Short Communication

Regulation of Neopterin Secretion during Human Monocyte to Macrophage Differentiation in Vitro¹⁾

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Introduction

Human mononuclear phagocytes (MP) can release neopterin upon induction of indoleamine 2,3-dioxygenase and subsequent degradation of L-tryptophan (1). While this pathway can be induced in a variety of other normal and neoplastic cells, MP are the only cell type able to release neopterin in significant amounts (2). They do so in response to various stimuli of which interferon-gamma (IFN γ) is the most potent one. Thus, neopterin secretion is regarded to sensitively indicate a successful activation of MP through T-lymphocyte mediated immune response (3) and neopterin has indeed found increasing attention as a marker of clinical disease activity in cell-mediated immunity (4). As the functional competence of MP is regulated both by activating and differentiation-inducing signals we were interested in studying the ability of MP at defined stages of differentiation to respond to activation by releasing neopterin. Peripheral blood monocytes (MO) represent only short-lived precursor cells able to differentiate into inflammatory or resident macrophages (MAC) upon migration from the vasculature to the various tissues (5). This MO to MAC differentiation can be studied in vitro where blood MO transform into exudate-type MAC under appropriate conditions in the presence of serum (6). It is shown that for both the IFN γ - and LPS-induced secretion of neopterin maturation of MO is required.

Material and Methods

Cell preparation

Leukapheresis was performed on healthy donors upon informed consent using a CS 3000 blood cell separator

(Baxter, Munich, FRG). Mononuclear cells were further separated from red blood cells by Ficoll Hypaque gradient centrifugation in a Cobe-2991 cell washer (Cobe, Colorado, USA). Monocytes were isolated by counter-current elutriation using a Beckmann J6-ME centrifuge (Beckmann, Munich, FRG). The MO fraction was > 95% pure by morphology and expression of CD14 molecules. Monocytes were cultured at 10⁶/ml for up to 14 days on hydrophobic teflon foils (Biofolie 25, Heraeus, Hanau, FRG) as described previously (6) in RPMI 1640 (Seromed, Berlin, FRG) supplemented with glutamin, non-essential amino acids, pyruvate, polyvitamins, streptomycin, penicillin, 2-ME and 2% pooled human AB-group serum. Maturation of MO was followed by morphology and expression of maturation-associated antigens as detected by immunoperoxidase-staining technique (7).

Supernatant production

Monocytes/macrophages were harvested at indicated days, washed once in RPMI 1640 and viability was determined by trypan blue exclusion test. 5 × 10⁵ viable cells/ml were seeded into 6-well plates (Falcon, Becton Dickinson, Heidelberg, FRG) in suppl. RPMI 1640 and 2% FCS with or without addition of 100 ng/ml LPS abortus equi (kindly provided by Dr. Galanos, Max-Planck Institut, Freiburg, FRG) or recombinant human IFN γ 200 U/ml (Bioferon, Laupheim, FRG), respectively. After 24 hours of culture, cell supernatants were harvested, filtered through 0.2 μ m membranes (Milipore, Molsheim, France) and stored at -20 °C.

Neopterin determination

Neopterin was measured using a commercially available radioimmunoassay (IBL, Hamburg, FRG).

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Results and Discussion

Primary cultures of blood MO were established from healthy volunteers and maturation was followed by morphology and expression of maturation-associated surface antigens (MAX antigens, CD16, CD71, CD51, see ref. 8). From these parameters typical MAC developed within about 7 days in culture (see descriptive analysis in Table 1; ref. 6–8).

Table 1. Comparative description of circulating blood monocytes and teflon-cultured, monocyte-derived macrophages

	Mono- cytes	Monocyte- derived MO ¹⁾
tumorcytotoxicity ²⁾	+	++
– stimulated by IFNg	++	+++
Procoagulant activity	–	+
– stimulated by IFNg	+	+++
O ₂ -Production (PMA-induced)	+	++
– stimulated by IFNg	++	+++
Surface expression of		
MAX.1/.3/.11	–	++
vitronectin receptor (CD51)	–	++
ICAM 1 (CD54)	(+)	++
Fc-ReceptorIII (CD16)	(+)	++
TF-Receptor (CD71)	–	+++
KiM-7 (CD68)	–	++
Secretion of		
TNF ³⁾	+	+++
M-CSF ⁴⁾	++	+++
GM-CSF ³⁾	–	++
IL-6 ³⁾	+++	+
IL-1 ³⁾	+++	–
IL-8 ³⁾	++	++

¹⁾ monocytes cultured for 7 days on teflon

²⁾ U937 cells as targets

³⁾ upon stimulation with LPS

⁴⁾ constitutive secretion

In unstimulated MO as well as in MO-derived MAC at all stages of culture only low levels of neopterin could be detected. As can be seen from Figure 1 neopterin release could be stimulated by IFNg but also by LPS as well. While LPS was in MO as potent as IFNg, IFNg became the more effective stimulus in MAC. During the first week of maturation the capacity of MAC to secrete neopterin upon stimulation increased severalfold from 0.32 ± 0.12 ng/ml using IFNg as stimulus and 0.39 ± 0.10 for LPS in 24 hour-supernatants of freshly isolated MO up to 4.44 ± 1.14 ng/ml and 2.45 ± 0.7 , respectively, in 7 day old MAC (results are expressed as mean of 3 different experiments).

Neopterin (ng/ml)

Figure 1. Regulation of constitutive and stimulated neopterin release during MO to MAC differentiation in vitro. Elutriated MO were cultured on teflon and harvested at consecutive days as indicated. 5×10^5 cells/ml were then cultured for 24 hours with or without LPS 100 ng/ml or IFNg 200 U/ml, respectively and supernatants analyzed for neopterin. Results of one representative out of 4 experiments are shown.

Induction of neopterin by IFNg as well as LPS was maximal around day 5 to 7 in culture and decreased upon further cultivation of MAC until day 14 to values of 1.65 ± 0.84 for IFNg and 0.95 ± 0.45 for LPS as stimulus (Fig. 1).

Our data are consistent with the results reported by others (9) that indoleamine 2,3-dioxygenase activity are seven times higher in mature MAC than in blood MO. It is of interest in this respect that the IFNg-mediated induction of tumorcytotoxicity (10), oxygen radical production and expression of procoagulant activity by human MAC (11) has been demonstrated to follow a similar dependence on the stage of maturation (summarized in Table 1). These findings demonstrate that the general ability of MP to respond to an activating signal may crucially depend on their stage of differentiation. The transformation of blood MO into tissue MAC is of special importance in the ontogeny of the MAC system as with this maturation step the site-specific phenotype of inflammatory MAC and thus their functional competence is determined. Monocytes migrating into inflamed or injured tissue need to undergo differentiation to interact with infiltrating lymphocytes. It seems to be necessary that during an ongoing inflammatory process the functional repertoire of infiltrating MO apparently is reg-

ulated not to shift from beneficial repair to detrimental induction of fibrosis and granuloma formation (12). Here, differentiation processes may play a dominant role not only to induce MAC responsiveness but also to suppress it at later stages of inflammation. The interpretation of neopterin levels in clinical situations and in experimental cell system should be re-evaluated in view of our findings. They may not only reflect activation of T-cell immunity (13) but may also be linked to the differentiation stage of the producing cells.

References

1. Shimizu, T., Nomiya, S., Hirath, F. & Hayaishi, O. (1978) *J. Biol. VChem.* 253, 4700–4707.
2. Werner-Felmayer, G., Werner, E. R., Fuchs, D., Hausen, A., Reibnegger, G. & Wachter, H. (1989) *Biol. Chem. Hoppe-Seyler* 370, 1063–1069.
3. Huber, C., Batchelor, J. R., Fuchs, D., Hausen, A., Lang, A., Niederwieser, D., Reibnegger, G., Swetly, P., Troppmair, J. & Wachter, H. (1984) *J. Exp. Med.* 160, 310–316.
4. Hausen, A., Fuchs, D., Reibnegger, G., Werner, E. R. & Wachter, H. (1989) *Pteridines* 1, 1–10.
5. van Furth, R. (1988) In: *Inflammation* (Gallen, J., Goldstein, M. & Snyderman, R., eds) pp. 281–295, Raven Press, New York.
6. Andreesen, R., Picht, J. & Löhr, G. W. (1983) *J. Immunol. Methods* 56, 295.
7. Andreesen, R., Bross, K. J., Osterholz, J. & Emmerich, F. (1986) Human macrophage maturation and heterogeneity: Analysis with a newly generated set of monoclonal antibodies to differentiation antigens. *Blood* 67, 1257.
8. Andreesen, R., Brugger, W., Scheibenbogen, C., Kreutz, M., Leser, H. G., Rehm, A. & Löhr, G. W. (1990) *J. Leuk. Biol.*, 47, 490–497.
9. Carlin, J. M., Borden, E. C., Sondel, P. M. & Byrne, G. I. (1989) *J. Leukoc. Biol.* 45, 29–34.
10. Andreesen, R., Brugger, W., Gadd, S., Löhr, G. W. & Atkins, R. C. (1988) *Immunobiol.* 177, 186.
11. Andreesen, R., Scheibenbogen, C., Moser, H., Krause, S. & Engler, H. (1990) *Proc. Am. Ass. Canc. Res.*, in press (abstract).
12. Martinet, Y., Rom, W. N., Grotendorst, G. R., Martin, G. & Crystal, R. (1987) *New Engl. J. Med.* 317, 202.
13. Werner, E. R., Bitterlich, G., Fuchs, D., Hausen, A., Reibnegger, G., Szabo, G., Dierich, M. P. & Wachter, H. (1987) *Life Sciences* 41, 273–280.