Immunology 1987 **62** 171–176

Quantitative immunocytochemical characterization of mononuclear phagocytes

II. MONOCYTES AND TISSUE MACROPHAGES

P. H. NIBBERING, P. C. J. LEIJH & R. VAN FURTH *Department of Infectious Diseases, University Hospital, Leiden, The Netherlands*

Accepted for publication 12 May 1987

SUMMARY

The purpose of the present study was to compare the monoclonal antibody (Mab) binding patterns of various tissue macrophages with each other and with blood monocytes. To allow recovery from the effects of the isolation procedure, or to obtain purified populations, macrophages were cultured for 24 hr and 48 hr. For comparison, blood monocytes were also cultured for 24 hr and 48 hr. Mab binding to individual cells, detected by the biotin avidin immunoperoxidase method, was quantified cytophotometrically and the results expressed as the median of the specific mean absorbance per 0·25 μm^2 cell surface area or as specific integrated absorbance per cell. Analysis of the quantitative data in relation to the results of subjective evaluation of the peroxidase reaction product, demonstrating Mab binding to cells, yielded three classes for description of the intensity of antigen expression by cells: weak (specific mean absorbance per unit cell surface less than 0·07), moderate (values between 0·07 and 0·14), and intense (values more than 0·14). No matter how the results were expressed, comparison of the Mab binding patterns of macrophages with those of blood monocytes showed that spleen macrophages bound significantly less F4/80 and more M5/114 (Ia antigen). Kupffer cells and skin macrophages bound either approximately the same amount or considerably less of the various Mabs than monocytes did. Pulmonary tissue and alveolar macrophages bound significantly more 30.G.12 (leucocyte antigen), M3/38 (Mac-2 antigen), and M3/84 (Mac-3 antigen) and comparable amounts or considerably less of the other Mabs than the monocytes did. Peritoneal macrophages bound significantly more F4/80, M1/70 (complement receptor III), and 2.4.G.2. (Fc receptor II) and comparable amounts or considerably less of the other Mabs than monocytes did. It is concluded that macrophages from different organs and different anatomical sites within one organ differ from one another, for example, peritoneal macrophages do not resemble any other population of macrophages and alveolar macrophages do not resemble pulmonary tissue macrophages, and differentiation of blood monocytes into tissue macrophages does not show a distinct pattern.

INTRODUCTION

Monocytes, which originate in the bone marrow, leave the circulation at random and migrate to tissues and body cavities where they differentiate into macrophages. Macrophages in tissues and body cavities exhibit a great diversity of phenotypic and functional characteristics (van Furth *et al.*, 1980; Gordon *et al.*, 1986).

Recently, we developed an immunocytochemical method, using the binding of monoclonal antibodies (Mabs), to quantify the expression of antigens on the surface of individual cells in relative terms (Nibbering, Leijh & van Furth, 1985). Employing a model system, we showed that the amount of the final immunocytochemical enzyme reaction product is proportional to the amount of cell antigen (Nibbering & van Furth, 1987). The quantitative approach allowed us to analyse changes in the expression of cell-surface antigens during differentiation of immature, dividing mononuclear phagocytes of the bone marrow (monoblasts, promonocytes) into monocytes, and of monocytes into alveolar and peritoneal macrophages (Nibbering, Leijh & van Furth, 1987).

The present study was undertaken to obtain a better understanding of the differentiation of monocytes into macrophages by comparing the binding patterns of an assortment of Mabs to blood monocytes and to macrophages of the spleen, liver, lung, skin, and peritoneal cavity. In addition, we compared the results of objective evaluation of the binding of Mabs to cells with those of subjective evaluation by visual inspection.

Abbreviations: A_{int}, integrated absorbance; EDTA, ethylenediamine tetraacetic acid, disodium salt; HBSS, Hanks' balanced salt solution; Mab, monoclonal antibody; PBS, phosphate-buffered saline; SPF, specific pathogen-free.

Correspondence: Dr P. H. Nibbering, Dept. of Infectious Diseases, University Hospital, Building 1, C5-P, PO Box 9600, 2300 RC Leiden, The Netherlands.

MATERIALS AND METHODS

Animals

Specific pathogen-free (SPF) male Swiss mice (Central Institute for the Breeding of Laboratory Animals, TNO, Zeist) weighing 20–25 grams were used.

Preparation of mononuclear phagocytes

Blood obtained by cardiac puncture was collected in heparinized syringes and subjected to differential centrifugation (20 min, 4°, 440 *g*) on Ficoll–Isopaque gradients ($\rho = 1{\cdot}077$ g/ml; Pharmacia Inc., Uppsala, Sweden). The interphase containing mononuclear cells was then washed four times with phosphate-buffered saline (PBS; pH 7·2) containing 0·5 U/ml heparin.

Briefly summarized, the isolation of tissue macrophages, described in detail elsewhere, was as follows. A suspension of spleen cells was obtained by gently pressing small pieces of spleen tissue through a stainless steel sieve (Nussenzweig *et al.*, 1981). For the isolation of Kupffer cells, blood monocytes were removed from the liver by perfusion of the organ vessels with 0·2% (w/v) pronase E (Merck, Darmstadt, FRG) in Hanks' balanced salt solution (HBSS; pH 7·2); the tissue was then cut into small pieces and incubated in 0·2% pronase and 0·5 mg/ml DNA-ase (Worthington, Freehold, NJ) in HBSS (Crofton, Diesselhoff-den Dulk & van Furth, 1978). For the isolation of alveolar macrophages, the circulation of the lungs was perfused to remove blood monocytes and the macrophages were harvested by lavage with a total of 15 ml PBS containing 0·6 mM EDTA, as described elsewhere (Blussé van Oud Alblas & van Furth, 1979). After the lavage, pulmonary tissue macrophages and the remaining alveolar macrophages were isolated from minced lung tissue by digestion with 0·2% (w/v) pronase and 0·5 mg/ml DNA-ase in HBSS (Blussé van Oud Alblas & van Furth, 1979). Skin macrophages were obtained by insertion of a subcutaneous glass coverslip (about 1·8 cm^2) that was left *in situ* for 24 hr (van Furth *et al.*, 1985). Peritoneal macrophages were harvested by lavage of the peritoneal cavity with 2 ml PBS containing 50 U/ml heparin as described elsewhere (van Furth & Cohn, 1968).

The cell suspensions obtained as mentioned above were cultured on glasss coverslips, at a concentration of about $1–5 \times 10^6$ cells/ml, in medium consisting of Medium 199 (Microbiological Associated, Walkersville, MD) containing 20% heat-inactivated new-born calf serum (NBCS; Gibco Europe Ltd., Paisley, Renfrewshire), 2000 U/ml sodium penicillin G, and 50 μg/ml streptomycin. After incubation for 2 hr at 37° in a humidified 5% CO_2 incubator, the non-adherent cells were removed by three washes with PBS and the adherent mononuclear phagocytes were cultured for an additional 22 or 46 hr.

Determination of monoclonal antibody binding to mononuclear phagocytes

Cell surface antigens on mononuclear phagocytes were detected with Mabs: F4/80 (Austyn & Gordon, 1981), M1/70 (Springer *et al.*, 1979), 2.4.G.2. (Unkeless, 1979), M5/114 (Bhattacharya, Dorf & Springer, 1981), 30.G.12 (Ledbetter & Herzenberg, 1979), M3/38 (Ho & Springer, 1983a), and M3/84 (Ho & Springer, 1983b). The cytophotometric method to quantify the relative amount of Mab bound to individual cells has been described in detail elsewhere (Nibbering *et al.*, 1985). Briefly, mononuclear phagocytes were fixed with 0·05% glutaraldehyde and successively incubated with 0·01 N HCl, 20% normal goat serum, and a saturating concentration of Mab. Monoclonal antibody binding was detected with biotinylated rabbit anti-rat IgG and horseradish peroxidase-conjugated avidin-D and visualized by incubation for 60 min in the presence of 0·6 mg/ml 3′–3′ diaminobenzidine-tetrahydrochloride in Tris-HCl buffer (pH 7·6) containing 0·01% H_2O_2. For the controls, incubation was performed with P3 X163 Ag8 653 plasmacytoma supernatant instead of Mab. The cells were counter-stained with 10 μg/ml ethidiumbromide (2,7 diamino-10-ethyl-9-phenyl-phenanthridiumbromide).

The integrated absorbance (A_{int}) of the peroxidase reaction product and the ethidiumbromide fluorescence were simultaneously detected with a microscope photometer (Zeiss, Oberkochen, FRG), interfaced to a PDP 11/10 microcomputer (Digital Equipment Corporation, Maynard, MA) provided with an epi-illuminator III RS (Zeiss). Scanning of the cells with photometric sampling at 0·5-μm intervals and computation of the A_{int} were performed with the ARRAYSCAN program of the HIDACSYS package (van der Ploeg *et al.*, 1977). In each population of mononuclear phagocytes the binding of Mab to 100 randomly selected cells was quantified and expressed as the median and range of the mean specific A_{int} per 0·25 μm^2 cell surface area of the Mab-binding mononuclear phagocytes. A cell was considered positive for a certain cell surface antigen when the mean observed A_{int} per 0·25 μm^2 cell surface was higher than the average of the mean non-specific A_{int} per 0·25 μm^2 cells surface of the control stained mononuclear phagocytes plus twice the SD.

Statistical analysis

The Fisher exact test was used to analyse the distributions of the mean specific A_{int} per 0·25 μm^2 cell surface and the sizes of mononuclear phagocytes; $P < 0{\cdot}01$ marked departure from normality. Differences in the mean specific A_{int} per 0·25 μm^2 cell surface and cell size between the various populations of mononuclear phagocytes were analysed with the Mann–Whitney *U*-test; $P < 0{\cdot}01$ was considered significant.

RESULTS

The cells isolated from spleen, liver, and lung tissue were cultured for 24 hr or 48 hr to obtain a pure population of macrophages and to let the cells recover from the procedure used for isolation, such as mechanical disruption or enzyme treatment of the tissue. For comparability with the results of 24- and 48-hr cultures, all other macrophages and blood monocytes were cultured for 24 hr and/or 48 hr as well.

Control experiments performed with peritoneal macrophages revealed no effect of exposure to Ficoll–Isopaque density centrifugation, mechanical disruption, pronase-DNA-ase treatment, or EDTA, all followed by culture on the binding of Mab (results not shown).

The percentages of mononuclear phagocytes expressing a given cell-surface antigen and the Mab binding patterns of 24- and 48-hr cultures of blood monocytes and macrophages are shown in Figs 1 and 2. Frequency histograms showed that the distribution of binding of Mabs to the various populations of mononuclear phagocytes was not normal, except in the case of alveolar and peritoneal macrophages cultured for 24 hr ($P > 0{\cdot}05$) (results not shown).

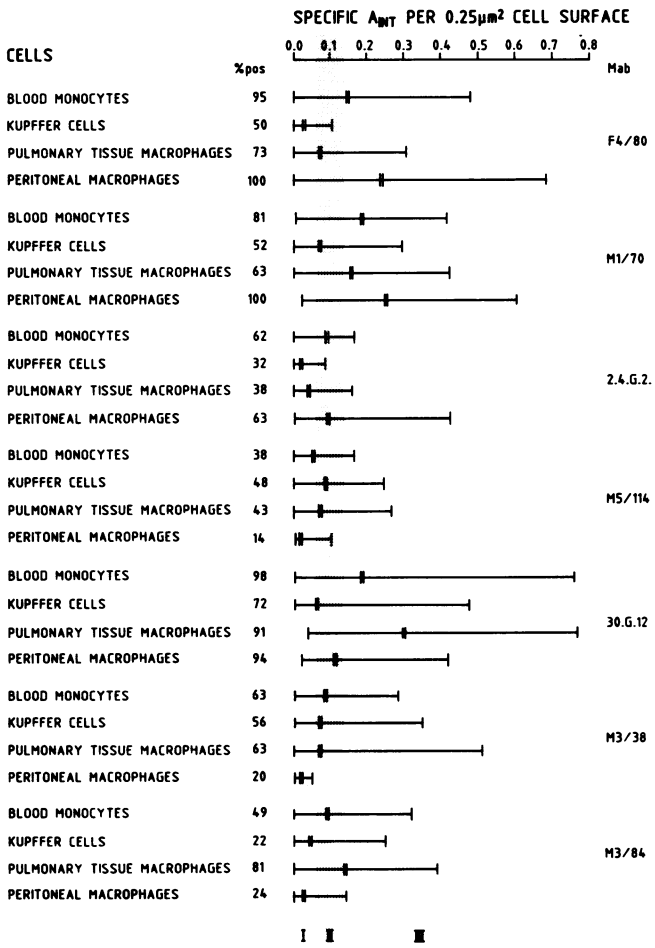

Figure 1. Mab binding patterns of blood monocytes and spleen, alveolar, skin, and peritoneal macrophages after 24 hr of culture. Mab binding to mononuclear phagocytes was quantified by photometric determination of the amount of peroxidase reaction product. The results are expressed as the percentage of cells expressing a given cell-surface antigen and the median and range of the specific mean A_{int} per 0·25 μm^2 cell surface area for the antibody-binding mononuclear phagocytes in three to four experiments. Specific mean A_{int} values per 0·25 μm^2 cell surface area lower than 0·07 reflect weak expression of the antigen (I); values between 0·07 and 0·14 represent moderate expression (II); and values above 0·14 intense expression of the antigen (III).

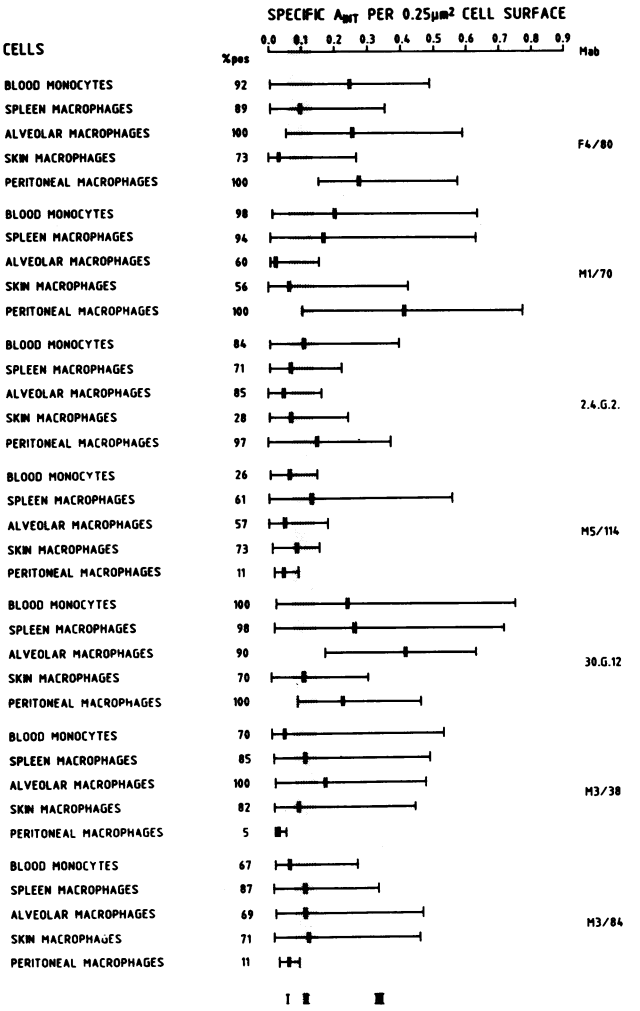

Figure 2. Mab-binding patterns of blood monocytes, Kupffer cells, pulmonary tissue macrophages (together with contaminating alveolar macrophages), and peritoneal macrophages after 48 hr of culture. The results are expressed as the percentage of cells expressing a given cell-surface antigen and the median and range of the specific mean A_{int} per 0·25 μm^2 cell surface area for the antibody-binding mononuclear phagocytes in three to four experiments. Specific mean A_{int} per 0·25 μm^2 cell surface area lower than 0·07 reflect weak expression of the antigen (I); values between 0·07 and 0·14 moderate expression (II); and values above 0·14 intense expression of the antigen (III).

Comparison of subjective and objective evaluations of the amount of peroxidase reaction product demonstrating monoclonal antibody binding to macrophages

In general, the results of detection of Mab binding to cells by peroxidase immunocytochemistry are evaluated by visual inspection. Cytophotometric quantification of the amount of peroxidase reaction product allows comparison of objective and subjective evaluation of the binding of Mab to cells.

The amount of immunoperoxidase reaction product on peritoneal and alveolar macrophages cultured for 24 hr and exposed to Mab, relative to that of control stained cells, was independently graded by three observers on the basis of a scale from 0 to +++. Comparison of these results with the corresponding medians of the specific mean A_{int} per 0·25 μm^2 cell surface area showed that weak expression of an antigen (+) corresponded with a median specific A_{int} per 0·25 μm^2 cell surface less than 0·07, moderate expression (++) with values between 0·07 and 0·14, and intense expression (+++) with values more than 0·14 (Table 1). The present results show good agreement between the subjective and objective determination of the percentage of peritoneal and alveolar macrophages in 24-hr cultures showing moderate to intense expression of a given antigen. However, in the case of weakly expressed antigens, considerable differences were found in the percentages of antigen-positive cells determined with cytophotometry and visual inspection and among the three observers (Table 1).

On the basis of these results, quantification of monoclonal antibody binding to cells will be described in the following sections as weak, moderate, and intense expression of an antigen.

Table 1. Comparison of the subjective and objective evaluation of the amount of the peroxidase reaction product on macrophages

Mab	Type of macrophage	Observer (% score)* 1		2		3		Cytometry % specific A_{int} per 0·25 μm^2 cell surface area† $\times 10^{-3}$	
F4/80	Peritoneal	100	+++	100	+++	100	+++	100	276
	Alveolar	100	+++	100	+++	100	+++	100	254
M1/70	Peritoneal	100	+++	99	+++	100	+++	100	412
	Alveolar	43	+	81	+	36	++	60	68
2.4.G.2.	Peritoneal	88	++	91	+++	96	++	97	145
	Alveolar	7	+	0	0	28	+	85	46
M5/114	Peritoneal	0	0	0	0	4	+	11	29
	Alveolar	23	+	16	+	33	+	57	46
30.G.12	Peritoneal	98	+++	100	+++	100	+++	100	212
	Alveolar	96	+++	93	+++	100	+++	90	371
M3/38	Peritoneal	0	0	7	+	0	0	5	14
	Alveolar	100	++	98	+++	100	+++	100	155
M3/84	Peritoneal	0	0	0	0	6	+	11	38
	Alveolar	51	+	43	+	24	+	69	63

* The amount of peroxidase reaction product on cells exposed to Mab exceeding that on cells exposed to plasmacytoma supernate was graded from 0 (no expression of antigen), + (weak expression of antigen), to +++ (intense expression of antigen).

† The results are expressed as the percentage of cells expressing a certain cell antigen and the median of the mean specific A_{int} per 0·25 μm^2 cell surface area of 100 mononuclear phagocytes from three to four experiments.

Antibody F4/80

Mab F4/80 detects an antigen present exclusively on mononuclear phagocytes (Austyn & Gordon, 1981). Irrespective of the duration of culture, expression of the F4/80 antigen by almost all blood monocytes and alveolar and peritoneal macrophages was intense, by about 80% of the spleen and pulmonary tissue macrophages was moderate, and by about 60% of the Kupffer cells and skin macrophages was weak (Figs 1 and 2).

If we compare the binding of Mab F4/80 to macrophages with that to identically cultured monocytes, the level of binding of Mab F4/80 to spleen and skin macrophages in 24-hr cultures was considerably higher ($P < 0{\cdot}001$) (Fig. 1). After 48 hr of culture, binding of Mab F4/80 to Kupffer cells and pulmonary tissue macrophages was significantly lower ($P < 0{\cdot}001$) and that to peritoneal macrophages considerably higher ($P < 0{\cdot}001$) than the binding to monocytes (Fig. 2).

Antibody M1/70

Mab M1/70 reacts with the complement receptor III (CR III) on mononuclear phagocytes, polymorphonuclear phagocytes, and natural killer cells (Beller, Springer & Schreiber, 1982). Irrespective of the duration of culture, Mab M1/70 labelled virtually all monocytes, spleen macrophages, and pulmonary tissue and peritoneal macrophages intensely, about half of the Kupffer cells and skin macrophages moderately, and 60% of the alveolar macrophages weakly.

Compared to 24-hr cultures of monocytes, binding of this antibody to spleen macrophages was about the same, that to alveolar and skin macrophages significantly lower ($P < 0{\cdot}001$), and to peritoneal macrophages considerably higher ($P < 0{\cdot}001$). After 48 hr of culture, binding of Mab M1/70 to Kupffer cells was significantly lower ($P < 0{\cdot}001$), to pulmonary tissue macrophages about the same as for monocytes, and to peritoneal macrophages significantly higher ($P < 0{\cdot}001$).

Antibody 2.4.G.2.

Mab 2.4.G.2. is directed against the Fc receptor II (FcR II) on mononuclear phagocytes and lymphocytes (Unkeless, 1979). Irrespective of the duration of culture, expression of FcR II by about 75% of the monocytes and spleen and peritoneal macrophages was moderate, and by the other macrophages weak.

In 24-hr cultures, Mab 2.4.G.2. binding to peritoneal macrophages was significantly higher ($P < 0{\cdot}001$) and to the other tissue macrophages considerably lower ($P < 0{\cdot}001$) than that to monocytes. After 48 hr of culture, binding of this Mab to peritoneal macrophages was about the same as for monocytes and to Kupffer cells and pulmonary tissue macrophages significantly lower ($P < 0{\cdot}001$).

Antibody M5/114

Mab M5/114 reacts with an epitope on $Ia^{b,d,q}$ $Ie^{b,d}$ molecules (Bhattacharya *et al.*, 1981). The Ia antigen was moderately expressed by about 50–60% of the spleen macrophages, Kupffer cells, and pulmonary tissue and skin macrophages, and weakly

by 25–50% of the monocytes and alveolar and peritoneal macrophages. Only spleen macrophages bound significantly more ($P < 0{\cdot}001$) Mab M5/114 than monocytes did.

Antibodies 30.G.12, M3/38, and M3/84

These Mabs are considered together because they react with a broad spectrum of cells: Mab 30.G.12 is directed against the common leucocyte antigen (Ledbetter & Herzenberg, 1979), Mab M3/38 against the Mac-2 and Mab M3/84 against the Mac-3 antigen (Ho & Springer, 1983a, b). Irrespective of the duration of culture, more than 70% of all mononuclear phagocytes expressed the common leucocyte antigen intensely, except Kupffer cells and skin macrophages, for which expression was moderate. Expression of the Mac-2 and Mac-3 antigens by almost all pulmonary tissue and alveolar macrophages was intense to moderate and that by 60–80% of the other mononuclear phagocytes was moderate, the only exception being weak expression of these antigens by about a fourth of the peritoneal macrophages.

Compared to monocytes cultured for 24 hr, binding of Mab 30.G.12 to spleen and peritoneal macrophages was about the same, that to skin macrophages considerably lower ($P < 0{\cdot}001$), and that to alveolar macrophages significantly higher ($P < 0{\cdot}001$). Binding of Mab M3/38 to spleen macrophages and alveolar and skin macrophages was significantly higher ($P < 0{\cdot}001$) and to peritoneal macrophages considerably lower ($P < 0{\cdot}001$) than binding to monocytes. Furthermore, there were no significant differences between the binding of Mab M3/84 to tissue macrophages and monocytes. In 48-hr cultures, binding of Mab 30.G.12 to Kupffer cells and peritoneal macrophages was considerably lower ($P < 0{\cdot}001$) and to pulmonary tissue macrophages significantly higher ($P < 0{\cdot}001$) than that to monocytes. Binding of Mabs M3/38 and M3/84 to Kupffer cells and pulmonary tissue macrophages was about the same as that to monocytes, and that to peritoneal macrophages considerably lower ($P < 0{\cdot}001$).

DISCUSSION

The main conclusion to be drawn from the results of the present study is that the expression of antigens on the surface of monocytes and macrophages from different sites does not follow a strict pattern. Both the percentages of antigen-positive cells and the relative amounts of Mab bound to cells show distinct differences between monocytes and the various types of macrophage.

In this report, binding of Mab to cultured monocytes and macrophages is expressed as the specific mean A_{int} per 0·25 μm^2 cell surface area. The relative amount of Mab bound per cell was calculated from the median specific mean A_{int} per 0·25 μm^2 cell surface area and the cell size (data not shown). Comparison of these data did not show any distinct pattern in the expression of cell-surface antigens. Thus it may be concluded that during differentiation of blood monocytes into tissue macrophages the expression of cell surface antigens does not follow a clearly defined pattern.

However, it must be taken into account that during *in vitro* culture of the cells for 24 hr or 48 hr, the expression of antigens by cells may change, and this would obscure patterns of antigen change during differentiation of monocytes into macrophages *in vivo*. Comparison of the Mab binding patterns of monocytes and alveolar and peritoneal macrophages cultured for 24 hr and 48 hr with those of similar cells immediately after the isolation procedure (Nibbering *et al.*, 1987) showed that the expression of all of the antigens under study, except the Ia antigen (Mab M5/114), altered during culture. During the first 24 hr of culture, binding of Mabs F4/80, M1/70, 2.4.G.2., and 30.G.12 to blood monocytes and peritoneal macrophages increased significantly ($P < 0{\cdot}007$) and during the second 24 hr of culture dropped to the initial level ($P < 0{\cdot}007$). The binding of Mabs M3/38 and M3/84 to these mononuclear phagocytes decreased significantly ($P < 0{\cdot}001$) or remained unchanged during culture for 48 hr. Further culture for up to 168 hr was accompanied by decreasing expression of all antigens, except a significantly higher ($P < 0{\cdot}001$) expression of Mac-2 antigen (Mab M3/38) by peritoneal macrophages (Nibbering *et al.*, 1987). Other authors have described the disappearance of Ia antigens on the surface of macrophages during culture (Shook & Niederhuber, 1981; Calamai, Beller & Unanue, 1982).

Another point to be considered is that the macrophages in an organ or body cavity might not be a homogeneous population of cells, since various stimuli in the local micro-environments within an organ might have different effects. This indeed seems to be the case because the distribution profiles of the mean specific A_{int} per 0·25 μm^2 cell-surface area for all Mab were not normal.

We expected that a given cell antigen would follow a given pattern during differentiation of mononuclear phagocytes from the immature stages (monoblasts and promonocytes) to the mature macrophages. However, from the present results and those of an earlier study performed with the same quantitative cytochemical method and Mab (Nibbering *et al.*, 1987) it is clear that regulation of the expression of cell surface antigens on mononuclear phagocytes during differentiation is very complex. For instance, expression of antigen F4/80 was weak to moderate for almost all of the bone marrow mononuclear phagocytes, moderate to intense for more than 90% of the blood monocytes, weak for 60% of the Kupffer cells and skin macrophages, moderate for about 80% of the spleen and pulmonary tissue macrophages, and intense for virtually all alveolar and peritoneal macrophages. The expression of other antigens, however, shows a completely different picture. In this respect it is of interest that at the cell-surface level the peritoneal macrophage, which is widely used for the study of phenotypical and functional characteristics of macrophages, is not a representative example of tissue macrophages.

An important aspect of the present study is the finding that subjective evaluation of the amount of immunoperoxidase reaction product based on visual inspection and the objective results obtained by cytophotometry are in excellent agreement except when the antigen is weakly expressed. Apparently it is difficult to differentiate by eye between weakly antigen-positive cells and antigen-negative cells showing background staining due to non-specific reactions. Therefore, in most, but not all, cases subjective evaluation to determine the percentage of antigen-positive cells, detected with peroxidase immunocytochemistry, is reliable. However, objective evaluation with cytophotometry has the advantage that the binding of Mab to the various types of cells can be quantitatively compared and statistically analysed.

ACKNOWLEDGMENTS

The authors thank Ms Th.L. van Zwet and Ms G. A. van der Heide for technical assistance and Ms E. M. Loovens for typing the manuscript. This work was supported by the Netherlands Asthma Foundation.

REFERENCES

AUSTYN J.M. & GORDON S. (1981) F4/80, a monoclonal antibody directed specifically against the mouse macrophage. *Eur. J. Immunol.* **11,** 805.

BELLER D.I., SPRINGER T.A. & SCHREIBER R.D. (1982) Anti-Mac-1 selectively inhibits the mouse and human type three complement receptor. *J. exp. Med.* **156,** 1000.

BHATTACHARYA A., DORF M.E. & SPRINGER T.A. (1981) A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: evidence for I region duplication. *J. Immunol.* **127,** 2488.

BLUSSÉ VAN OUD ALBLAS A. & VAN FURTH R. (1979) Origin, kinetics, and characteristics of pulmonary macrophages in the normal steady state. *J. exp. Med.* **149,** 1504.

CALAMAI E.T., BELLER D.I. & UNANUE E.R. (1982) Regulation of macrophage populations. IV. Modulation of Ia expression by bone marrow derived macrophages. *J. Immunol.* **128,** 1692.

CROFTON R.W., DIESSELHOFF-DEN DULK M.M.C. & VAN FURTH R. (1978) The origin, kinetics and characteristics of the Kupffer cells in the normal steady state. *J. exp. Med.* **148,** 1.

VAN FURTH R. & COHN Z.A. (1968) The origin and kinetics of mononuclear phagocytes. *J. exp. Med.* **128,** 415.

VAN FURTH R., DIESSELHOFF-DEN DULK M.M.C., RAEBURN J.A., VAN ZWET TH.L., CROFTON R.W. & BLUSSÉ VAN OUD ALBLAS A. (1980) Characteristics, origin and kinetics of human and murine mononuclear phagocytes. In: *Mononuclear Phagocytes. Functional Aspects* (ed. R. van Furth), p. 279. Martinus Nijhoff Publishers, The Hague, Boston, London.

VAN FURTH R., NIBBERING P.H., VAN DISSEL J.T. & DIESSELHOFF-DEN DULK M.M.C. (1985) The characterization, origin, and kinetics of skin macrophages during inflammation. *J. invest. Dermatol.* **85,** 398.

GORDON S., CROCKER P.R., MORRIS L., LEE S.H., PERRY V.H. & HUME D.A. (1986) Localization and function of tissue macrophages. In: *Biochemistry of Macrophages* Ciba Foundation Symposium 118, p. 54. Pitman Publishing Ltd., London.

HO M.K. & SPRINGER T.A. (1983a) Mac-2, a novel 32,000 Mr macrophage subpopulation-specific antigen defined by monoclonal antibody. *J. Immunol.* **128,** 1221.

HO M.K. & SPRINGER T.A. (1983b) Tissue distribution, structural characterization, and biosynthesis of Mac-3, a macrophage surface glycoprotein exhibiting molecular weight heterogeneity. *J. biol. Chem.* **258,** 636.

LEDBETTER J.A. & HERZENBERG, L.A. (1979) Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* **47,** 63.

NIBBERING P.H., LEIJH P.C.J. & VAN FURTH R. (1985) A cytophotometric method to quantitate the binding of monoclonal antibodies to individual cells. *J. Histochem. Cytochem.* **33,** 53.

NIBBERING P.H., LEIJH P.C.J. & VAN FURTH R. (1987) Quantitative immunocytochemical characterization of mononuclear phagocytes. I. Monoblasts, promonocytes, monocytes, and peritoneal and alveolar macrophages. *Cell. Immunol.* **105,** 374.

NIBBERING P.H., VAN FURTH R. (1987). Quantitative study of peroxidase immunocytochemical reactions demonstrating monoclonal antibody binding to antigens immobilized on nitrocellulose. *Histochem. Cytochem* (in press).

NUSSENZWEIG M.C., STEINMAN R.M., UNKELESS J.C., WITMER M.D., GUTCHNIKOV B. & COHN Z.A. (1981) Studies of the cell surface of mouse dendritic cells and other leucocytes. *J. exp. Med.* **154,** 168.

VAN DER PLOEG M., VAN DEN BROEK K. SMEULDERS A.W.M., VOSSEPOEL A.M. & VAN DUIJN P. (1977) HIDACSYS: Computer programs for interactive scanning cytophotometry. *Histochemistry,* **54,** 273.

SHOOK L.B. & NIEDERHUBER J.E. (1981) Induction of an *in vitro* antibody response using Ia positive bone marrow derived macrophages. In: *Heterogeneity of Mononuclear Phagocytes* (ed. O. Forster and M. Landy), p. 208. Academic Press, New York.

SPRINGER T.A., GALFRÉ G., SECHER D.S. & MILSTIEN C. (1979) Mac-1: a differentiation antigen identified by a monoclonal antibody. *Eur. J. Immunol.* **9,** 301.

UNKELESS J.C. (1979) Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. *J. exp. Med.* **150,** 580.