The Carbohydrate Sialyl Lewis\textsuperscript{x} (sLe\textsuperscript{x}) Sulfated Glycomimetic GM2941 Attenuates Glucan-Induced Pulmonary Granulomatous Vasculitis in the Rat\textsuperscript{1}

KENNETH S. KILGORE, KAREN L. POWERS, MICHELLE M. IMLAY, ANU MALANI, DOUGLAS I. ALLEN, JENNIFER T. BEYER, MARK B. ANDERSON\textsuperscript{3} and JEFFREY S. WARREN

Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan

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ABSTRACT

We examined the protective effects of GM2941, a sulfated glycomimetic of the complex carbohydrate sialyl Lewis\textsuperscript{x}, in a model of pulmonary granuloma development. This study was based on the rationale that formation of glucan-induced lung granulomas is dependent on neutrophils and that sialyl Lewis\textsuperscript{x} glycomimetic (GM2941) interferes, in vitro, with P-selectin-dependent neutrophil-endothelial adhesive interactions. Infusion of particulate yeast cell wall glucan into rats results in the rapid (48 hr) formation of monocyte/macrophage-rich angiocentric pulmonary granulomas. Development of granulomas exhibits a temporal pattern characterized by the early, transient influx of neutrophils into blood vessel walls at sites of glucan embolization, followed by accumulation of monocytes and macrophages that constitute the definitive angiocentric lesions. Within 1 hr after the infusion of glucan, immunohistochemical analysis revealed up-regulation of blood vessel wall-associated P-selectin. Previous studies utilizing neutrophil-depleted animals have revealed that neutrophils, although not present in definitive lesions, are required for full granuloma development. The potential of GM2941 to inhibit neutrophil-endothelial cell adhesive interactions was demonstrated by the ability of the compound to inhibit P-selectin-mediated adhesion to histamine-stimulated HUVECs. Infusion of GM2941 retarded pulmonary granuloma development in a dose-dependent manner. Whole-lung myeloperoxidase activity, measured at the time of peak neutrophil accumulation, was significantly reduced in animals pre-treated with GM2941 (30 mg/kg, 24 \( \mu \)M/kg), which suggests that this compound affords protection, at least in part, through impedance of neutrophil recruitment. These data indicate that GM2941 affords a significant degree of protection against granuloma formation associated with glucan infusion, probably through the interruption of neutrophil recruitment.

A large body of data indicates that neutrophils play a central role in the pathogenesis of diverse pulmonary diseases, including the ARDS, emphysema and a variety of acute vasculitic diseases (Gadek, 1992; Mulligan \textit{et al}., 1992a; Henderson \textit{et al}., 1991). The recruitment of neutrophils into sites of acute inflammation is mediated through the expression of a series of intercellular adhesion molecules expressed on both neutrophils and endothelial cells (reviewed, Pardi \textit{et al}., 1992; Zimmerman \textit{et al}., 1992). The initial events in neutrophil adhesion encompass an early transient interaction that is mediated by members of the selectin family. These include P-selectin, which is expressed on endothelial cells, and L-selectin, which is expressed on neutrophils (Lasky, 1992). Upon activation of endothelial cells by an agonist such as thrombin or histamine (Zimmerman \textit{et al}., 1992; Lorant \textit{et al}., 1991) or by complement-derived factors including C5a and the membrane attack complex (Foreman \textit{et al}., 1994; Hattori \textit{et al}., 1989), P-selectin is rapidly translocated from Weibel-Palade bodies to the cell surface where it can interact with its counterligand on the neutrophil (Varki, 1992; Moore \textit{et al}., 1992). This counterligand is believed to be a sialylated O-linked oligosaccharide-containing glycoprotein (\textit{e.g.}, sLe\textsuperscript{x}) or other related sulfated glycoproteins and glycolipids (Moore \textit{et al}., 1992; Norgard \textit{et al}., 1993). Once weakly bound to the endothelium, the neutrophil can establish a firm, irreversible adhesive interaction mediated by members of the selectin family.

ABBREVIATIONS: sLe\textsuperscript{x}, sialyl Lewis\textsuperscript{x}; ARDS, adult respiratory distress syndrome; MCP-1, monocyte chemoattractant protein-1; ROIs, reactive oxygen intermediates; MPO, myeloperoxidase; HUVECs, human umbilical vein endothelial cells; BCECF-AM, 2',7'-bis-(2-carboxyethyl)-5(6)-carboxyfluorescein, acetoxymethyl; WBC, white blood cell; ELISA, enzyme-linked immunoabsorbant assay; PBS, phosphate-buffered saline; HBSS, Hanks’ balanced salt solution; BSA, bovine serum albumin.
The i.v. infusion of particulate yeast cell wall glucan into rats results in the rapid, synchronous development of foreign body-type lung granulomas composed almost entirely of monocytes and macrophages (Flory et al., 1993; Jones and Warren, 1992). A number of systemic diseases, including Wegener’s granulomatosis, sarcoidosis and several occupational lung diseases (e.g., berylliosis and talcosis), are characterized by the presence of monocyte-rich granulomas that resemble those found in rats after glucan infusion (Shellito et al., 1987; Lugano et al., 1982; van Oud Albas et al., 1983a; Kilgore et al., 1997). Glucan-induced granuloma formation exhibits a characteristic temporal pattern that involves the local synthesis of chemotactic cytokines and the recruitment of leukocytes into sites of glucan deposition (Flory et al., 1993; Jones and Warren, 1992; Flory et al., 1994). We have previously observed that MCP-1 is required for full granuloma development (Flory et al., 1993; Flory et al., 1994). Early (within 1 hr) in the evolution of granuloma formation, there is a rise in MCP-1 expression that is associated with blood vessel wall cells. Later (24 hr), there is a second rise in MCP-1 expression that is associated with granuloma cells per se. The early rise in MCP-1 activity is temporally and anatomically associated with the transient influx of neutrophils into vessel walls (at sites of glucan embolization)—hence the hypothesized relationship between locally elaborated neutrophil products and granuloma formation. The transient, early influx of neutrophils into blood vessel walls at sites of glucan embolization suggests that neutrophils may play a role in formation of these lesions, despite the fact that mature granulomas do not contain neutrophils.

Recent studies from our laboratory have focused on the role of the neutrophil in the pathogenesis of granuloma formation. Depletion of circulating neutrophils through the use of a selective antibody directed against rat neutrophils results in a significant decrease in granuloma size and number, which suggests a relationship between neutrophil influx and subsequent granuloma development (Kilgore et al., 1997). This hypothesis is supported by the observations that neutrophil depletion is associated with decreased expression of MCP-1 at both the mRNA and protein levels and that both activated neutrophils and H2O2 can induce MCP-1 production by isolated endothelial cells (Kilgore et al., 1997). The temporal and anatomic linkage between localized neutrophil influx and the subsequent expression of chemotactic cytokines, in conjunction with a growing literature indicating that ROIs can induce chemokine expression (Satriano et al., 1993; DeForge et al., 1993), suggests that neutrophils may play an important role in mediating this inflammatory process.

Inhibition of neutrophil-endothelial adhesion by interrupting P-selectin-dependent neutrophil-endothelial cell interactions has retarded the development of tissue injury in a number of pathologic conditions, including complement-mediated lung injury and myocardial reperfusion injury (Mulligan et al., 1992a; Kilgore et al., 1995; Weyrich et al., 1993). The purpose of the present study was to assess the potential of carbohydrate-based therapeutics (Musser et al., 1995a; Oppenheimer-Marks and Lipsky, 1996; Musser et al., 1996; Rao et al., 1994), specifically a novel glycomimetic of sLeX (Rao et al., 1994), to prevent the development of glucan-induced pulmonary granulomas in the rat. In this study, GM2941 (fig. 1), a sulfated form (Musser et al., 1995b) of a previously reported glycomimetic (Anderson et al., submitted for publication), was examined. The results of this study indicate that GM2941 significantly retards granuloma development in a dose-dependent manner. The observation that GM2941 infusion reduces whole-lung MPO activity, coupled with morphologic studies revealing decreased numbers of blood vessel wall neutrophils at sites of glucan embolization, suggests that interruption of neutrophil recruitment is an important mechanism of action for this compound.

Materials and Methods

Preparation of GM2941. GM2941 was a gift from Glycomed Incorporated (Alameda, CA). The compound is soluble when dissolved in sterile saline (pH 7.4). GM2941 was prepared immediately before use in a stock solution from which aliquots were taken and added to the glucan solution to achieve the desired dose. The final volume administered was 1.1 ml, which was infused over 1 min.

Endothelial cell culture. HUVECs were isolated from umbilical veins by treatment with 0.1% collagenase in Dulbecco’s modified Eagle’s medium (Jaffee, 1984). Cells were plated in gelatin-coated 48-well plates and incubated at 37°C, 5% CO2. Cells were grown in M199 medium supplemented with 20% heat-inactivated fetal calf serum, L-glutamine (4 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), 25 μg/ml endothelial cell growth supplement (Collaborative Research, Bedford, MA) and 15 U/ml bovine heparin. Cells were characterized by a cobblestone appearance and utilized between the first and third passages.

Isolation and fluorescence labeling of human neutrophils. Human neutrophils were isolated by Ficoll/Hypaque (Pharmacia, Uppsala, Sweden) centrifugation and dextran (MW 200,000; Sigma Chemical Co.) sedimentation as described previously (Foreman et al., 1994). Contaminating erythrocytes were removed by hypotonic lysis. Cells preparations were >90% pure with a viability of approximately 95% as determined by trypan blue exclusion. Neutrophils were fluorochrome-labeled as described previously, using BCECF-AM (Molecular Probes, Eugene, OR) (Vaporciyan et al., 1993). Briefly, BCECF-AM was dissolved in dimethyl sulfoxide to achieve a final concentration of 1 μg/μl. One microliter of BCECF-AM was added per 1.0 ml of cells (final concentration of 1 μM) and incubated for 30 min at 37°C. The cells were washed two times in HBSS/BSA and resuspended in serum free HUVEC medium to achieve a final concentration of 1 × 106 cells/ml.

Neutrophil endothelial cell adhesion assay. The ability of GM2941 to decrease P-selectin-mediated neutrophil adherence to
To a Macintosh II FX computer with NIH Image 1000 software. The granuloma was measured using a Sony video image camera coupled from the conversion of H2O2 in the presence of and the supernatants removed. Myeloperoxidase activity was determined by a modified whole-cell ELISA assay as described previously (Foreman et al., 1994). Monolayers were treated with 10 mM histamine for 10 min in the presence or absence of GM2941 (3 mg/ml). Cells were washed with PBS and fixed with 1% paraformaldehyde for 30 min, followed by the addition of nonfat dry milk (5% in PBS). Cells were incubated with the anti-P-selectin monoclonal antibody PB1.3 (1 µg/ml) or with an isotype-matched murine antibody for 45 min, followed by incubation with the peroxidase-conjugated goat anti-mouse secondary antibody (Dako Corporation, Carpenteria, CA) for 45 min. Cells were washed with PBS and then exposed to the substrate (o-phenylenediamine dihydrochloride, Sigma) for 30 min. The reaction was halted by addition of 3 M sulfuric acid. Optical density was determined by an automated microplate reader (EL340, Bio-Tek Instruments, Winooski, VT) set to a wavelength of 495 nm.

**Statistical data.** The data presented in the text, figures and tables are expressed as mean ± S.E.M. for the indicated number of determinations. A one-way ANOVA (repeated-measures) was used to assess differences over time within groups. One-way analysis of variance (factorial) was used for group comparisons. If significance was determined, Sheffe’s test was used as a post-hoc analysis. Student’s t test was used to examine statistical differences between treatment groups (Linton and Gallow, 1975). A P value less than .05 was considered significant. All statistical evaluations were performed using a Macintosh 8500 computer (Apple Computer, Cupertino, CA) with Statview software (Abacus Concepts Inc., Berkeley, CA).

**Results**

GM2941 decreases P-selectin-mediated neutrophil adhesion to histamine-stimulated HUVEC monolayers. To determine the potential of GM2941 to inhibit P-selectin-mediated neutrophil-HUVEC adhesive interactions, we stimulated HUVEC monolayers with histamine (10 mM) for 10 min. After incubation of HUVECs with histamine, GM2941 (3 mg/ml; 2.4 µM) was added to monolayers for 5 min, followed by the addition of BCECF-labeled human neutrophils. Neutrophils were allowed to incubate with the HUVECs at 37°C for 15 min. Incubation with GM2941 decreased neutrophil adhesion by 86% compared with monolayers treated with vehicle alone (fig. 2; P < .05, one-way ANOVA). Use of the anti-P-selectin antibody Throm/6 (10

**Effect of GM2941 on expression of P-selectin.** The ability of GM2941 to inhibit histamine-mediated P-selectin expression was determined by a modified whole-cell ELISA assay as described pre-viously (Foreman et al., 1994). Monolayers were treated with 10 mM histamine for 10 min in the presence or absence of GM2941 (3 mg/ml). Cells were washed with PBS and fixed with 1% paraformaldehyde for 30 min, followed by the addition of nonfat dry milk (5% in PBS). Cells were incubated with the anti-P-selectin monoclonal antibody PB1.3 (1 µg/ml) or with an isotype-matched murine antibody for 45 min, followed by incubation with the peroxidase-conjugated goat anti-mouse secondary antibody (Dako Corporation, Carpenteria, CA) for 45 min. Cells were washed with PBS and then exposed to the substrate (o-phenylenediamine dihydrochloride, Sigma) for 30 min. The reaction was halted by addition of 3 M sulfuric acid. Optical density was determined by an automated microplate reader (EL340, Bio-Tek Instruments, Winooski, VT) set to a wavelength of 495 nm.

**Immunohistochemical analysis of P-selectin expression.** The anatomic distribution of P-selectin and MCP-1 expression was examined by immunohistochemistry as previously described (Mulligan et al., 1992b). The murine monoclonal antibody to human P-selectin, PB1.3 (Cytel Corporation, San Diego, CA), was utilized for P-selectin immunohistochimistry and has previously been shown to recognize rat P-selectin (Mulligan et al., 1992a). Controls included sections treated with a nonimmune primary antibody and sections in the absence of primary antibody. Detection of the primary antibodies was accomplished using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA). The peroxidase substrate was 3-amino-9-ethyl-carbazole.

**Lung MPO assay: quantitation of neutrophil accumulation.** A whole-lung MPO assay was used to quantitate pulmonary neutrophil recruitment (Mulligan et al., 1992b). Lungs harvested for MPO analysis were immediately frozen in liquid N2. At the time of MPO assay, tissue was placed in 2 volumes of homogenization buffer (50 mM sodium phosphate, pH 6.0) and homogenized (4 × 10 sec at setting 5) with a Polytron homogenizer (Tekmar Co., Cincinnati, OH). The homogenates were centrifuged for 30 min (3000 × g, 4°C) and the supernatants removed. Myeloperoxidase activity was determined by measuring the change in absorbance at 460 nm resulting from the conversion of H2O2 in the presence of o-dianisidine (Sigma) as previously described (Mulligan et al., 1992b). MPO activity measurements were normalized to the wet weight of the tissue.

**Fig. 2. In vitro analysis of the ability of GM2941 to decrease P-selectin-mediated human neutrophil-endothelial interactions.** Endothelial cells were treated with histamine (10 mM) for 10 min, and the degree of human neutrophil adhesion was determined. The presence of GM2941 (3 mg/ml; 2.4 µM) was associated with a significant decrease in neutrophil adhesion as compared with control. Results are representative of one experiment (n = 6). *P < .05 vs. vehicle control (one-way ANOVA). **P < .05 vs. histamine-treated monolayers (one-way ANOVA).
μg/ml) resulted in a similar degree of inhibition. Use of a nonspecific isotype-matched antibody did not decrease the degree of neutrophil adhesion (results not shown). These data demonstrate that GM2941 has the ability to decrease P-selectin-mediated neutrophil adhesion in vitro.

Pulmonary vascular expression of P-selectin after glucan infusion. Immunohistochemical analysis using the anti-P-selectin murine monoclonal antibody PB1.3 was carried out in order to examine the temporal and anatomic distributions of P-selectin expression after glucan infusion. As shown in figure 3A, positive staining for P-selectin was evident in the vascular walls of rats by 30 min after glucan infusion. In contrast, little immunoreactivity was present in sections removed immediately after glucan infusion (time zero; fig. 3B). Sections of tissue removed 30 min after the infusion of glucan but incubated with nonspecific IgG revealed no staining for P-selectin (results not shown). These results indicate that infusion of glucan is associated with a rapid (30-min) increase in P-selectin expression in the walls of pulmonary blood vessels.

Effect of GM2941 on circulating WBC concentrations. In order to determine the effect of GM2941 on circulating cell WBC concentrations, we conducted WBC and differential counts on whole-blood samples obtained 48 hr after GM2941 (30 mg/kg) administration (table 1). The concentrations of circulating neutrophils, monocytes and lymphocytes were not significantly different in animals treated with GM2941 compared with vehicle-treated animals. In addition, the concentration of circulating red blood cells was not affected by the compound (results not shown). Thus, infusion of GM2941 into rats is not associated with alterations in the concentrations of circulating blood cells.

GM2941 infusion inhibits glucan-induced pulmonary granuloma formation. GM2941 (30 mg/kg, 24 μM) resulted in reduced glucan-induced formation (table 2). An 80% reduction in average granuloma profile area was observed after 48 hr in GM2941-treated rats as compared with the vehicle control group (mean granuloma sizes 2734 ± 353 μm² and 13,728 ± 1104 μm², respectively (P < .05). Preliminary studies revealed that similar degrees of inhibition against granuloma formation were achieved in rats that received a single infusion of GM2941 as compared with those that also received a booster injection 24 hr after glucan infusion (results not shown).

Granulomas (48 hr after glucan infusion) in vehicle-treated animals were large and cellular, composed of numerous monocytes and macrophages with few neutrophils (fig. 4, A, B and C). In contrast, granulomas in GM2941-treated animals were significantly smaller and exhibited less cellularity (fig. 4, D, E and F). The reduction in both granuloma size and number are indicative of the ability of GM2941 to prevent full granuloma development.

The maximal degree of inhibition was seen at a dose of 30 mg/kg (24 μM; fig. 5). When the dose was increased to 90 mg/kg, no further decrease in granuloma size was noted.

GM2941 infusion prevents glucan-induced pulmonary neutrophil accumulation after glucan infusion. Quantitative measurements of whole-lung MPO activity, a quantitative measurement of neutrophil accumulation (Bradley et al., 1982), were obtained from GM2941-treated and vehicle-treated control animals to determine the effect of GM2941 on neutrophil recruitment (fig. 6). Rat lungs were removed 6 hr after infusion of glucan. Lungs from GM2941-treated animals (30 mg/kg) exhibited a significant decrease in MPO activity when compared with the lungs of vehicle-treated animals. These data suggest that GM2941 infusion

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**TABLE 1**

<table>
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<tr>
<th>Condition</th>
<th>Number</th>
<th>Neutrophils</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
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</thead>
<tbody>
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<td>Saline infusion (vehicle control)</td>
<td>3</td>
<td>59.3 ± 2.91%</td>
<td>2.7 ± 0.67%</td>
<td>36.7 ± 2.6%</td>
</tr>
<tr>
<td>GM2941 (30 mg/kg; 24 μM)</td>
<td>3</td>
<td>58.5 ± 1.5%</td>
<td>2.5 ± 0.5%</td>
<td>40.5 ± 1.5%</td>
</tr>
</tbody>
</table>

* Peripheral blood samples (100 μl) were obtained 48 hr after glucan infusion.

**TABLE 2**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of Rats</th>
<th>Granuloma Number/10× Field</th>
<th>Mean Granuloma Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline infusion (vehicle control)</td>
<td>5</td>
<td>1.99 ± 0.31</td>
<td>13,728 ± 1104 μm²</td>
</tr>
<tr>
<td>GM2941 (30 mg/kg; 24 μM)</td>
<td>3</td>
<td>1.18 ± 0.27*</td>
<td>2,734 ± 353 μm²</td>
</tr>
</tbody>
</table>

* Rats were sacrificed 48 hr after glucan infusion.

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Fig. 3. P-selectin immunoreactivity in rat lung sections removed 30 min after glucan infusion. A) P-selectin staining in sections removed at time zero reveals little localization of P-selectin associated with the pulmonary vasculature. B) However, 30 min after glucan infusion, P-selectin immunoreactivity is present along the lumina of the pulmonary blood vessels.
significantly reduces pulmonary neutrophil accumulation measured at 6 hr.

**GM2941 does not directly influence P-selectin expression.** A whole-cell ELISA assay was performed to examine potential direct effects of GM2941 on P-selectin expression by HUVECs. Endothelial cell monolayers were treated with histamine (10 mM) for 10 min in the absence or presence of GM2941 (3 mg/ml), and P-selectin expression was quantified. As shown in figure 7, histamine significantly increased P-selectin expression as compared with control. Incubation of HUVEC monolayers with histamine in the presence of GM2941 was also associated with a significant increase in P-selectin. There was no difference in P-selectin expression between monolayers treated with histamine plus GM2941 and those treated in the absence of the glycomimetic compound. These results indicate that GM2941 does not directly affect the degree of expression of P-selectin.

**Discussion**

Neutrophils are important in the pathogenesis of a wide variety of human lung diseases, including emphysema, ARDS and disease states incurred through the inhalation of particles (e.g., silica, quartz) (Henderson et al., 1991; Lugano...
treated with vehicle. For each group, n = 5 mg/kg) rats after removal 6 hours after glucan infusion. MPO content in the lungs of control and GM2941-treated (30 mg/kg) animals was significantly decreased as compared with the lungs of animals treated with vehicle (saline). *P < .05 vs. vehicle control (one-way ANOVA). For each group, n = 4.

**Fig. 5.** The protective effects of GM2941 are dose-dependent. Granulomas formed in the presence of GM2941 (30 mg/kg) exhibited decreased granuloma size. Animals pretreated with a dose of 3 mg/kg did not show a decrease in granuloma size. The results shown are from one experiment that is representative of three separate experiments (six animals per group). *P < .05 vs. vehicle control (one-way ANOVA).

**Fig. 6.** Lung MPO content in the lungs of control and GM2941-treated (30 mg/kg) rats after removal 6 hours after glucan infusion. MPO content in the lungs of GM2941-treated animals was significantly decreased as compared with the lungs of animals treated with vehicle (saline). *P < .05 vs. vehicle control (one-way ANOVA). For each group, n = 4.

**Fig. 7.** Effect of GM2941 on P-selectin expression as determined by a whole-cell ELISA assay. HUVEC monolayers were treated with histamine (10 mM) for 10 min in the presence or absence of GM2941 (3 mg/ml). No significant difference in P-selectin expression was observed between cells treated with histamine with or without GM2941. *P < .05 vs. cells treated with vehicle.

et al., 1982; Bowden and Adamson, 1978). Neutrophils have recently been shown to play an essential role in pulmonary granuloma development after the i.v. infusion of yeast cell wall glucan into rats (Kilgore et al., 1997). The requirement for neutrophils in granuloma development suggests that the glucan-induced granulomatous vasculitis model may be both useful and relevant in the assessment of pharmacologic interventions that limit the degree of neutrophil accumulation at sites of acute inflammation. In the present study, we examined the potential of the carbohydrate-based sLeα glycomimetic GM2941 to prevent the development of glucan-induced pulmonary granulomas in the rat.

As revealed in the present study, the expression of the adhesion molecule P-selectin is increased after glucan infusion. Furthermore, ICAM-1 has previously been shown to be up-regulated (Kishimoto et al., 1994). Direct evidence implicating neutrophils in glucan-induced granuloma formation has been obtained from studies that employed rats depleted of circulating neutrophils through the use of an antineutrophil antibody (Kilgore et al., 1997). Neutrophil depletion results in a significant reduction in granuloma size and number. A similar reduction in granuloma size was noted when animals were pretreated with the ROI scavenger catalase, a result that suggests a role for H2O2 in granuloma development. Both neutrophil depletion and pretreatment with catalase cause a marked decrease in pulmonary MCP-1 mRNA and protein expression (Kilgore et al., 1997). These data, in conjunction with in vitro data indicating that H2O2 and activated neutrophils can directly induce MCP-1 secretion, suggest that neutrophils (or their secreted products, such as H2O2) play a role in granuloma formation through the induction of proinflammatory mediators (e.g., MCP-1). Accordingly, pharmacologic approaches designed to disrupt adhesive interactions between circulating neutrophils and pulmonary endothelial cells may be useful in retarding pulmonary granuloma formation.

The sLeα sulfated glycomimetic GM2941 significantly reduced both granuloma size and number measured 48 hr after glucan infusion. These protective effects were associated with a marked decrease in early (6 hr) neutrophil accumulation within the lungs of treated rats. The primary mechanism by which GM2941 reduces granuloma size and number has yet to be fully elucidated, but both in vitro and in vivo data from this study suggest that this compound interrupts P-selectin-mediated neutrophil-endothelial adhesive interactions. Several investigators have proposed a two-step model of neutrophil-endothelial adhesion whereby L- and P-selectin act in concert to facilitate the recruitment of neutrophils into the microenvironment of the vasculature (von Andrian et al., 1991; Lorant et al., 1991). The initial adhesive interaction between neutrophils and endothelium is mediated by endothelium-derived P-selectin and its carbohydrate ligand sLeα, which is located on the surface of neutrophils. Another member of the selectin family, L-selectin, which is found on the neutrophil surface, is also involved in tethering the neutrophil to the endothelial cell surface (Lasky, 1992). The relatively weak “rolling” adhesive interaction that occurs between neutrophils and the endothelial surface is a prerequisite for a more firm adhesion mediated by the interaction of the β2 integrins (e.g., CD11b/CD18) with their endothelial counterligands (ICAM-1) (Lawrence and Springer, 1991).

The observation that neutrophil adhesion and subsequent migration from the vasculature is a multistep process suggested that disruption of one or more of these processes may serve to prevent or reduce neutrophil-mediated tissue injury.
In this study cannot be attributed to a general leukopenia, as evidenced by the similar numbers of circulating neutrophils in treated and control animals.

Prevention of neutrophil adhesion through the use of GM2941 may provide both direct and indirect protection against pulmonary granuloma formation after glucan infusion. The direct action of the compound, as we have noted, probably occurs through the inhibition of neutrophil adhesion/function and a resulting reduction in the extent of cellular damage mediated by the secretion of neutrophil-derived products (e.g., ROI, proteolytic enzymes). GM2941 and similar agents may also act indirectly to protect against granuloma formation by reducing the expression of proinflammatory mediators. Monocyte chemoattractant protein-1 is a monocyte-specific chemokine that has previously been shown to be required for full granuloma formation (Flory et al., 1993; Jones and Warren, 1992). Within 1 hr after glucan infusion, there is an increase in MCP-1 expression localized primarily to blood vessel wall cells. A second peak of MCP-1 expression is seen 24 hr after glucan infusion and is associated with lesional cells per se. The early rise in MCP-1 activity is associated with the transient influx of neutrophils into vessel walls and perivascular space (Flory et al., 1994). The temporal relationship between neutrophils and MCP-1 expression suggests that neutrophils may play a role in mediating the induction and expression of MCP-1 by resident alveolar cells. Previous in vitro studies using LPS-stimulated HUVECs have demonstrated that GM2941 does not directly alter the up-regulation of MCP-1 production (unpublished observation), which suggests that the compound is not exerting its protective effects by directly decreasing MCP-1 secretion. Thus, modulating neutrophil function with GM2941 may have an important influence on the up-regulation of MCP-1 and/or other proinflammatory cytokines. The importance of neutrophils in mediating monocyte recruitment is further exemplified by Doherty et al. (1988), who demonstrated, using a rabbit model of C5 fragment-induced lung inflammation, that the recruitment and migration of monocytes are neutrophil-dependent. That neutrophils are required for subsequent monocyte recruitment/migration, as demonstrated by previous studies (Kilgore et al., 1997; Doherty et al., 1988), underscores the potential value of inhibiting neutrophil adhesion in the setting of pulmonary inflammation.

The results of this investigation demonstrate that the sulfated sLe\textsuperscript{a} glycomimetic GM2941 has a salutary effect in reducing the degree of granuloma formation after the i.v. infusion of glucan. Moreover, the results derived from this study agree with the concept that the early, selectin-mediated adhesion of neutrophils at sites of glucan embolization is an important prerequisite for full granuloma formation. Thus these observations not only provide an understanding of the potential pharmacologic applications for GM2941 and similar agents but also offer further insight into the pathologic basis for pulmonary granuloma formation.

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References


Send reprint requests to: Jeffrey S. Warren, M.D., Department of Pathology, Box 0602, University of Michigan Medical School, 1301 Catherine Road, Ann Arbor, MI 48109-0602.