Surface Phenotype Analysis of Human Monocyte to Macrophage Maturation

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Cells of the mononuclear phagocyte system arise from circulating blood monocytes. Upon emigration from the vasculature, monocytes differentiate into macrophages, a process that monocytes similarly undergo in vitro. We have established primary cultures from elutriated or adherence-purified blood monocytes and analyzed the antigenic modulation during monocyte to macrophage transformation, which could be followed by the expression of specific antigens and which required as yet unknown inducer signals present in the serum. It is shown that in the absence of serum monocytes only survive in vitro when cultured adherent to plastic but rapidly die in suspension culture. Starting at 0.5%, serum induced maturation dose-dependently, with the optimal concentration being 2 to 5%. Of those antigens not present on monocyte, the low-affinity Fc receptor (CD16), the α-chain of the vitronectin receptor (CD51), gp65-MAX.1, and gp68-MAX.3 were expressed only upon serum-induced macrophage differentiation, whereas the transferrin receptor (CD71), MAX.28, and to some degree also gp65-MAX.11 appeared to be independent of maturation and were also found on primary cultures of adherent monocytes under serum-free conditions. In addition, the rapid induction of HLA class II antigens (within 24 hr) was similar with and without serum, as was the continued high-density expression in long-term culture. The monocyte-specific CD14 antigen was down-regulated in the absence of serum but kept its level of expression on differentiated macrophages. In comparison, alveolar and peritoneal macrophages, respectively, differed in their antigenic phenotype: Alveolar macrophages expressed high HLA class II antigens but low CD14, whereas for peritoneal macrophages the opposite was found. Both interferon-γ and -α suppressed macrophage maturation in vitro but had contrary effects on HLA class II and CD16 expression: Interferon-γ up-regulated the two types of antigens, which, in contrast, were down-regulated by interferon-α.

Key words: serum-induced differentiation, HLA class II antigens, interferon

INTRODUCTION

The many biological functions that are attributed to cells of the mononuclear phagocyte system (MPS) [1–5] all depend on the successful completion of the differentiation pathway from immature precursor cells to the mature macrophage (MAC). Circulating blood monocytes (MO) emigrate into extravascular tissue sites either to become resident organ-specific MAC or to be recruited as immune effector cells at sites of inflammation, injury, allograft, or tumor rejection, respectively. Monocyte to macrophage differentiation also occurs in vitro [6,7] in the presence of as yet undefined serum factors [8–10]. As cell differentiation is modulated in situ by the site-specific microenvironment that is responsible for the development of cell heterogeneity within the MPS, MAC derived in vitro from blood MO may represent yet another distinct differentiation stage. However, in vitro maturation has proven to be a very useful model for studying possible regulatory mechanisms involved in MAC terminal differentiation as well as the functional and phenotypic changes that accompany it [6,7,10–14]. In this report we have analyzed the antigenic phenotype of MAC derived from MO in culture on plastic or hydrophobic teflon foils and have evaluated those antigens indicative of serum-induced MAC differentiation and not expressed on primary MO cultures established in the absence of serum. We also investigated the influence of interferons (IFN) on MAC maturation in vitro. It was found that these mediators, while inducing functional activation, inhibited cell differentiation.

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MATERIALS AND METHODS

Monocyte Isolation

Mononuclear cells (MNC) were isolated fromuffy coat preparations or cytapheresis concentrates of healthy blood donors by density gradient centrifugation over Ficoll-Hypaque. They were washed twice in RPMI 1640 (supplemented with 20 μM 2-mercaptoethanol, 2 mM L-glutamin, 50 U/ml penicillin, and 5 μg/ml streptomycin), and the monocytes were separated either by countercurrent centrifugal elutriation (CCE) in a Beckman J2/21 or by adherence to plastic surfaces, respectively [10]. For CCE, cells were resuspended in phosphate-buffered saline (PBS) with 5% clinical grade human albumin and loaded in a volume of 50 ml onto the elutriator at 10 ml/min and 2,500 rpm using a standard elutriation chamber. With stepwise increase of flow (0.5 ml/min every 50 ml), MO elutriated at 15 ml/min with a purity of more than 90% as judged by morphology and CD14 expression. Alternatively, 5 × 10⁶ MNC/ml were cultured in suppl. RPMI 1640 plus 10% fetal calf serum (FCS) for 60 min in plastic tissue culture flasks (Fa. Greiner, Nürtingen, FRG) before the nonadherent cells were removed with two washes in warm medium. The adherent cells were cultured overnight in suppl. RPMI 1640 plus 5% human AB-group serum pooled from different pretested donors. The cells could then be washed completely off the flasks after cooling them down to 4°C for 30 min. They proved to be MO with a purity of more than 90%, and they accounted for about 65% of the circulating monocyte population.

Explanted Macrophages

Cells were isolated from the bronchoalveolar lavage fluids of healthy volunteers (both smokers and nonsmokers) or from ascites fluids secondary to portal hypertension or malignancy. Volunteers and patients had given informed consent prior to cell collection.

Monocyte Culture

Elutriated or adherence-purified MO were seeded either into hydrophobic teflon bags at a concentration of 3 × 10³ cells/ml or into 96 well microtiter plates (Greiner) at 10⁵ cells/0.2 ml suppl. RPMI 1640 with or without AB serum at a concentration of 2% or as indicated [10,15]. In some experiments human heparinized plasma was used as culture additive. Cultures were set up in some experiments with different concentrations of recombinant human interferon (rhIFN)-γ (IMMUNERON™, Biogen, Geneva, Switzerland) and rhIFN-α2 (Emil-Böhringer Institut f. Arzneimittelforschung, Vienna, Austria). The units of recombinant materials were calculated based on the information provided by the manufacturers. In some experiments serum was replaced by 0.1 mg/ml lactalbumin hydrolysate, 0.2 mg/ml fetuin, and 0.15 IU/ml bovine insulin (LIF; materials all from Sigma Chemicals, St. Louis, MO).

Surface Antigen Expression Analysis With the Enzyme-Linked Immunosorbent Assay (ELISA)

As described previously [15], MO and MAC were analyzed at indicated days of culture for antigen expression in the microtiter plates in which the cultures were initiated. For freshly seeded MO (at day 0 for elutriated cells and at day 1 for adherence-purified cells) as well as peritoneal and alveolar lavage MAC, respectively, cells were first incubated for 30 min in suppl. RPMI 1640 plus 2% AB serum (elutrated MAC from alveolar space and peritoneal cavity at 2 × 10⁴ per ml, MO at 10⁴ per ml) before being subjected to cell ELISA. Cells were then fixed at 4°C with 0.05% glutaraldehyde for 10 min. Triplicates were incubated with the following monoclonal antibodies (mAb) diluted in gelatin (0.2%)-containing medium: Anti-β₂-microglobulin (b2M) and Leu10 (HLA-DQ; Becton and Dickinson, Rödermark, FRG); My4 (CD14; Coulter Immunology, Hialeah, FL); OKT9 (CD71; Ortho Diagnostics, Raritan, NJ), Tu22 (HLA-DQ) and Tu39 (HLA-DR/DP; Biostest, Dreieich, FRG); MAX.1 (gp65), MAX.3 (gp68), MAX.11 (gp65), MAX.21 (HLA-DR), MAX.26 (our laboratory) [12,16,17]; 13C2 and 23C6 (CD51; gift of Dr. M. Horton, Imperial Cancer Research Fund Laboratories, London, England) [18]; and CLB FcR gran 1 (CD16; Dr. P. Tetteroo, Netherlands Red Cross Blood Transfusion Service, Amsterdam) [19]. As controls, mAb of different immunoglobulin (1g) types raised against cytoplasmic antigens of Salmonella enteritidis 11RX [20] were used. Immunoperoxidase staining was done using phenyl-diamine-dichloride as the substrate. Data are expressed as OD₄₅₀ corrected by the values measured in control wells without the first antibody, mean of triplicate values, S.D. >15%, but in most experiments an antigen expression index (AEI) was calculated as percentage of b2M expression.

Immunoperoxidase Staining on Adhesion Slides

At different stages of culture MAC of more than 95% purity were recovered from the teflon bags by needle aspiration. As described recently [12], cells were attached to Alcian blue-coated slides, prefixed in 0.05% glutaraldehyde, and incubated with mAb. A four-layer peroxidase-antiperoxidase (PAP) technique was applied followed by postfixation with OsO₄.

RESULTS

Monocytes were isolated from other mononuclear blood cells either by countercurrent elutriation or by ad-
TABLE 1. Cell Survival in Primary Human Monocyte Cultures: Dependence on the Culture Substrate

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>LIF</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teflon cultures</td>
<td>69^b</td>
<td>9</td>
<td>0.37</td>
</tr>
<tr>
<td>Plastic cultures</td>
<td>2.18^c</td>
<td>1.81</td>
<td>2.06</td>
</tr>
</tbody>
</table>

^aMonocytes purified by centrifugal elutriation were cultured at 10^5 per 0.2 ml for 7 days in supli. RPMI 1640 with or without 5% AB group serum or defined additives (LIF), respectively.

^bData from teflon-cultured cells are expressed as percentages of cells recovered from the total cell number seeded initially.

^cCultures in plastic microtiter plates were subjected to cell ELISA analysis of b2M expression. Data are given as optical density (OD), mean of triplicates.

herence to plastic surfaces at a purity of <90%. Recovery of MO from buffy coat preparations was consistently higher by elutriation than by adherence separation, suggesting the existence of a considerable portion of non-adherent MO. Survival and recovery of MO-derived MAC after a culture period of 7 days depended on the substrate.

Serum was essential for MAC survival in cultures on hydrophobic surfaces and induced the development of fully differentiated MAC as judged by typical morphology and the expression of maturation-associated antigens [12,14] (shown below). In the absence of serum, however, cells died; this could only slightly be prevented by the addition of serum-replacement additives (Table 1). If MO were cultured on plastic surfaces (96 well microtiter plates), cells survived as seen from the expression values of b2M (Table 1) but did not undergo transformation to MAC and retained their monocyoid morphology. Monocyte to MAC differentiation occurred in cultures of either unseparated MNC and elutriation- or adherence-purified MO, with the latter preparation resulting in the poorest recovery rate and the lowest density of the maturation-associated antigen gp65-MAX.1 as detected by the cell ELISA technique (Table 2).

We have analyzed more than 200 mAbs regarding their differential reactivity with freshly isolated MO and teflon-cultured MAC, respectively. We have reported that only 6 of 150 antmyeloid mAbs submitted to the Third Leukocyte Differentiation Antigen Workshop reacted selectively with mature MAC [21]. Here we demonstrate the α-chain of the vitronectin receptor (CD51, 13C2, and 23C6) [18] to be expressed on MAC derived from MO in culture (Fig. 1) as well as the low-affinity receptor for monomeric IgG (CD16; Fig. 2). In figures 1 and 2 values for antigen expression are depicted as both percentage of positive cells and antigen density determined by cell ELISA. Independent of the process of maturation, i.e., whether MO were cultured with or without serum, expression of HLA class II molecules increased rapidly already after 24 hr of culture and retained their level of b2M-related expression during the 7 day culture period (Fig. 3) and beyond (data not shown in detail). When, in comparison, HLA expression was quantitated on exudate-type MAC obtained from the peritoneal cavity and the bronchoalveolar space, respectively, it was evident that these cells represent two different subpopulations with distinct antigenic profiles (Table 3). Lung MAC had much higher HLA class II expression, whereas CD14 expression was consistently higher in peritoneal MAC.

To distinguish antigens that are associated with MO to MAC maturation in vitro from those that (in the absence of cell maturation) are induced by the culture environment, we compared antibody reactivity in plastic adherent monolayers of MO cultured in the presence or absence of serum, respectively. As can be seen from Figure 3, the early up-regulation and the persistent high expression of HLA class II antigens are similar for serum-containing and serum-free MO cultures. For the other antigens tested it was found that MAX.26 and the transferrin receptor are independent of MAC maturation, whereas gp65-MAX.1, gp68-MAX.3, CD51 (13C2), CD16, and gp65-MAX.11, though to a lesser degree, are specifically associated with serum-induced cell differentiation (Table 4). The MO-specific CD14 molecule decreases in the absence of serum but retains its expression in serum-containing cultures (Peripheral blood MO expressing CD14 at about 70 AEI [15]). Thus MAX.1 but not CD71 expression can be used to quantify the degree of maturation induced by different concentrations of serum and plasma, respectively, as can be seen from Figure 4.

When MO were cultured in the presence of IFN, profound effects on maturation and cell phenotype were observed: Both IFN-α and IFN-γ suppressed the development of the maturation-associated phenotype (MAX.1*13C2*) with the extent of inhibition varying consistently from experiment to experiment, especially for IFN-γ. This, we believe, most probably reflects donor-to-donor variation, a phenomenon that is frequently encountered also by groups studying human macrophage biology [22]. Figure 5 shows a representative experiment in which a substantial inhibition of MO to MAC maturation by IFNs could be observed. IFN-γ in parallel to its suppressive effect on cell differentiation substantially activated MAC tumor cytotoxicity [13] (data not shown in detail here) and induced an increased expression of HLA class II (Fig. 5) and CD16 (Fig. 6) antigens. In contrast, IFN-α down-regulated the expression of these antigens (Figs. 5, 6).
DISCUSSION

Within the MPS the circulating blood MO represents an immature precursor cell that is ready to undergo further differentiation upon migration from the vasculature into the various tissues and body cavities [1,2]. Most likely initiated through attachment to and interaction with endothelial cells [23], MO migration may be directed by chemotactic signals originating from sites of infection or tumor growth, or it may follow intrinsic programs of differentiation [24] to result in the generation of organ-specific populations of resident MAC [25]. Maturation of MAC from MO in vitro in many aspects resembles the in vivo situation and facilitates investigation of the modulation and the functional significance of the differentiation process [6,7,11-13].

In the present investigation we show that considerable changes occur in the antigenic phenotype when MO are induced to differentiate in vitro and confirm the results of others [8,9] that serum is essential for this MO to MAC transformation, though its active fraction(s) have not been successfully identified. In the absence of serum, MO do not develop into MAC; they only survive when cultured adherent to plastic surfaces and rapidly die in suspension cultures on hydrophobic teflon membranes (Table 1). Autocrine cell survival factors are possibly

**TABLE 2. Phenotype Analysis of Human MAC Maturation on Hydrophobic Teflon: Dependence on Monocyte Isolation Techniques**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>MNC</th>
<th>Adherent MO</th>
<th>Elutriated MO</th>
</tr>
</thead>
<tbody>
<tr>
<td>b2M</td>
<td>58h</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>55</td>
<td>38</td>
<td>66</td>
</tr>
<tr>
<td>HLA-DQ</td>
<td>46</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>gp65-MAX.1</td>
<td>52</td>
<td>38</td>
<td>63</td>
</tr>
</tbody>
</table>

*MNC or MO purified either by adherence or by centrifugal elutriation were cultured in suppl. RPMI 1640 plus 2% AB serum for 7 days at \(5 \times 10^9/ml\) for MO and \(2 \times 10^9/ml\) for MNC, respectively.

*Data are expressed as percentage of positive cells recovered from the total cell number seeded in the teflon bags initially.

*Antigen expression was evaluated by cell ELISA on \(2 \times 10^4\) MAC.

*Data are given as OD values corrected by cell recovery rate in percentage, mean of triplicate values.

**Fig. 1.** Expression of vitronectin receptor (CD51) on in vitro differentiating MO-derived MAC. Adherence-purified MO were cultured in teflon bags at \(3 \times 10^6\) cells/ml suppl. RPMI 1640 with 5% AB serum, harvested at indicated days, and subjected to surface antigen analysis by immunoperoxidase staining of single cells using the mAb 13C2 and 23C6 (left). Data are given as mean percentage of antigen-positive cells. Also, \(2 \times 10^4\) MO-derived MAC per well on day 9 of culture were subjected to cell ELISA (right). Data are given as antigen expression index (AEI).

**Fig. 2.** Expression of the CD16 molecule (low affinity Fc receptor) on in vitro-differentiating MO-derived MAC. Adherence-purified MO were cultured either in teflon bags (at \(3 \times 10^6\) cells/ml) or in plastic microwell plates (at \(10^4\) cells/0.2 ml) in suppl. RPMI 1640 plus 5% AB serum. At indicated days antigen expression was analyzed on single cells and on cell monolayers grown in microwells, respectively. Data are given as percentages of positive cells and as specific OD per monolayer, respectively.
TABLE 4. Primary Cultures of Human MO-MAC: Expression of Maturation-Associated Antigens as Distinct From Culture-Induced Antigens*

<table>
<thead>
<tr>
<th>Antigen</th>
<th>AB serum</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcRIII (CD16)</td>
<td>22(\pm)6</td>
<td>8</td>
</tr>
<tr>
<td>gp65-MAX.1</td>
<td>42</td>
<td>6</td>
</tr>
<tr>
<td>gp68-MAX.3</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>gp65-MAX.11</td>
<td>94</td>
<td>39</td>
</tr>
<tr>
<td>MAX.26</td>
<td>95</td>
<td>86</td>
</tr>
<tr>
<td>TFR (CD71: OKT9)</td>
<td>78</td>
<td>84</td>
</tr>
<tr>
<td>VNR (CD51: 1JC2)</td>
<td>43</td>
<td>9</td>
</tr>
</tbody>
</table>

*Monocytes were elutriated and cultured for 7 days at 10^6/0.2 ml suppl. RPMI 1640 in microtiter plates with and without 2% AB serum. Cell ELISA was used to evaluate antigen expression as described.

Data are given as percentage of b2M expression (antigen expression index [AEI]).

![Image](https://example.com/image.png)

**Fig. 3.** Expression of β_2_ microglobulin (b2M) and HLA class II antigens on freshly isolated MO, cultured for 24 hr and after long-term cultivation (8 days). Elutriated MO were seeded at 10^6 cells/0.2 ml in plastic microplates and analyzed immediately or after various times of culture without and with 2% AB serum, respectively. Data are given as specific OD for the expression of b2M and as antigen expression index (AEI) for HLA class II antigens.

**Fig. 4.** Expression of gp65-MAX.1 (A) and transferrin receptor (OKT9; CD71; B) on MO/MAC cultured in the presence of various concentrations of serum/plasma. Elutriated MO were seeded at 10^6 cells/0.2 ml suppl. RPMI 1640 with indicated concentrations of serum or heparinized plasma and cultured for 7 days. Antigen expression was then analyzed by cell ELISA and given as the antigen expression index, mean of triplicate values, SD <15%.

**TABLE 3. Expression of HLA and CD14 Antigens on Human Alveolar and Peritoneal Macrophages**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Alveolar MAC (n = 10)</th>
<th>Peritoneal MAC (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>b2M</td>
<td>1.628 ± 0.44(^*)</td>
<td>1.498 ± 0.17</td>
</tr>
<tr>
<td>CD14</td>
<td>48 ± 27(^*)</td>
<td>85 ± 8</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>115 ± 12</td>
<td>72 ± 13</td>
</tr>
<tr>
<td>HLA-DR/DP</td>
<td>129 ± 21</td>
<td>81 ± 11</td>
</tr>
<tr>
<td>HLA-DQ (TU22)</td>
<td>96 ± 12</td>
<td>33 ± 13</td>
</tr>
<tr>
<td>HLA-DQ (Leu10)</td>
<td>41 ± 5</td>
<td>11 ± 6</td>
</tr>
</tbody>
</table>

\(^*\)Cells were isolated from the bronchoalveolar lavage fluids of healthy volunteers (both smokers and nonsmokers) or from ascites fluids secondary to portal hypertension or malignancy.

\(^\text{MAC 2×10}^6\) was seeded into microtiter plates and subjected to cell ELISA analysis. Data are given as specific OD, mean of triplicate values per test sample.

\(^\text{Data are expressed as percentage of b2M expression (antigen expression index).}\)

induced by cell adherence, as has been shown recently for CSF-1 [26], c-fos [26], TNF [26], IL-1 [27], and many other, as yet unidentified proteins [28]. CSF-1 is likely to be involved in MO survival, as addition of neutralizing antibodies to serum-containing cultures led to cell death [29]. However, addition of exogenous CSF-1 to serum-free cultures cannot induce maturation but prevents cell death in teflon cultures [29]. Thus cell survival in vitro is clearly different from cell differentiation. It is noteworthy that adherence per se may not be sufficient to induce autocrine factor secretion, as the majority
Phenotype of Macrophage Maturation

Fig. 5. Phenotype analysis of MO/MAC cultured in the presence of recombinant interferon-γ and -α. Elutriated MO were cultured for 7 days at 10^6 cells/0.2 ml suppl. RPMI 1640 with 1% AB serum in the absence or presence of 200 IU/ml rIFN-γ or rIFN-α2, respectively. Expression of b2M, CD14 (My4), HLA class II (MAX.21, T022, Leu10), MAX.1, and 13C2 was analyzed by cell ELISA. Data are given as the antigen expression index calculated on the basis of b2M expression (see Materials and Methods).

Fig. 6. Differential effects of interferon-γ and -α on the expression of CD16 by in vitro matured MAC. Elutriated MO were cultured for 7 days in suppl. RPMI 1640 with 1% AB serum and various concentrations of rIFN-γ and rIFN-α2, respectively. For other details, see Figures 4 and 5.

of MO also adheres (though loosely) to the teflon material. Similarly, different effects were observed for MO adherent to plastic vs. adherent to connective tissue cells [26]. Also, the type of surface coating can modify the process of MO to MAC maturation in vitro [30].

In studying MO to MAC transformation in vitro it is essential to distinguish those antigenic changes that are associated with serum-induced true maturation from those that are culture induced. As depicted in Figure 3A, MAC maturation can be exactly quantified by the expression of an antigen reactive with the MAX.1 mAb. This antigen is also detected, though at a different epitope, by the MAX.11 mAb, has a molecular weight of 65 kD, and, like CD14 [31], seems to be a phosphatidylinositol-linked membrane protein (S. Gadd, personal communication) with an as yet unknown function. In contrast, the transferrin receptor (CD71), though also absent from MO [14], is expressed independently of serum-induced maturation and is obviously induced by as yet unknown autocrine factors or signals generated from the culture environment (Fig. 3B). Similarly, other antigens originally believed to be restricted to MO-derived MAC were observed to be expressed on cultured MO to the same density (defined by the mAb MAX.26) as on mature MAC or at least at detectable levels (gp65-MAX.11). Likewise, the rapid increase in b2M and HLA class II expression already after a 24 hr culture period is independent of serum, as is their continued high expression over long-term culture (Fig. 4). This may reflect the functional requirements that MO are subjected to in vivo when they enter inflammatory sites, i.e., to be able to cooperate effectively with the specific immune system by processing and presenting antigen [3]. It is also possible that MO to MAC maturation proceeds via distinct differentiation stages, with the early, serum-independent phase being more related to functional activation as reflected by the induction of HLA antigens, i.e., the inflammatory-type of MAC. This step may be consistent with the "veiled accessory cell" described by Peters et al. [32]. A further serum-dependent progression may then lead to the mature resident MAC type of cell. In this respect, activating signals like the T-lymphokine IFN-γ or the monokine IFN-α suppress this progression from the early ("inflammatory") differentiation stage to the more mature form and, at the same time, induces HLA class II expression even further. Thus, activation may exclude MO from maturation. IFN-α, when compared with IFN-γ, exerts similar and in most experiments stronger inhibitory effects on MAC maturation but, in contrast to IFN-γ, does not induce HLA class II antigens and may fix cells at the differentiation stage of the circulating MO.

Whereas IFN-γ therefore primes MAC to act within the specific immune network, IFN-α seems to have the opposite effect. Thus our work confirms early observations of the inhibitory effects of IFN on MAC maturation in vitro [33,34]. In addition, very effective inhibition of
MO to MAC transformation is also seen with bacterial endotoxins [35], another stimulus for MAC functional activity. Unlike other investigators [36,37], we did not observe down-regulation of CD14 antigens by IFN-γ. A decrease in CD14 expression, however, was most pronounced after 24 hr cultivation and might thus be a consequence of functional activation. In contrast, we studied the long-term effects of IFN-γ on MAC cell differentiation over a period of 7 days.

It is of interest that in situ matured MAC from the alveolar space and the peritoneal cavity differed considerably. The alveolar MAC, which are in constant contact with inhaled immunogens, seem to be highly competent for immunological cooperation as reflected by their high expression of HLA class II molecules. The peritoneal MAC express much lower HLA class II antigens but nearly double as much as the alveolar MAC of the constitutive MAC antigen CD14.

Upon culture MAC obviously can express some lineage-associated antigens still being repressed on circulating MO [12,14,16]. The appearance on mature MAC of antigens originally described to be specific for osteoclasts [18] supports the hypothesis of these cells belonging to the MPS and sharing a common precursor cell [2]. A phenotype analysis of MO to MAC maturation thus can assist in the uncovering of as yet unproven lineage assignments.

Phenotype analysis can also help to understand the functional changes that occur in situ upon MAC maturation. For example, mAbs 13C2 and 23C6 both have been characterized at the Fourth Leukocyte Differentiation Antigen Workshop [38] to recognize the α-chain of the vitronectin receptor (VNR). This molecule mediates cell–cell adhesion, is involved in the binding of coagulation factors, and seems to modulate the lytic activity of complement [39,40]. In the interaction of MAC with endothelial, mesenchymal, but also lymphoid immune effector cells the expression of the vitronectin receptor might be of importance, especially during local inflammatory processes. It should be noted here that, similarly, fibronectin, as another cyoadhesive molecule also appeared to be a maturation-associated MAC product [13,41].

In addition, the low-affinity receptor for IgG (FcRIII; CD16) is expressed on end-stage differentiated MAC (Table 2; P. Tetteroo, personal communication) [42,43]. This molecule seems to be important for that part of MAC tumor cytotoxicity that is triggered upon Fc receptor-mediated binding to tumor-associated antibodies [42]. Thus any impairment of MO to MAC maturation might cause a deficient cytotoxic effector cell function of intratumoral MAC derived from immigrated blood MO. Furthermore, the suppression of CD16 expression on cells of the MAC system by IFN-α could well be considered the causative effect of the treatment of steroid-resistant immune thrombocytopenia by IFN-α [44].

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