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A NOVEL INTERLEUKIN STIMULATING FREE RADICAL PRODUCTION BY GRANULOCYTES

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Polymorphonuclear granulocytes (PMN) are potent producers of free oxygen-derived radicals. Since other granulocyte functions are affected by interleukins, we investigated whether free-radical production can be initiated by a similar mediator. For estimation of free radical production, SOD-inhibitable lucigenin-dependent chemiluminescence and SOD-inhibitable cytochrome C reduction were used. As a source of interleukins, serum-free 24 h culture supernatants of human mononuclear cells (MNC) stimulated with bacterial lipopolysaccharide were prepared. Addition of such supernatants to PMN caused stimulation of sod-inhibitable chemiluminescence and superoxide production. Studies with separated MNC showed that monocytes were the cellular source of the activity. Biochemically, this activity of the supernatants was due to a heat-labile glycoprotein with a MW of approx. 60 KDa. This mediator, termed granulocyte chemiluminescence inducer (GCI), appears to be distinct from interleukin 1 (α and β) and interferon (α and γ).

In conclusion we describe a novel monokine, granulocyte chemiluminescence inducer (GCI), which initiates granulocyte free radical production. This interaction of monocytes and granulocytes may also in vivo constitute a new and potent pathway leading to stimulation of free oxygen production by granulocytes.

KEY WORDS: Interleukins, Granulocytes, Free Radicals.

ABBREVIATIONS: PMN: polymorphonuclear granulocytes, GCI: granulocyte chemiluminescene inducer, SOD: superoxide dismutase, MNC: mononuclear cells, IL-1: Interleukin 1, IFN: Interferon, GM-CSF: granulocyte-macrophage colony stimulating factor, MW: molecular weight, SN: supernatant, LPS: lipopolysaccharide, BSA: bovine serum albumin, FCS: fetal calf serum, MEM: minimal essential medium, DCDHFDA: dichloro-dihydro-fluorescin-diacetate, HPLC: high performance liquid chromatography, PBS: phosphate buffered saline, ConA: Concanavalin A.

INTRODUCTION

Polymorphonuclear leukocytes (PMN) can be activated (e.g. by particles, immune complexes or complement activation products) to produce free oxygen radicals, which are important mediators of their anti-infective action, but also participate in inflammatory tissue damage, immunosuppression and DNA damage.[1–3] Of immune cells, T-lymphocytes and natural killer cells are particularly sensitive to functional impairment by oxygenating agents.[4,5]

Other PMN functions have been shown to be controlled by cytokines: PMN migration is affected by Interleukin 1 and leukocyte inhibitory factor,[6,7] and colony stimulating factor has been shown to increase the antibody-dependent cytotoxic action of PMN.[8] We have investigated whether their production of reactive oxygen species can also be directly induced by a cytokine-like mediator in the absence of phagocytosis.

Here we demonstrate that supernatants (SN'S) of LPS-stimulated mononuclear cells induce an oxidative burst as evidenced by chemiluminescence,[9] superoxide and hydrogen peroxide production. The factor(s) responsible, termed granulocyte chemiluminescence inducer (GCI), appear to be a novel monocyte-derived peptide(s) different from interleukin 1.

MATERIALS AND METHODS

Ficoll-Hypaque solution was obtained from Pharmacia, Freiburg FRG. Purified bovine serum albumin (BSA), trypsin and trypsin inhibitor attached to agarose, superoxide dismutase, lucigenin, phorbol myristate acetate were obtained from Sigma, Munich, FRG.

Culture media and fetal calf serum (FCS) were obtained from Biochrom Seromed, Berlin, FRG.

LPS from salmonella abortus equi, prepared by electrodialysis as described previously,[10] was a generous gift by Dr. C. Galanos, Max Planck-Institut für Immunobiologie, Freiburg, FRG.

Interferon: Human recombinant IFN α 2 (3×10^8 IU/mg protein) and human IFN ($1\text{–}2 \times 10^7$ Iu/mg protein) were a gift from Dr. W. Berthold, Thomae GmbH, Biberach a.d.Riss, FRG.

Interleukin 1: Human recombinant IL-1 (α and β) were kind gifts from Drs. C. Henney and S. Gillis, Immunex Corp. Seattle, USA.

Monoclonal antibodies: Leu M3 (human monocytes/macrophages), and Leu 7 (HNK-1, Large Granular Lymphocytes) were obtained from Becton Dickinson, Rutherford, PS. Anti-T11 (T-lymphocytes, E receptor) and anti B1 (B-lymphocytes) were from Coulter Clone, Nialeah, FL.

Production of GCI-containing supernatant: Mononuclear cells were isolated from citrated blood of healthy donors by ficoll gradient (1.077)[11] and cultured in MEM with BSA (100 μg/ml). To stimulate mediator production, LPS (S. abortus equi) was added (optimal level 500 ng/ml). After 24 h, SN'S were collected, sterilfiltered and stored at -80°C.

Granulocytes were isolated by ficoll gradient and hypotonic lysis with over 95% purity (giemsa stain) and over 98% viability (trypan blue exclusion).

Chemiluminescence: 1×10^6 PMN in 250 μl MEM with 1 mM lucigenin were placed in a thermostasized (37°C) luminometer (Berthold LB 9505) and test sample added.

Superoxide production was determined as SOD-inhibitable cytochrome C reduction using 4×10^6 PMN/tube and supernatants concentrated 10 × over Amicon membranes (10 kDa cutoff).

Production of H_2O_2 was followed by flow-cytometric determination of production of fluorescent oxidation products of dichlorodihydro-fluorescin-diacetate (DC-DHFDA).

HPLC gel filtration: Supernatant of mononuclear cells cultured with LPS was concentrated as described previously.[12] Concentrated sample (100 μl) was applied to a bio-sil TSK 125 gel filtration column (300 × 7.5 mm, Biorad, Richmond, CA, USA). Elution of the column was carried out isocratically with PBS, pH 7.2, at a flow rate of 1 ml/min.[12] Fractions were tested for interleukin 1 activity in a murine thymocyte costimulator assay as described[6] and for their activity to induce granulocyte chemiluminescence.

TABLE I
Production of superoxide by GCI-treated PMN

Stimulant used		Superoxide production (nmoles/4 × 10^6 PMN × 60 min.)
MEM	10 × conc.	5.01
MEM + LPS	10 × conc.	6.37
MNC – SN without LPS	10 × conc.	7.25
LPS-MNC-SN	10 × conc.	23.59
Phorbol myristate acetate	10 ng/ml	57.15
Phorbol myristate acetate	1 ng/ml	11.79

Superoxide production was determined as SOD-inhibitable cytochrome C reduction. Values are means of triplicates.

Counterflow elutriation was employed to prepare monocyte-depleted and monocyte-enriched fractions.[13] Cellular composition of elutriated preparations was analysed by flow-cytometry using monoclonal antibodies.

RESULTS

Induction of PMN oxy-radical production by mononuclear cell supernatants

In PMN exposed to 10 × concentrated LPS-MNC-SN, stimulation of superoxide production was observed, while concentrates of either medium, medium + LPS or SN of unstimulated mononuclear cells were not stimulatory (Table I). It was, however, not possible to demonstrate such stimulation with unconcentrated LPS-MNC-SN, possibly due to medium constituents interfering with the assay. Therefore, other assays for the detection of free oxy-radicals or oxygenating agents respectively, were employed.

When following the intracellular production of fluorescent oxidation products from DCDHF-DA by flow-cytometry, unconcentrated LPS-MNC-SN was stimulatory, while controls were not (Fig. 1a). Furthermore, when applying lucigenin-dependent chemiluminescence, activity could also be detected in unconcentrated supernatants: when supernatants of LPS-stimulated mononuclear cells were added to granulocytes, long-lasting intensive chemiluminescence could be observed in the presence of lucigenin (Fig. 1b) or luminol (data not shown). Superoxide dismutase (100 μg/ml) inhibited more than 95% of the response (data no shown), suggesting that superoxide production was responsible for the observed chemiluminescence.

Since lucigenin-dependent chemiluminescence proved more sensitive than cytochrome C reduction and allowed for kinetic measurements, further studies were carried out with this technique. Also, the substance(s) responsible for the observed activity was termed Granulocyte Chemiluminescence Inducer (GCI).

Supernatant of mononuclear cells not treated with LPS was marginally stimulatory. Also, medium + LPS or medium along elicited only background chemiluminescence indicating that at the concentration used (500 ng/ml), LPS by itself was not stimulatory for granulocytes (Tables II). Therefore, GCI-activity was produced at high level if the mononuclear cells had been treated with LPS, while unstimulated mononuclear cells spontaneously produced low but significant GCI-activity. Supernatant of LPS-

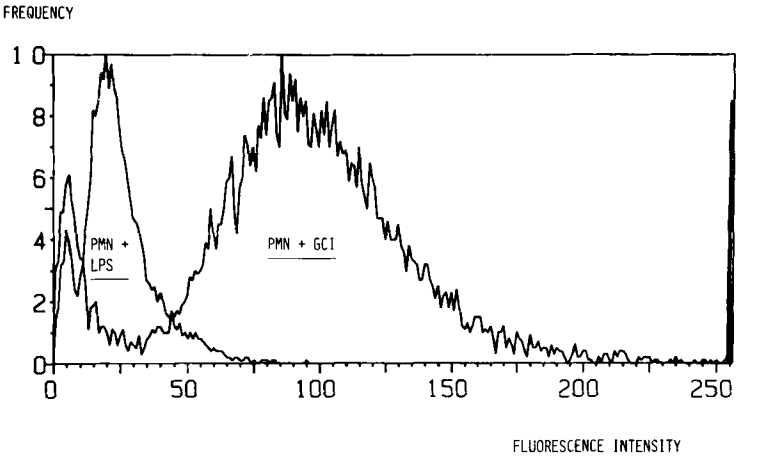

FIGURE 1(a) Oxidation of DCDHF-DA to fluorescent products by GCI-treated granulocytes. Granulocytes were loaded with non-fluorescent dichloro-dihydrofluorescin-diacetate (DCDHF-DA) and exposed to LPS-induced mononuclear cell supernatant ("PMN + GCI") or to medium + LPS ("PMN + LPS") as control. After 1 h, production of fluorescent oxidation products was estimated by flow cytometry.

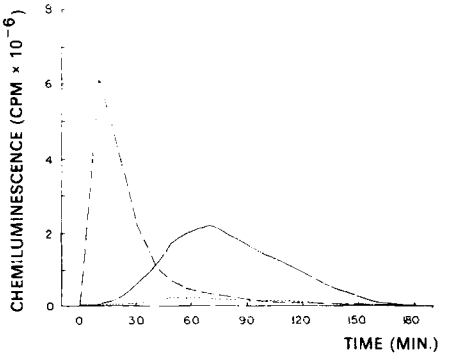

FIGURE 1(b) Granulocyte chemiluminescence induced by culture supernatant of LPS-stimulated mononuclear cells. 1 × 10^6 granulocytes were stimulated with supernatant from LPS-stimulated (solid line) or unstimulated mononuclear cells (pointed line) at a final dilution of 1/2. Phorbol myristate acetate (100 ng/mg) served as a positive control (dashed line).

TABLE II
Production of GCI-activity by adherent and non-adherent mononuclear cells

Cell preparation	GCI-activity (integral counts × 1/1000)	
	unstimulated	+ LPS 500 ng/ml
unseparated	61'470 ± 26'289	289'860 ± 196'544
medium alone	23'097 ± 10'880	35'836 ± 11'690
adherent population	76'314 ± 37'722	371'248 ± 135'506
non-adherent population	40'062 ± 15'827	108'440 ± 51'840

Values represent mean + sem from 3 experiments tested on granulocytes from 3 different donors.

stimulated mononuclear cells was about as potent as phorbol myristate acetate as an inducer of chemiluminescence: at a 1:2 dilution of supernatants, the 2 hr-integral of chemiluminescence was comparable to the one induced by phorbol myristate acetate at 100 ng/ml. However, the kinetic of the chemiluminescence induced by the supernatant was quite different from the chemiluminescence elicited with the phorbol ester (Fig. 1b).

Conditions for production of GCI-activity in dependence on the number of mononuclear cells and on the concentration of LPS were studied. As shown in Fig. 2(a), already 1.25×10^6 mononuclear cells/ml produced GCI-activity. GCI-activity of the supernatant increased with the number of mononuclear cells and reached a plateau at 5×10^6 cells/ml.

The effect of LPS concentration is shown in Fig. 2(b). LPS was a very effective stimulus, inducing appearance of GCI-activity even at concentrations as low as 25 ng/ml. With increasing concentration of LPS, GCI-activity of supernatant also increased. However, at concentrations over 800 ng/ml LPS was itself stimulatory for granulocytes. In 24 hr-cultures, optimal production of GCI-activity under serum-free conditions was found with $2.5–5 \times 10^6$ mononuclear cells/ml and 500 ng/ml of LPS. Such supernatants still induced chemiluminescence at dilutions of 1:80.

If either human or fetal calf serum were included in the culture media, yield of GCI-activity was even improved (data not shown). However, addition of serum components other than bovine serum albumin was normally avoided since serum factors can influence chemiluminescence.[14]

Cellular origin of GCI-activity

To define the cellular origin of GCI-activity, mononuclear cells were fractionated and then cultured in the presence of LPS. When cells were fractionated according to their capacity to adhere to plastic, adherent cells, constituted to over 95% of monocytes, were capable of producing substantial GCI-activity, pointing to the monocyte as the producer of GCI-activity (Table II). However, the non-adherent, monocyte-depleted population produced less, but still significant GCI-activity. Since a subpopulation of small monocytes is known to be immature in terms of adherence capacity, production of GCI-activity by the non-adherent population could still be due to monocytes. In a second aproach, mononuclear cells were fractionated by counterflow elutriation, which allows to deplete lymphocytes from monocytes, and vice versa, to a much higher degree. Using this technique, mononuclear cell fractions were prepared which consisted of practically pure monocytes or lymphocytes as estimated by cytofluorography using monoclonal antibodies. If LPS-induced supernatants were produced with these fractions, the capacity to produce GCI-activity in response to LPS was found in the monocyte fraction, but not in the lymphocyte fraction (Table III). Also admixture of lymphocytes did not change production of GCI-activity by monocytes (data not shown). Therefore, the cellular origin of GCI-activity appear to be the monocytes.

Biochemical properties of the GCI-activity of supernatants

To characterize the molecule(s) responsible for GCI-activity of LPS-induced mononuclear cell supernatants (LPS-MNC-SN), biochemical properties of GCI-activity were investigated. GCI-activity was found unchanged after freezing and thawing and

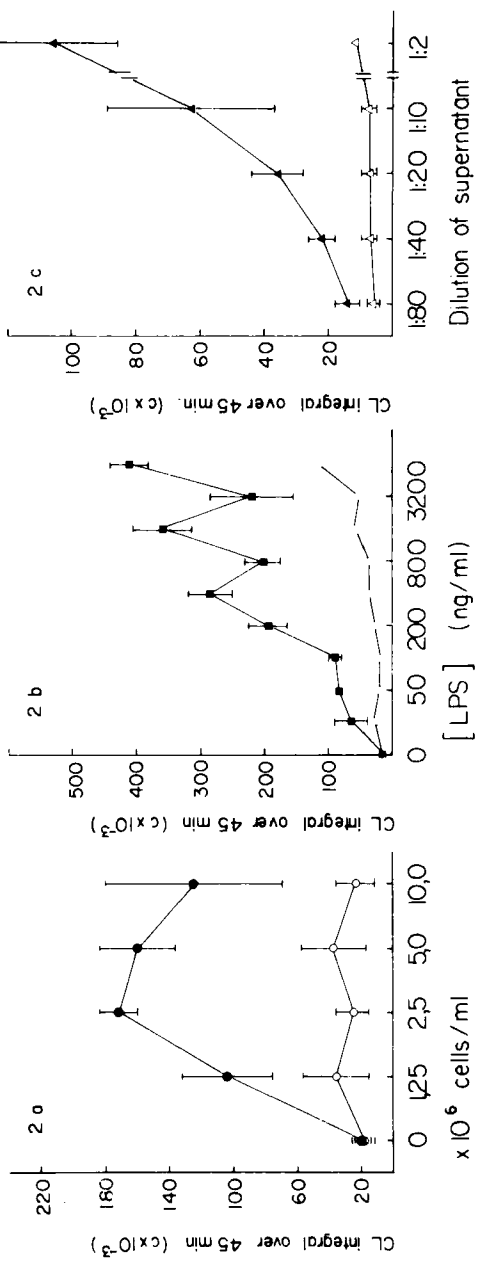

FIGURE 2 Influence of culture conditions on GCI-activity. **2a** Number of mononuclear cells in culture. Mononuclear cells were cultured at different densities with 500 ng/ml LPS (closed circles or without LPS (open circles). **2b** Concentration of lipopolysaccaride 5×10^6 mononuclear cells were cultured at different concentrations of LPS and 24 hr-supernatants were tested for GCI-activity (solide squares). As a control, LPS was diluted in medium and tested for induction of granulocyte chemiluminescence (open squares). **2c** Dose-dependence of chemiluminescence induced by culture supernatant. Supernatant from mononuclear cells cultured at 5×10^6 cells/ml and stimulated with 500 ng/ml of LPS was diluted in medium and tested for GCI-activity (solid triangles). Supernatant from mononuclear cells cultured without added LPS was diluted and tested in the same way (open triangles).

TABLE III
Production of GCI-activity by mononuclear cells fractions obtained by counterflow elutriation

Cellular composition					GCI-activity of supernatant (45 min. int. counts × 1/1000)	
% cells	T	B	NK	MO	unstimulated	LPS-stimulated
Experiment 1						
total mnc	47	12	12	24	2'246 ± 2'200	41'599 ± 8'036
monocyte-depleted mnc	45	40	14	1	0	0
monocyte-enriched mnc	1	1	1	87	0	58'144 ± 10'991

Data from a representative experiment.

Counterflow-elutriation was used to obtain practically pure monocyte and monocyte-depleted lymphocyte populations. Cellular composition was determined cytofluorographically. Data are given as % of cells staining with anti-t11 (T-lymphocytes), anti B_1 (B-lymphocytes), Leu-7 (NK-cells) or Leu-m3 (monocytes). 1×10^6 cells were cultured 24 hours with or without LPS (500 ng/ml), supernatants were harvested and tested for GCI-activity. Background values of medium resp. medium + LPS (16'000 ± 3'000 c/45 min.) were subtracted from values obtained with cell culture supernatants. Data are given as mean ± sem of determinations done on 3 different granulocyte donors.

was stable at 4 C and 20 C for several days. At temperatures over 56 C, GCI-activity was labile, suggesting peptide nature of the molecule(s) underlying GCI-activity (Table IV). Therefore, trypsin sensitivity of GCI-activity was studied. It proved important to remove trypsin before testing for GCI-activity, because trypsin by itself could stimulate chemiluminescence.[15] GCI-activity was reduced to 40% after 2 hrs of incubation with trypsin (100 μg/ml) at 37 C. This partial sensitivity of GCI-activity to trypsin supports the hypothesis of peptide nature of the molecule(s) responsible for GCI-activity. Inhibition of production of GCI-activity by cycloheximide also supports this view. The possibility that some of the tryptic fragments retain stimulatory capacity may account for the apparently trypsin-insensitive remainder of GCI-activity.

To address the question whether GCI-activity was due to one single or to several molecular species, the molecular weight of the active molecule(s) was estimated. Dialysis on a cellophane membrane with a cutoff-point of 3500 Dalton resulted in a

TABLE IV
Biochemical properties of GCI-activity

Treatment	Residual activity (%)
Untreated LPS-MNC-SN	100[1]
56 C, 30 min.	40
100 C, 30 min.	17
pH-change (2–12)	95
Trypsin treatment	41
Dialysis (against MEM, cutoff 3.5 kDa)	81
Cycloheximide present during culture (20 μg/ml)	22
Absorption on ConA-Sepharose	11

Supernatants form 5×10^6 MNC/ml stimulated with LPS (0,5 μg/ml) were treated as indicated and tested for GCI-activity in CL. Values represent mean ± sem of 3 experiments using 3 different supernatants.

[1] Absolute activity at 1:2 dilution: 260'055 ± 49'211 × 10^3 counts/45 min.

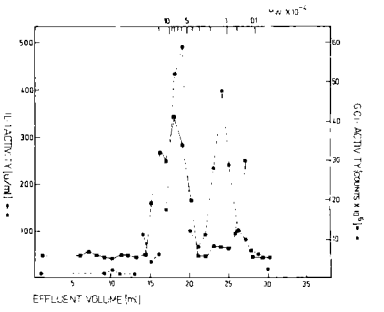

FIGURE 3 Determination of molecular weight of GCI by HPLC gel filtration and comparison with interleukin-1. Culture supernatant from LPS-stimulated mononuclear cells was concentrated and fractionated by HPLC on TSK 125. Fractions were tested for induction of granulocyte chemiluminescence (solid line) and interleukin-1-activity (broken line).

20% loss of activity, either due to binding to membrane material, or to the presence of an active molecule smaller than 3500 Dalton. On HPLC gel filtration of LPS-induced mononuclear cell supernatant, GCI-activity eluted as a peak corresponding to a m.w. of 60 $\pm$ 10 KDa and with a smaller peak between 1 and 5 KDa (Fig. 3), explaining the behaviour of GCI-activity on dialysis.

GCI-activity, interleukin 1 and interferon

The purpose of this study was to demonstrate the basic fact that a mononuclear cell derived mediator can enhance granulocyte oxygenation activity substantially. To further delineate GCI-activity, its relationship to interferon-α and -γ and interleukin 1 was addressed. As can be seen from Table 5, recombinant human interferon-α and -γ were devoid of any GCI-activity.

In addition, HPLC-fractions of LPS-stimulated human mononuclear cell supernatants were concurrently tested for interleukin 1-activity in a thymocyte costimulator assay. As shown in Fig. 3, GCI-activity and interleukin 1-activity overlap in the high m.w. region, however, peak activities do occur in different fractions. Furthermore, peak activities in the low m.w. region are well separated. Finally, neither recombinant human interleukin 1-α nor -β had any GCI-activity (Table V).

DISCUSSION

From the results presented above, it appears that supernatants of LPS-treated mononuclear cells can in fact stimulate production of reactive oxygen species, as evidenced by production of superoxide, intracellular H_2O_2-dependent oxidation production and chemiluminescence.

To establish whether this activity is due to a molecule(s) qualifying as a cytokine, studies of the cellular origin and biochemical properties were performed.

When mononuclear cells were fractionated by adherence to plastic, adherent cells were found to be more active than nonadherent, suggesting that monocytes were generating the observed activity. With the aid of counterflow elutriation, it was possible to enrich or deplete for monocytes to a much higher degree than possible with

TABLE V
Activation of PMN chemiluminescence by recombinant materials

Substance tested	GCI-activity (counts per 45 min. × 1/000)	Thymocyte costimulator assay (cpm)
medium alone (MEM)	10'874	2'211
LPS-MNC-SN	249'631	n.d.
rec. hum. IL-1 (alpha)		
1/500	12'300	32'073
1/12'500	14'100	27'063
rec. hum. IL-1 (beta)		
1/500	13'100	36'346
1/12'500	9'120	10'172
IL-2	n.d.	137'170
rec. hum. interferon (alpha)		
10 ng/ml	14'620	n.d.
100 ng/ml	10'930	n.d.
rec. hum. interferon (gamma)		
1 ng/ml	14'050	n.d.
10 ng/ml	12'272	n.d.

n.d. = not tested. Values represent the mean of triplicates.

an adherence procedure. Studies with so obtained monocyte-enriched and monocyte-depleted populations showed that monocytes were sufficient to produce GCI-activity. Therefore it appears that monocytes are the origin of GCI-activity in supernatants of LPS-treated mononuclear cells.

Biochemically, the factor(s) responsible for GCI-activity appears to be a glycoprotein, as evidenced by inhibition of production by cycloheximide, sensitivity of the activity to trypsin treatment and loss of activity after absorption onto ConA-sepharose. The apparent greater stability against trypsin than against cycloheximide may be due to tryptic fragments still possessing biological activity.

On gel filtration (HPLC), activity eluted with a major peak at 60 ± 10 KDa and a minor peak at 1–5 KDa. Whether the low m.w. peak represents a split product, of the large molecule, or the large molecule an aggregated form, has not yet been explored. However, since other cytokines like interleukin 1 or colony stimulation factor exhibit similar heterogeneity, these are attractive possibilities.[6]

Monocytes are known to have the potential to produce a variety of biologically active molecules which could account for the described effect of supernatants on granulocytes.[1,6,8] Molecule weight delineates GCI-activity from the complement components C1q and C3b, both activators of granulocytes. The complement split product C5a, can also not account for GCI-activity, since it only causes a very short-lived chemiluminescence response quite different from the long-lasting kinetic described here.

Furthermore, interleukin 1 and GM-colony stimulating factor can both be produced by monocytes and are known to affect granulocyte function and must therefore be considered as potential causes of GCI-activity. When HPLC fractions of LPS-MNC-SN were tested for GCI-activity and interleukin 1, activity profiles were partly overlapping in the m.w. region, but well separated in the 10–20 KDa region

— particularly, no GCI-activity was present in those fractions containing the monomeric interleukin 1-peak. Also, none of the recombinant interleukin 1 preparations (α and β) possessed any GI-activity, despite good activity in the thymocyte costimulator assay. Therefore it appears quite unlikely that interleukin 1 should be responsible for GCI-activity. GM-Colony stimulating factor, which can also be produced by monocytes, is known to enhance the oxidative burst potential,[16] but to have no stimulatory capacity per se. Also, GM-CSF would elute at lower m.w. than GCI-activity. Taken together, it is unlikely, though not yet formally excluded, that GM-CSF is primarily responsible for the stimulatory activities of LPS-MNC-SN described by us. However, it is possible that the presence of GM-CSF could contribute to GCI-activity of LPS-MNC-SN by enhancing the response of granulocytes.

Finally, we were able to show that neither recombinant interferon-α nor -γ possess any GCI-activity, allowing delineation of GCI-activity from these cellular mediators.

In summary, we demonstrate stimulation of production of reactive oxygen species by an interleukin-like mediator. This substance appears to be produced by monocytes and to be distinct from other known activators of the granulocyte oxidative burst. Furthermore, on the basis of the data presently available, it can be delineated from other monokines and therefore appears to be a novel interleukin. Full resolution of this issue will depend on further biochemical characterization and, ultimately, cloning of the molecule.

From a functional point of view, our finding of stimulation of granulocyte free radical production by a monokine represents evidence for a novel pathway of granulocyte activation, which may also be operative *in vivo* and lead to enhanced fluxes of reactive oxygen species. Conceivably, production and action of GCI *in vivo* could locally complement anti-microbial actions of granulocytes and monocytes. However, depending on location and quantity, impairment of host cell function and integrity could also ensue.

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References

1. Badwey, J.A. and Karnovsky, M.L. *Annu. Rev. Biochem.*, **49**, 695, (1980).
2. Allen, R.C. In: *The Reticulo-endothelial system.* Sbarra and Strauss (eds), Plenum Publishing Corp. (1980) Vol. *8.* p. 309.
3. Sacks, T., Moldow, C.F., Craddock, P.R., Bowers, T.K. and Jacobs, H.S. *J. Clin. Invest.*, **81**, 1161, (1978).
4. Metzger, Z., Hoffeld, J.T. and Oppenheim, J.J. *J. Immunol.*, **124**, 983, (1980).
5. El-Hag, A. and Clark, R.A. *J. Immunol.*, **133**, 3291, (1984).
6. Luger, T.A., Charon, J.A., Colot, M., Micksche, M. and Oppenheim, J.J. *J. Immunol.*, **131**, 816, (1983).
7. Rocklin, R.E. *J. Immunol.*, **114**, 1161, (1975).
8. Vadas, M.A., Nicola, N.A. and Metcalf, D. *J. Immunol.*, **130**, 795, (1983).
9. Allen, R.C. In *Chemical and Biological Generation of Excited Stages.* eds. W. Adam and G. Cilento, (1982). Academic Press, p. 310.
10. Galanos, C. and Luederitz, O. *Eur. J. Biochem.*, **54**, 603, (1975).
11. Boeyum, A. *Scan. J. Lab. Clin. Med.*, **21** (spp. 97) (1986).

12. Koeck, A. and Luger, T.A. *J. Chromat.*, **296**, 293, (1984).
13. Clementson, K.J., McGregor, J.L, McEver, R.P., Jacques, Y.V., Bainton, D.F., Domzig, W. and Baggiolini, M. *J. Exp. Med.* (in press).
14. Maly, F.E., Kapp, A. and Rother, U. *Immunobiol.*, **164**, 90, (1983).
15. Kitagaura, S., Takaka, F. and Sakamoto, S. *FEBS Lett.*, **99**, 275, (1979).
16. Weisbart, R.H., Golde, D.W., Clark, S.C., Wong, G.G. and Gasson, J.C. *Nature*, **314**, 361, (1985).

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