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# Macrophage-released neutrophil chemotactic factor (MNCF) induces PMN-neutrophil migration through lectin-like activity

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## Abstract

We have previously reported that rat peritoneal macrophages stimulated with LPS release a factor (MNCF) which induces neutrophil migration that is not blocked by glucocorticoids. The supernatant of macrophage monolayers stimulated with LPS was submitted to affinity chromatography on immobilized sugar columns. We observed that the D-gal binding fraction retained MNCF activity. This fraction, consisting of four protein components, was submitted to chromatography on Superdex 75, yielding a homogeneous preparation of the active component. MNCF has a MW of 54 KDa (gel filtration and SDS–PAGE) and pI < 4.0 (isoelectrofocusing and chromatofocusing). D-gal did not interfere with the behaviour of known interleukins (IL-1$\beta$, IL-6, IL-8 TNF-$\alpha$), but blocked MNCF activity in an *in vitro* migration assay. The present results reinforce our previous suggestion that MNCF may correspond to a novel monokine which induces neutrophil migration through a direct mechanism involving the D-gal binding site of the molecule.

## Introduction

In an inflammatory process, resident macrophages have been considered to act as "alarm" cells responsible for initiating neutrophil migration to the site of injury [1–4]. One argument consistent with this idea was the demonstration that macrophage monolayers stimulated by LPS release a chemotactic factor for neutrophils, MNCF, which was active when tested in the Boyden chamber and when injected into the peritoneal cavities of rats. The neutrophil migration induced *in vivo* by the MNCF was not reduced by pretreatment with dexamethasone [4] or by peritoneal resident cell depletion of test rats [5]. These observations have led to the hypothesis that MNCF does not correspond to any known monokine and acts as a resident cell-independent inducer of neutrophil migration.

To warrant a peritoneal influx of white blood cells to an inflammatory site, the adhesive interactions between circulating leukocyte populations and the endothelium are considered critical [6]. A number of adhesion molecules involved in this phenomenon have been characterized as glycoproteins with a lectin-like domain and for some the adhesive functions are inhibited by sugars. The members of this adhesion family are named selectins (formerly known as LEC-CAMs, lectin cell adhesion molecules) [7–8]. The discovery of selectins provides compelling evidence for the potential role of carbohydrate–protein interactions in white blood cell adhesion and, consequently, in white blood cell

migration. The possibility that MNCF may have a carbohydrate binding property is investigated in the present study.

## Materials and methods

The supernatant of rat peritoneal macrophage monolayers stimulated by LPS was obtained as previously described [4] and subjected to affinity chromatography on D-mannose- or D-galactose-immobilized columns (Pierce Chemical Co., Rockford, Illinois, USA). The bound fractions were eluted with 0.4 *M* D-mannose or D-galactose and tested for their ability to induce neutrophil migration when injected into the dorsal air pouch or peritoneum of dexamethasone-pretreated rats (0.5 mg/kg). The bound active fraction was analysed by SDS–PAGE and submitted to gel filtration on a Superdex 75 HR 10/30 column (Pharmacia Fine Chemicals, Uppsala, Sweden), previously calibrated with known MW markers, equilibrated with PBS and operated at 20 °C, 0.5 ml/min. Each chromatographic fraction (0.5 ml) was monitored using an *in vitro* neutrophil chemotactic assay and the active fraction (confirmed by *in vivo* tests), was analysed by SDS–PAGE, isoelectrofocusing (Phast gel, Pharmacia Fine Chemicals, Uppsala, Sweden) and chromatofocusing (MONO-P HR 5/5 column eluted with polybuffer 96, Pharmacia Fine Chemicals, Uppsala, Sweden). The effect of D-galactose on the *in vitro* chemotactic activity of MNCF purified as described above, on FMLP and on human recombinant cytokines was determined as described in Fig. 1.

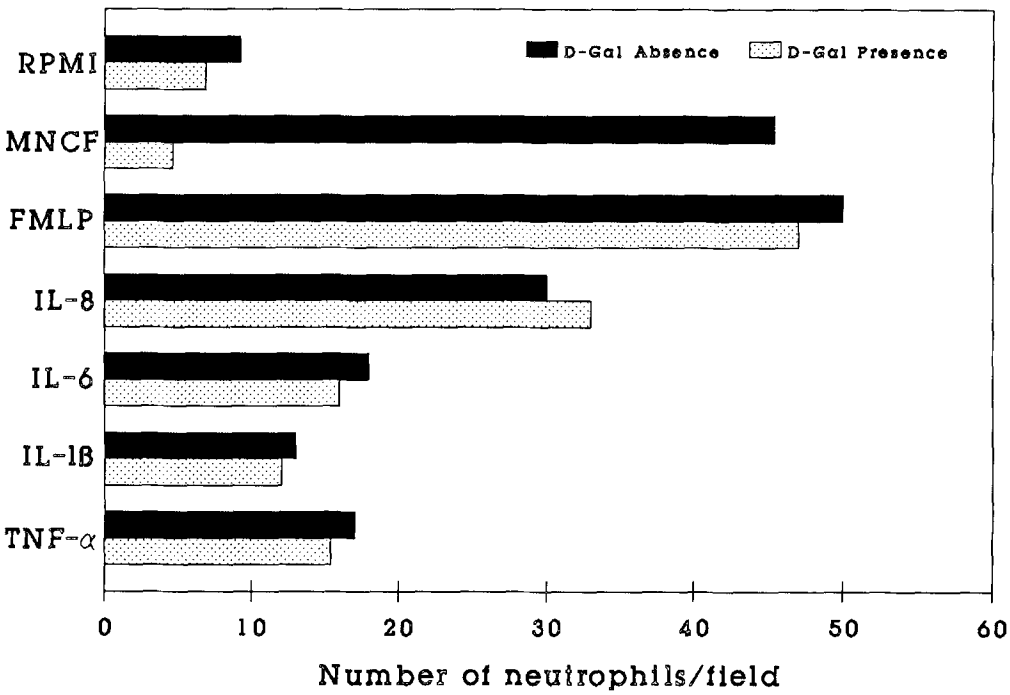


**Figure 1**
Effect of D-gal presence on *in vitro* human neutrophil migration induced by MNCF. Test samples in the presence or absence of D-gal, 0.4 *M*, were applied (29 µl/well) to the lower chamber in triplicate. The amount of MNCF used originated from the supernatant obtained by the incubation of $1.07 \times 10^9$ peritoneal macrophages. The other substances tested in the following amounts: FMLP, 44 pg; IL-8, 10 ng; IL-6, 10 ng; IL-1$\beta$, 10 ng; TNF-$\alpha$, 10 ng. Human neutrophils ($5 \times 10^4$) were added to each upper chamber well. After incubation at 37 °C, 5% $CO_2$, for 1 h, the polycarbonate membrane was washed, fixed and stained. The number of neutrophils which migrated to the lower surface of the membrane was determined. The results represent the average of triplicate measurements.

## Results and discussion

In order to investigate the lectin property of MNCF, we have submitted the crude supernatant of macrophage monolayers stimulated with LPS to affinity chromatography on sugar-immobilized columns. MNCF activity was detected in the fraction bound to the D-galactose–agarose column eluted with a 0.1 M D-galactose solution. Electrophoretic analysis of the bound fraction (D-gal+) showed four protein bands corresponding to 39, 45, 54 and 68 KDa MW. The D-gal+ fraction was submitted to gel filtration on Superdex 75 HR 10/30 and the active material was eluted in a volume corresponding to 54 KDa. SDS–PAGE of this material shows only one band with a migration corresponding to the same MW. An MNCF pI below 4 was indicated by isoelectrofocusing or chromatofocusing. Thus, these results characterize MNCF as an acidic protein, MW 54 KDa, which is able to bind D-galactose. This sugar binding property was utilised to purify MNCF. The purified MNCF was able to induce neutrophil migration both *in vivo* and *in vitro*. The activity was inhibited by D-gal, as illustrated in the figure, whereas the presence of sugar had no effect on the behavior of FMLP, IL-8, IL-6, IL-1$\beta$ or TNF-$\alpha$ in the same assay. Thus, a direct involvement of the lectin binding property of MNCF in the induction of neutrophil migration has been demonstrated. Furthermore, MNCF was distinguishable from other known chemotactic agents and/or monokines. The observation that MNCF acts directly on neutrophils confirms the previous suggestion that a resident cell independent mechanism is responsible for the induction of migration [4, 5]. However, it is not yet known if the *in vivo* action of MNCF also involves adhesion of neutrophils to endothelial cells, for example, through a double binding of MNCF to glycoproteins of both cell surfaces. This hypothesis is now under investigation in order to evaluate the possibility that MNCF may be classified as a member of the selectin family [7] and to create new opportunities for the development of carbohydrate anti-inflammatory drugs [8].

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