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The identification and partial characterization of a human mononuclear cell-derived neutrophil chemotactic factor apparently distinct from IL-1, IL-2, GM-CSF, TNF and IFN-γ

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SUMMARY

We have identified a neutrophil chemotactic factor (NCF) in supernatants from human blood mononuclear cells (MNC) cultured in the presence of phytohaemogglutinin (PHA). Maximal activity was observed 48 hr after culture. Following gel filtration, NCF eluted as a single major peak, together with proteins, having a molecular size of approximately 10,000 MW. The material gave a single band on SDS–PAGE but was heterogeneous following chromatofocusing (pIs approximately 6·8–7·0, 5·5–6·0 and 5·0). The biological activity of the partially purified material was abolished by trypsin and chymotrypsin treatment. NCF was heat stable (70°, 60 min) and promoted both directional migration (chemotaxis) of neutrophils and, to a lesser extent, stimulated random locomotion (chemokinesis). The factor was not associated with detectable amounts of IL-1, IL-2 or interferon-gamma (IFN-γ). MNC-derived NCF had a molecular size lower than recombinant granulocyte–monocyte colony-stimulating factor (rGM-CSF) and recombinant tumour necrosis factor (rTNF), and was considerably more active in chemotaxis. Optimal chemotactic concentrations of partially purified MNC-derived NCF were of comparable potency to FMLP and LTB_4 and had about 60% of the activity of optimal concentrations of C5a, C5a-des-Arg and platelet-activating factor (PAF). These experiments indicate that human MNC-derived NCF is a potent chemo-attractant distinct from other cytokines previously reported to promote neutrophil locomotion.

INTRODUCTION

Although mononuclear phagocytes and lymphocytes are prominent in cell-mediated immunity, and early infiltration of neutrophils is also characteristic of many delayed-type hypersensitivity reactions the *in vitro* production of chemotactic factors by stimulated mononuclear cells (MNC) has been demonstrated in a number of species, including man. These 'lymphocyte-derived chemotactic factors' (LDCF) were generated from MNC preparations stimulated both with polyclonal mitogens such as PHA, as well as specific antigen (Altman *et al.*, 1973) or with serotonin (Foon *et al.*, 1976). LDCF was investigated primarily in terms of monocyte chemotaxis, whereas the neutrophil chemotactic activity of stimulated MNC supernatant does not appear to have been documented in any detail in man.

More recently a number of cytokines have been described with chemotactic activity for neutrophils and/or monocytes. These include IL-1 (Sauder *et al.*, 1984), tumour necrosis factor (TNF) (Ming, Bersani & Mantovani, 1987) and granulocyte–monocyte colony-stimulating factor (GM-CSF) (Wang *et al.*, 1987). Since some or all of these factors may have been present, to a greater or lesser extent, in supernatants from MNC stimulated with mitogen or antigen, it seems important to clarify the relationship between neutrophil chemotactic factor (NCA) and a number of currently recognized cytokines. In the present report we have established that the major chemo-attractant for neutrophils in PHA-stimulated MNC supernatants is a protein of approximately 10,000 MW that is apparently distinct from IL-1, IL-2, GM-CSF, TNF and IFN-γ.

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MATERIALS AND METHODS

Generation of neutrophil chemotactic activity

Human peripheral blood MNC were prepared from heparinized venous blood by Ficoll–Paque (Pharmacia AB, Uppsala, Sweden) centrifugation. The MNCs at the interface were washed three times with RPMI-1640 culture medium (Gibco Ltd, Paisley, Renfrewshire) supplemented with 25 mM HEPES, 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin (Gibco Ltd) and resuspended at a concentration of 2×10^6 cells/ml. One millilitre of cell suspension in 12 × 75 mm cell tissue

culture tubes (Falcon 2054, Becton-Dickinson Laboratories, Lincoln Park, NJ) was incubated with different concentrations of PHA (Sigma Chemical Co., Poole, Dorset) for varying lengths of time in a 5% CO_2 atmosphere at 37° and 95% humidity. After incubation, the cell suspensions were centrifuged at 400 *g* for 10 min at 4° and the sterile cell-free supernatants aliquoted and stored at −20° until tested for chemotactic activity. Cell viability, as assessed by trypan blue dye exclusion, was >90%. Controls included (i) medium alone, (ii) cell supernatants incubated in the absence of PHA and (iii) supernatants from unstimulated cultures to which PHA was added at the end of culture. A large batch ('Batch II') was prepared from the leukapheresis packs of seven different donors. These cells were separated, stimulated with 5 μg/ml of PHA and cultured for 48 hr as described above. Cell-free supernatants were pooled and concentrated 100-fold using a hollow fibre cartridge (HIP 5-20) with a molecular weight cut-off of 5000 (MWCO 5000), followed by Diaflo ultrafiltration on a YM5 membrane, MWCO 5000 (Amicon Ltd, Stonehill, Gloucestershire).

Cell proliferation assay

Proliferation was assessed by incubating 2×10^5 MNC in triplicate in 96-well round-bottomed microtitre plates (Flow Laboratories, Rickmansworth, Herts) under the same experimental conditions described above. Tritiated thymidine ([^{3}H]TdR) (Amersham International plc, Amersham, Bucks, 0·66 μCi/well), was added during the final 6 hr of incubation. The cells were collected on glass fibre paper, GF/C (Ilacon Ltd, Tonbridge, Kent) using a multi-harvester apparatus (Titertek Cell Harvester, Skatron, Lier, Norway). Radioactivity was determined in a liquid scintillation counter.

Neutrophil locomotion

Neutrophil locomotion was described with a modified Boyden technique (Boyden, 1962) using a 48-well microchemotaxis assembly (Falk, Goodwin & Leonard, 1980) (Neuro-Probe Inc., Cabin John, MD) and nitro-cellulose filters (Sartorius membrane filters, 8 μm pore size, 24 Göttingen, FRG). Leucocyte-rich plasma, obtained by dextran sedimentation (Dextraven 110, Fisons plc, Loughborough, Leicester) of heparinized blood from normal healthy donors, was applied to a Ficoll–Paque cushion. After centrifugation at 400 *g* for 30 min at 20°, the cell pellet was retained, erythrocytes lysed using 0·153 M ammonium chloride buffered with 0·01 M potassium bicarbonate pH 7·3, the granulocytes washed in Hank's balanced salt solution (HBSS) (Gibco Ltd) and resuspended in HBSS containing 30 mM HEPES and 0·4% ovalbumin (Grade VI, Sigma Chemical Co.). This procedure routinely gave neutrophil purity of >96%.

Twenty-five microlitres of test solutions and diluent controls were placed in the lower compartment of the chemotaxis chamber. Fifty microlitres of neutrophil suspension, adjusted to 5×10^6/ml, were placed in the upper compartment.

In each assay formyl-methionyl-leucyl-phenylalamine (FMLP) (Sigma Chemical Co.), at a concentration of 10^{-7} M, was used as a positive control. Following 90 min incubation at 37°, the filters were removed, fixed and stained as previously described (Turnbull & Kay, 1976), and the number of cells that had migrated through the entire thickness of the filter counted and the results were expressed as the total number of cells in 10 random high-power fields (HPF). The samples were assayed in triplicate and coded in such a way that the investigator did not have prior knowledge of the protocol. The cell counts of each triplicate did not differ by >20%.

The relative potency of the fast-protein liquid chromatography (FPLC)-purified NCF was compared with a number of recognized neutrophil chemoattractants, including complement C5a and C5a-des-arg (a gift from Dr P. J. Jose, Clinical Research Centre, Harrow, Middlesex), platelet-activating factor (PAF-acether, C16; Bachem U.K. Ltd, Saffron Walden, Essex) and leukotriene B_4 (LTB_4) (Miles Laboratories, Slough, Bucks).

Gel filtration

Gel-filtration chromatography was performed on columns of Superose 12 prep. grade in a FPLC system (Pharmacia AB). Two millilitres of three-fold concentrated MNC supernatants (Speed Vac Concentrator 200H, Savant Instruments Inc., Hicksville, NY) were applied to an HR 16/50 column (Pharmacia) previously equilibrated with phosphate-buffered saline (PBS) (0·43 g/l KH_2PO_4, 1·16 g/l Na_2HPO_4 and 6·8 g/l NaCl, pH 7·35). Chromatography was performed at 19° with a flow rate of 0·5 ml/min and 1-ml fractions were collected. The column was calibrated with molecular weight markers [Blue Dextran (2,000 MW), thyroglobulin (669,000 MW), aldolase (158,000 MW), bovine serum albumin (BSA) (67,000 MW), ovalbumin (43,000 MW), chymotrypsinogen (25,000 MW), ribonuclease A (13,700 MW, vitamin B12 (1,300 MW); Pharmacia AB]. In other experiments 2-ml aliquots of Batch II were loaded to a Superose 12 prep. grade column in an FPLC system and eluted in PBS as described above. After testing in the neutrophil locomotion assay, the fractions (6 ml) corresponding to the peak of NCA at a molecular size of 10,000 MW were pooled and used in the experiment shown in Fig. 5 and Tables 2 and 3. The protein content in Batch II-pooled fractions was determined by Bio-Rad protein microassay using bovine-gamma-globulin as a standard (Bio-rad Laboratories Ltd, Watford, Herts).

Chromatofocusing

Three millilitres of the peak of NCA obtained from FPLC analysis were concentrated to 1 ml and dialysed for 24 hr at 4° against 0·025 M bis-Tris-imino diacetic acid buffer (Sigma), pH 7·1, and applied to a Mono-P HR 5/20 column (Pharmacia AB) that had been equilibrated previously with the same buffer. A 10% solution of Polybuffer 74-amino diacetic acid (Pharmacia) pH 4, was used for elution at a flow rate of 0·5 ml/min. A linear pH gradient of 40 ml from pH 7 to 4 was generated and 1-ml fractions collected. The fractions were dialysed for 24 hr against two changes of 100 times volumes PBS, pH 7·35, and subsequently tested for chemotactic activity. The column was regenerated with 2 M imino diacetic acid disodium salt and re-equilibrated with the starting buffer before each experiment.

Polyacrylamide gel electrophoresis

SDS–PAGE was performed according to the method of Laemmli (1970). One millilitre of the peak of NCA obtained from FPLC analysis was dialysed against distilled water (Spectra/Por 1, Spectrum medical Industries Inc., Los Angeles, CA) and lyophilized. The sample was resuspended in 50 μl of 3% SDS 0·01 M Tris (pH 8·1) and separated on 15% SDS–PAGE. The gel was stained by a protein silver stain method (Bio-Rad

Laboratories Ltd, Watford, Herts). Molecular weight markers were run in adjacent lanes. Ovalbumin (43,000 MW), carbonic anhydrase (30,000 MW), soybean trypsin inhibitor (20,100 MW), alpha-lactalbumin (14,400 MW) (Pharmacia AB) and insulin (6,000 MW) (Sigma Chemical Co.) were used as molecular markers.

IL-2 bioassay

IL-2 activity of supernatants and FPLC fractions was assayed by the inability to support the growth of a human IL-2-dependent T-cell line (Gillis *et al.*, 1978). For bioassay, 2×10^4 cells were washed and resuspended to 100 μl RPMI-1640 culture medium, 50 μl of PBS containing 1 mg/ml BSA (Sigma Chemical Co.) and 50 μl of test sample were mixed to test the activity. After 24 hr incubation, cultures were pulsed with 0·66 μCi of [^{3}H]TdR in 10 μl. After further 16–18 hr incubation the well contents were harvested using a multi-harvester onto glass fibre paper and the incorporated radioactivity was determined by liquid scintillation counting.

Recombinant IL-2 (rIL-2) was tested at serial two-fold dilutions starting at 1 U/ml, and the results generated were evaluated by reference to a double logarithmic plot of the counts per minute of [^{3}H]TdR uptake versus the dilution of rIL-2. Culture medium and PHA 5 μg/ml were included in the assay as controls. Experiments were performed in triplicate.

IL-1 bioassay

IL-1 bioactivity was tested by Dr R. A. Wolstencroft (The Rayne Institute, St. Thomas' Hospital, London) in a conventional mouse-thymocyte co-mitogenic assay (Mizel, Oppenheim & Rosenstreich, 1978). The samples were assayed at a final dilution of 1:20 in quadruplicate.

IFN-γ radioimmunoassay

IFN-γ was measured with SUCROSEP® IFN-gamma immunoradiometric assay (Boots-Celltech Diagnostic Ltd, Slough, Bucks). Samples and dilutions of IFN-γ standard, and the appropriate controls were incubated with an ^{125}I-labelled anti-IFN-γ monoclonal antibody. The monoclonal antibody/IFN-γ complex was immobilized by incubation with a sheep anti-IFN-γ antibody coupled to solid phase. The separation of bound from unbound labelled monoclonal antibody was obtained by a sucrose layering system (SUCROSEP®). The radioactivity in the assay tubes was counted using a gamma-scintillation counter. A standard curve was constructed by plotting log ^{125}I counts versus log IFN-γ standard concentrations. The assays were performed in triplicate.

Enzyme experiments

FPLC-purified NCF was incubated with insoluble trypsin or chymotrypsin (Sigma Chemical Co.) for 60 min at 30° and pH 7·3. The insoluble enzyme was removed by centrifugation at 1500 *g*, 4°, 10 min, and chemotactic activity was determined in the supernatants.

Cytokines

Ultrapure human nIL-1 (normal IL-1) (Koch-Light Ltd, Haverhill, U.K.). rIL-1, IL-2 rGM-CSF, nTNF and rIFN-γ were generous gifts from Biogen Inc. Cambridge, MA, and rIL-1β was a generous gift from Biogen Inc.

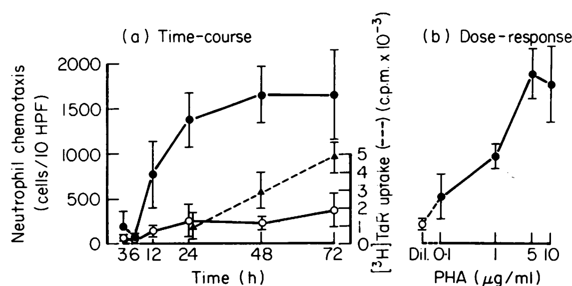

Figure 1. The time–course (a) and dose–response (b) of PHA-induced MNC-derived NCA. (a) MNCs (2×10^6 cells) were incubated with PHA (5 mg/ml) (closed circles) or without PHA (open circles). [^{3}H]TdR uptake (closed triangles) in parallel cultures of PHA-stimulated MNC is also indicated. All cell supernatants were tested at a dilution of 1:5. The points represent the mean (±SEM) of four experiments. (b) MNCs (2×10^6 cells) were incubated with (closed circles) or without (open circles) PHA for 48 hr. All cell supernatants were tested at a dilution of 1:5. The points represent the mean (±SEM) of four experiments.

RESULTS

Time–course and dose–response of NCA generation

MNC were incubated with and without PHA at time intervals for up to 72 hr and the supernatants harvested and tested for NCA. In the presence of PHA, chemotactic activity was detectable at 12 hr, increased at 24 hr and reached a maximum at 48 hr, with no further increases being observed at 72 hr (Fig. 1a). The supernatants from five different 48-hr cultures in the presence of 5 μg/ml PHA were tested undiluted and at dilutions of 1/2·5, 1/5 and 1/10. the mean chemotactic responses (±SEM) were 171±92, 288±108, 873±422 and 195±131 neutrophils/10 HPF, respectively. High levels of NCA were found consistently in the 1/5 dilution, although on two occasions maximal levels of activity were detected at 1/2·5 and 1/10. In the absence of PHA, small but insignificant amounts of activity were observed at the various time-points studied. In separate experiments (data not shown) NCA was not detectable at earlier time-points, i.e. 15 min, 30 min, 1 hr. Proliferation of PHA-stimulated MNC was minimal at 24 hr, increased at 48 hr and maximal at 72 hr. In dose–response studies (Fig. 1b) chemotactic activity was directly related to the concentration of PHA, maximal activity being observed with a concentration of 5 μg/ml.

Gel filtration

Supernatants from PHA-stimulated and unstimulated MNCs were applied to columns of Superose 12 prep. grade in an FPLC system (Fig. 2). A major peak of NCA, corresponding to molecular size of approximately 10,000 MW, was observed in the PHA-stimulated but not in the unstimulated MNC supernatants. Other smaller peaks of activity were also observed. This experiment was performed on at least four occasions and the major peak at (approximately) 10,000 MW was always observed; the smaller peaks on the other hand were variable in their

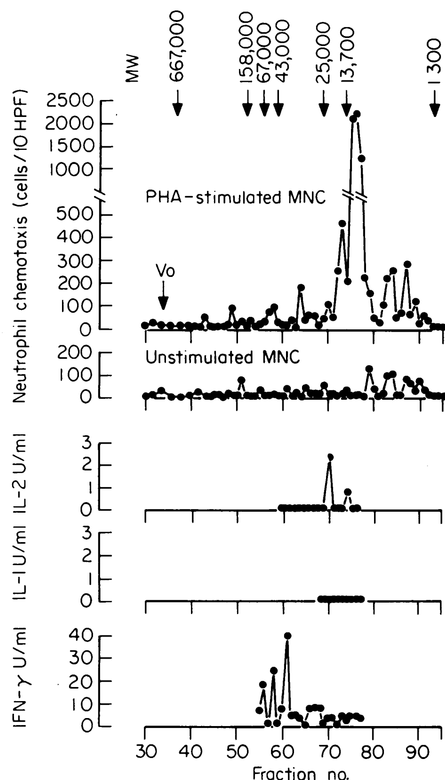

Figure 2. Gel filtration (FPLC, Superose 12 prep. grade) of supernatants from PHA-stimulated or unstimulated MNCs. The molecular markers are indicated at the top of the figure and described in the Materials and Methods. NCA was measured in each fraction, diluted 1:5. FPLC fractions from PHA-stimulated MNC supernatant were tested for the presence of IL-1, IL-2 and IFN-γ.

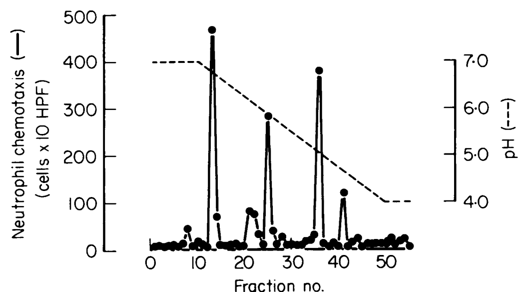

Figure 3. Chromatofocusing of PHA-stimulated MNC supernatants. The samples subjected to this procedure were partially purified by FPLC gel filtration (Fig. 2). FPLC fractions 75–77 were pooled and concentrated by lyophilization and dialyzed against 0·025 M bis-Tris buffer, pH 7·1, before being applied to the column.

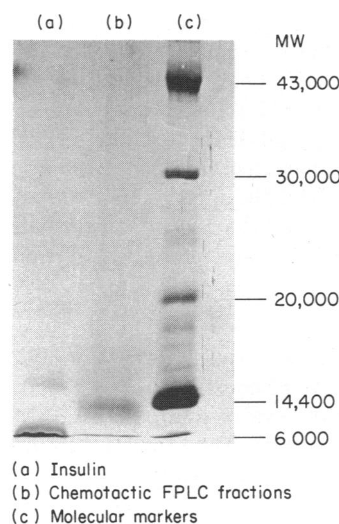

Figure 4. SDS–PAGE of MNC supernatants partially purified by FPLC gel filtration. Fractions 75–77 (Fig. 2) were dialysed against water, lyophilized and resuspended in 50 μl of 3% SDS 0·01 M Tris buffer (pH 8·1) and separated on 15% polyacrylamide.

elution profiles. There was no loss of chemotactic activity on gel filtration in as much as the crude supernatant and the 10,000 MW peak of NCF tested at dilutions of 1/5 gave comparable levels of activity. FPLC fractions were also tested for the presence of IL-1, IL-2 and IFN-γ. None of these cytokines co-eluted with the NCF. A small amount of IL-2 eluted before the NCF peak and IFN-γ eluted with molecules having an approximate MW of 40,000–70,000; IL-1 was undetectable in the region of the NCF peak.

Further characterization: chromatofocusing, SDS–PAGE, physicochemical treatment, chemotaxis and chemokinesis

MNC-derived NCF, partially purified by gel filtration, was subjected to chromatofocusing. In three experiments, using material from different donors, major peaks of chemotactic activity were observed in association with proteins eluting at pH 6·8–7·0, 5·5–6·0 and 5·0. Smaller peaks were also observed at pH 4·5. When these various peaks of activity were recombined there was no evidence of synergism. A representative experiment is shown in Fig. 3.

Fractions 75–77, which contained the peak of NCA, were then concentrated and analysed by SDS–PAGE (Fig. 4). A single protein staining band was observed at 12,000 MW.

NCF, partially purified by FPLC, was heat resistant, with no loss of activity occurring after incubation at 56° for 30 min and at 70° for either 30 or 60 min (Table 1). Incubation of MNC-derived NCF with insoluble trypsin or chymotrypsin for 1 hr at 30° completely abolished the biological activity of the material.

In order to separate the relative contribution of directional movement (chemotaxis) and stimulated random motility (chemokinesis), we compared locomotion observed with equal concentrations of MNC-derived NCF above and below the filter (migration due to chemokinesis) with locomotion observed with MNC-derived NCA only in the lower wells (migration due to a combination of chemokinesis and chemotaxis). Partially purified NCF promoted both chemotaxis as well as chemokinesis, although the dose–response for chemokinesis was unimpressive compared with that observed for chemotaxis (Fig. 5).

Comparison with other lymphokines and neutrophil chemo-attractants

The potency of FPLC-purified NCF was compared with a number of recognized neutrophil chemo-attractants, namely

Table 1. Effect of temperature on MNC-derived NCF*

Treatment	Neutrophil chemotaxis (cells × 10 HPF)†	%
20°, 60 min	851 ± 229	100
56°, 30 min	1087 ± 322	128
70°, 30 min	976 ± 265	115
70°, 60 min	900 ± 343	106

* MNC-derived NCF was partially purified by FPLC gel filtration (Fig. 2) and used undiluted.

† Four experiments (± SEM).

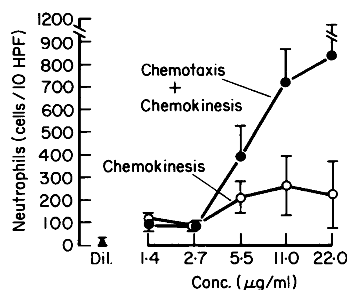

Figure 5. The relative contribution of chemokinesis (open circles) and chemotaxis + chemokinesis (closed circles) to MNC-derived NCF prepared by FPLC gel filtration. The diluent control (closed triangle) is indicated. The points represent the mean (± SEM) of four experiments.

Table 2. A comparison of MNC-derived NCA with other recognized neutrophil chemotactic factors.

Chemo-attractant	Neutrophil chemotaxis* (cells × 10 HPF)	Optimal concentration (M)
MNC-derived NCF†	1239 ± 297	—‡
C5a	2045 ± 146	10^{-8}
C5a-des-Arg	1873 ± 318	10^{-7}
PAF	1871 ± 224	10^{-6}
LTB_4	1350 ± 240	10^{-7}
FMLP	1176 ± 483	10^{-7}
Diluent§	23 ± 10	—

* Four experiments (± SEM).

† The MNC-derived NCA was Batch II, prepared from multiple leucocyte donors as described in the Materials and Methods.

‡ MNC-derived NCA was tested at a final protein concentration of 11·0 μg/ml.

§ HBSS + 0·2% BSA.

Table 3. Neutrophil chemotactic activity of human rIL-1, IL-2, IFN-γ, GM-CSF, TNF and 'natural' IL-1 compared with MNC-derived NCA

Chemo-attractants		Neutrophil chemotaxis (cells × 10 HPF*)
MNC-derived NCF†		613 ± 147
Diluent‡		5 ± 1
rIL-1β	5 Uml	8 ± 5
	10 Uml	2 ± 1
	20 Uml	2 ± 1
	40 Uml	8 ± 3
rIL-2	1 Uml	12 ± 7
	10 Uml	7 ± 4
	30 Uml	6 ± 2
	100 Uml	4 ± 1
rIFN-γ	10^0 Uml	12 + 7
	10^1 Uml	25 ± 12
	10^2 Uml	8 ± 2
	10^3 Uml	4 ± 1
rGM-CSF	0·5 ng/ml	10 ± 3
	1 ng/ml	197 ± 151
	2 ng/ml	20 ± 9
	4 ng/ml	13 ± 5
rTNF	10^0 Uml	23 ± 4
	10^1 Uml	9 ± 5
	10^2 Uml	67 ± 44
	10^3 Uml	5 ± 2
nIL-1	Diluent + 2% FCS§	30 ± 2
	5 Uml	33 ± 5
	10 Uml	20 ± 1
	20 Uml	37 ± 8
	40 Uml	24 ± 2

* Three experiments (± SEM), except rIL-1, rIL-2 and rIFN-γ (five experiments)

† MNC-derived NCF was 'Batch II' as described in the Materials and Methods. It was tested at a protein concentration of 22·0 μg/ml.

‡ HBSS + 0·2% ovalbumin.

§ nIL-1 = 'natural' IL-1. This was contained in 2% heat-inactivated FCS (Gibco Ltd.).

C5a, C5a-des-Arg, PAF, LTB_4 and FMLP. When tested over a wide dose-range, all of these agents gave a characteristic 'bell-shaped' curve with high dose inhibition. The apparent variation in 'Batch II' NCA, reflected in the data in Tables 2 and 3 and Fig. 5, was attributable to inter-donor variation in neutrophil chemotactic responsiveness. 'Batch II' NCA tested against neutrophils from eight different donors gave neutrophil counts ranging from 438 to 1900 cells/10 HPF with a mean value (± SEM) of 842 ± 169 cells/10 HPF. Similar variations were observed for FMLP, zymosan-activated serum and other concentrations of NCA. Comparisons between optimal concentrations of NCF and other chemotactic agents (Table 2) indicated that NCF appeared to be equipotent with LTB_4 and FMLP and gave approximately 60% of the activity of C5a-des-Arg, C5a and PAF.

rIL-1β, IL-2, IFN-γ, GM-CSF and TNF were also tested for neutrophil chemotaxis over a wide concentration range and compared with MNC-derived NCA and 'natural' IL-1 for their ability to attract neutrophils in directional migration (Table 3). GM-CSF and TNF had weak neutrophil chemotactic activity which gave a poor dose–response relationship. The activity of all the recombinant material and the natural IL-1 was insignificant compared with MNC-derived NCF.

DISCUSSION

In the present study we show that preparations of human MNC, stimulated with PHA in a serum-free culture medium, produced a NCF. The activity was clearly detectable at 12 hr, with appreciable amounts observable at 24 hr. In contrast, DNA synthesis, as estimated by [^{3}H]TdR uptake, was minimal at 24 hr but maximal at 72 hr, at which time the experiment was terminated. Thus, there was a dissociation between the elaboration of NCF and DNA synthesis. A similar finding was reported by Altman *et al.* (1973) who described a monocyte chemotactic factor detectable before maximal thymidine incorporation, and by Foon *et al.* (1976) who produced a chemotactic factor from human peripheral blood leucocytes stimulated with serotonin in the absence of a proliferative response. On the other hand, the time–course suggests that MNC-derived NCF is different from the preformed chemotactic factor associated with the granules of large granular lymphocytes (Greenberg *et al.*, 1986).

Most of the chemotactic activity was associated with protein of molecular size approximately 10,000 MW as estimated by SDS–PAGE (Fig. 2). Smaller peaks of NCA were sometimes observed in the higher molecular weight region, but this was an inconsistent finding and may have been due to partial aggregation of the bioactive material.

IL-1 is a 12,000–18,000 MW protein that is produced by macrophages and stimulated lymphocytes (Dinarello, 1984). MNC-derived NCF, partially purified by gel filtration, was unlikely to have been IL-1 for several reasons. NCF was resistant to high temperature (Table 1), whereas IL-1 activity is destroyed when heated at 70° for 60 min (Rosenwasser & Dinarello, 1981). No IL-1 was detectable when partially purified NCA was tested in the standard mouse thymocyte co-mitogenic assay. Furthermore, rIL-1 and natural IL-1, tested over a wide concentration range, did not evoke neutrophil chemotaxis (Table 2). IL-1 has been reported to have chemotactic or chemokinetic properties (Sauder *et al.*, 1984; Camp *et al.*, 1986). However, in these studies semi-purified preparations of IL-1 were tested, and contamination might have been responsible for the chemotactic activity attributed to IL-1. Our results are in agreement with a very recent report which showed that there was no chemotactic activity associated with recombinant and ultrapure IL-1 (Yoshimura *et al.*, 1987). NCF was also unlikely to be IL-2 because of the difference in molecular size (Robb, 1984), the partial heat sensitivity of IL-2 (Mochizuki, Watson & Gillis, 1980), the absence of IL-2 activity from active fractions and the fact that rIL-2 did not elicit neutrophil chemotaxis (Table 2). Similarly, IFN-γ has a different molecular size (Nathan *et al.*, 1981), is very heat-labile (Wheelock, 1965), was not detected in the chemotactic fractions (Fig. 2) and the recombinant protein was without chemotactic activity (Table 2).

Human rGM-CSF has been shown to promote monocyte and neutrophil directional migration (Wang *et al.*, 1987). We were able to confirm these observations, but noted that the activity of GM-CSF was considerably less than that of partially purified MNC-derived NCA (Table 3). Furthermore, it was unlikely that our material contained GM-CSF since the latter has been reported to have a higher molecular weight (22,000 MW) (Gasson *et al.*, 1984) and the conditions of culture were not optimal. Production of colony-stimulating factor (CSF) in PHA-stimulated lymphocyte cultures required serum and had a different time–course, with a delay of 2 days before a significant increase of CSF was observed (Parker & Metcalf, 1974). We were able to confirm a previous report that TNF was chemotactic for monocytes and polymorphonuclear leucocytes, but observed that MNC-derived NCA was considerably more active and was unlikely to have contained TNF since that monokine has a higher molecular size (17,000 MW; Wang *et al.*, 1985) and occurs naturally in a dimeric form which elutes from gel-filtration columns together with proteins having a molecular weight of 34,000 (Matthews, 1981). Moreover, human MNC stimulated with PHA produced very low lymphotoxin activity (Stom-Wolf *et al.*, 1984) and in serum-free medium, TNF release was not enhanced by the addition of endotoxin (Matthews, 1981). We did not test IL-3 and IL-4 activity but they differ from MNC-derived NCA on the basis of molecular weight (Yokota *et al.*, 1985; Inle *et al.*, 1983).

Preliminary experiments (data not shown) indicated that partially purified NCF (Superose 12) increased the complement-dependent cytotoxic capacity of neutrophils towards the schistosomula of *Schistosoma mansoni* using the method of Moqbel *et al.* (1983), but had no effect upon IgG-dependent leukotriene B_4 generation by neutrophils studied using the method of Shaw *et al.* (1985).

The material described here bears some similarity to the LDCF described previously for human monocytes (Altman *et al.*, 1973), as we have found that it is also chemotactic for human peripheral blood monocytes (P. Maestrelli, unpublished observations). On the other hand, LDCF produced in a serum containing medium gave major activity at pI 10 (Altman, Chassy & Mackler, 1975). A recent report indicates that MNC spontaneously release neutrophil chemotactic activity with a molecular weight of approximately 10,000 MW (Kownatzki, Kapp & Uhrich, 1986). However, it is intriguing to note that the same amount of chemotaxis was detected in unstimulated MNC supernatants as in the supernatants of MNC stimulated with lipopolysaccharide (LPS), silica or PHA. A plausible explanation for this was suggested by Yoshimura *et al.* (1987), who reported that LPS contamination of 10 ng/ml was sufficient to generate significant chemotactic activity.

The maximal chemotactic activity seen with optimal concentrations of semi-purified MNC-derived NCF had a potency comparable with that of FMLP and LTB_4 and had about 60% of the activity of C5a, C5a-des-Arg and PAF. SDS–PAGE indicated that 80–90% of the applied protein was associated with a major band at 10,000–12,000 MW, with only two or three faint bands present.

A MNC-derived NCF may be important within the broad context of cell-mediated immunity in a number of diverse conditions, including rheumatoid arthritis, polyarteritis nodosa, inflammatory bowel disease and bronchial asthma (Stites, Stobo & Wells, 1987). In all these conditions acute inflammatory cells, including neutrophils, have been observed and MNC-derived NCF represents a potential link between cellular immunity and the acute inflammatory process. The development of inhibitors of MNC-derived NCF (or its actions) may have therapeutic potential in the treatment of the clinical conditions mentioned above.

The precise origin of MNC-derived NCF (i.e. T cells, B cells or monocytes) is yet to be established and is the subject of an ongoing study.

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