The Macrophage-derived Lectin, MNCF, Activates Neutrophil Migration through a Pertussis Toxin-sensitive Pathway

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SUMMARY The macrophage-derived neutrophil chemotactic factor (MNCF) is a D-galactose-binding lectin that induces neutrophil migration in vitro and in vivo. Neutrophil recruitment induced by MNCF is resistant to glucocorticoid treatment and is inhibited by the lectin-specific sugar, D-galactose. In the present study, we characterized the binding of MNCF to neutrophils and the responses triggered by this binding. Exposure to MNCF resulted in cell polarization, formation of a lamellipodium, and deep ruffles on the cell surface. By confocal microscopy, we observed that MNCF was evenly distributed on the cell surface after 30 min of incubation. The labeling intensity progressively diminished with longer incubations. Internalization kinetics showed that MNCF/ligand complexes were rapidly internalized, reaching maximum intracellular concentrations at 120 min and then decreased thereafter. The binding and internalization of MNCF were selectively inhibited by D-galactose. MNCF-induced neutrophil chemotaxis was inhibited by pertussis toxin. This fact strongly suggests that the MNCF–ligand on the neutrophil surface is a G-protein-coupled receptor (GPCR), similar to receptors for well-established neutrophil attractants. Our observations on the ability of MNCF to activate neutrophils are consistent with the increasing evidence for the participation of animal lectins in the innate immune response.

KEY WORDS mammalian lectin neutrophil migration neutrophil polarization inflammation

NEUTROPHILS play a crucial role in inflammation, and their accumulation within injured tissue is a hallmark of the acute inflammatory response. The movement of neutrophils from the circulation to sites of injury is initiated and directed by numerous exogenous and endogenous attractants that bind and activate seven-transmembrane G-protein-coupled receptors (GPCR) (Springer 1994; Baggiolini et al. 1997; Locati and Murphy 1999; Mantovani 1999). The response of neutrophils to attractants is largely due to the presence and level of expression of GPCRs on the cell surface. The number of GPCRs expressed on the cell surface is regulated by internalization of the receptor and its rapid recycling back to the plasma membrane (Van Koppen 2001). Although GPCRs have been found evenly distributed on the surface of neutrophils during chemotaxis, studies have demonstrated that a gradient of attractant can induce an asymmetry in the localization of phosphatidylinositol-3,4,5-triphosphate, with enhancement at the leading edge of the migrating neutrophil (Xiao et al. 1997; Servant et al. 1999, 2000; Tian et al. 2003). This implies that GPCR activation leads to changes in membrane organization and recruitment of signaling intermediates. In fact, neutrophils stimulated by attractants, such as chemokines, C5a, and N-formyl peptide, rapidly change their morphology from rounded cells covered with microvilli to elongate cells with ruffles. The activated cells also have pseudopodia that form broad lamellipodia that are extended anteriorly in the direction of the increasing concentration of attractant, whereas a contractile

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uropod is formed posteriorly. The result is a morphologically polarized cell that is efficient at vectorial migration (Grob et al. 1990; Chuntharapai and Kim 1995; Ray and Samanta 1997; Sánchez-Madrid and Del Pozo 1999).

Few recent studies show that lectins can attract neutrophils. Lectins can act as cell recognition mediators in a wide range of biological systems because of their great versatility and diversity in binding to carbohydrates (Sharon and Lis 1989). Plant lectins, such as KM+ (Santos-de-Oliveira et al. 1994), and mammalian lectins, such as macrophage-derived neutrophil chemotactic factor (MNCF) (Dias-Baruffi et al. 1995a,b) and galectin-3 (Sano et al. 2000; Sano and Liu 2001), can directly attract leukocytes and induce their movement. The neutrophil migration-inducing activity of MNCF has two unique characteristics. First, the neutrophil recruitment triggered in vivo by MNCF occurs even in animals pretreated with dexamethasone (Cunha and Ferreira 1986; Dias-Baruffi et al. 1995a,b). Second, incubation of MNCF with D-galactose blocks its ability to attract neutrophils both in vivo and in vitro (Dias-Baruffi et al. 1993, 1995a,b). MNCF is obtained from the supernatant of rat adherent peritoneal cells, following LPS stimulation. Supernatant chromatography on D-galactose column provides the recovery of chemokine-free MNCF in the D-galactose bound fraction (unpublished results). In fact, neutrophil attractants whose activity is inhibited by dexamethasone were recovered in the column flow-through (Dias-Baruffi et al. 1995a).

In the present study, we demonstrate that MNCF binds to glycans of a GPCR on the surface of human neutrophils and induces neutrophil polarization. Following incubation of neutrophils with MNCF, the lectin bound to the cell surface and was rapidly detected in the cytoplasm, indicating internalization of lectin-glycosylated ligand complexes. The response of the neutrophils to MNCF is comparable to that seen with the binding of known neutrophil attractants to their specific receptors. This report provides evidence for the significant role animal lectins play in the inflammatory response.

Materials and Methods

Neutrophil Purification

Heparinized human blood from healthy volunteers was layered on a neutrophil isolation medium (Cardinal Associates; Santa Fe, NM) density gradient and centrifuged at 400 × g for 30 min. The neutrophils were washed by centrifugation and suspended in RPMI medium (Gibco-BRL, Life Technologies; Gaithersburg, MD), at a concentration of 10^6 cells/ml. Samples were subjected to hypotonic lysis to eliminate remaining erythrocytes. Resulting preparations were 98% pure, and more than 95% of the neutrophils were viable as measured by trypan blue.

Preparation of the Lectin MNCF

MNCF was obtained and purified as previously described (Cunha and Ferreira 1986; Dias-Baruffi et al. 1995a). Briefly, the supernatant from LPS-stimulated rat macrophage monolayers was submitted to affinity chromatography on an agarose-D-galactose column (Pierce Chemical Co.; Rockford, IL). Neutrophil attractants whose activity is inhibited by dexamethasone were recovered in the column flow-through. The adsorbed fraction was eluted with 0.4 M D-galactose, yielding the MNCF preparation that was dialyzed against water, concentrated using a YM-10 membrane (Amicon Division, W.R. Grace and Co.; Beverly, MA), and stored at −70°C. Protein concentration was estimated through absorbance measurements at 280 nm by arbitrarily considering that 1.0 OD is equal to 1 mg/ml. All MNCF preparations were analyzed by SDS-PAGE and tested for biological activity.

Polarization Assays

Human neutrophils were plated at 10^6 cells/ml in RPMI medium. The neutrophils were incubated with MNCF (10 μg/ml RPMI), fMLP (10^-6 M/well), casein (15 μg/ml RPMI), or RPMI medium alone for 45 min at 37°C. In some experiments, the attractants were incubated with 0.2 M D-galactose or 0.2 M D-mannose for 30 min at room temperature (RT) before incubation with neutrophils. The percentage of neutrophil polarization was determined by counting the number of polarized and non-polarized cells in a Neubauer chamber. In some experiments, cells incubated with the attractants were also processed for transmission electron microscopy.

Transmission Electron Microscopy

Samples were rinsed twice in PBS and once in 0.1 M cacodylate (EM Sciences; Fort Washington, PA) buffer (pH 7.4) and then fixed at room temperature for 40 min in 2% glutaraldehyde, 2% paraformaldehyde in the cacodylate buffer containing 0.05% CaCl₂. After rinsing twice in PBS and once in cacodylate buffer, the cell preparations were embedded in 2% agar and postfixed at room temperature for 1 hr in 2% osmium tetroxide (EM Sciences). The samples were rinsed in distilled water, dehydrated through a graded series of ethanol and acetone, and embedded in Embed 812 (EM Sciences). Thin sections were cut with a diamond knife, mounted on copper grids, stained with uranyl acetate, and then incubated with streptavidin–FITC (Pierce Chemical Co.) for 30 min. The slides were mounted in Fluormount-G (EM Sciences) and examined by confocal microscopy using a
Leica TCS NT confocal microscope (Leica Microsystems; Heidelberg, Germany).

Internalization of MNCF–Ligand Complexes by Human Neutrophils

Non-fixed human neutrophils were placed on coverslips coated with Biobond (EM Sciences) and incubated with biotinylated MNCF (10 µg/ml RPMI) or with RPMI medium alone at 37°C for 30, 60, 120, and 180 min. After being rinsed with PBS, the cells were treated for 30 min at 4°C with 0.2 M D-galactose to quench the MNCF present on the cell surface and fixed for 20 min at RT with 2% formaldehyde. The cells were then incubated for 30 min at RT with streptavidin–FITC in PBS containing 0.01% saponin (Sigma; St Louis, MO). The slides were mounted in Fluormount-G (EM Sciences) and examined by confocal microscopy.

Chemotaxis Assays

Neutrophil migration was assayed in a modified Boyden chamber (a microchamber with 48 wells; NeuroProbe, Cabin John, MD) (Bignold 1988). One of the attractants, MNCF (10 µg/ml well), IL-8 (40 ng/well), or fMLP (10⁻⁶ M/well) was added to the lower wells of the chamber, separated from the upper wells by a 5-µm polycarbonate membrane filter (25 × 80 mm) (Poretics Corporation; Livermore, CA). After placing human neutrophils (5 × 10⁴ cells/well) in the upper wells, the chambers were incubated for 45 min at 37°C in a humidified incubator containing 5% CO₂ in air. Cells that migrated through the entire width of the membrane were counted. Random migration was assessed by using RPMI medium in the lower chamber. To examine the role of GPCRs on the MNCF chemotactic activity, neutrophils were preincubated with pertussis toxin (PTx, 500 ng/ml; List Biological Laboratories, Campbell, CA) for 90 min at 37°C, washed twice with RPMI medium, and added to the upper wells (5 × 10⁴ cells/well). The cells treated with PTx had a similar viability to that of the untreated cells (>95%). Five fields were counted for each assay, and each sample was assayed in triplicate. All results were expressed as mean ± SD.

Scanning Electron Microscopy

Human neutrophils were incubated for 45 min at 37°C with MNCF (10 µg/ml RPMI), which was preincubated for 45 min at 37°C with or without 0.2 M D-galactose. Some cells were preincubated with pertussis toxin (PTx, 500 ng/ml; List Biological Laboratories, Campbell, CA) for 90 min at 37°C and then incubated with MNCF (10 µg/ml RPMI) or medium alone for 1 hr at 37°C. Cells (1 × 10⁶) were plated onto 13-mm round glass coverslips coated with Biobond (EM Sciences) in RPMI medium. After being rinsed twice in PBS at 37°C, the cells were fixed for 4 hr in 2% glutaraldehyde in PBS, rinsed in 0.1 M cacodylate buffer (pH 7.4), and postfixed with 2% OsO₄ in cacodylate buffer for 30 min. The samples were then treated with a saturated aqueous solution of thioacetamide (EM Sciences) for 10 min and osmicated again for 30 min. The two latter steps were repeated once, the samples dehydrated in ethanol, critical-point dried in liquid CO₂, and coated with gold. Samples were examined in a JSM-5200 Scanning Electron Microscope (JEOL; Tokyo, Japan).

Statistical Analysis

Data are presented as the mean ± SD. Statistical determinations of the difference between means of experimental groups were performed using one-way ANOVA with Bonferroni posttest for multiple comparisons. Differences that provided p < 0.001 were considered as being statistically significant. All experiments were performed at least three times, each one using neutrophils from a different donor.

Results

MNCF Induces Human Neutrophil Polarization

The ability of MNCF to induce neutrophil polarization, a well-known characteristic of neutrophil activation by chemoattractants (Sánchez-Madrid and Del Pozo 1999), was examined. In a previous study (Dias-Baruffi et al. 1995b), we showed that the dose-response curve for MNCF-induced neutrophil migration was bell shaped with a maximum response at 10 µg/ml, an observation that was confirmed in the present study. MNCF (10 µg/ml) was able to induce the polarization of 85% of the neutrophils. The chemoattractants fMLP and casein induced polarization in 70% and 78% of neutrophils, respectively, whereas only 18% of cells incubated with medium alone were polarized (Figure 1). To verify if neutrophil polarization induced by MNCF was dependent on sugar recognition, assays were per-
formed using MNCF preincubated with either of the monosaccharides D-galactose or D-mannose (0.2 M). The percentage of neutrophils polarized by MNCF was reduced by 65% in the presence of D-galactose and was not affected by D-mannose (Figure 1). These monosaccharides had no effect on neutrophil polarization induced by fMLP or casein or on the spontaneous polarization that occurs in the absence of stimuli (not shown).

Transmission electron microscopy was also used to examine the polarization of neutrophils induced by MNCF. The cells incubated with MNCF (Figure 2A) were elongate, contained abundant electron-dense granules in the cytoplasm, and had lamellipodium free of organelles. Similar structural changes were observed with neutrophils stimulated with fMLP (B) or casein (C) (positive controls). Cells that were incubated with RPMI medium (negative control) showed no morphological changes (D). Bar = 2 μm.

Figure 2 MNCF induces ultrastructural changes in neutrophils. (A) Isolated human neutrophils incubated for 45 min at 37°C with MNCF (10 μg/ml) were elongate, contained abundant electron-dense granules in the cytoplasm, and had lamellipodium free of organelles. Similar structural changes were observed with neutrophils stimulated with fMLP (B) or casein (C) (positive controls). Cells that were incubated with RPMI medium (negative control) showed no morphological changes (D). Bar = 2 μm.
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organelles. Similar effects on neutrophil morphology were seen in cells treated with either fMLP (Figure 2B) or casein (Figure 2C). Neutrophils incubated with RPMI medium only (Figure 2D) maintained their spherical shape with characteristic granules.

MNCF Binds to the Neutrophil Cell Surface and Is Internalized

To visualize the MNCF bound to glycoligands on the surface of human neutrophils, cells were incubated with biotinylated MNCF followed by streptavidin–FITC. After a 30-min incubation, confocal microscopy showed that biotinylated MNCF was evenly bound to the cell surface (Figure 3A). Fluorescent labeling on the cell surface clearly decreased with longer incubation periods (Figures 3C, 3E, and 3G).

Lectin internalization was then investigated. Neutrophils were incubated with biotinylated MNCF for varying times, rinsed with 0.2 M d-galactose, and fixed. The cells were permeabilized and incubated with streptavidin–FITC. These cells displayed punctuate intracellular labeling after 30- and 60-min incubation with MNCF (Figures 3B and 3D). Maximum labeling occurred at 120 min of incubation (Figure 3F). At this time there was little or no labeling of the cell surface, suggesting that the majority of lectin–ligand complexes formed on the neutrophil surface were internalized. After 180 min, the intracellular labeling decreased (Figure 3H) and was close to that observed after a 60-min incubation. Cells incubated with RPMI medium only did not show any labeling (Figures 3I and 3J), similar to the cells incubated with MNCF pretreated with 0.2 M d-galactose (not shown).

Pertussis Toxin (PTx) Inhibits Neutrophil Migration Induced by MNCF

To determine if MNCF induces neutrophil migration by interacting with GPCRs, we investigated the effect of PTx on cell polarization and migration induced by this lectin. Pretreatment of neutrophils with PTx abolished the MNCF-induced neutrophil migration (Figure 4). The original response to MNCF (97.87 ± 1.337) decreased drastically (9.94 ± 0.665) and was similar to that found in the absence of the stimuli (13.06 ± 0.72). As expected, IL-8 and fMLP were also unable to induce migration of neutrophils that had been pretreated with PTx (Figure 4). This finding is in agreement with other studies showing that chemokines and other known neutrophil attractants trigger directional cell surface at 30 min of incubation (A). The cell surface labeling progressively decreased at 60, 120, and 180 min of incubation (C,E,G). After incubation with biotinylated MNCF, some neutrophil suspensions were rinsed with 0.2 M d-galactose and incubated with streptavidin–FITC and 0.01% saponin. At 30 (B) or 60 (D) min of incubation, an intense punctuate intracellular labeling was detected, attributable to MNCF internalization. After 120 min of incubation with MNCF (F), the cells showed maximum intracellular labeling. The intracellular labeling was decreased at 180 min (H). Neutrophils incubated with RPMI medium (negative control) were not labeled. (I,J) show the fluorescence and differential interference contrast images, respectively, of neutrophils incubated with medium only.
cell movement by binding to GPCRs (Bagnolinni et al. 1997; Locati and Murphy 1999; Mantovani 1999).

Scanning electron microscopy showed that after a 30-min incubation with MNCF the neutrophils were spread over the substratum, exhibited deep membrane ruffling, and lost their fine microvilli (Figure 5A). When neutrophils were pretreated with PTx and then incubated with MNCF, no morphological changes were observed (Figure 5B). The neutrophils maintained their spherical shape, and their surface was covered with microvilli. Neutrophils and neutrophils pretreated with PTx incubated with RPMI medium only also showed no morphological changes (Figures 5C and 5D).

**Discussion**

In the present study, we have expanded our characterization of the inflammatory properties of the mammalian lectin MNCF, which is secreted by activated macrophages (Dias-Baruffi et al. 1995a,b; Moreno et al. 2003). When MNCF binds to the cell surface, it induces neutrophil polarization and migration. This neutrophil activation, as well as migration, can be inhibited by exposure to pertussis toxin, suggesting that MNCF acts through a GPCR. MNCF induces neutrophil migration by binding to D-galactose-containing glycans, even in animals pretreated with dexamethasone (Cunha and Ferreira 1986; Dias-Baruffi et al. 1993, 1995a).

Following short exposures to MNCF, human neutrophils became polarized, contrasting with non-stimulated cells and similarly to cells incubated with well-characterized neutrophil attractants such as fMLP and casein (Sánchez-Madrid and Del Pozo 1999; Wagner and Roth 2000; Lindbom and Werr 2002). As observed in response to other known neutrophil attractants (Lauffenburger and Horwitz 1996), the transition from a spherical to a typically polarized shape induced by MNCF is associated with the formation of a broad, ruffled lamellipodium that is free of organelles. Large electron-dense granules were observed in the cell body, indicating the movement of secretory granules, which might reflect the reorganization of cell compartments that follows neutrophil activation after a wide variety of inflammatory stimuli (Sengelov et al. 1993a,b; Faurschou and Borregaard 2003).

The fact that D-galactose selectively inhibits the MNCF-induced neutrophil polarization, but not in the case of fMLP or casein-induced polarizations, suggests that the response to MNCF is due to its interaction with D-galactose-containing glycans on the neutrophil surface. The specific inhibitory effect of D-galactose was expected because this monosaccharide blocked all MNCF activities described until now, including induction of neutrophil migration in vivo and in vitro (Dias-Baruffi et al. 1995b), mast cell degranulation, and binding to rat IgE (Moreno et al. 2003). These findings indicate that the carbohydrate recognition domain of MNCF is responsible for its biological properties. Because our results show that MNCF is a neutrophil activator, we suggest that the MNCF glycoligands, like cell ligands for other well-established chemoattractants (Gerard and Gerard 1994; Horuk 1994), correspond to cell surface receptor(s) able to initiate cell signaling.

The MNCF binding pattern on the cell surface corresponds to the uniformly dispersed localization of receptors for well-known chemoattractants (Servant et al. 1999; Jin et al. 2000). The kinetics of MNCF binding and internalization in neutrophils, as reported here, is consistent with previous studies on the internalization of attractant–receptor complexes by neutrophils (Grob et al. 1990; Samanta et al. 1990; Chintharlapai and Kim 1995; Sabroe et al. 1997). Feniger-Barish et al. (1999) demonstrated that IL-8 internalization by neutrophils is initiated within 5 min of contact with the cells, and the intracellular signal is more intense at 30 and 120 min. A rapid and reversible disappearance of attractant receptors from the neutrophil surface, upon ligand binding, involves receptor clustering on the plasma membrane, receptor internalization, and recycling and accounts for the chemoattractant desensitization of neutrophils (Sabroe et al. 1997), a process that regulates neutrophil accumulation during an inflammatory response in vivo.

Receptors for well-established chemoattractants belong to the serpine family characterized by seven-transmembrane domains. They transmit intracellular
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signals by receptor phosphorylation dependent G proteins (Chuang et al. 1996; Ferguson et al. 1996; Böhm et al. 1997; Koenig and Edwardson 1997), a process blocked by pertussis toxin (Becker et al. 1987; Berger et al. 2002). In our experiments, pertussis toxin drastically inhibited MNCF, as well as fMLP and IL-8 without affecting cell viability. Among lectins, this effect is not unique to MNCF, once it has been demonstrated that galectin-3 attracts mononuclear leukocytes through the activation of a specific GPCR (Sano et al. 2000).

IL-8 and other CXC chemokines activate human neutrophils through their interaction with the CXCR1 and CXCR2 receptors, which are coexpressed on the cell surface (Holmes et al. 1991; Murphy and Tiffany 1991). These receptors are seven-transmembrane domain-type proteins functionally coupled to G proteins. Because they are glycoproteins (Besemer et al. 1989; Grob et al. 1990), these receptors are potential ligands for MNCF. The CXCR2 amino acid sequence contains consensus sites for N-glycosylation at asparagine residues, located within the N-terminal part of the receptor (Asn\textsuperscript{17}) and within the second extracellular loop (Asn\textsuperscript{186} and Asn\textsuperscript{197}) (Murphy and Tiffany 1991). The second extracellular loop is a critical domain for agonist binding and for initiation of the signal transduction pathway (Vaidehi et al. 2002). At least two of these sites are used, each carrying an N-linked sugar moiety of 9 kDa. Indeed, two distinct CXCR-2 glycoforms were identified in neutrophils, each with a distinct subcellular distribution. The highly glycosylated variants are expressed on the cell surface, whereas a less-glycosylated form is found as an intracellular pool of CXCR-2 molecules. Full glycosylation of the receptor is required for its resistance to neutrophil proteases (Ludwig et al. 2000) but not for its interaction with

Figure 5 Neutrophil pretreatment with pertussis toxin inhibits morphological changes induced by MNCF. Isolated human neutrophils incubated for 30 min at 37°C with MNCF (A) showed significant morphological changes; these cells spread and showed deep membrane ruffling. Neutrophils pretreated with pertussis toxin and then incubated with MNCF (B) remained unchanged and had the same morphology as the negative controls (incubated with RPMI). Neutrophils pretreated with pertussis toxin (D) or without (C) and incubated for 30 min at 37°C with RPMI medium retained their spherical shape.
IL-8. This observation goes against the general idea that N-glycans on cell-surface receptors influence their ligand binding (Paulson 1989). On the other hand, our observations strongly suggest that when agonists are lectins, a direct involvement of receptor glycans in binding and cell signaling may be required. This is true for KM+, a plant lectin that induces neutrophil migration (Santos-de-Oliveira et al. 1994) by interacting with CXCR-2 glycans (Pereira-da-Silva G et al., unpublished data). Highly glycosylated CXCR-2 forms are likely targets for MNCF recognition on the neutrophil surface and are probably able to initiate cell activation.

Relatively high concentrations of MNCF are required for the demonstration of optimal chemotactic and cell-polarizing activities, when compared with chemokine concentrations necessary to trigger similar responses in neutrophils (Feniger-Barish et al. 1999). High local concentrations of MNCF could be attained if there is a burst of proteins released from stimulated macrophages as postulated galectin-3 (Sano et al. 2000). We observed that macrophages present strong labeling for MNCF in their cytoplasm and that the lectin is rapidly mobilized to the cell surface and to the extracellular medium following LPS stimulus (unpublished data). One hour after stimulation, the cells no longer expressed MNCF, and the lectin could be captured from the supernatant by d-galactose conjugated to agarose beads (Dias-Baruffi et al. 1995a).

The resistance of the MNCF activity to glucocorticoids makes the lectin a very special inflammatory mediator and justifies the interest in its full biological and structural characterization. Until now, this task has been seriously limited by the fact that only one MNCF source has been identified, i.e., rat peritoneal macrophages requiring a large number of animals to yield a low amount of purified lectin. The obtained preparations were demonstrated to be free of chemokines. Other macrophage products able to induce neutrophil migration, such as IL-8, were recovered in a different chromatography fraction obtained from MNCF, i.e., they were not adsorbed on immobilized d-galactose. In contrast to MNCF, their inflammatory activity was inhibited by pretreating the test animals with dexamethasone and was maintained in the presence of d-galactose. In addition, anti-IL-8 neutralizing antibodies only blocked the neutrophil chemotaxis induced by the non-bound fraction but exerted no effect on the MNCF attractant activity (Dias-Baruffi et al. 1995a,b). Our laboratory recently obtained MNCF from macrophage cell lines (unpublished data), which will probably allow us to complete the lectin characterization.

In conclusion, the present study shows that MNCF exerts its neutrophil migration-inducing property by recognizing d-galactose-containing glycans, most likely on G-protein-coupled receptors on the cell surface. This interaction results in neutrophil activation, as demonstrated by morphological changes in the cells, accompanied by internalization of MNCF complexes. Highly glycosylated CXCR-2 forms are likely targets for MNCF binding on the neutrophil surface. Our observations are consistent with increasing evidence for the participation of animal lectins in the innate immune response.

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