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In Vitro Proliferation of Mononuclear Phagocytes from Murine and Human Bone Marrow

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Abstract

Techniques for liquid culture of proliferating mononuclear phagocytes from bone marrow of mice and men are described. Mouse bone marrow must be cultured in the presence of colony-stimulating factor, whereas proliferation of human mononuclear phagocytes occurred in medium with 50 % serum but without colony-stimulating factor. The number of mononuclear phagocytes that can be determined in mouse bone marrow cultures is higher than that in cultures of human bone marrow. However, the number of mononuclear phagocytes found for the human system is an underestimation, because the immature mononuclear phagocytes cannot be recognized at the light-microscopical level. These precursor cells (monoblasts and promonocytes) can be recognized with the electron microscope. The characteristics of the various types of mononuclear phagocyte, especially in cultures of murine bone marrow, are reviewed.

Introduction

Techniques for the culture of proliferating hematopoietic cells have been available since 1966 (1, 2). Proliferation of these cells is dependent on critical culture conditions; for the proliferation of precursors of granulocytes and of mononuclear phagocytes, factors possessing colony-stimulating activity are necessary, whereas erythroid precursors require erythropoietin for proliferation *in vitro*. Most investigators use semisolid media (i.e. soft agar, methylcellulose) for cell support in these cultures, and proliferation is quantitated by counting the colonies. However, the presence of agar or methylcellulose hampers the study of the characteristics of the cultured cells. Therefore for the study of the proliferation of mononuclear phagocytes from mouse bone marrow, GOUD *et al.* (3, 4) modified the original culture method: in a liquid culture system the cells were cultured on a glass surface. This method made it possible to study the morphology, cytochemistry, function, and proliferation of the cells in question. The characteristics and proliferative behaviour of monoblasts, promonocytes, and the various types of macrophage could be established (3–6). Certain studies require recovery of the mononuclear phagocytes cultured in suspension, and this can be accomplished most conveniently by culture of the cells on hydrophobic Teflon® film to which even mononuclear phagocytes adhere poorly (7, 8).

For the culture of proliferating mononuclear phagocytes from human bone marrow, the conditions for liquid culture according to GOUD et al. (3) proved unsatisfactory: only a few glass adherent mononuclear phagocytes were found, and the embryonic mouse fibroblast conditioned medium, the source of the colony stimulating activity used in the mouse system, did not seem to be active for the human system. Therefore the method for the culture of rabbit bone marrow (9) was used in the Teflon system for the culture of human bone marrow.

In the present paper the methods used for liquid culture of murine and human bone marrow in our laboratory will be reviewed, and the results will be discussed in relation to the data in the literature.

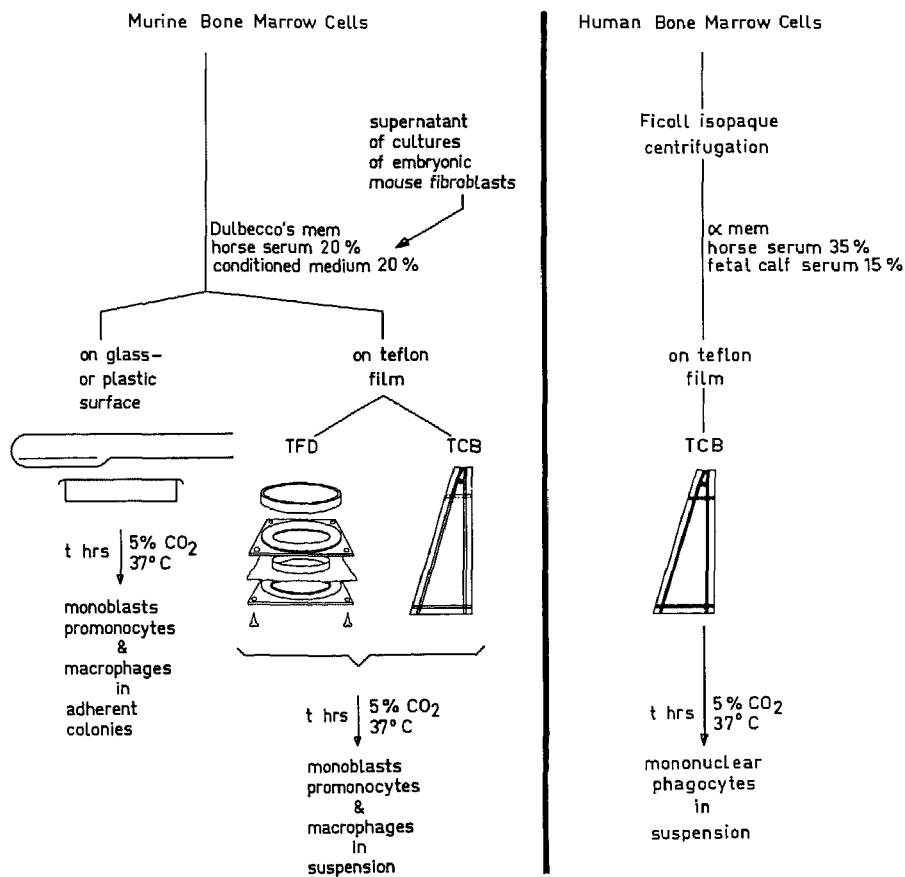


Fig. 1. Techniques for the culture of murine and human bone marrow mononuclear phagocytes in liquid culture.

TFD = Teflon film dish (ref. 7)

TCB = Teflon culture bag (ref. 8)

Methodology

The methods used for culturing mononuclear phagocytes from murine and human bone marrow are shown in Figure 1. The techniques for the culture of murine bone marrow mononuclear phagocytes on a glass or plastic surface have been described by GOUD et al. (3) and for culture in a Teflon film dish (TFD) or Teflon culture bag by VAN DER MEER et al. (7, 8). The embryonic fibroblast-conditioned medium was prepared as reported elsewhere (10). The culture technique for human marrow will be published in detail elsewhere (11); in short, human bone marrow cells are centrifuged over Ficoll Hypaque and incubated in 35 % horse serum and 15 % foetal calf serum (both heat-inactivated) in alpha modified Eagles' medium. No source of colony-stimulating factor is added.

Results and Discussion

Proliferation of murine bone marrow mononuclear phagocytes

The proliferation of mononuclear phagocytes on glass and Teflon surfaces is very similar. Per 10^3 nucleated bone marrow cells, which include approximately 1 monoblast, a progeny of more than 5×10^3 mononuclear phagocytes is found at day 14 (Fig. 2). The rate of proliferation is initially very high and levels off after two weeks of culture. After 3 to 4 weeks of culture without replating, the cells round up, the nucleus becomes pycnotic, and the cells detach from the glass. Proliferation declines despite the addition of fresh conditioned medium twice weekly. The decline in proliferation is reflected by a fall in the ^3H -thymidine labeling index. Proliferation can be maintained for prolonged periods (longer than 180 days) by replating cells cultured in Teflon culture bags at regular intervals.

If the culture conditions for human bone marrow are applied to the mouse system, no growth occurs.

Characteristics of mononuclear phagocyte murine bone marrow cultures

Cultures on a glass or plastic surface show two kinds of colony, i.e. mononuclear phagocyte colonies and granulocyte colonies. Mixed granulocyte-mononuclear phagocyte colonies, which have been claimed to be present in agar (12), are not observed in the liquid culture system. Thus this system provides no evidence for a stem cell committed for both lineages (3, 4). Granulocyte colonies grow in a very compact form; they constitute a minority and gradually disappear after the first week of culture; after day 10 of culture, only mononuclear phagocyte colonies can be recognized easily: they grow in a single layer with crowding of round cells in the centre of the colony and more elongated cells in the periphery. When cells are cultured on a Teflon surface, no colony formation is seen because the cells grow in suspension.

In the mononuclear phagocyte colonies, three types of cell are distinguished at the light-microscopical level: monoblasts, promonocytes, and

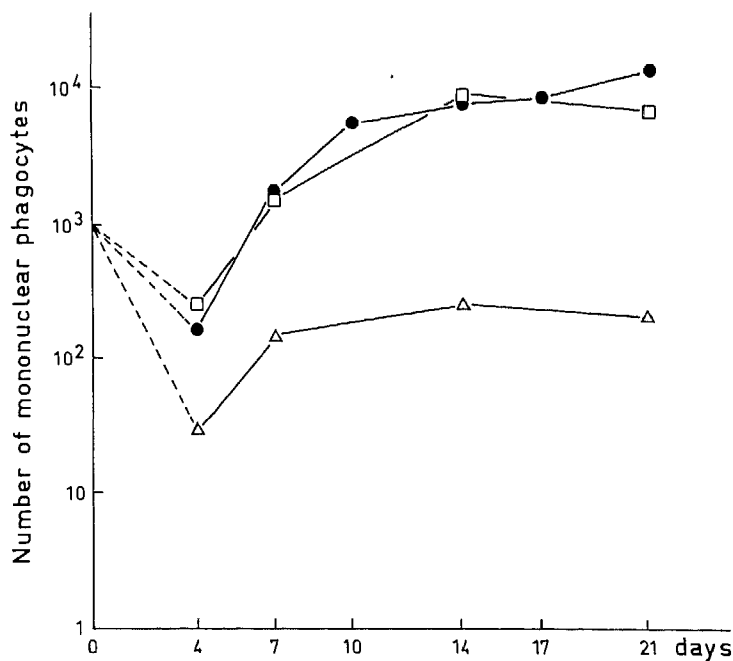


Fig. 2. Proliferation of mononuclear phagocytes.

- murine bone marrow on a glass surface
- murine bone marrow in a Teflon culture bag
- △ human bone marrow in a Teflon culture bag

The curves are standardized to 10^3 nucleated bone marrow cells on day 0. The curve for human mononuclear phagocytes represents an underestimation, because immature mononuclear phagocytes cannot be recognized by light microscopy.

macrophages (3). With the electron microscope and the peroxidatic-activity marker, the macrophages can be subdivided into early macrophages (cells resembling monocytes or exudate macrophages), transitional macrophages (cells resembling exudate-resident macrophages (13)), mature macrophages (cells with the characteristics of resident macrophages), and peroxidase-negative macrophages (6). The morphological, cytochemical, and functional characteristics of the various types of mononuclear phagocyte are summarized in Table 1. In all respects, the bone marrow mononuclear phagocytes in culture have characteristics quite similar to those of freshly harvested mononuclear phagocytes. With respect to a number of functions, such as the low activity of the ectoenzymes (5' nucleotidase and leucine-aminopeptidase), the uptake of opsonized red cells via the complement (C3b) receptor, the degradation of immune aggregates (14), and the secretion of plasminogen activator (15), these cultured mononuclear phagocytes can be considered to be activated. Colony-stimulating factor seems to play a crucial role in this activation (14, 15).

Table 1. Characteristics of murine bone marrow mononuclear phagocytes in culture¹

	Monoblast	Promonocyte	Early macrophage	Transitional macrophage	Mature macrophage
Diameter	10 × 12 μm	13 × 34 μm	17 × 69 μm	17 × 69 μm	17 × 69 μm
Nuclear-to-cytoplasm ratio	> 1	~ 1	< 1	< 1	< 1
Shape	round	slightly elongated	elongated	elongated	elongated
Surface	smooth	few micro-extensions 1 pseudopod	several micro-extensions 2 or more pseudopods	many micro-extensions 2 or more pseudopods	many micro-extensions 2 or more pseudopods
Organelles	RER scarce many poly-ribosomes	few strips of RER considerable number of polyribosomes	several strips of RER few polyribosomes	several strips of RER few polyribosomes	several strips of RER few polyribosomes
Peroxidatic activity (EM)	RER, granules, NE	RER, Golgi, granules, NE	granules	RER, granules, NE	RER, NE
α-naphthyl butyrate esterase	+	+		+	
Lysozyme	± → +	± → +		+	
β-glucuronidase	+	+		+	
Acid phosphatase	+	+		+	
5' nucleotidase	- → -	- → ±		- → ±	
Leucine aminopeptidase	- → ±	- → +		- → +	
Adherence	±	+		+	
Fc receptors	+	+		+	
C receptors	± → +	± → +		+	
Phagocytosis	±	+		+	
Pinocytosis	±	+		+	

¹ compiled from references 3, 5, 6, 11, and unpublished observations

- no cells show activity

→ change of activity during culture

+ majority of the cells show activity

± minority of the cells show activity

Proliferation of human bone marrow mononuclear phagocytes

In human bone marrow cultures, proliferating cells of the granulocytic series are in the majority during the first week of culture and remain present even after prolonged culture. The numbers of mononuclear phagocytes recognized in these cultures are shown in Figure 2; this number is, however, an underestimation, since the immature cells of the mononuclear phagocyte series cannot be recognized with certainty at the light-microscopical level and thus are not included in the counts. The cells recognized as mononuclear phagocytes have the appearance of mature macrophages, and few of them synthesize DNA. The large population of blast cells, which includes the unrecognizable mononuclear phagocyte precursors, does synthesize DNA. Primary cultures of human bone marrow have been maintained for more than 30 days.

Characteristics of human bone marrow mononuclear phagocytes

The mononuclear phagocytes in cultures of human bone marrow are positive for α -naphthyl butyrate esterase, have Fc receptors, and phagocytose opsonized red cells and bacteria. Some of the blast cells are also positive for α -naphthyl butyrate esterase, but these cells do not ingest opsonized red cells or bacteria. At the ultrastructural level, monoblasts, promonocytes, and macrophages can be recognized with the help of the peroxidatic activity marker (11). The peroxidatic activity patterns of monoblasts and promonocytes are identical with those of the murine monoblasts and promonocytes (5, 6). The macrophage population can only be subdivided into early macrophages (with peroxidase-positive granules) and mature macrophages (which do not show peroxidatic activity in their rough endoplasmic reticulum and nuclear envelope).

Concluding Remarks

The techniques for the *in vitro* culture of mononuclear phagocytes from mouse bone marrow are such that mononuclear phagocytes of good quality and a high degree of purity can be obtained, either adherent to glass or plastic or in suspension when cultured on glass. The culture techniques for human bone marrow mononuclear phagocytes are much more cumbersome. Furthermore, the precursor cells of the human mononuclear phagocyte series cannot be recognized by light microscopy, it is more difficult to obtain high numbers of mononuclear phagocytes, and it is difficult to remove granulocytes and lymphocytes. Comparison of the growth characteristics of murine and human mononuclear phagocytes shows that better growth is obtained in the culture of mouse bone marrow. However, it must be kept in mind that the difficulties encountered in the recognition of the human monoblasts and promonocytes lead to an underestimation of the numbers of human mononuclear phagocytes in the cultures.

Comparison of the characteristics of immature and mature mononuclear phagocytes grown in vitro from mouse and human marrow shows that there is great similarity between the murine and human mononuclear phagocytes.

References

1. BRADLEY, T. R., and D. METCALF. 1966. The growth of mouse bone marrow cells in vitro. *Austr. J. Exp. Biol. Med. Sci.* 44: 287.
2. PLUZNIK, D. H., and L. SACHS. 1965. The cloning of normal mast cells in tissue culture. *J. Cell. Comp. Physiol.* 66: 319.
3. GOUD, TH. J. L. M., C. SCHOTTE, and R. VAN FURTH. 1975. Identification and characterization of the monoblast in mononuclear phagocyte colonies grown in vitro. *J. Exp. Med.* 142: 1180.
4. GOUD, TH. J. L. M., and R. VAN FURTH. 1975. Proliferative characteristics of monoblasts grown in vitro. *J. Exp. Med.* 142: 1200.
5. VAN FURTH, R., and M. FEDORKO. 1976. Ultrastructure of mouse mononuclear phagocytes in bone marrow colonies grown in vitro. *Lab. Invest.* 34: 440.
6. VAN DER MEER, J. W. M., R. H. J. BEELEN, D. M. FLUITSMA, and R. VAN FURTH. 1979. Ultrastructure of mononuclear phagocytes developing in liquid bone marrow cultures – a study on peroxidatic activity. *J. Exp. Med.* 149: 17.
7. VAN DER MEER, J. W. M., D. BULTERMAN, T. L. VAN ZWET, I. ELZENGA-CLAASSEN, and R. VAN FURTH. 1978. Culture of mononuclear phagocytes on a Teflon surface to prevent adherence. *J. Exp. Med.* 147: 271.
8. VAN DER MEER, J. W. M., J. S. VAN DE GEVEL, I. ELZENGA-CLAASSEN, and R. VAN FURTH. 1979. Suspension cultures of mononuclear phagocytes in the Teflon culture bag. *Cell. Immunol.* 42: 208.
9. HAUSER, P., and G. VAES. 1978. Degradation of cartilage proteoglycans by a neutral proteinase secreted by rabbit bone marrow macrophages in culture. *Biochem. J.* 172: 275.
10. GOUD, TH. J. L. M. 1975. Identification and characterization of the monoblast. Thesis, Leiden, The Netherlands.
11. VAN DER MEER, J. W. M., J. S. VAN DE GEVEL, R. H. J. BEELEN, D. M. FLUITSMA, and R. VAN FURTH. Culture of human bone marrow in the Teflon culture bag, identification of the human monoblast. To be published.
12. METCALF, D. 1973. Regulation of granulocyte and monocyte-macrophage proliferation by colony stimulating factor (CSF). A review. *Exp. Hematol.* 1: 185.
13. BEELEN, R. H. J., D. M. BROEKHUIS-FLUITSMA, C. KOM, and E. CH. M. HOEFMITS. 1978. Identification of exudate-resident macrophages on the basis of peroxidatic activity. *J. Reticuloendothel. Soc.* 23: 103.
14. VAN DER MEER, J. W. M., N. KLAR-MOHAMED, J. S. VAN DE GEVEL, M. R. DAHA, and R. VAN FURTH. Degradation of immune aggregates by mouse mononuclear phagocytes – stimulation by colony-stimulating factor, submitted for publication.
15. LIN, H. S., and S. GORDON. 1979. Secretion of plasminogen activator by bone marrow derived mononuclear phagocytes and its enhancement by colony stimulating factor. *J. Exp. Med.* 150: 231.

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