Ann Hematol (1992) 64: 190–195

Annals of
Hematology

Original article

Developmental regulation of granulocyte-macrophage colony-stimulating factor production during human monocyte-to-macrophage maturation*

S. W. Krause[1], M. Kreutz[1], G. Zenke[2], and R. Andreesen[1]
[1] Medizinische Klinik I der Universität Freiburg, Freiburg, Federal Republic of Germany
[2] Sandoz AG, Basel, Switzerland

Received October 22, 1991 / Accepted March 3, 1992

Summary. Cells of the macrophage lineage are a major source of various cytokines and hematopoietic growth factors. With regard to the growth factors acting on cells of their own lineage, macrophage colony-stimulating factor (M-CSF) has been proven to be secreted by monocytes (MO) and macrophages (MAC), whereas the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) by human MO/MAC is under debate. Here we report that in elutriation-purified MO, as well as in MAC derived from cultured MO, GM-CSF m-RNA was regularly induced by LPS. In MO the GM-CSF message was still detectable 18 h after stimulation under serum-free conditions, but in contrast was already lost at this time point in MAC. Secreted GM-CSF protein was detected in the culture medium using a sandwich ELISA. Furthermore, a factor-dependent cell line (M-07) was used for a biological assay. Here, a neutralizing anti GM-CSF antibody specifically blocked the proliferation-inducing activity of MO/MAC supernatants. Whereas only small amounts of GM-CSF were detected in MO, its secretion increased severalfold upon MO-to-MAC differentation in vitro. A similar increase upon in vitro maturation of MO was observed for the production of granulocyte colony-stimulating factor. The highest amounts of GM-CSF (up to 2.8 ng/10^6 cells) were produced by MAC that had been derived from MO cultured under serum-free conditions in the presence of 0.5 mg/ml albumin as the only medium supplement.

Key words: Monocytes – Macrophages – Cell differentiation – Granulocyte colony-stimulating factor – Granulocyte-macrophage colony-stimulating factor

Address for correspondence: R. Andreesen, Medizinische Klinik und Poliklinik I, Universität Regensburg, Franz Joseph Strauß-Allee 1, W-8400 Regensburg, Federal Republic of Germany

* This work was supported by the *Deutsche Forschungsgemeinschaft* (AN 111)

Introduction

Besides their historically well-known property of phagocytosis, cells of the macrophages lineage produce a variety of cytokines, colony-stimulating factors (CSF), and other secretory products. Through the secretion of these factors, monocytes (MO) and macrophages (MAC) are involved in the regulation of the function of both hematopoietic and nonhematopoietic cell systems [6, 10, 21]. The pattern of secreted proteins differs between blood MO and tissue MAC, as is evident from in vivo and in vitro studies [26, 30]. At least two of the known growth factors could be involved in the autocrine regulation of cells of the MAC lineage: macrophage-CSF (M-CSF) and granulocyte-macrophage-CSF (GM-CSF). M-CSF is produced both by adherent MO and MAC [14, 18, 26]. Currently, M-CSF is recognized to be a factor which not only stimulates committed progenitor cells but also acts as a survival and differentiation factor for MO [5, 7, 20]. GM-CSF, a glycoprotein of 18–23 kD [31], is a stimulator of stem-cell proliferation, leading to granulocyte colonies, granulocyte-MAC colonies, and some MAC colonies [12]. Similar to M-CSF, it seems to be involved in the modulation and activation of differentiated MO and MAC [13, 16, 24], in addition to its action on neutrophils. The main source of GM-CSF are activated T-cells, endothelial cells, and fibroblasts [10, 22, 27]. It is not clear whether MO and MAC are also GM-CSF-producing cells; published results so far are contradictory [18, 22, 27]. Here we report that in highly purified MO/MAC the production of GM-CSF is detected on the RNA and protein level, and that its secretion is modulated during in vitro maturation of MO into MAC. Besides GM-CSF that acts on granulocytes and MO/MAC as a pluripotent growth factor, other factors (e.g., IL-8 and granulocyte colony-stimulating factor, G-CSF) with major effects upon the granulocyte lineage are produced by MAC [4, 18, 22]. Here we report that the producing of G-CSF is also developmentally regulated during MAC differentation.

Materials and methods

Cell preparation

Mononuclear cells were collected by leukapheresis of healthy donors and Ficoll-separation as described previously [18]. MO and lymphocytes (LY) were isolated by counter-current elutriation using a Beckmann J6-ME centrifuge with a JE 5.0 rotor and a large elutriation chamber (Beckmann, Munich, FRG). Elutriation was performed in 2% human albumin in Hank's salt solution at constant 2500 rpm with LY being elutriated at a flow rate of 64 ml/min and MO at a flow rate of 110 ml/min. The monocyte fraction was >90% pure, the lymphocyte fraction was >90% pure, as determined by the detection of CD 14, CD 4, CD 8, and CD 20 antigens. For in vitro maturation, MO were cultured at 10^6 cells/ml for up to 14 days on hydrophobic Teflon foils (Biofolie 25, Heraeus, Hanau, FRG) in the presence of 2% pooled human AB-serum [1]. In some experiments parallel cultures were performed with 0.5 mg/ml human albumin (Biotest, Dreieich, Germany) instead of serum. On the days indicated, cells were harvested from the Teflon bags, and washed, and viability was assessed by trypan-blue exclusion. MAC maturation was followed by morphology and the expression of maturation-associated antigens [2].

Northern blot analysis

Either LY, MO, or MO-derived MAC obtained after different culture periods were seeded into 60 mm plasic Petri dishes (Greiner, Solingen, Germany, or Falcon, distributed by Becton-Dickinson, Heidelberg, Germany) at a density of 10^6 cells/ml and cultured either with or without 100 ng/ml bacterial lipopolydsacchride from *Salmonella abortus equii* (LPS, kindly provided by C. Galanos, MPI, Freiburg, Germany) or phorbolmyristate acetate (PMA, 5×10^{-10} *M*) and phytohemagglutinin (PHA, 5 μg/ml). At the indicated time the supernatants (SN) were collected, filtered through 0.22 μm filters (Millipore, Bedford, MA) in order to remove cell debris, and stored at −20°C.

After aspiration of SN, extraction of total cellular RNA was performed according to Chomzynski and Sacchi [8]. Cells were lysed directly in the dishes with guanidium thiocyanate solution; 6 μg total RNA were dissolved in 10 μl loading buffer containing 50% formamide, 2.2 *M* formaldehyde, 20 m*M* MOPS, 5 m*M* sodium acetate, 1 m*M* EDTA, 0.04% bromephenol blue, 1% Ficoll, and 50 μg/ml ethidium bromide. Samples were run on a 1% agarose gel containing 2.2 *M* formaldehyde, 20 m*M* MOPS, 5 m*M* sodium acetate, 1 m*M* EDTA, without ethidium bromide, and transferred to nylon membranes (Nytran, Schleicher & Schuell, Dassel, Germany) by capillary blotting in 20×SSC. The ethidium bromide-stained RNA was visualized on the wet filters by transillumination with UV light and photographed to verify that all lanes contained equal amounts of RNA. GM-CSF mRNA was detected by hybridization with a cDNA probe (kindly provided by D. Krumwieh, Behringwerke, Marburg, Germany) labeled with [^{32}P] dCTP (3000 Ci/mmol, Amersham, Buckinghamshire, England) by the random prime method with a commercially avaiable kit (Amersham). Hybridization conditions were 500 m*M* sodium phosphate pH 7.2, 7% SDS, 1 m*M* EDTA, 150 μg/ml tRNA (adapted from Church and Gilbert [9]) at 65°C over night. Washing conditions were 0.1×SSC, 1% SDS at 52°C (three times for 30 min).

Quantitation of GM-CSF and G-CSF in cell culture supernatants

Biological assay Supernatants of MO/MAC were tested for GM-CSF and Interleukin-3 (IL-3) using the leukemia cell line M-07 [3]. These cells depend on GM-CSF and/or IL-3 for cell growth. Cell were maintained in DMEM supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 m*M* L-glutamine, 13 μg/l α-thioglycerol (all from Gibco, Paisley, Scotland), 10% heat-inactivated fetal bovine serum (Boehringer, Mannheim, FRG), and 10 U/ml CHO-expressed rhu IL-3 (Sandoz, Basel, Switzerland). The M-07 cells were washed once in culture medium without IL-3 immediately before the assay. Washed cells were seeded at 2×10^4 per well in 96-well plates (Falcon, Lincoln Park, NJ) in the presence of either purified CHO-expressed rhu GM-CSF (2000 pg/ml, Sandoz, Basel, Switzerland), purified CHO-expressed rhuIL-3 (0.3–30 ng/ml), serial dilutions of MO/MAC SN, or control medium containing the same amount of LPS as the MO/MAC SN (all in duplicates). Neutralizing anti-GM-CSF and anti-IL-3 monoclonal antibodies were included at 25 μg/ml and 5 μg/ml, respectively. At these antibody concentrations the effect of approximately 1 ng/ml of GM-CSF or 2 ng/ml of IL-3, respectively, was blocked. After 68 h incubation at 37°C, 0.5 μCi ^{3}H-thymidine (15 Ci/mmol, Amersham) was added to each well and incubation continued for additional 4 h. Cells were harvested on filter papers (Inotech, Wohlen, Switzerland). After addition of scintillation fluid, radioactivity was determined with a scintillation counter (LS 3801, Beckman). Data given are cpm of M-07 in conditioned medium subtracted by cpm M-07 in control medium (the latter always below 3000 cpm).

ELISA. Sandwich ELISA's (MRL for GM-CSF and Amgen for G-CSF, both distributed by Biermann, Bad Nauheim, Germany) were performed according to the manufacturer's protocols.

Results

At first we tested cell culture supernatants by a bioassay with the growth factor-dependent cell line M-07. Supernatants of LPS-stimulated MO and MAC induce the growth of M-07 cells. Bioactivity in the SN was blocked by the addition of an anti-GM-CSF antibody, but only to minimal degrees by the addition of an anti IL-3 antibody, indicating that MO and MAC secrete biologically active GM-CSF upon stimulation with LPS. A typical experiment is shown in Fig. 1. On comparison of the activity of the MAC-conditioned media with serial dilutions of recombinant GM-CSF, the bioactivity in the SN corresponded to 1.1 ng/ml recombinant GM-CSF. In several other

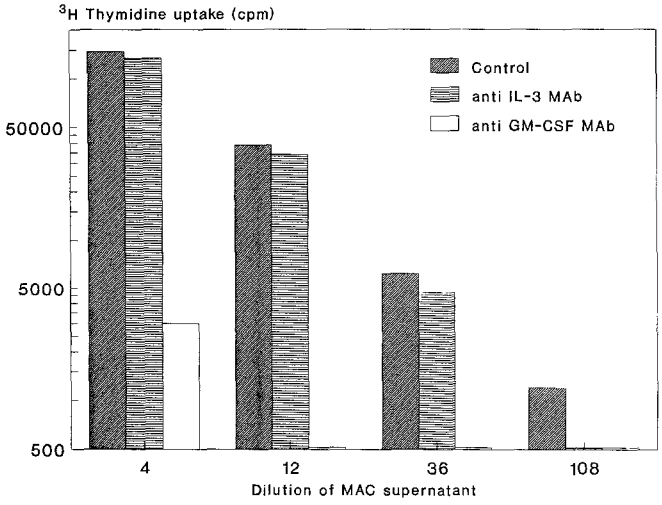

Fig. 1. Growth promotion of factor-dependent M-07 cells by MAC supernatants and its inhibition by anti-GM-CSF antibodies. Serial dilutions of conditioned media of LPS-stimulated, MO-derived MAC were incubated with M-07 cells, either alone (*control*), with neutralizing anti-IL-3 antibody (*MAb*), or with neutralizing anti-GM-CSF antibody

experiments GM-CSF bioactivity in the range of 3.0–1.1 ng/ml was detected. No GM-CSF bioactivity was found in the conditioned media of unstimulated MO or MAC.

In addition to the findings in the bioassay, we detected and quantitated GM-CSF protein by sandwich ELISA. In preliminary experiments, not shown in detail here, MO and MAC constantly produced higher amounts of GM-CSF when stimulated with LPS under serum-free culture conditions. This is in contrast to other factors such as TNF, IL-1, and G-CSF that are produced in higher amounts in the presence of serum. Therefore, for measurements of GM-CSF, stimulation of MO/MAC was carried out in the absence of serum, unless indicated otherwise. The amount of G-CSF was determined in parallel cultures that had been stimulated in the presence of 2% human serum. Neither G-CSF nor GM-CSF was detected in unstimulated MO/MAC cultures. During in vitro maturation of MO into MAC the amount of GM-CSF secretion increased severalfold. The time course, as well as the extent to which GM-CSF production was up-regulated during MO-to-MAC maturation, differed in individual experiments. The results of two representative experiments are shown (Fig. 2). The capability of mature MAC to secrete higher amounts of GM-CSF compared with freshly isolated MO was confirmed with cells of several different donors. While stimulated MO produced up to 100 pg/ml GM-CSF (in some experiments below the detection threshold of 10 pg/ml), GM-CSF was secreted in the range of 200–700 pg/ml by MAC that had been allowed to mature for 8–14 days (the GM-CSF values determined by ELISA tended to be slightly lower than the values determined by bioassay with the M-07 cell line; not shown in detail).

The secretion of G-CSF increased to a similar extent as did the secretion of GM-CSF during MO-to-MAC maturation. We detected up to 0.3 ng/ml G-CSF in MO SN and up to 5 ng/ml G-CSF in MAC SN. As for GM-CSF,

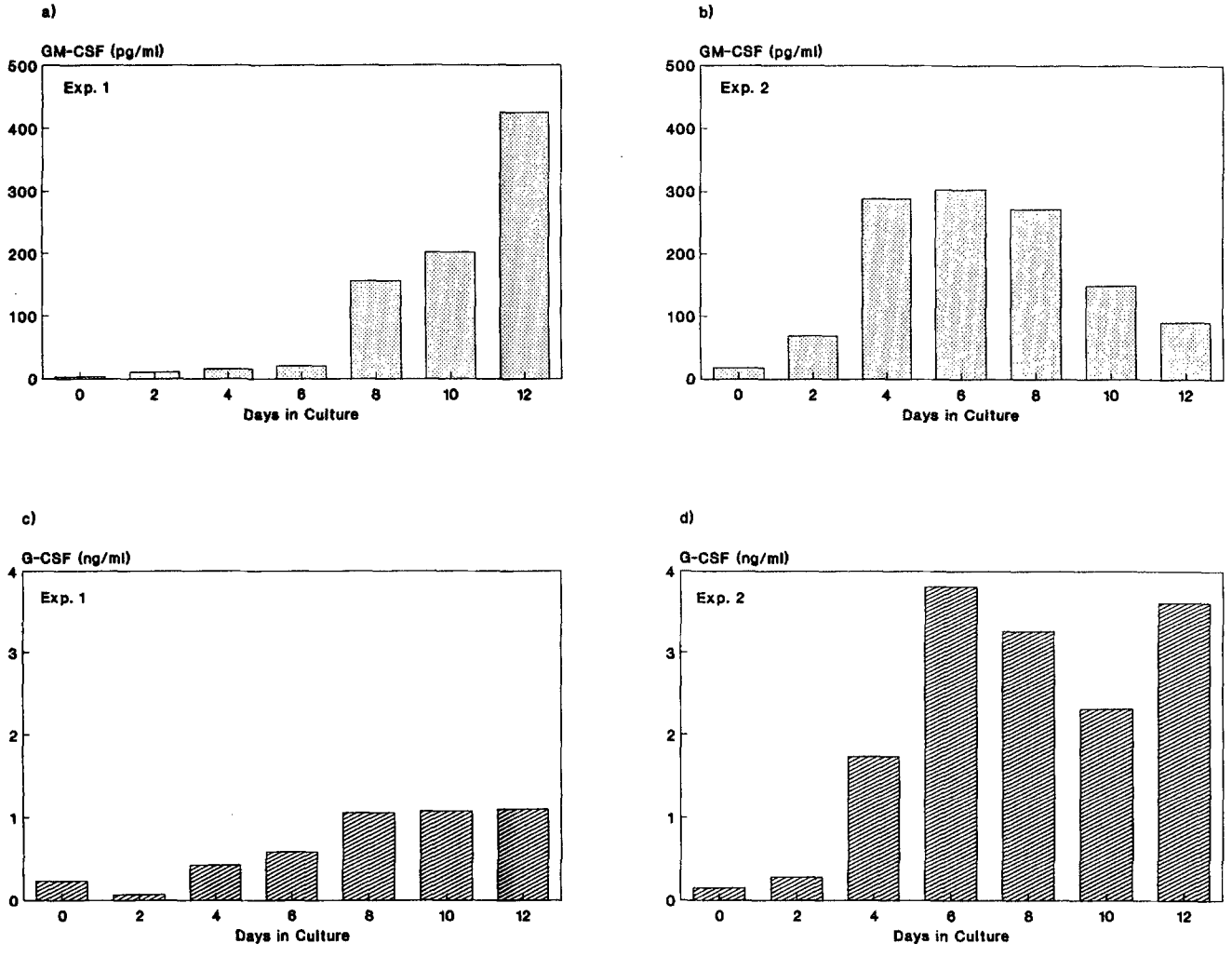

Fig. 2a–d. Influence of MO-to-MAC differentiation on stimulated GM-CSF and G-CSF secretion. Purified MO were either used directly or cultured in Teflon bags. After the indicated period of time, cells were detached from the Teflon bags, seeded into plastic dishes at 10^6 cells/ml, and incubated with LPS for 24 h in serum-free medium for the measurement of GM-CSF, or in the presence of 2% human serum for the detection of G-CSF. Conditioned media were tested for GM-CSF and G-CSF by ELISA. Two independent experiments are shown (Exp 1 and Exp 2)

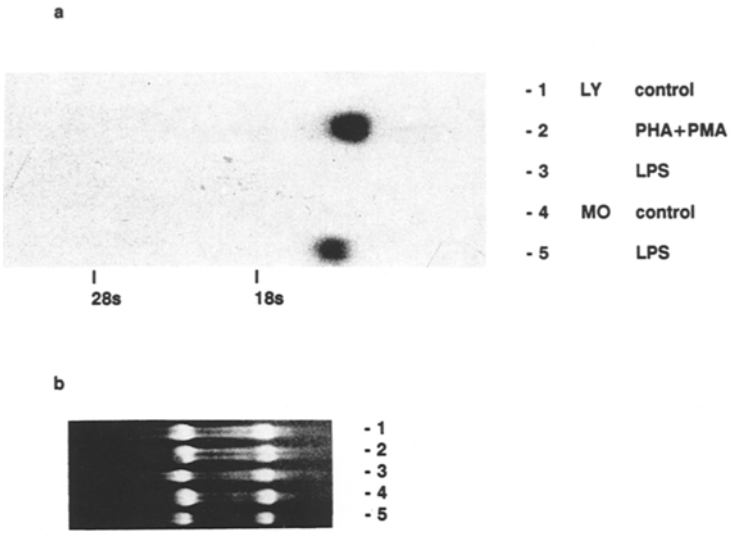

Fig. 3 a, b. Detection of GM-CSF mRNA in purified lymphocytes and monocytes. LY and MO were elutriated from the same batch of mononuclear cells. Total RNA was extracted 4 h after stimulation with either LPS or PMA and PHA, respectively. **a** Detection of GM-CSF RNA; **b** ethidium bromide staining. *Lane 1:* LY, unstimulated; *lane 2:* LY, stimulated with PMA and PHA; *lane 3:* LY, stimulated with LPS; *lane 4:* MO, unstimulated; *lane 5:* MO, stimulated with LPS

the time course of the increase in G-CSF production during the maturation process varied between different experiments (Fig. 2).

To further support our findings we performed nothern blots and hybridization with a GM-CSF-specific probe. In LY, only upon stimulation with PMA and PHA but not upon stimulation with LPS was the typical GM-CSF signal at about 1.1 kb detected, whereas LPS induced the GM-CSF message in purified MO of the same donor (Fig. 3). For neither of the cell populations was GM-CSF mRNA detected without previous stimulation. In an indepent experiment after stimulation with LPS a higher level of GM-CSF mRNA was detected in purified MO than in a mixed population of mononuclear cells (data not shown in detail). These observations rule out the possibility of contaminating LY as the source of GM-CSF in MO.

Our next goal was to determine the time course of the GM-CSF mRNA induction in MO and MAC. RNA was extracted 2, 6, and 18 h after stimulation with LPS either in the presence or in the absence of human serum. GM-CSF mRNA appeared later in MO than in MAC but persisted longer (Fig. 4). In MO, the GM-CSF message was detected ealier and disappeared earlier when the cells were stimulated in the presence of serum. In three similar time-course experiments, elevated GM-CSF mRNA levels were detected under serum-free conditions in MO at the 18 h time point; however, in two of the experiments a strong signal was already observed in these cells at the 6 h time point. In MAC no difference in the time kinetics of GM-CSF mRNA was observed in the presence or absence of serum.

Finally, we wanted to determine whether the GM-CSF production of MAC was modulated by the culture conditions during the in vitro maturation process. Serum-induced MAC are phenotypically and functionally different from cells cultivated serum free with human serum albumin as the only medium supplement, i. e., the latter survive at a lower percentage, produce less TNF, and express maturation-associated antigens at a lower density [17]. In the majority of experiments, MAC obtained from

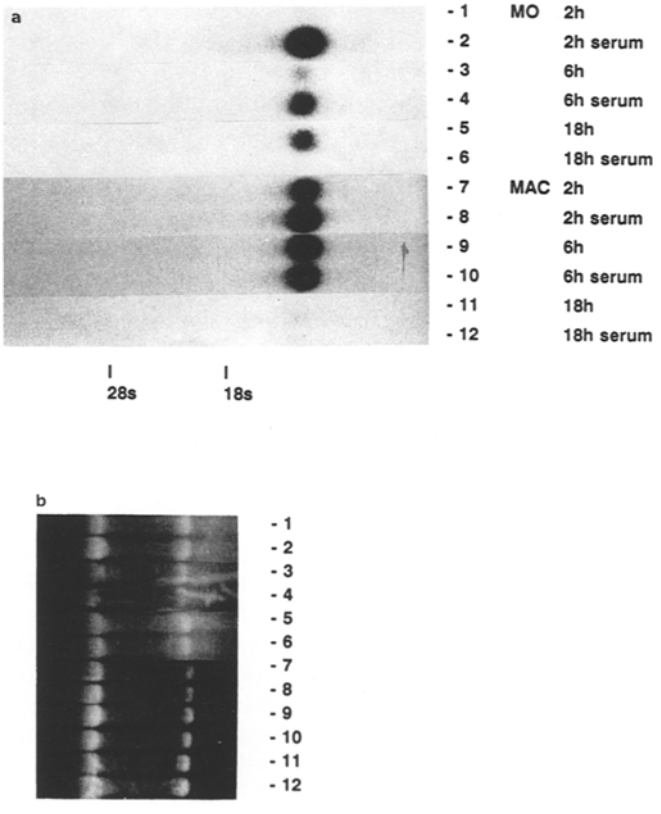

Fig. 4 a, b. Time course of GM-CSF message in MO and MO-derived MAC. MO and MO-derived MAC (7 days old) derived from a single leukapheresis product were stimulated with LPS either in serum-free culture medium (*lanes 1, 3, 5, 7, 9, 11*) or in the presence of 2% human serum (*lanes 2, 4, 6, 8, 10, 12*). RNA was extracted 2, 6, and 18 h after stimulation. **a** GM-CSF RNA; **b** ethidium bromide staining

albumin cultures showed a higher GM-CSF mRNA level and secreted larger quantities of GM-CSF into the culture medium than MAC obtained from serum cultures (Fig. 5).

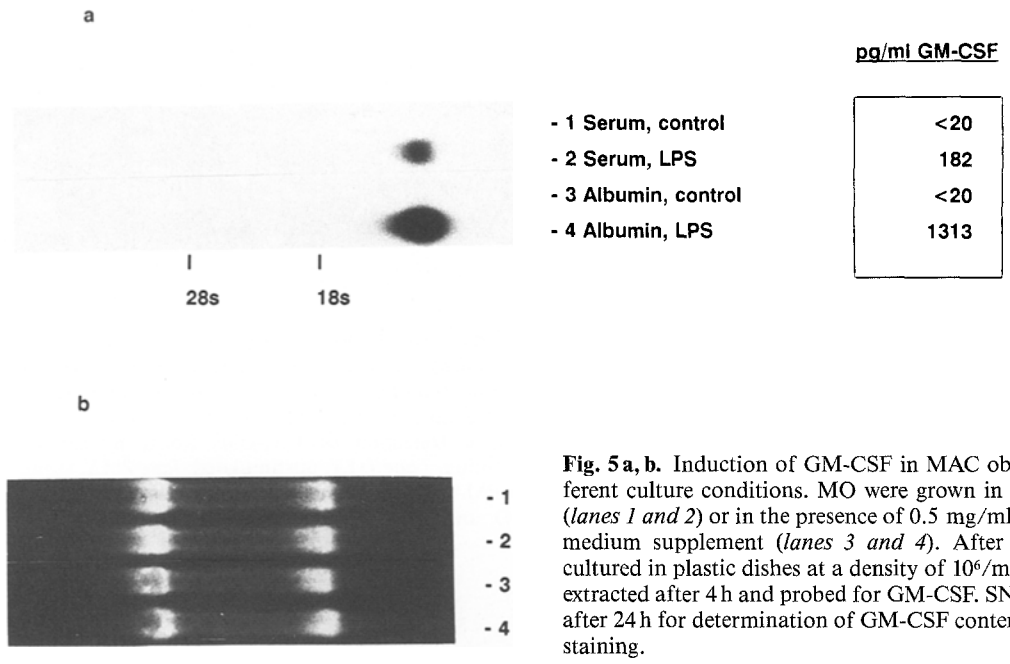

Fig. 5 a, b. Induction of GM-CSF in MAC obtained from MO grown under different culture conditions. MO were grown in the presence of 2% human serum (*lanes 1 and 2*) or in the presence of 0.5 mg/ml human serum albumin as the only medium supplement (*lanes 3 and 4*). After 7 days, cells were harvested and cultured in plastic dishes at a density of 10^6/ml with or without LPS. **a** RNA was extracted after 4 h and probed for GM-CSF. SN of parallel cultures were harvested after 24 h for determination of GM-CSF contents by ELISA. **b** Ethidium bromide staining.

Discussion

In several indepedent experiments the production of GM-CSF by LPS-stimulated MO and MAC was a consistent finding. Our results support the findings made by other groups [18, 27] and it is likely that MO/MAC can produce this factor also in vivo. Most importantly, the GM-CSF production of mononuclear phagocytes depends upon their state of differentiation. The increase in the production of GM-CSF during MO-to-MAC maturation correlates with the increased capacity of mature MAC to secrete M-CSF, TNF, and neopterin, whereas other factors (e. g., IL-1) are down-regulated [26, 30]. As we show here, G-CSF production rises in a similar fashion during this maturation process. On the mRNA level there is no clearly visible difference in GM-CSF content between MO and MAC, probably because MAC contain about five times the amount of total RNA compared with MO; however, on a per-cell level the production of GM-CSF protein by mature MAC is reproducibly several times higher than that by blood MO. This massive increase in GM-CSF secretion had led us to earlier findings [25]; we detected GM-CSF only in the SN of MAC and not in that of blood MO due to the less sensitive detection methods avaiable at that time. Other groups who did not detect GM-CSF when experimenting whith freshly isolated MO might have dealt with the same problem.

The increase of GM-CSF secretion in MAC is accompanied by the faster induction of GM-CSF mRNA in MAC: more GM-CSF is produced in a shorter period of time, but the down-regulation of GM-CSF mRNA occurs faster that in MO, too. Both the slower increase and the decrease of the GM-CSF message in MO are accelerated in the presence of serum. In MAC, the GM-CSF mRNA response is already faster than in MO and cannot be further accelerated by serum. Therefore, in terms of the GM-CSF mRNA kinetics, the presence of serum as a fast modulatory effect partially mimics the slow process of MO-to-MAC differentation. Whether this similar effect is achieved by similar intracellular mechanims affecting mRNA production and/or stability remains to be clarified. However, only the time course of GM-CSF expression in MO is shifted to a MAC-like pattern in the presence of serum, whereas the cumulated secretion of GM-CSF tends to decrease as compared with serum-free conditions. In contrast to the results of Thorens et al. [28], who performed their experiments with mouse macrophages cultured in fetal calf serum, we never found an induction of GM-CSF in the presence of serum alone.

At the present time we have no explanation for the high GM-CSF levels produced by MAC derived from albumin-containing cultures. As we used pyrogen-free clinical-grade albumin, contaminating endotoxin or other pyrogens in the Teflon cultures can be ruled out. However, priming events through denatured compounds that are not present in total serum are still possible. Nevertheless, it is temping to speculate that cells kept under suboptimal culture conditions are more easily stimulated to produce GM-CSF as a growth factor of their own lineage; in this picture, missing signals instead of positive signals would lead to the priming event. The modulation of responsiveness by the cultivation of MO under different culture conditions is an interesting model system, useful for studying the influence of microenvironmental signals during MAC maturation in vivo [11, 17]. Attempts to characterize such modulatory factors in detail are under way.

By the secretion of GM-CSF, MO and MAC can help to induce the production of cells of their own lineage as well as the production of granulocytes. In addition to its function of cell recruitment, MAC-activating properties of GM-CSF [13, 15, 24] are well documented. Furthermore, GM-CSF leads to enhanced accessory function for T-cell activation in MAC [16, 23], and it is an important

survival factor for circulating dendritic cells [19]. Via the secretion of GM-CSF, MAC not only effect hematopoiesis but also induce the functional activity of mature granulocytes in a concerted action with other monokines like IL-8 [4]. If the developmental regulation of these factors observed in vitro also occurs in vivo, interaction of mononuclear phagocytes with other leukocytes might be similarly potentiated when blood MO migrate into inflamed tissues and transform into reactive MAC.

In conclusion, our results prove that GM-CSF is a constituent of the secretory repertoire of human MAC. Thus, stimulated by bacterial endotoxins and probably other factors, and acting synergistically with such factors as G-CSF and IL-8, the production of GM-CSF by MO and MAC might stimulate the inflammatory infiltrate and play a role in the further recruitment of mononuclear phagocytes and other cells involved in the pathogenesis of acute and chronic inflammation.

Acknowledgements. The authors want to thank A. Rehm for her expert technical assistance, and A. Pollack, D. Eick, and E. Koch for their valuable advice in setting up the molecular biology techniques.

References

1. Andreesen R, Picht J, Löhr GW (1983) Primary cultures of human blood-borne, macrophages grown on hydrophobic Teflon membranes. J Immunol Meth 56: 295–304
2. Andreesen R, Brugger W, Scheibenbogen C, Kreutz M, Leser HG, Rehm A, Löhr GW (1990) Surface phenotype analysis of human monocyte-to-macrophage maturation. J Leukoc Biol 47: 490–497
3. Avanzi GC, Lista P, Giovinazzo B, Miniero R, Saglio G, Benetton G, Coda R, Cattoretti G, Pegoraro L (1988) Selective growth response to IL-3 of a human leukaemic cell line with megacarioblastic features. Br J Haematol 69: 359–366
4. Baggiolini M, Walz A, Kunkel SL (1989) Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest 84: 1045–1049
5. Becker S, Warren MK, Haskill S (1987) Colony-stimulating factor induced monocyte survival and differentiation into macrophages in serum-free cultures. J Immunol 139: 3703–3709
6. Becker S, Devlin RB, Haskill JS (1989) Differential production of tumor necrosis factor, macrophage colony-stimulating factor, and interleukin 1 by human alveolar macrophages. J Leukoc Biol 45: 353–361
7. Brugger W, Kreutz M. Andreesen R (1991) Macrophage colony-stimulating factor is required for human monocyte survival and acts as a cofactor for their terminal differentiation to macrophages in vitro. J Leukoc Biol 49: 483–488
8. Chomzynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156–159
9. Church GM, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci 81: 1991–1995
10. Clark, SC, Kamen R (1987) The human hematopoietic colony-stimulating factors. Science 236: 1229–1237
11. Eierman DF, Johnson CE, Haskill JS (1989) Human monocyte inflammatory mediator gene expression is selectively regulated by adherence substrates. J Immunol 142: 1970–1976
12. Gough NM (1990) The hematopoietic growth factor, granulocyte-macrophage colony-stimulating factor. In: Habenicht A (ed) Growth factors, differentiation factors, and cytokines. Springer, Berlin, Heidelberg, New York, p 177
13. Hart PH, Whitty GA, Piccoli DS, Hamilton JA (1988) Synergistic actions of human monocytes by granulocyte-macrophage colony-stimulating factor and IFN-gamma. J Immunol 141: 1516–1521
14. Haskill S, Johnson C, Eierman D, Becker S, Warren K (1988) Adherence induces selective mRNA expression of monocyte mediators and proto-oncogenes. J Immunol 140: 1690–1694
15. Heidenreich S, Gong JH, Schmidt A, Nain M, Gemsa D (1989) Macrophage activation by granulocyte-macrophage colony-stimulating factor. J Immunol 143: 1198–1205
16. Kato T, Inaba K, Ogawa Y, Inaba M, Shimizu S, Ikehara S, Sudo T, Muramatsu S (1990) Granulocyte-macrophage colony-stimulating factor enhances macrophage accessory function in Con-A-stimulated T-cell proliferation. Cell Immunol 130: 490–500
17. Kreutz M, Krause SW, Hennemann B, Rehm A, Andreesen R (1992) Macrophage heterogeneity and differentiation: defined serum-free culture conditions induce different types of macrophages in vitro. Res Immunol 143: 107–115
18. Lee MT, Kaushansky K, Ralph P, Ladner MB (1990) Differential expression of M-CSF, G-CSF, and GM-CSF by human monocytes. J Leukoc Biol 47: 275–282
19. Markovicz S, Engelman EG (1990) Granulocyte-macrophage colony-stimulating factor promotes differentiation and survival of human peripheral blood dendritic cells in vitro. J Clin Invest 85: 955–961
20. Munn DH, Cheung NKV (1989) Antibody-dependent antitumor cytotoxicity by human monocytes cultured with recombinant macrophage colony-stimulating factor. J Exp Med 170: 511–526
21. Nathan CF (1987) Secretory products of macrophages. J Clin Invest 79: 319–326
22. Oster W, Lindemann A, Mertelsmann R, Herrmann F (1989) Production of macrophage-, granulocyte-, granulocyte-macrophage- and multi-colony-stimulating factor by peripheral blood cells. Eur J Immunol 19: 543–547
23. Reske-Kunz AB, Fischer HG, Reske K (1988) Induction of antigen presentation capacity and MHC class-II gene expression in bone marrow macrophages derived from GM-CSF-supplemented in vitro cultures. Behring Inst Mitt 9(83): 258–264
24. Roberts PJ, Devereux S, Pilkington GR, Linch DC (1990) Fc gamma RII-mediated superoxide production by phagocytes is augmented by GM-CSF without a change in Fc gamma RII expression. J Leukoc Biol 48: 247–257
25. Scheibenbogen C, Zenke G, Faggs B, Motoyoshi K, Kanz L, Sawert H, Brugger W, Andreesen R (1990) Secretion of colony-stimulating factor for macrophages (M-CSF) and granulocyte/macrophages (GM-CSF) is developmentally regulated in human macrophages. In: Freund M, Link H, Welte K (eds) Cytokines in hemopoiesis, oncology, and AIDS. Springer, Berlin, Heidelberg, New York, p 45
26. Scheibenbogen C, Andreesen R (1991) Developmental regulation of the cytokine repertoire in human macrophages: IL-1, IL-6, TNF-α, and M-CSF. J Leukoc Biol 50: 35–42
27. Sieff CA, Niemeyer CM, Mentzer SJ, Faller DJ (1988) Interleukin-1, tumor necrosis factor, and the production of colony-stimulating factors by cultured mesenchymal cells. Blood 72: 1316–1323
28. Thorens B, Mermod JJ, Vassalli P (1987) Phagocytosis and inflammatory stimuli induce GM-CSF mRNA in macrophages through posttranscriptional regulation. Cell 48: 671–679
29. Welte K, Platzer E (1990) Granulocyte colony-stimulating factor. In: Habenicht A (ed) Growth factors, differentiation factors, and cytokines. Springer, Berlin, Heidelberg, New York, p 201
30. Wewers MD, Herzyk DJ (1989) Alveolar macrophages differ from monocytes in human IL-1β release. J Immunol 143: 1635–1641
31. Wong GG, Witek JS, Temple PA, Wilkens KM, Leary AC, Luxenberg DP, Jones SS, Brown EJ, Kay RM, Orr EC, Shoemaker C, Golde DW, Kaufman RJ, Hewick RM, Wang EA, Clark SC (1985) Human GM-CSF: molecular cloning of the complementary DNA and purification of the natural and recombinant proteins Science 228: 810–815