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Macrophage Colony-Stimulating Factor Is Required for Human Monocyte Survival and Acts as a Cofactor for Their Terminal Differentiation to Macrophages In Vitro

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Functional competence as well as phenotype heterogeneity of macrophages depend on the completion of their maturation pathway. Differentiation of committed myeloid progenitor cells is induced by colony-stimulating factors (CSF), but no consistent data exist on which factor(s) induce the terminal maturation from the circulating blood monocyte to the mature macrophage. In vitro, monocyte to macrophage transformation occurs in the presence of serum and can be followed by the expression of the maturation-associated antigens gp65-MAX.1, gp68-MAX.3, and CD51. We describe that the differentiation-inducing activity in serum cannot be replaced by any of the known and available purified recombinant cytokines. In the absence of serum monocytes die in suspension cultures while surviving as non-differentiating cells when cultured adherent to plastic. In serum-free suspension cultures survival can be significantly improved by the addition of recombinant human macrophage (rhM)-CSF whereas other cytokines do not. At any stage of serum-free adherent culture, monocyte to macrophage differentiation can be induced rapidly by the addition of serum, whereas cytokines (rhM-CSF, recombinant human granulocyte macrophage [rhGM]-CSF, recombinant human granulocyte [rhG]-CSF, recombinant human interleukin [rhIL]-1, rhIL-3, rhIL-4, rhIL-6, tumor necrosis factor [TNF]-α, interferon [IFN]-α, IFN-γ) alone or in combination are not effective. Serum-induced maturation, however, was suppressed in the presence of neutralizing anti-M-CSF antibodies. In addition to phenotype analysis, the secretory repertoire of rhM-CSF cultured monocytes was analyzed in comparison to serum cultured monocytes which further characterized them to be immature cells, i.e., low release of maturation-associated products such as α-2-macroglobulin, neopterin, fibronectin, and TNF-α, but high IL-6 secretion, an attribute of blood monocytes. We conclude that for monocyte survival in vitro the presence of endogenous M-CSF and possibly other autocrine factors elicited by cell adherence are required for the induction of macrophage maturation; however, yet undefined additional factor(s) are necessary. They are present in serum and may act in conjunction with M-CSF but are distinct from all known cytokines. Our in vitro system may be useful in the screening and discovery of these serum factor(s).

Key words: macrophage maturation, TNF-α, IL-6

INTRODUCTION

Cells of the mononuclear phagocyte system (MPS) are involved in the immune response, in host defense against microorganisms, in control of tumor development and spread, inflammatory reactions, regulation of hematopoiesis, bone formation, iron metabolism, and in the removal of senescent cells [24,25,28,30]. Ubiquitous distribution and phenotype variability are characteristic attributes of this cell system consisting of resident and inflammatory types of tissue macrophages (MAC), free MAC in serous cavities, and a pool of circulating precursor cells, the blood monocytes (MO). As for other cell lineages, complete transition of a committed bone marrow precursor cell to the mature MAC is of crucial importance for the acquisition of full functional competence. Upon migration from the vasculature into the various tissues and body cavities, MO undergo terminal maturation modulated by site-specific signals. Here, the remarkable heterogeneity within the MPS in terms of morphology, biochemistry, function, and antigenic phenotype [5,12–15, 19,31] is created. While colony-stimulating factors (CSF) induce proliferation of committed bone marrow progenitor cells and their differentiation into promonocytes and MO [26], factors which are responsible for the terminal maturation beyond the blood MO stage of differentiation are not known. Migration of blood MO into tissues seems to be a random phenomenon in the absence of localized stimuli (e.g., inflammation)

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and no conclusive data exist to show that circulating MO leaving the marrow are predestined for any particular tissue. In vivo, one of the first events to induce MO differentiation is adherence to the vascular endothelium [11,33] and adherence itself seems to be a priming signal for MO gene expression such as those coding for proto-oncogenes or various growth factors including macrophage (M)-CSF [16]. Recently, M-CSF was reported to induce monocyte differentiation in vitro [8]. In vitro, serum is well known to induce MO to MAC differentiation. The aim of this study was to investigate the effect of adherence and whether the differentiation-inducing capacity of serum could be replaced by any of the various cytokines to induce terminal differentiation of elutriation-purified human blood MO. We used a culture system as described elsewhere [7] where this differentiation step is followed by the expression of maturation-associated antigens (e.g., MAX antigens [2] or other differentiation markers [3] and by characteristic changes in the secretory repertoire of cultured MO.

MATERIALS AND METHODS

MO Isolation and Culture

Mononuclear cells (MNC) were isolated from buffy coat preparations or cytapheresis concentrates of healthy blood donors by density gradient centrifugation over Ficoll/Hypaque (Pharmacia, Freiburg, FRG). MO were separated from lymphocytes by counter-current centrifugal elutriation in a J6M-E Beckman centrifuge with a standard chamber and a JE-5 rotor at 2,500 rpm and a flow rate of 20–25 ml/min as described elsewhere [3] and washed twice in RPMI 1640 medium (Biochrom, Berlin, FRG) supplemented with polyvitamins, 5×10^{-5} M 2-mercaptoethanol, antibiotics, pyruvate, and non-essential amino acids. Elutriated MO were more than 95% pure and contaminating T-lymphocytes were less than 3% as estimated by morphology and antigenic phenotype (CD14, CD3, CD4, CD8 expression). MO were cultured in suspension on hydrophobic teflon foils (Biofolie 25, Heraeus, Hanau) at 10^6/ml or in 96 well microtiter plates (Greiner, Nürtingen, FRG) as adherent monolayers at 5×10^5/ml supplemented RPMI 1640 medium with or without heat inactivated human AB-group serum for up to 13 days. Cultures were set up with or without recombinant interleukin human(rh)IL 1-α, rhIL-β, and rh tumor necrosis factor (TNF)-α (Boehringer, Mannheim, FRG), rh granulocyte macrophage (GM)-CSF, rhG-CSF, rhIL-3 (Behringwerke, Marburg, FRG), rhM-CSF (Cetus Corporation, Emeryville, CA), rhIL-4 and rhIL-6 (Immunex Corporation, Seattle, WA), rhIFN-α (Ernst Boehringer Institut, Wien, Austria), and rhIFN-γ (Thomae, Biberach, FRG), respectively. Neutralizing antibodies were kindly provided by Cetus Corporation (rabbit anti-human M-CSF), Immunex Corporation (rabbit anti-human GM-CSF, rabbit anti-human IL-6), and by the National Institute for Biological Standards (NBSB), Blanche Lane, South Mimms, Potters Bar (UK) (sheep anti-human IL-1α, sheep anti-human IL-1β).

Analysis of MO to MAC Maturation by Immunoperoxidase Staining of Surface Antigens

MO/MAC were attached to alcian blue coated slides and prefixed in 0.05% glutaraldehyde on ice. They were incubated with the following mouse monoclonal antibodies (mAb): anti-β-2-microglobulin (b2M) (Beckton Dickinson, Rödermark, FRG); anti-CD14 (My4; Coulter, Krefeld, FRG); anti-gp65 (MAX.1), anti-gp68 (MAX.3), anti-gp65 (MAX.11), MAX.21 (HLA-DR) (our own laboratory); anti-CD51 (13C2 [17]) and anti-CD71 (OKT9; Ortho-Diagnostics, Neckargemünd, FRG). A peroxidase-anti-peroxidase (PAP) technique was applied followed by postfixation with OsO_4 as described [9].

Analysis of Semiquantitative Antigen Expression in the Cell-ELISA [6]

MO/MAC cultures were analyzed for antigen expression in the microtiter plates in which the cultures originally were initiated. MAC from teflon bags were recovered and seeded in supplemented RPMI 1640 medium in microtiter plates (10^5/ml) and incubated for 30 min before being subjected to the cell-enzyme-linked immunoassay (ELISA). Surface antigen expression was performed as described recently [9]. The optical density (OD) was measured at 492 nm, corrected by the OD of specificity controls, mean of triplicates. Data are given as specific OD or in some experiments as antigen expression index (AEI) which was calculated by dividing the OD values of the respective antigen by the OD value for b2M expression, ×100.

Analysis of Secretory Products in Culture Supernatants

Five × 10^5 MO/MAC/ml supplemented RPMI 1640 were seeded in plastic dishes and cultured for 24 h with or without addition of 1 μg/ml *Salmonella abortus equi* lipopolysaccharides (LPS) or 200 IU/ml rhIFN-γ, respectively. Supernatants were collected and specific ELISA techniques were used to measure the amount of fibronectin (own development), α-2-macroglobulin (own development), and TNF-α (T-cell Science, Hamburg, FRG). Neopterin was measured in a radioimmunoassay (RIA) (IBL, Hamburg, FRG), IL-6 in a bioassay using the IL-6 dependent cell line B9 (kindly provided by Dr. Lucien Aarden, Amsterdam, The Netherlands [32]).

Statistical Methods

The statistical significance of the data obtained was analyzed by the Wilcoxon's rank-sum test; a P value of less than 0.05 was considered significant.

RESULTS

Blood MO differentiate into cells which share many functional criteria of mature MAC when cultured in the presence of serum either on hydrophobic teflon foils [7,29] or as adherent cells on plastic substrates [35]. This MAC differentiation can be followed by morphological criteria or changes in enzyme activities [18] but is more specific and sensitive by the expression of lineage-restricted maturation-associated antigens of the MAX series [2] and other differentiation markers (CD51 [3]), which are absent on circulating blood MO but are strongly expressed on in vitro matured MAC and on some exudate-type MAC subsets in situ [5].

Typically, human serum promoted MO to MAC transformation in a dose-dependent manner as measured by the expression of MAX.1 and MAX.3 antigens (Fig. 1). Without serum no cellular differentiation occurred and maturation-associated MAX antigens could not be detected. Plastic adherent cells stayed viable in serum-free cultures for up to 10 days. Upon addition of serum, MAC maturation could be induced rapidly at any stage of culture as shown in Figure 2. It was a consistent observation in repeated experiments that differentiation was more rapid and MAX expression higher when serum was added on day 2 or 3 as compared with cultures where serum was added from the beginning (Fig. 2). This is at present not understood and might be suggestive of priming events induced during the lag phase. When MO were cultured in suspension on hydrophobic teflon foils, however, MO died in serum-free medium and needed serum or defined cytokines for better survival (Fig. 3). Only addition of rhM-CSF and to a much lesser extent rhGM-CSF increased MO survival in serum-free suspension cultures. Recovery rates of MO cultured in 2% AB-group serum were 41.6 ± 15.2% whereas recovery rates of serum-free cultured MO were always less than 3%. Recovery of rhM-CSF cultured MO, however, was significantly higher being 14.8 ± 2.0% of MO originally initiated. Both rhM-CSF and rhGM-CSF, however, could not induce MAC maturation as measured by antigen expression (Fig. 3). Addition of neutralizing antibodies to rhM-CSF into serum-containing cultures (2% AB-serum) inhibited MO survival and subsequently differentiation, whereas neutralizing antibodies to rhGM-CSF or rhIL-6 only had marginal effects on MO survival and differentiation (Fig. 4). This anti-M-CSF effect could be abrogated by the addition of 500 ng/ml rhM-CSF (not shown in detail). None of the other cytokines tested (rhIL-1α, -β, rhIL-3, rhIL-4, rhIL-6, rhG-CSF, rhIFN-α, rhIFN-γ, rhTNF-α) either alone or in combination induced MAC maturation in serum-free cultures at doses of 0.5 to 500 ng/ml as measured by the expression of maturation-associated MAX.1 antigens (Fig. 5). They also did not enhance MAC recovery from teflon cultures

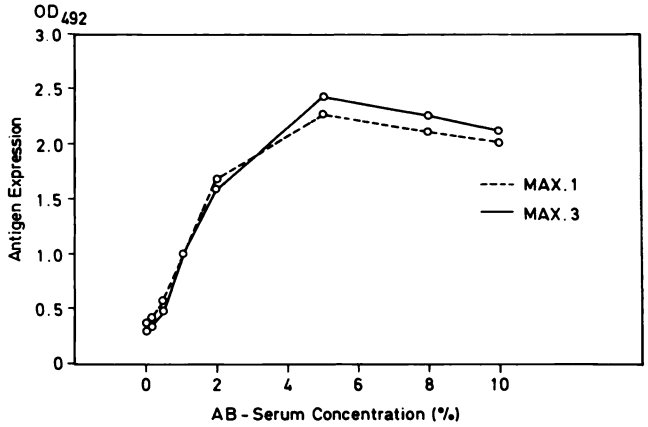

Fig. 1. Serum induces terminal MO to MAC maturation in vitro. Dose-dependence of the serum effect. 5 × 10^5 elutriation-purified MO/ml was cultured in supplemented RPMI 1640 medium for 7 days in microtiter plates at different concentrations of human AB-group serum. Maturation-associated antigen expression was evaluated by cell-ELISA, data are given as the specific optical density (OD_{492} nm) for MAX.1 and MAX.3 expression.

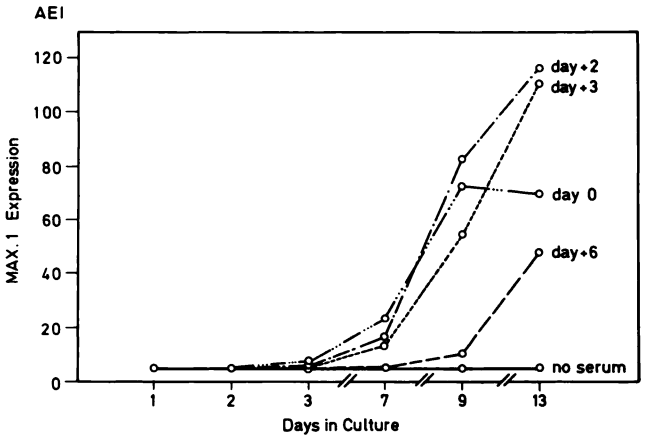

Fig. 2. Induction of MAC maturation upon addition of serum to primarily serum-free cultured MO. 5 × 10^5 elutriation-purified MO/ml were cultured in supplemented RPMI 1640 medium in microtiter plates for up to 13 days. AB-serum (2%) was added as indicated. Cultures were subjected to phenotype analysis by cell-ELISA on days 1, 3, 5, 7, 9, or 13, respectively. AEI are shown for MAX.1 expression. Data are given from one representative experiment.

supplemented with suboptimal serum concentrations (not shown in detail).

When culture-derived MO/MAC were tested for their secretory activity, a characteristic difference was noticed for rhM-CSF cultured MO in comparison to serum cultured MAC (Table 1). The maturation-associated products fibronectin and α-2-macroglobulin were secreted only by cells cultured in 2% AB-serum but not by MO cultured in rhM-CSF or rhGM-CSF. MO cultured in rhM-CSF produced neopterin and TNF-α only in amounts comparable to blood MO, whereas MO cultured

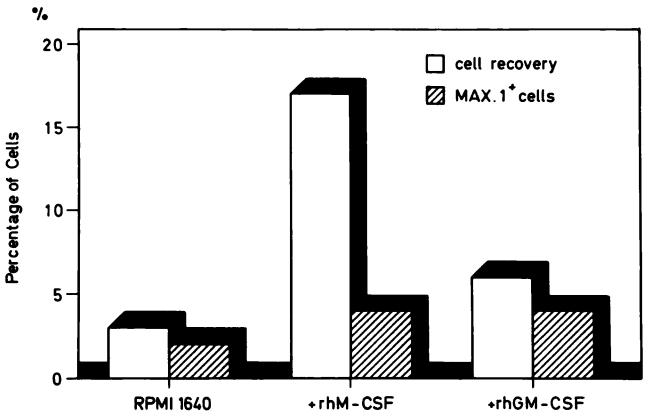

Fig. 3. Effect of M-CSF and GM-CSF on MAC survival and maturation in serum-free cultures. 5×10^5 elutriation-purified MO/ml were cultured for 7 days on hydrophobic teflon foils in supplemented RPMI 1640 medium without or with 50 ng/ml rhGM-CSF or 50 ng/ml rhM-CSF, respectively. Survival of cells was evaluated as % cell recovery, viability of recovered cells was more than 90% as estimated by trypan blue dye exclusion. Maturation was measured by analysis of MAX.1 expression on single cells, data are given as percentage of positive cells. Data are given from one representative experiment, out of three.

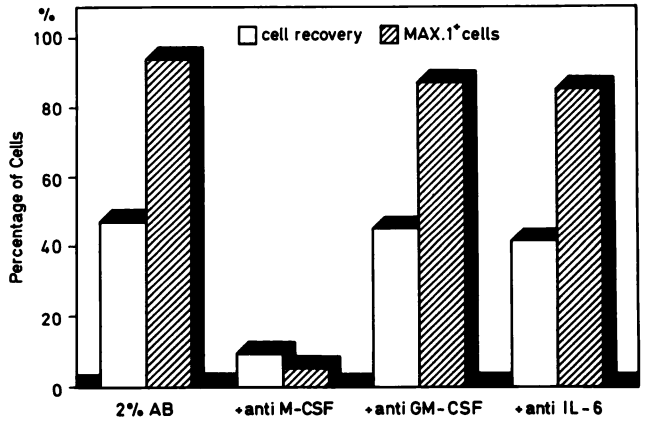

Fig. 4. Effect of neutralizing antibodies on MAC survival and maturation in serum containing suspension cultures. 5×10^5 elutriation-purified MO/ml were cultured for 7 days on teflon foils in supplemented RPMI 1640 medium plus 2% AB-serum without or with neutralizing antibodies (final dilution 1:500) directed to M-CSF, GM-CSF, or IL-6. For further details see legend to Figure 4.

in 2% serum produced large amounts. In contrast, secretion of IL-6 was lower in mature MO-derived MAC whereas MO cultured in rhM-CSF produced still large amounts of IL-6 which was comparable to the secretion of IL-6 by peripheral blood MO (Table 1).

DISCUSSION

The molecular identity of the regulatory signals responsible for the terminal maturation of blood MO into

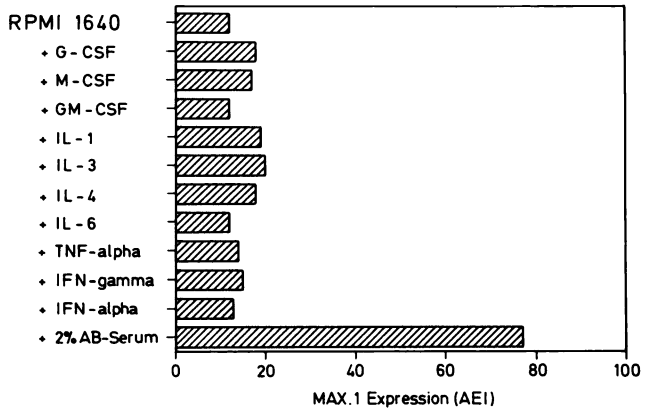

Fig. 5. Induction of MO to MAC maturation in vitro: effect of recombinant human cytokines. 5×10^5 elutriation-purified MO/ml were cultured in supplemented RPMI 1640 medium for 7 days in microtiter plates either with 2% AB-serum or rhG-CSF, rhM-CSF, rhGM-CSF (all 50 ng/ml), rhIL-3, rhIL-1β, rhIL-4, rhIL-6, rhIFN-α, rhIFN-γ, or rhTNF-α (all 100 U/ml), respectively. Phenotype analysis was performed by cell-ELISA, data are given as AEI for MAX.1. Data are from one representative experiment.

MAC in vitro is yet unresolved. Up to now, the only source for this activity is serum or plasma [1–3,7,18,22,23]. In this study, we analyzed the effect of cell adherence and certain cytokines in comparison to serum on survival and maturation in primary long-term cultures of peripheral human blood MO. Whereas serum was shown to be essential to promote MO survival in suspension cultures, only MO cultured adherent to plastic were able to survive in the *absence* of serum. Confirming earlier work by Becker et al. [8], M-CSF was able to partially replace serum in suspension cultures; however, neither M-CSF nor adherence alone was sufficient to induce MO to mature MAC transformation as evident from surface antigen and functional analysis. Apparently, other serum factors in addition to M-CSF are needed to induce MAC differentiation. Unlike M-CSF, other cytokines with hematopoietic activity (IL-3, IL-6, G-CSF, GM-CSF) did not induce MO survival and similarly failed to induce MO differentiation. Also, inhibition of serum-induced MAC differentiation was seen only in the presence of neutralizing antibodies to M-CSF. Adherence may be an essential competence signal for MO to MAC maturation also in vivo where MO migration is initiated by their adherence to the capillary endothelium [11,33]. It is of interest in this respect that adherence has been shown to induce gene expression for several cytokines including that for M-CSF [16]. Own experiments confirm these results and further demonstrate the constitutive expression of M-CSF mRNA during MO to MAC differentiation (manuscript in preparation). In vitro, about 2 ng/10^6 MO per 24 h are secreted in plastic adherent MO cultures (own unpublished results, measured courtesy by Peter Ralph, Cetus

TABLE 1. Analysis of Secretory Products and Maturation-Associated Antigens

		Primary MAC cultures[b]			
	MO[a]	None	2% AB	+ M-CSF	+ GM-CSF
Functional analysis					
Fibronectin[c] (ng/ml)	<10	<10	540	27	<10
α-2-macroglobulin[c] (ng/ml)	<10	<10	284	28	22
Neopterin[d] (ng/ml)	0.16	0.24	2.72	0.29	0.33
TNf-α[e] (ng/ml)	1.3	<0.2	12.0	<0.2	<0.2
IL-6[e] (U/ml)	1,700	1,840	680	2,090	1,800
Antigen expression (AEI)					
MAX.1[f]	<5	<5	69	<5	8
MAX.3	<5	6	76	10	<5
CD51	<5	<5	52	<5	<5

[a]Freshly elutriated blood MO (5×10^5/Ml) were cultured for 24 h in plastic dishes without ([c]) or with addition of 1 μg/ml *S. abortus equi* ([e]) or 200 IU/ml rhIFN-g ([d]), respectively. The supernatants were collected and secretory products were measured as described in Materials and Methods.
[b]Elutriated blood MO were cultured for 7 days on hydrophobic teflon foils in supplemented RPMI 1640 medium in the absence or presence of 2% AB-serum, 50 ng/ml rhM-CSF, and 50 ng/ml rhGM-CSF, respectively. 5×10^5 MO/MAC/ml were cultured for 24 h without ([c]) or with addition of 1 μg/ml *S. abortus equi* ([e]) or 200 IU/ml rhIFN-g ([d]), respectively for the analysis of secretory products.
([f])Maturation-associated phenotype analysis was measured by cell-ELISA. Data are given as AEI. All data are from one representative experiment, out of four.

Corp., Emeryville). Additional autocrine factors elicited by adherence may also be required for MO survival or, alternatively, M-CSF may prime MO for the production of other monokines [34], which in turn could help to induce terminal MO maturation.

Further evidence for an important role of M-CSF in the development of a functional competent MAC comes from the work of Munn and Cheung [21] who described the induction of antibody-dependent cellular cytotoxicity (ADCC) by human blood MO cultured with M-CSF. Their claim, however, to have induced mature MAC by M-CSF is not substantiated by their results [21].

Thus, the question still remains: which serum-derived factor(s) operate with M-CSF in induction of cell differentiation?

Akiyama et al. [1] attempted to characterize certain serum proteins and found human IgG, fibronectin, and fibrinogen to induce MAC maturation. We also tested these and other serum components but could not confirm their results (not shown here). Te Velde et al. [27] suggested IL-4 to be a MAC differentiation factor, an observation which again could not be confirmed by us (Fig. 5). It should be noticed that definition of cell maturation is rather poor in both reports, e.g., increased expression of MHC class II molecules was correlated to MAC maturity. This, however, occurs in MO cultures even in the absence of any proteins and, accordingly, any evidence of MAC differentiation [3]. IFN-γ rather suppressed MO to MAC differentiation in our system as shown recently [4]. This may be in line with a previous study by Clement et al. [10] who postulated that T-cell-derived lymphokines distinct from IFN-γ or GM-CSF are responsible for MO differentiation.

In conclusion, we believe that M-CSF may be required only for cell survival but not for the induction of MO to MAC differentiation. It may activate distinct MO/MAC functions at a defined stage of differentiation (as shown for ADCC or monokine production), but in MO to MAC differentiation it serves only as a competence factor which needs additional mediator molecules. These are present in serum or induced by serum and seem to be distinct from all yet defined cytokines. Preliminary evidence indicates that the active metabolite of vitamin D_3, 1,25-dihydroxyvitamin D_3, may be a true differentiation factor as it has been shown recently to induce MAC maturation from blood MO in serum-free cultures [20].

Our system may be useful for further analysis of vitamin D_3-induced MAC maturation and molecules with similar activity, which should be of great importance for the understanding of MAC physiology but which may also be targets for new treatment strategies of immunological disorders.

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REFERENCES

1. Akiyama, Y., Griffith, R., Miller, P., Stevenson, G.W., Lund, S., Kanapa, D.J., and Stevenson, H.C. Effects of adherence, activation and distinct serum proteins on the in vitro human monocyte maturation process. J. Leukoc. Biol. 43,224, 1988.
2. Andreesen, R., Bross, K.J., Osterholz, J., and Emmrich, F. Human macrophage maturation and heterogeneity: analysis with a newly generated set of monoclonal antibodies to differentiation antigens. Blood 67,1257, 1986.
3. Andreesen, R., Brugger, W., Scheibenbogen, C., Kreutz, M., Leser, H.G., Rehm, A., and Löhr, G.W. Surface phenotype analysis of human monocyte to macrophage maturation. J. Leukol. Biol. 47,490, 1990.
4. Andreesen, R., Brugger, W., Thomssen, C., Rehm, A., Speck, B., and Löhr, G.W. Defective monocyte-to-macrophage maturation in aplastic anemia. Blood 74,2150, 1989.
5. Andreesen, R., Gadd, S., Costabel, U., Leser, H.G., Speth, V., Cesnik, B., and Atkins, R.C. Human macrophage maturation and heterogeneity: restricted expression of late differentiation antigens in situ. Cell Tissue Res. 253,271, 1988.
6. Andreesen, R., Mackensen, A., Osterholz, J., Brugger, W., and Löhr, G.W. Microculture assay for human macrophage maturation in vitro. Cell-ELISA analysis of differentiation antigen expression. Int. Arch. Allergy Appl. Immunol. 86,281, 1988.
7. Andreesen, R., Picht, J., and Löhr, G.W. Primary cultures of human blood-born macrophages grown on hydrophobic teflon membranes. J. Immunol. Methods 56,295, 1983.
8. Becker, S., Warren, M.K., and Haskill, S. Colony-stimulating factor-induced monocyte survival and differentiation into macrophages in serum-free cultures. J. Immunol. 139,3703, 1987.
9. Bross, K.J., Pangalis, G.A., Staatz, C.G., and Blume, K.G. Demonstration of cell surface antigens and their antibodies by the peroxidase-antiperoxidase method. Transplantation 25,331, 1978.
10. Clement, L.T., Roberts, R.L., and Martin, A.M. Characterization of a monocyte differentiation factor distinct from gamma-interferon, tumor necrosis factor, or G-, M-colony-stimulating factor that regulates the growth and functional capabilities of the U937 monocytic cell line. J. Leukol. Biol. 44,101, 1988.
11. Doherty, D.E., Haslett, C., Tonnesen, M.G., and Henson, P.M. Human monocyte adherence: a primary effect of chemotactic factors on the monocyte to stimulate adherence to human endothelium. J. Immunol. 138,1762, 1987.
12. Dougherty, G.J., and McBride, W.H. Macrophage heterogeneity. J. Clin. Lab. Immunol. 14,1, 1984.
13. Forster, O., and Landy, M. Heterogeneity of Mononuclear Phagocytes. San Diego: Academic Press, 1981.
14. Franklin, W.A., Mason, D.Y., Pulford, K., Falini, B., Bliss, E., Gatter, C., Stein, H., Clarke, L.C., and McGee, J.O.D. Immunohistological analysis of human mononuclear phagocytes and dendritic cells by using monoclonal antibodies. Lab. Invest. 54, 322, 1986.
15. Hancock, W.W., Zola, H., and Atkins, R.C. Antigenic heterogeneity of human mononuclear phagocytes: immunohistological analysis using monoclonal antibodies. Blood 62,1271, 1983.
16. Haskill, S., Johnson, C., Eierman, D., Becker, S., and Warren, K. Adherence induces selective mRNA expression of monocyte mediators and proto-oncogenes. J. Immunol. 140,1690, 1988.
17. Horton, A.H., Lewis, D., McNulty, K., Pringle, J.A.S., and Chambers, T.J. Monoclonal antibodies to osteoclastomas (giant cell bone tumors): definition of osteoclast-specific cellular antigens. Cancer Res. 45,5663, 1985.
18. Johnson, W.D., Mei, B., and Cohn, Z.A. The separation, long-term cultivation and maturation of the human monocyte. J. Exp. Med. 146,1613, 1977.
19. Johnston, R.B. Monocytes and macrophages. N. Engl. J. Med. 318,747, 1988.
20. Kreutz, M., and Andreesen, R. Induction of human monocyte to macrophage maturation in vitro by 1a,25 dihydroxy vitamin D_3. Blood, in press, 1990.
21. Munn, D.H., and Cheung, N.K. Antibody-dependent antitumor cytotoxicity by human monocytes cultured with recombinant macrophage colony-stimulating factor. J. Exp. Med. 170,511, 1989.
22. Musson, R. Intracellular levels and stimulated release of lysosomal enzymes from human peripheral blood monocytes and monocyte-derived macrophages J. Reticuloendothel. Soc. 28, 249, 1980.
23. Musson, R.A. Human serum induces maturation of human monocytes in vitro. Changes in cytolytic activity, intracellular lysosomal enzymes, and non-specific esterase activity. Am. J. Pathol. 111,331, 1983.
24. Nathan, C.F., and Cohn, Z.A. Cellular components of inflammation: monocytes and macrophages. In Textbook Rheumatology, (Kelly, W., et al., Eds). Philadelphia: W.B. Saunders, p. 144, 1985.
25. Rosenthal, A.S. Regulation of the immune response—role of the macrophage. N. Engl. J. Med. 303,1153, 1980.
26. Sieff, C.A. Hematopoietic growth factors. J. Clin. Invest. 79, 1549, 1987.
27. Te Velde, A.A., Klomp, J.P.G., Yard, B.A., De Vries, J., and Figdor, C.G. Modulation of phenotypic and functional properties of human peripheral blood monocytes by IL-4. J. Immunol. 140, 1548, 1988.
28. Unanue, E. The basis for the immunoregulatory role of macrophages and other accessory cells. Science 236,551, 1987.
29. van der Meer, J.W.M., van de Gevel, J.S., Bluuse van Oud Alblas, A., Kramps, J.A., van Zwet, T.L., Leijh, P.C.J., and van Furth, R. Characteristics of human monocytes cultured in teflon bags. Immunology 47,617, 1982.
30. van Furth, R. Current view on the mononuclear phagocyte system. Immunobiology 161,178, 1982.
31. van Furth, R., Diesselhoff-den Dulk, M.M.C., Sluiter, W., and van Dissel, J.T. New perspectives on the kinetics of mononuclear phagocytes. In Mononuclear Phagocytes: Characteristics, Physiology and Function, (van Furth, R. Ed.) Dordrecht: Martinus Nijhoff, p. 201, 1985.
32. van Oers, M.H.J., van der Heyden, A.A., and Aarden, L.A. Interleukin-6 in serum and urine of renal transplant recipients. Clin. Exp. Immunol. 71,314, 1988.
33. Wallis, W.J., Beatty, P.G., Ochs, H.D., and Harlan, J.M. Human monocyte adherence to cultured vascular endothelium: monoclonal antibody-defined mechanisms. J. Immunol. 135, 2323, 1985.
34. Warren, K.M., and Ralph, P. Macrophage growth factor CSF-1 stimulates human monocyte production of interferon, tumor necrosis factor, and colony stimulating activity. J. Immunol. 137, 2281, 1986.
35. Zuckermann, S.H., Ackerman, S.K., and Douglas, S.D. Long-term human peripheral blood monocyte cultures: establishment, metabolism and morphology of primary human monocyte-macrophage cell cultures. Immunology 38,401, 1979.