Arch Dermatol Res (1988) 280:346–353

Archives of Dermatological Research

Granulocyte-activating mediators (GRAM): III. Further functional characterization of monocyte-derived GRAM*

A. Kapp[1], G. Zeck-Kapp[2], A. Möller[3], and R. Putz[4]

Departments of [1] Dermatology, [2] Pathology, and [4] Anatomy III, University of Freiburg, and [3] BASF AG, Hauptlabor, Ludwigshafen, FRG

Summary. As shown previously monocytes upon stimulation with bacterial lipopolysaccharides (LPS) release granulocyte-activating mediator(s) (M-GRAM) which induced a long-lasting chemiluminescence (CL) response in human granulocytes. M-GRAM could be separated from interleukin-1α and β, interleukin-2, interferon α and γ, granulocyte colony stimulating factor (G-CSF) and macrophage colony stimulating factor (M-CSF), since these cytokines were shown to be unable to induce a significant CL response. In contrast, granulocyte macrophage colony stimulating factor (GM-CSF) and particularly tumor necrosis factor (TNF) are important triggers of the oxidative burst and they are capable of inducing a CL response. TNF activity but not lymphotoxin (LT) activity could be demonstrated in M-GRAM samples. A polyclonal rabbit IgG as well as a monoclonal antibody to recombinant human TNF which neutralized the TNF activity in M-GRAM preparations did not substantially block the CL signal. Furthermore, M-GRAM-induced CL response was not significantly inhibited by a polyclonal calf antiserum to human recombinant GM-CSF. For further functional characterization of M-GRAM-induced granulocyte activation different assays were performed in order to compare GM-CSF and TNF: (a) SOD-inhibitable cytochrome C-reduction ($^{\cdot}O_2^-$); (b) horse radish peroxidase-mediated oxidation of phenol red (H_2O_2); (c) the release of peroxidase; (d) ultrastructural detection of hydrogen peroxide production; and (e) scanning and transmission electron microscopy (SEM and TEM). Significant release of $^{\cdot}O_2^-$ was induced by M-GRAM, TNF, and GM-CSF, whereas H_2O_2 production was significantly stimulated only by M-GRAM and TNF, as shown by functional and ultrastructural assays. In contrast, only M-GRAM was able to induce significant release of peroxidase. Granulocyte activation could be visualized by SEM and TEM. Upon stimulation with M-GRAM polymorphonuclear neutrophilic granulocytes (PMN) showed an increased adherence to the substratum, developing an increased number of intracytoplasmic vacuoles and short filopodia, whereby the morphological pattern was different from that induced by GM-CSF and TNF. Based on our results we suggest that M-GRAM activity is mediated, in addition to TNF, by a possible new cytokine which is capable to specifically activate granulocytes turning them into scavengers of invading microbes and parasites.

Key words: Granulocyte – Cytokines – Superoxide – Oxygen radicals

As has been shown previously monocytes as well as epidermal cells (ECs) are capable of producing granulocyte-activating mediators (GRAM) [4–6] which induce a long-lasting chemiluminescence (CL) signal in isolated human polymorphonuclear neutrophilic granulocytes (PMNs). These mediators can be separated from interferon α and γ, interleukin-1 α and β, interleukin-2, granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), and from additional relevant cytokines which have been suggested to influence human PMN functions [4, 6]. Only granulocyte-macrophage colony-stimulating factor (GM-CSF) [7], tumor necrosis factor/cachectin (TNF), and lymphotoxin (LT) [8] are potent activators of PMN oxidative metabolism. In the present study we, therefore, investigated whether these mediators are responsible for GRAM activity released by monocytes. Furthermore, the biological

* The paper was presented in part at the 17th meeting of the ESDR, March 29 – April 1, 1987, Amsterdam, and the 15th meeting of the ADF, November 13–15, 1987, Munich

Offprint requests to: Dr. Alexander Kapp, Hautklinik, Hauptstr. 7, D-7800 Freiburg, FRG

activity of these relevant mediators is compared in different assay systems by using functional as well as ultrastructural criteria.

Materials and methods

Cytokines

E. coli-derived recombinant GM-CSF was a kind gift from Dr. J. DeLamarter (Biogen, Geneva, Switzerland), and had a specific activity of 1.1×10^5 U/μg in the CML assay [11]. The material had an endotoxin concentration of 0.5 EU/10 μg in the limulus amebocyte lysate assay.

E. coli-derived recombinant human TNF and recombinant human lymphotoxin (LT) were produced by BASF (Ludwigshafen, FRG) with a specific activity of 8.5×10^6 U/mg for TNF and 6×10^7 U/mg protein for LT. Biologic activity of TNF and LT was monitored by a standard 48-h cell lytic assay using mouse L-929 fibroblast cells grown in absence of actinomycin D [1, 2]. Furthermore, TNF and LT concentrations were determined using a double sandwich ELISA with monoclonal antibodies.

Recombinant human G-CSF and M-CSF/CSF-1 were a kind gift from Dr. Seiler (Behring, Marburg, FRG). Recombinant human interleukin-3 and interleukin-4/BSF-1 were purchased from Genzyme (Boston, USA).

Before testing all cytokines were diluted in PBS containing 50 μg/ml bovine serum albumin (BSA).

Concentrated semipurified M-GRAM and EC-GRAM were prepared as described previously [4, 6].

Reagents and antibodies

Ficoll-Paque solution was obtained from Pharmacia (Freiburg, FRG); purified BSA, lucigenin, phorbol-myristate-acetate (PMA), f-met-leu-phe, zymosan A, ferricytochrome C (type III), cytochalasain B, phenosulfonphtalein (phenol red, sodium salt), *D*-mannitol, dimethylthiourea (DMTU), o-phenylen-diamine (OPD), cetyltrimethylammonium bromide (CETAB), horseradish peroxidase (HRPO, type II), superoxide dismutase (SOD, type I), catalase (bovine liver), normal rabbit and calf serum, and zymosan were obtained from Sigma (Munich, FRG). Culture media and antibiotics, were purchased from Biochrom-Seromed (Berlin, FRG). $CeCl_3$ and 3-Amino-1,2,4 triazole (AT) were from Fluka (Buchs, Switzerland). Reagents and media for electron microscopy were obtained from Roth (Karlsruhe, FRG). A calf and a rabbit antiserum to recombinant human GM-CSF wre a gift from Dr. P. Moonen (Biogen, Geneva, Switzerland). A rabbit antiserum, rabbit anti-IgG, and a neutralizing monoclonal mouse anti-IgG against recombinant human TNF were from BASF (Ludwigshafen, FRG).

Isolation of human PMNs and bioassays

Human PMNs were isolated as previously described [7, 8]. Following isolation, PMNs were suspended in different media as indicated.

Lucigenin-dependent CL, detection of superoxide and hydrogen peroxide production, release of peroxidase, and ultrastructural detection of granulocyte activation: all bioassays were performed as described previously [7, 8].

Statistical analysis

Statistical significance of the data was calculated using Student's *t*-test and Mann-Whitney's *U*-test.

Table 1. Effect of preincubation with M-GRAM on the chemiluminescence (CL) response of polymorphonuclear neutrophilic granulocytes (PMNs) to different stimuli

A.	Preincubation with[a]		
Stimulus	M-GRAM	Medium	
M-GRAM (50 μl)	16± 9[b,c]	350±24	$P < 0.001$
EC-GRAM (50 μl)[d]	59± 1	236±17	$P < 0.001$
Medium (50 μl)	35± 2	30±12	N.S.[d]
PMA (25 ng/ml)	588±25	850±68	$P < 0.05$
Zymosan (1 mg/ml)	939±52	1171±33	$P < 0.02$
B.	Preincubation with[a]		
Stimulus	M-GRAM	Medium	
f-met-phe (10^{-4} *M*)	27± 1	5± 1	$P < 0.001$
Medium	1.2±0.1	0.1± 0.02	

[a] PMNs were preincubated with 50 μl M-GRAM or Medium for 120 min at 37° C and subsequently stimulated with the stimuli indicated

[b] Values represent the mean ± SEM of 3 experiments

[c] Values are expressed as 60-min integral counts (counts × 10^{-6}), only following stimulation with f-met-phe the peak CL response within 10 min (cpm × 10^{-6}) was calculated

[d] Not significant

Table 2. Effect of different growth factors on CL response of human PMNs.

CL response (counts × 10^{-6})[a]				
(U/ml)	G-CSF[b]	M-CSF	IL-3	IL-4
10^5	15.7 ± 5.1[c]	7.5 ± 1.3	–	–
10^4	8.6 ± 1.3	6.8 ± 1.0	–	–
10^3	6.3 ± 1.0	7.3 ± 1.3	51.4 ± 15.4	9.7 ± 2.4
10^2	6.6 ± 1.6	7.5 ± 1.3	11.3 ± 2.3	10.1 ± 1.2
10	6.3 ± 1.7	7.6 ± 1.4	6.0 ± 0.8	9.6 ± 1.8
1	–	–	7.7 ± 1.8	–
0.1	–	–	7.0 ± 1.2	–
BG	6.6 ± 1.2	7.9 ± 1.3	6.9 ± 1.1	9.9 ± 1.6
PMA (10 ng/ml)	1 001.9 ± 61.6			

[a] 60-min integral counts were measured

[b] PMNs were incubated with different concentrations of the growth factors indicated and the CL response was measured for 60 min at 37° C. Cytokinases were diluted in PBS containing 50 μg/ml BSA

[c] Values represent the mean ± SEM of 4 to 5 experiments on 5 donors

Results

Modulating effect of M-GRAM on cellular reactivity of PMNs

To further evalutate the effect of M-GRAM on the CL response of PMNs to subsequent stimulation, PMNs

Table 3. Biological and immunological detection of tumor necrosis factor (TNF) and lymphotoxin (LT), detection of colony stimulating factor (CSF)-activity in M-GRAM and EC-GRAM preparations

	TNF[a] (ng/ml)	LT[a] (ng/ml)	Cytotoxic[b] activity (U/ml)	CSF activity (U/ml)
M-GRAM	9.3	0	205	0
EC-GRAM	0	0	0	3×10^3

[a] Immunologic detection of TNF and LT was done with a double sandwich ELISA using monoclonal antibodies against recombinant human TNF and LT
[b] Biological activity was determined using a cytotoxicity assay as described in Methods
[c] CSF activity was detected using the CML assay (11)

Table 4. Effect of an antiserum against granulocyte-macrophage colony stimulating factor (GM-CSF) on the M-GRAM-induced CL response of human PMNs

CL response (counts $\times 10^{-6}$)[a]			
Stimulus	+ Calf serum[b]	+ Calf anti-GM-CSF	
GM-CSF	166.8 ± 26.4[c]	32.1 ± 4.0	$P < 0.001$
M-GRAM	554.3 ± 67.9	394.6 ± 49.1	N.S.
Medium	26.1 ± 3.2	25.9 ± 3.5	N.S.

[a] 60-min integral counts were measured
[b] 30 µl M-GRAM, GM-CSF (10^4 U/ml), or Medium were incubated with 30 µl normal calf serum or calf antiserum to recombinant human GM-CSF (1:10) for 30 min at room temperature, subsequently 50 µl were added to the PMNs for testing the CL response
[c] Values represent the mean ± SEM of 4 to 6 experiments

Table 5. Effect of rabbit anti-TNF on the M-GRAM-induced CL response of human PMNs

CL response (counts $\times 10^{-6}$)[a]			
Stimulus	+ Rabbit serum[b]	+ Rabbit anti-TNF	
TNF	311.6 ± 24.7[c]	23.3 ± 7.7	$P < 0.01$
M-GRAM	346.0 ± 45.5	359.0 ± 47.8	N.S.
Medium	14.3 ± 2.2	10.0 ± 0.4	N.S.

[a] 60-min integral counts were measured
[b] 30 µl M-GRAM, TNF (10^4 U/ml), or Medium were incubated with 30 µl normal rabbit serum or rabbit antiserum against recombinant human TNF (1:20) for 30 min at room temperature, subsequently 50 µl were added to the PMNs for testing the CL response
[c] Values represent the mean ± SEM of 4 experiments on 2 donors

Table 6. Effect of different antibodies against TNF on the M-GRAM-induced CL response of human PMNs

CL response (counts $\times 10^{-6}$)[a]		
	M-GRAM (1:2)	Medium
Rabbit IgG (10 µg/ml)[b]	370.7 ± 71.1[c]	33.2 ± 11.9
Rabbit anti-TNF IgG (10 µg/ml)	275.9 ± 79.9	28.1 ± 10.2
Mouse IgG_{2a} (1 µg/ml)	320.4 ± 69.2	30.0 ± 10.5
Mouse Ak 195 (1 µg/ml)	241.5 ± 44.4	24.7 ± 7.4

[a] 60-min integral counts were measured
[b] 30 µl M-GRAM (1:2) or Medium were incubated with 30 µl of the antibodies indicated for 120 min at room temperature, subsequently 50 µl were added to the PMNs for testing the CL response
[c] Values represent the mean ± SEM of 4 experiments on 4 donors

were preincubated with M-GRAM and stimulated with different triggers of the oxidative burst (Table 1). Following preincubation with M-GRAM, the signal induced by PMA or zymosan particles was significantly decreased (Table 1 A). In addition, PMNs preincubated with M-GRAM were completely deactivated to further stimulation with the same mediator and with EC-GRAM. In contrast, preincubation with M-GRAM resulted in a significantly enhanced response following stimulation with the chemotactic peptide f-met-phe (Table 1 B).

Relationship of M-GRAM to other cytokines

Since GM-CSF was shown to be a potent activator of PMNs [7] different additional growth factors (recombinant human G-CSF, M-CSF, interleukin-3, and interleukin-4) were tested for their effect on PMN oxidative metabolism (Table 2). Neither G-CSF and M-CSF (10 to 10^5 U/ml) nor interleukin-4 (10 to 4×10^3 U/ml) exhibited any GRAM activity. Only interleukin-3 induced a detectable CL signal at a concentration as high as 10^3 U/ml, whereas lower concentrations were ineffective.

In addition to GM-CSF, TNF and LT [8] could be possible candidates for M-GRAM activity. Therefore, the presence of these mediators was determined in preparations of M-GRAM and EC-GRAM (Table 3). Cytotoxic activity could only be detected in M-GRAM samples. This biologic activity was apparently due to TNF since no LT was measured by ELISA. In contrast, CSF activity was only present in EC-GRAM preparation.

For further characterization, the effect of antibodies specific for recombinant human GM-CSF and

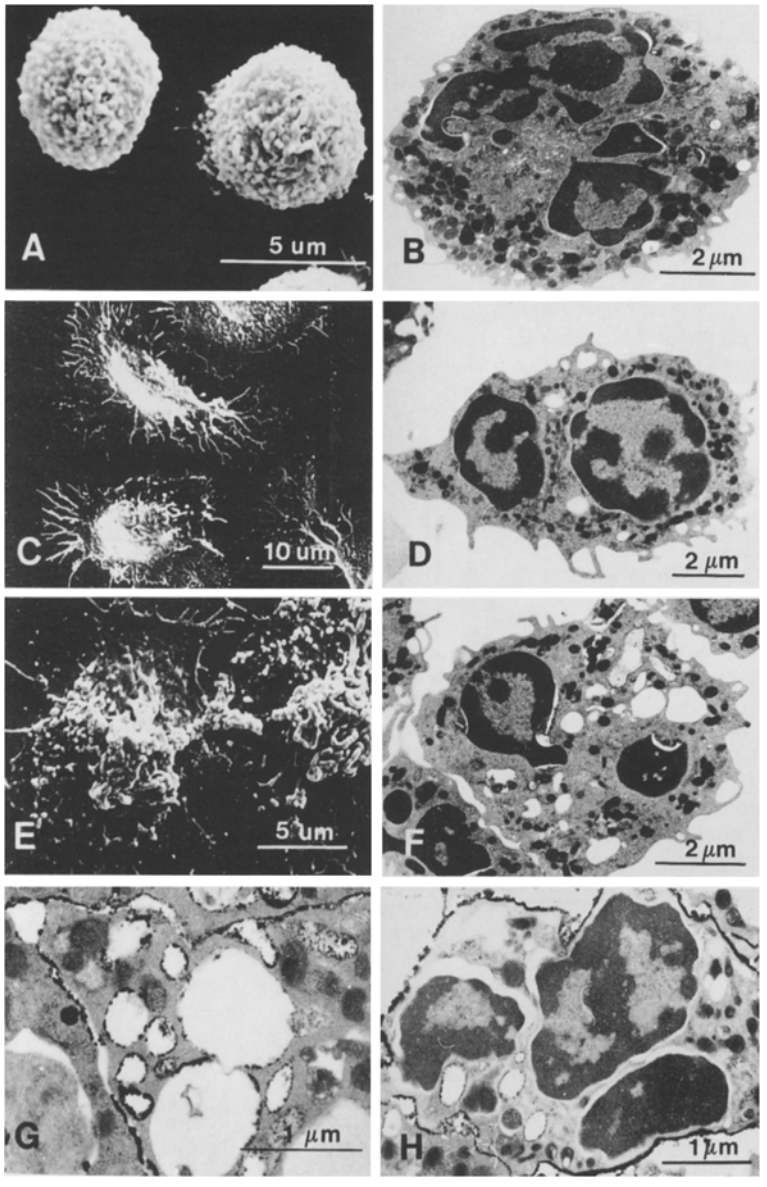

Fig. 1 A–H. Ultrastructural detection of granulocyte activation induced by M-GRAM. **A** Nonstimulated PMNs, 30 min at 37° C, scanning electron microscopy (SEM); **B** nonstimulated PMNs, 30 min at 37° C, transmission electron microscopy (TEM); **C** M-GRAM stimulated PMNs, 30 min at 37° C, SEM; **D** M-GRAM stimulated PMNs, 30 min at 37° C, TEM; **E** PMNs stimulated with PMA (10 ng/ml), 15 min at 37° C, SEM; **F** PMNs stimulated with PMA (10 ng/ml), 15 min at 37° C, TEM; **G** ultrastructural detection of hydrogen-peroxide production following stimulation of PMNs with PMA (10 ng/ml), 15 min at 37° C, TEM; **H** ultrastructural detection of hydrogen-peroxide production following stimulation with M-GRAM, 30 min at 37° C, TEM

TNF on M-GRAM-induced CL response was tested. Of EC-GRAM activity 75% was blocked by the addition of anti-GM-CSF, as previously shown [6]. The response to M-GRAM, however, was unaffected (Table 4). A rabbit antiserum to TNF did not show any blocking effect on M-GRAM-induced CL signal (Table 5). To further substantiate this observation, experiments were done with the purified IgG fraction of the antiserum and with a neutralizing monoclonal antibody against TNF at a concentration which completely blocked the cytotoxic activity contained in the tested M-GRAM preparation (Table 6). In addition, inhibition experiments with TNF and anti-TNF to which inactivated heated M-GRAM was added clearly demonstrated that M-GRAM did not influence antibody binding (data not shown).

Ultrastructural changes and ultrastructural detection of hydrogen peroxide production

Nonstimulated PMNs remained rounded with ruffled surface membranes (Fig. 1 A). The cells showed the characteristic morphology of PMNs, which induces a multilobulated nucleus, a large population of morphologically diverse granules, few mitochondria, and scattered deposits of glycogen (Fig. 1 B). Within 30 min following stimulation with M-GRAM, PMNs were flattened, showing dendritic protrusions in the

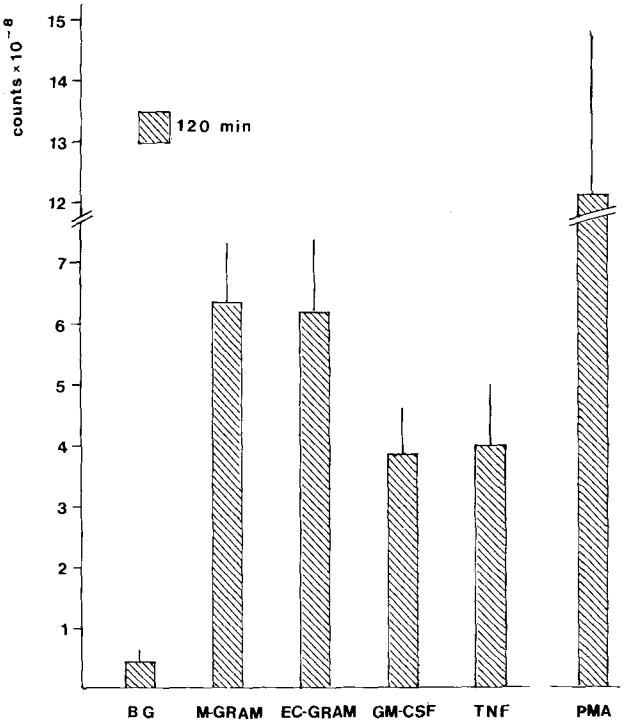

Fig. 2. Comparison of PMN chemiluminescence (CL) response upon stimulation with M-GRAM, EC-GRAM, GM-CSF, and TNF. Medium (BG), M-GRAM (1:5), EC-GRAM (1:5), recombinant human GM-CSF (10^3 U/ml), recombinant human TNF (10^3 U/ml), and PMA (10 ng/ml) as a control stimulus were added to the PMNs; 60-min integral counts were measured (counts $\times 10^{-8}$). Values represent the mean $\pm$ SEM of 3 to 5 experiments on 3 to 5 donors

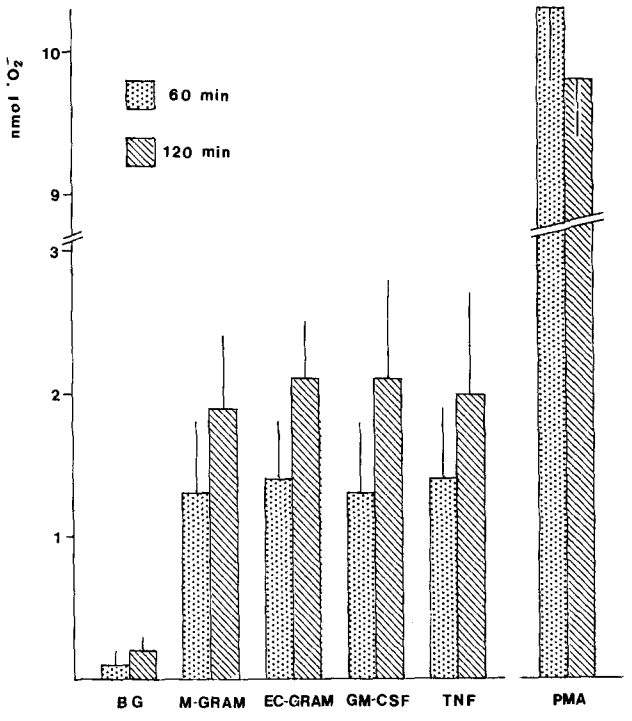

Fig. 3. Comparison of superoxide production by human PMNs upon stimulation with M-GRAM, EC-GRAM, GM-CSF, and TNF. Medium (BG), M-GRAM (1:5), EC-GRAM (1:5), recombinant human GM-CSF (10^3 U/ml), recombinant human TNF (10^3 U/ml), and PMA (10 ng/ml) as a control stimulus were added to the PMNs. The SOD-inhibitable part of cytochrome-C reduction was measured for 60 or 120 min at 37°C (nM $\cdot O_2^-$/well). Values represent the mean $\pm$ SEM of 3 to 5 experiments on 3 to 5 donors

rounded central portion in contact with the plastic surface (Fig. 1C). Clear intracytoplasmic vesicles were almost seen at 15 min and were visible to a major extent within 30 min (Fig. 1D). EC-GRAM, in contrast, increased the adherence of PMNs to the plastic layer significantly within 30 min, whereby the cells exhibited a long dendritic protrusion mainly seen at one side of the cells (data not shown). Upon stimulation with PMA, which was used as a control stimulus, attached cells were firmly adherent to the plastic layer within 15 min, short filopodia were seen in rounded central portion of the PMNs (Fig. 1E) and large intracytoplasmic vesicles could be detected (Fig. 1F).

Production of H_2O_2 in PMNs was visualized by detection of the electron-dense reaction product cerium perhydroxide at the outer surface of the plasma membrane and the luminal part of almost all intracytoplasmic vacuoles within 15 min upon stimulation with PMA (Fig. 1G) and similarly, but to a lower extent, following stimulation with M-GRAM (Fig. 1H).

Chemiluminescence, production of superoxide and hydrogen peroxide, release of myeloperoxidase — comparison of M-GRAM with EC-GRAM, GM-CSF, and TNF

When testing the capacity of the different factors to induce a CL response in human PMNs, comparable results were obtained (Fig. 2). Similar results were found with superoxide production (Fig. 3). Rates of hydrogen peroxide production were almost identical following stimulation with M-GRAM, EC-GRAM, and TNF. GM-CSF, however, had a significantly lower effect (Fig. 4). Whereas EC-GRAM, GM-CSF, and TNF represented weak inducers of the release of myeloperoxidase, M-GRAM proved to be a significant stimulus (Fig. 5).

Discussion

The present paper is considering the question whether M-GRAM represents a new cytokine. First, it was investigated whether well-known cytokines represent

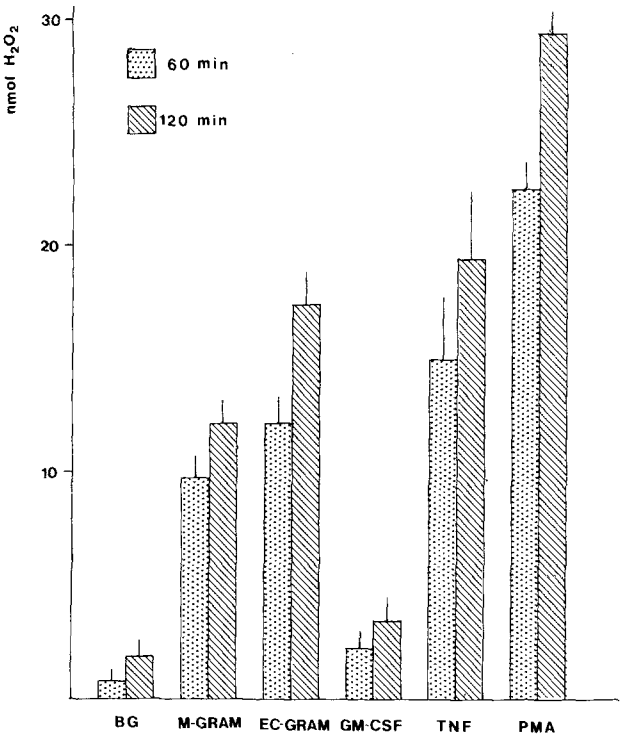

Fig. 4. Comparison of hydrogen-peroxide production by human PMNs upon stimulation with M-GRAM, EC-GRAM, GM-CSF, and TNF. Medium (BG), M-GRAM (1:5), EC-GRAM (1:5), recombinant human GM-CSF (10^3 U/ml), recombinant human TNF (10^3 U/ml), and PMA (10 ng/ml) as a control stimulus were added to the PMNs. HRPO-mediated oxidation of phenol red was measured for 60 or 120 min at 37°C (nM H_2O_2/ml). Values represent the mean ± SEM of 3 to 5 experiments on 3 to 5 donors

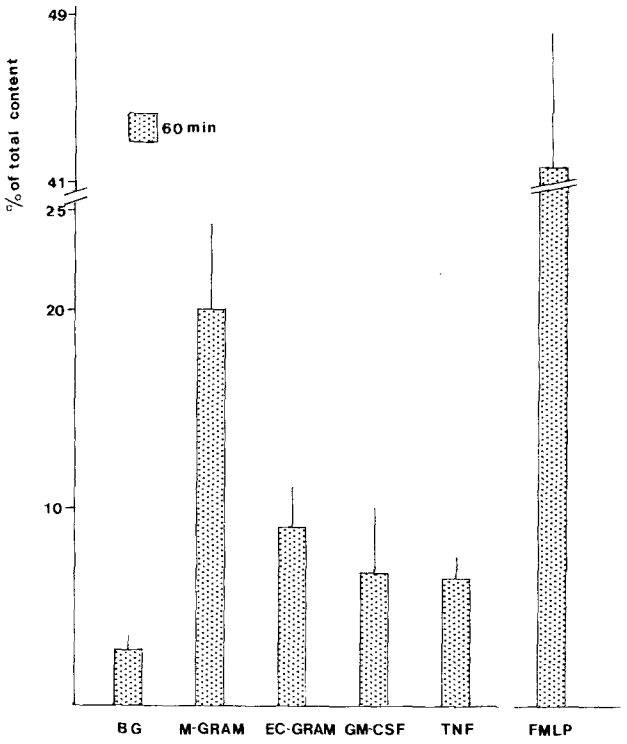

Fig. 5. Comparison of the release of myeloperoxidase from human PMNs upon stimulation with M-GRAM, EC-GRAM, GM-CSF, and TNF. Medium (BG), M-GRAM (1:5), recombinant human GM-CSF (10^3 U/ml), recombinant human TNF (10^3 U/ml), and PMA (10 ng/ml) as a control stimulus were added to the PMNs. Release of myeloperoxidase was measured for 60 min at 37°C (% of total cellular content released). Values represent the mean ± SEM of 3 to 5 experiments on 3 to 5 donors

candidates for M-GRAM. For this purpose mediators which were suggested to affect PMN functions were tested for their capacity to elicit a CL response in human PMNs.

Based on our findings IFN α and γ [4], IL-1α and β [6], IL-2 [6], IL-3 and EC-IL-3 [3, 10], IL-4, G-CSF, M-CSF, and the chemotactic factors MOC/MONAP [9, 14], and ANAP [6, 13] were apparently not responsible for M-GRAM activity. At highest and unphysiologic concentrations, however, IFNγ, IL-1β, MOC/MONAP, ANAP (anionic neutrophil-activating peptide), and particularly IL-3, were able to induce a distinct CL response in human PMNs. Therefore, the effect observed was not of any relevance for our question. In addition, M-GRAM as well as EC-GRAM could be easily separated from MOC/MONAP and ANAP during biochemical purification (A. Kapp and J. Schröder, unpublished observation). Furthermore M-GRAM and EC-GRAM preparations used were devoid of chemotactic activity [4, 6].

Based on previous investigations [7, 8], GM-CSF, TNF, and LT were suggested to be the most relevant characterized cytokines with GRAM activity. Their effect on PMN oxidative metabolims has been previously described in detail [7, 8]. We, therefore, tested whether these cytokines are present in preparations of M-GRAM or EC-GRAM by using biological and immunological assay systems. CSF activity was only detected in EC-GRAM samples, but not in M-GRAM. In contrast, M-GRAM samples exhibited a significant cytotoxic activity. Using the ELISA technique no LT, but TNF at significant concentration could be detected in semipurified M-GRAM preparations. Therefore, the observed cytotoxic effect appeared to be due to TNF. The results confirmed the previously proposed relationship between EC-GRAM and GM-CSF [6] and suggested a relation between M-GRAM and TNF.

Thus, the effect of different neutralizing antibodies against GM-CSF and TNF on M-GRAM-induced CL response was tested. Whereas anti-GM-CSF inhibited

75% of the effect induced by EC-GRAM [6], the CL signal following stimulation with M-GRAM remained unchanged. This was very likely because, no CSF activity could be detected in M-GRAM samples. Interestingly, a rabbit antiserum against TNF was ineffective in blocking the effect of M-GRAM on human PMNs. Therefore, the purified IgG fraction of the antiserum as well as a neutralizing monoclonal antibody were tested. All antibodies were tested at concentrations which completely blocked the cytotoxic activity present in the M-GRAM samples tested. Even under the described conditions, no significant inhibition of the M-GRAM-induced CL response was observed. In addition, the inhibiting effect of contaminants in M-GRAM samples on antibody binding was excluded. Hence the effect of M-GRAM on PMNs was not simply due to TNF although this mediator was present in the preparations tested.

Therefore, it appears likely that LPS-stimulated monocytes release at least two granulocyte-activating mediators which are capable of activating the oxidative burst in PMNs. One of these is TNF, the other probably represents a new cytokine.

In examining the ultrastructural changes induced by EC-GRAM, similar effects were found as with TNF [8] and GM-CSF [7]. PMNs firmly adherent at the plastic surface were typically polarized, showing very long protrusions. M-GRAM also increased the adherence of PMNs, but induced – comparable to PMA – development of small filopodia. Although M-GRAM samples contained TNF, the effects induced by M-GRAM preparations were significantly different from the ultrastructural changes upon stimulation with TNF alone. M-GRAM activity apparently "dominated" in the semipurified preparations.

Whereas preincubation of PMNs with GM-CSF resulted in deactivation to further stimulation with M-GRAM, EC-GRAM, and TNF, PMNs could be restimulated with GM-CSF and TNF, but not with M-GRAM following prestimulation with TNF (data not shown). This finding possibly suggests that different subcellular events are triggered by M-GRAM.

No significant differences between M-GRAM and the other mediators tested were found when they were tested for their capacity to induce a CL response or to produce superoxide. When the production of hydrogen peroxide was measured, GM-CSF released significantly lower amounts in comparison to M-GRAM, EC-GRAM, and TNF. These results have been confirmed by ultrastructural detection of H_2O_2 release. M-GRAM, EC-GRAM, and TNF [8] induced effects comparable with PMA, only GM-CSF was significantly less active [7]. Distinct effect of GM-CSF and EC-GRAM may be due to the glycosylation state of EC-derived GM-CSF – in contrast to the recombinant *E. coli*-derived GM-CSF – or could be caused by EC IL-3/ELSA (epidermal cell derived leukocyte stimulating activity) present in semipurified EC-GRAM preparations. Further biochemical characterization of EC-GRAM to homogeneity is necessary in order to answer the question.

M-GRAM released significant amounts of myeloperoxidase from PMNs, in contrast to EC-GRAM, GM-CSF, and TNF. Release of myeloperoxidase is essential for microbicidal action of PMNs [9]. TNF and GM-CSF, however, do not represent important triggers of peroxidase release. Thus, M-GRAM could play a role as a cofactor of TNF. It is speculated that TNF-induced cytotoxicity via oxidative mechanisms in vivo is dependent on the simultaneous release of these two factors. It is to be noted that PMNs which were prestimulated with TNF could be restimulated with M-GRAM, but not with other cytokines in the CL assay.

M-GRAM probably represents a new immunomodulating cytokine which – in addition to TNF – directly activates the oxidative metabolism of human PMNs.

Acknowledgements. We gratefully acknowledge the expert technical assistance of Mrs. A. Piskorski. We wish to thank Dr. J.-J. Mermod, Glaxo Institute for Molecular Biology, Geneva, for performing the CML assay.

References

1. Aggarwal BB, Moffat B, Harkins RN (1984) Human lymphotoxin: production by a lymphoblastoid cell line, purification, and initial characterization. J Biol Chem 259:686–691
2. Aggarwal BB, Kohr WJ, Hass PE, Moffat B, Spencer SA, Henzel WJ, Birgman TS, Nedwin GE, Goeddel DV, Harkins RN (1985) Human tumor necrosis factor: production, purification and characterization. J Biol Chem 260:2345–2354
3. Danner M, Kapp A, Micksche M, Luger TA (1987) Human epidermal cell derived leukocyte stimulating activity (ELSA): a distinct unique cytokine with multiple immunological functions. Lymphokine Res 6:Abstr 1309
4. Kapp A, Luger TA, Maly FE, Schöpf E (1986) Granulocyte-activating mediators (GRAM): I. Generation by LPS-stimulated mononuclear cells. J Invest Dermatol 86:523–528
5. Kapp A, Luger TA, Danner M, Wokalek H (1987) Generation of granulocyte chemiluminescence by a mediator derived from human epidermal cells. In: Schölmerich J, Andreesen R, Kapp A, Ernst M, Wood WG (eds) Bioluminescence and chemiluminescence – new perspectives. Wiley, Chichester, pp 117–120
6. Kapp A, Danner M, Luger TA, Hauser C, Schöpf E (1987) Granulocyte-activating mediators (GRAM). II. Generation by human epidermal cells. Arch Dermatol Res 279: 470–477
7. Kapp A, Zeck-Kapp G, Danner M, Luger TA (1988) Human granulocyte-macrophage colony stimulating factor: an effective direct activator of human polymorphonuclear neutrophilic granulocytes. J Invest Dermatol (in press)

8. Kapp A, Zeck-Kapp G, Blohm D (1988) Human tumor necrosis factor is a potent activator of the oxidative metabolism in human polymorphonuclear neutrophilic granulocytes (PMN) – comparison with human lymphotoxin. J Invest Dermatol (in press)
9. Kownatzki E, Kapp A, Uhrich S (1986) Novel neutrophil chemotactic factor derived from human peripheral blood mononuclear leucocytes. Clin Exp Immunol 64:214–222
10. Luger TA, Kapp A, Micksche M, Danner M (1987) Characterization of a distinct epidermal cytokine with multiple immuno-regulatory properties. J Invest Dermatol 88:504
11. Moonen P, Mermod JJ, Ernst JF, Hirschi M, DeLamarter JF (1987) Increased biological activity of deglycosylated recombinant human granulocyte/macrophage colony-stimulating factor produced by yeast or animal cells. Proc Natl Acad Sci USA 84:4428–4431
12. Movat HZ (1985) Phagocytosis and release of lysosomal contents. In: The inflammatory reaction. Elsevier, Amsterdam, pp 273–310
13. Schröder JM, Christophers E (1986) Identification of $C5a_{des\ arg}$ and an anionic neutrophil-activating peptide (ANAP) in psoriatic scales. J Invest Dermatol 87:53–58
14. Schröder JM, Mrowietz U, Morita E, Christophers E (1987) Purification and partial biochemical characterization of a human monocyte-derived, neutrophil-activating peptide that lacks interleukin-1 activity. J Immunol 139:3474–3483

Received March 3, 1988