of astrocytes to respond to GXM was not due to an inability of these glial cells to produce IL-8, since astrocytes stimulated with IL-1β released appreciable amounts of IL-8 (figure 1B).

Chemotaxis. To test the hypothesis that GXM blocks the migration of PMNL toward IL-8, it was first determined that PMNL (added to the upper chamber of the chemotaxis device) migrated toward IL-8 (add to the lower chamber) in a dose-dependent manner (data not shown). On the basis of these findings, a concentration of 50 ng/mL IL-8 was selected for all subsequent experiments. GXM was then added in different concentrations directly to the PMNL (upper chamber). When GXM was added to the PMNL, it blocked migration (>65%) toward IL-8 at all concentrations tested (range: 0.1 – 3 mg/mL; data not shown). No significant difference in the inhibitory activity was found between GXM serotypes A and D at these concentrations. Figure 2A shows the inhibitory effect of GXM (500 μg/mL, both serotypes) on IL-8–induced migration of PMNL. Inhibition of PMNL migration by GXM was not specific for IL-8, since GXM, added to the PMNL (upper chamber), could also block migration toward GXM and GXM plus IL-8 (figure 2A).

To determine whether the blocking effect of GXM on PMNL migration could be related to a cross-desensitization phenomenon [10], we tested whether the chemoattractant IL-8 itself would also block PMNL migration. Figure 2B shows that addition of IL-8 directly to the PMNL (upper chamber) blocked migration toward IL-8 or GXM (lower chamber), in a way similar to that shown with GXM.

Discussion

The recent clinical observation of Chaka et al. (unpublished data) that the CSF of patients with cryptococcal meningitis contains few PMNL despite high IL-8 levels prompted the hypotheses that the major cryptococcal capsular polysaccharide component GXM is capable of both stimulating glial cells to release IL-8 and blocking migration of PMNL toward IL-8. The findings that cryptococcal GXM serotype A (but not D) induced IL-8 production by human fetal microglial cells (but not astrocytes) and that both GXM serotypes inhibited PMNL migration toward IL-8 support these two hypotheses.

To our knowledge, this is the initial description of IL-8 production by microglial cells. Microglia are found in about equal numbers as neurons within the cerebral cortex. Moreover, 4%–13% of the cell processes forming the glia limitans of the BBB have been shown to be of microglial origin [11]. Thus, it is possible that GXM within the brain parenchyma or subarachnoid space elicits IL-8 production by microglia. The concentrations of GXM used in this study to induce IL-8 production reflect values that have been found in CSF and serum of AIDS patients with cryptococcal meningitis [12].

Variations in virulence of different cryptococcal isolates are thought to be related to the chemical structure of the polysaccharide capsule [1]. Our results show that different GXM serotypes vary in their ability to induce IL-8 production. Most clinically important isolates of *C. neoformans* var *neoformans* are of serotype A. Little is known about clinical differences between serotypes A and D, although patients with serotype D tend to have a lower incidence of meningitis and a higher incidence of skin lesions. [13]

Cryptococcal capsular polysaccharide has been shown to inhibit PMNL migration toward FMLP and C5a [3, 9]. In the present study, both GXM A and D were found to block PMNL migration toward IL-8, the chemokine found in CSF of patients with cryptococcal meningitis, thus mimicking the clinical situation more closely. Our study confirmed the observation of others that GXM also has chemoattractant...
activity [9]. The precise mechanism is not known, but there are several possible explanations for the observed dual effects of GXM on PMNL migration, which have been described for IL-8 as well [14]. The simplest explanation is that, in the presence of GXM, migration of PMNL does not occur because there is no chemotactic gradient across the semipermeable membrane barrier. Another potential explanation for the inhibition of chemotaxis by GXM is a phenomenon recently described for other neutrophil chemotactic factors (FMLP and C5a) in relation to IL-8, namely cross-desensitization of the IL-8 receptor [10]. The IL-8 receptor on PMNL belongs to the seven transmembrane G protein–coupled receptor family. Two IL-8 receptor types (A and B) have been identified on PMNL. Calcium-mobilization studies showed rapid desensitization for IL-8 if PMNL were preexposed to FMLP, C5a, or IL-8 itself. The mechanism involved was believed to be related to receptor phosphorylation and internalization of IL-8 receptors with partial or no reexpression of either receptor type [10]. In our chemotaxis studies, both GXM and IL-8 were able to inhibit PMNL migration toward IL-8, indicating that a similar mechanism of cross-desensitization of the IL-8 receptor by GXM could be involved.

The pathophysiologic significance of GXM-mediated blockade of PMNL migration is not clear. PMNL can readily kill opsonized cryptococci [15], and even though neutropenia is not a risk factor for the development of cryptococcal meningitis, non-AIDS patients have a better prognosis if their CSF leukocyte count is >20 cells/μL [1]. The lack of PMNL in the subarachnoid space, frequently seen in AIDS patients, might also contribute to the high relapse rate of cryptococcal meningitis in these patients, despite antifungal therapy.

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References