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Cell surface antigens on the guinea-pig macrophage: identification by monoclonal antibodies and association with the activation state

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Summary. Ten monoclonal antibodies (moAb) directed against cell surface antigens of guinea-pig monocytes and macrophages (mph) were produced and characterized. The corresponding antigens are not present on granulocytes, T lymphocytes, an Ia-positive B-cell line or other haematopoietic cells. In binding or cytotoxicity assays, the moAb demonstrated characteristic patterns of reactivity, with mph being in different stages of differentiation or activation. Three moAb (342, 322, 249) recognized 'lineage antigens' (i.e. antigens continuously expressed during maturation of monocytes to mph and after stimulation or activation of the cells). MoAb 342 possibly defines a major cell surface determinant, being present on 90% of mph. The antigens detected by moAb 305, 320, 321 and 344 characterize mature mph. They were not expressed on monocytes, but were expressed on the majority of resident, elicited or activated peritoneal mph. MoAb 253, 310 or 257 defined discrete subpopulations of elicited and—with the exception of moAb 257—activated mph. The corresponding antigens were not present on monocytes or resident mph, but appeared on the cell surface during *in vivo* or *in vitro* stimulation of the cells. There was no indication of a contribution of the moAb-defined antigens to the presentation of antigen, mitogen or alloantigen by the mph to T cells. The functional significance of the antigens thus remains to be elucidated. Our studies indicate that cells committed to the monocyte/mph lineage share a family of differentiation antigens, distinguishing them from other cell lines. The moAb provide useful tools for further investigation of the activation of mph and allow the rapid detection of mph in different tissues.

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Abbreviations: Ab, antibody; Con A, concanavalin A; FACS, fluorescence-activated cell sorter; [^{3}H]TdR, tritiated thymidine; Ia, I-region associated; LNL, column-purified T lymphocytes from lymph nodes; MLR, mixed leucocyte reaction; moAb, monoclonal antibody; mph, macrophages; OVA, chicken albumin; PAF, platelet activating factor; PEC, peritoneal exudate cells; PMA, phorbol myristate acetate; PPD, purified protein derivative of tuberculin.

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INTRODUCTION

Mononuclear phagocytes form a particular line of myeloid differentiation. Haematopoietic precursors proliferate and differentiate into monocytes; these are distributed via the blood stream and develop into mature macrophages (mph) in the periphery. Mph display heterogeneous properties, depending on their particular site and adaptation to local inflammatory and microbial agents (van Furth, 1980). In all likeli-

hood, this process of differentiation is accompanied by qualitative and quantitative variations in the expression of cell surface molecules, as conclusively demonstrated during maturation of T lymphocytes (Reinherz & Schlossman, 1980).

For the identification and characterization of individual cell surface antigens, monoclonal antibodies (moAb) are useful tools, allowing the analysis of the functional role(s) of the cells or the delineation of their pathway of differentiation. MoAb recognizing antigens on mouse mph or human monocytes have been described recently (Todd & Schlossman, 1983). Only one moAb with specificity for guinea-pig mph has been raised (Mathew *et al.*, 1983). This Ab apparently recognizes an antigen expressed on mph independent of their state of differentiation. To our knowledge, moAb defining differentiation antigens or subpopulations of guinea-pig mph are still lacking.

In this communication, we describe ten moAb, each specifically reacting with guinea-pig mph, and some of them also reacting with blood-derived monocytes. The qualitative and quantitative expression of the distinct antigens is examined on mph derived from different anatomical sites or at different stages of activation. A possible functional role of these antigens during stimulation of T cells by mph is evaluated.

MATERIALS AND METHODS

Animals

BALB/c mice were purchased from the Zentral Institut für Versuchstierkunde, Hannover, FRG. Inbred strain 2 and strain 13 guinea-pigs were originally obtained from the Division of Research Services, National Institutes of Health (NIH), Bethesda, MD, and were then bred at the Institute of Medical Microbiology, Mainz, FRG.

Cell lines

The non-secretor mouse myeloma line P3-NS1/Ag 4-1 (NS-1) was used for fusion. The Ia-bearing guinea-pig B-cell leukaemia, EN-L2C, was serially transplanted in strain 2 guinea-pigs and obtained from the peripheral blood of moribund animals (Forni, Shevach & Green, 1976). The leukaemic cells were separated from the erythrocytes as previously described (Clement *et al.*, 1980).

Media

Media for maintenance of the NS-1 line and for cultivation of cells after fusion were prepared as described (Burger *et al.*, 1981). For antigen- or mitogen-induced proliferation of antigen-primed T cells, or for alloantigen-induced T-cell proliferation, RPMI-1640 medium, supplemented with L-glutamine (2 mM), penicillin/streptomycin (50 IU/ml; 50 μg/ml) and 5-fluorocytosine (1 μg/ml) was used. Ten per cent heat-inactivated FCS or 5% normal guinea-pig serum (NGpS) was added to the medium. The *in vitro* stimulation of resident mph was performed in RPMI-1640 medium supplemented with L-glutamine, penicillin/streptomycin and 2% FCS. Platelet activating factor (PAF), (1-*o*-octadecyl-2-acetyl-sn-glycero-3-phosphorylcholine), kindly provided by Dr H. Betzing (Nattermann & Cie, Cologne, FRG), was added at a final concentration of 10^{-6} M. Phorbol myristate acetate (PMA) (Sigma-Chemie, München, FRG) was used at a final concentration of 10^{-8} M. If not otherwise indicated, media and supplements were obtained from Boehringer, Mannheim, FRG or Flow Laboratories, Meckenheim, FRG.

Identification of mph

The percentage of mph or monocytes in different cell preparations was determined by staining for α-naphtyl butyrate esterase (non-specific esterase, NSE) (Tucker, Pierre & Jordon, 1977) and by Wright-Giemsa morphology. In addition, phagocytosis of latex beads was used for characterization of peritoneal mph.

Cell preparations

Peritoneal exudate cells (PEC). Non-stimulated (i.e. resident peritoneal cells) were collected from untreated animals by lavage of the peritoneal cavity using Hanks' balanced salt solution (HBSS) (Gibco, Karlsruhe, FRG) containing 5 IU/ml heparin (Nordmark, Hamburg, FRG). Oil-elicted PEC were obtained by injecting guinea-pigs intraperitoneally with 25 ml of sterile mineral oil (EGA-Chemie, Steinheim, FRG) and harvesting the cells 3–4 days later. In order to yield activated PEC, guinea-pigs were immunized with BCG-vaccine (Behringwerke, Marburg, FRG), followed by PPD injection as described (Hopper & Geczy, 1980). The PEC preparations consisted of 60–70% mph, 15–20% granulocytes and 15–20% lymphocytes independently from *in vivo* stimulation or activation. PEC were used as a source of mph in most tests, and if not otherwise indicated are designated as mph. Cell viability was estimated by trypan blue dye exclusion and showed > 95% viability

by this criterion. For the immunization of BALB/c mice and for cytotoxicity assays, mph were purified from oil-elicited PEC by adherence to plastic dishes. Adherent cells were removed by incubation for 30 min with cold (4°) PBS containing 0·5 mM EDTA, followed by gently scraping with a rubber policeman. The adherent cells consisted of 85–95% mph. Trypan blue dye was excluded by 80–90% of the adherent cells.

Peripheral blood mononuclear cells (PBMC) and granulocytes. PBMC were isolated from guinea-pig heart blood by Ficoll–Hypaque density gradient centrifugation (Pollack, Brandhorst & Hanna, 1981). Between 12 and 15% of PBMC were monocytes, as defined by positive staining for NSE. Granulocytes were obtained from the pellet formed during the density gradient centrifugation or from the peritoneal cavity after injection of 2% starch solution.

Thymocytes. Guinea-pig thymus was obtained from 8-week-old animals. Suspensions of thymocytes were prepared in the cold by mincing thymus tissue and passing it gently through a stainless steel mesh.

Thrombocytes and erythrocytes. Thrombocytes were isolated from guinea-pig heart blood by centrifugation at 420 *g* as described (Meuer *et al.*, 1982). Erythrocytes were obtained from the pellet.

Lymph node T lymphocytes (LNL). LNL were obtained from guinea-pigs immunized with soluble protein antigens, i.e. chicken albumin (OVA) (Sigma, Taufkirchen, FRG) or PPD (Behringwerke, Frankfurt, FRG) as described (Burger & Shevach, 1980). For further depletion of remaining mph, LNL were treated with the monoclonal anti-Ia Ab 22C4 (Burger *et al.*, 1981; Burger & Shevach, 1980) at a dilution of 1:5000 and rabbit complement (final dilution 1:10). After this procedure, no proliferation of LNL in response to soluble antigens was observed, unless mph were added.

Production of monoclonal antibody

Female BALB/c mice were immunized with oil-elicited, adherence-purified guinea-pig mph. Different immunization schedules were applied as indicated in Table 1. Three to four days after the final injection of mph, a spleen cell suspension of an immunized mouse was fused with NS-1 mouse myeloma cells at a ratio of 1:1. The fusion was performed according to standard methods (Fazekas, de St Groth & Scheidegger, 1980). Supernatants of growing hybrids were tested 10–14 days after fusion using indirect radioimmunoassay (RIA). Binding to oil-elicited PEC (60–70% mph), thymocytes and B cells (Ia-positive EN-L2C B-cell leukaemia line) was assessed. Hybrids which secreted Ab with selective reactivity for mph were cloned by limiting dilution. Cloning was performed in HT medium with 20% FCS and mouse thymocytes as feeder cells (Burger *et al.*, 1981; Mishell & Shigii, 1980). Ascites were produced with selected clones by injecting cells i.p. into BALB/c mice primed with pristane, and ascitic fluid was used as the source of moAb in the following tests.

Indirect radioimmunoassay (RIA). Binding of Ab to the cells was detected using ^{125}I-labelled rabbit F(ab′)$_2$ anti-mouse Ig (Burger *et al.*, 1981). For the detection of Ab reactive with thrombocytes, the described procedure was slightly changed. Thrombocytes were resuspended in Tyrode solution with 30 mM EDTA at a cell number of 10^9/ml. Six-hundred μl of the cell suspension were mixed with 200 μl ascites dilution. After incubation for 1 hr, the cells were washed four times with Tyrode solution plus 30 mM EDTA, and were then incubated for 1 hr with radioiodinated second-stage Ab. The thrombocytes were then washed twice with RIA buffer and the radioactivity was counted. Different controls were employed to ensure the specific binding of moAb contained in culture supernatants or ascites fluid. HAT medium supplemented with 20% FCS was used as a negative control for culture supernatants. Non-specific effects of the ascitic fluid were excluded by control ascites produced with a hybrid line, which secreted an IgM Ab directed to inulin in addition to the parental (IgG1, K) Ab of the P3X63 Ag8 myeloma line. Additionally, control ascitic fluids containing IgG2a Ab, directed against an irrelevant bacterial protein, were employed.

Complement-mediated lysis of mph. Oil-elicited, adherence-purified mph were labelled by ^{51}Cr-chromate (New England Nuclear, Dreieich, FRG) and used as target cells in the cytotoxic assay. The assay was performed as previously described (Shevach, Rosenstreich & Green, 1973), except that rabbit complement (1/10 final dilution) which had been extensively absorbed with guinea-pig lymph node and spleen cells was used in place of guinea-pig complement. For indirect cytotoxicity, cells were incubated firstly with ascites or control ascites. The cells were then washed twice, and 1:40 diluted rabbit anti-mouse

Ig (as facilitating antibody) was added at the second stage. After two more washes, cells were incubated with rabbit complement and the radioactivity was counted.

Fluorescent-activated cell sorter (FACS) analysis. Binding of moAb to peritoneal mph or monocytes was quantified by indirect immunofluorescence using fluorescein isothiocynate (FITC)-conjugated rabbit $F(ab')_2$ anti-mouse Ig (Dynatech, Denkendorf, FRG). Controls were incubated with different control ascites and FITC conjugate. Labelled cells were analysed on a Becton-Dickinson FACS IV. Detailed methodology and instrument settings have been described previously (Mauer *et al.*, 1984). Analysis was performed on unfractionated PEC (60–70% mph) and on PBMC (12–15% monocytes) without gating mph, because of a substantial overlap between the mph and the lymphocyte peak in the low-angle light scatter profile. As staining of PEC represented binding of moAb to mph only, data are expressed as percent fluorescent mph. One-hundred percent mph fluorescent corresponds to about 70% PEC-staining, reflecting the percentage of mph in the individual PEC population.

Proliferative response of immune T lymphocytes to soluble protein antigens or mitogen. Mitomycin *c*-treated, oil-elicited PEC (1×10^5) were incubated in round-bottomed microtitre plates (Dynatech) with syngeneic lymph node T lymphocytes (2×10^5) in 0·2 ml RPMI medium supplemented with 10% FCS (Burger & Shevach, 1980). LNL were isolated from immunized guinea-pigs as described above. The cells were incubated in the presence of OVA (50 μg/ml), PPD (25 μg/ml) or Con A (1 μg/ml) (Pharmacia, Freiburg, FRG). MoAb (ascites fluid at a final dilution of 1:200) was either present from the start of the cultures or, in another series of experiments, oil-elicited and mitomycin *c*-treated PEC were depleted of mph by treatment with cytotoxic moAb (ascites fluid 1:200 final dilution) and complement (1:10 final dilution) prior to incubation with antigen or mitogen and LNL. In parallel, irrelevant control ascites was used in the corresponding controls. After culture for 3 days, 0·5 μCi of tritiated thymidine ([^{3}H]TdR) (New England Nuclear) was added to each well. Cells were harvested 3 days later, and the amount of radioactivity incorporated into cellular DNA was measured. The results of triplicate cultures are expressed as total counts per minute/culture. The SEM of triplicate samples was never greater than 10% of the mean and, for simplicity, only the mean of triplicate samples is reported.

Mixed leucocyte reaction (MLR). The MLR was performed as described (Burger & Shevach, 1980), except that LNL were purified by passage over a nylon-wool column. MoAb was added at the start of the cultures or was used to deplete antigen-bearing mph. [^{3}H]TdR incorporation was measured as described above.

RESULTS

Production of monoclonal antibodies and analysis of their reactivity pattern

Three fusions were carried out: for each fusion, mice were immunized with oil-induced, adherence-purified mph, applying different immunization schedules (Table 1). The culture supernatants of growing hybrids were examined in an indirect RIA for binding to mph, B cells and thymocytes. Hybridoma cultures containing Ab reactive with mph, but negative for thymocytes or B cells, were selected for further cultivation. By using the Ia-positive EN-L2C B-cell leukaemia line, a reactivity of the moAb for Ia antigens was excluded.

As shown in Table 1, a high proportion of cultures obtained from fusion 101 reacted with B cells. In order to reduce this reactivity, we attempted to induce tolerance against B cells in newborn mice (fusion 105). The animals were injected with EN-L2C cells within 24 hr of birth. Compared to fusion 101, this procedure led to a three-fold increase in the percentage of cultures recognizing mph antigens only. Fusion 110 was carried out to yield IgM Ab preferentially. After a single injection of mph, a few hybrids were obtained which secreted Ab selectively reacting with mph. The hybrid-derived Ab, as expected, all belonged to the IgM isotype.

Ten moAb were chosen for further characterization because of their strong binding to mph. Eight of these moAb did not react with granulocytes, erythrocytes, lymph node T lymphocytes and thrombocytes, as demonstrated for three of the Ab in Fig. 1. A non-specific binding of moAb to the different cell populations could be excluded by employing control ascites of different specificities. Two moAb (249, 253), which showed no reactivity with all other guinea-pig cell types tested so far, bound to mph and thrombocytes. The isotypes of the moAb were defined by

Table 1. Reactivity of culture supernatants obtained from three fusions in the initial screening test

Fusion	101	105	110
Tolerance-induction	–	2×10^7 B cells i.p.	–
Immunization	2×10^7 mph i.p. Day 1 2×10^7 mph i.p. Day 15 1×10^7 mph i.v. Day 30	1×10^7 mph i.p. Day 1 1×10^7 mph i.p. Day 15	2×10^7 mph i.p.
Hybrids obtained	272	177	180
Positive* B cells/thymocytes	208 (76·5%)	79 (44·6%)	35 (19·0%)
mph	232 (85·2%)	125 (70·6%)	10 (5·5%)
mph only	26 (9·5%)	46 (26·0%)	6 (3·3%)

* The supernatants of hybrids obtained after different fusions were examined for binding to B cells (Ia-positive EN-L2C B-cell leukaemia line), thymocytes and mph (oil-elicited PEC) using the indirect RIA. The number of superantants reactive with these cell types is additionally expressed as percentage of hybrids grown after fusion.

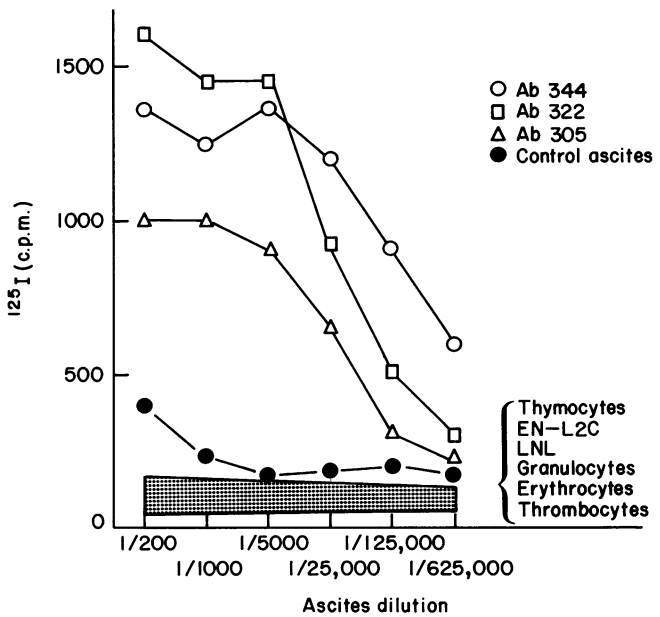

Figure 1. Specific binding of moAb 305, 322 and 344 to oil-elicited macrophages. Oil-elicited PEC were incubated with serial dilutions of moAb (ascites fluid) or control ascites. Bound Ab was detected with ^{125}I-labelled F(ab')$_2$ anti-mouse Ig. The shaded area represents the range of binding of the moAb with cell types other than mph, as indicated in the figure.

specific anti-mouse Ig reagents in an Ouchterlony analysis. Ab 249, 253, 322, 320 and 257 were IgG1; Ab 321, 344 and 342 belonged to the IgM subclass, and Ab 305 and 310 proved to be of the IgG2b isotype.

Complement-mediated lysis of macrophages

MoAb 321, 342 and 344 (IgM isotype) lysed high numbers of mph, up to an ascites dilution of 10^{-3} in a ^{51}Cr-release assay. The percentage of mph lysed by one moAb varied between different experiments, ranging from 68 to 98% (Ab 321 and 342, respectively) and from 57 to 86% (Ab 344). These variations in cytotoxicity might reflect a heterogeneity of antigen expression within the peritoneal mph population.

In contrast to the moAb of IgM subclass, none of the IgG Ab were cytotoxic for mph, even in an indirect cytotoxicity assay.

Expression of antigens on blood-derived monocytes and peritoneal macrophages stimulated *in vivo* by different agents

We examined the reaction of the moAb with non-stimulated (i.e. resident), inflammatory (i.e. oil-elicited) or immunologically activated peritoneal mph using the indirect RIA (data not shown) and indirect immunofluorescence staining followed by FACS analysis. As shown in Table 2, and as an example for two moAb (Ab 342, 257) in Fig. 2, the cellular distribution of the antigens defined by the moAb was quite different, and additionally depended on the activation stage of the mph. MoAb 342 recognized a membrane determinant present on all resident, elicited and activated mph. Similar to Ab342, moAb 305, 344 and 321 reacted with a high percentage of resident and stimulated mph. In contrast to these Ab, moAb 253, 257 and 310 did not react with resident mph. The corresponding antigens were expressed on discrete subpopulations of oil-elicited and—with the exception of Ab 257—on activated mph. MoAb 257 defined a cell surface antigen present only on inflammatory mph. Applying the indirect RIA, we found that moAb 320 and 322 bound strongly to mph, independent of their state of activation.

Out of the ten moAb, only three (namely moAb 249, 322 and 342) reacted with monocytes. These Ab stained between 10 and 17% cells of PBMC when subjected to FACS analysis; in the control, 1·5% cells were labelled.

Table 2. Expression of antigens on peritoneal macrophages stimulated *in vivo*

Ab	Percentage of antigen-bearing mph		
	Resident	Oil-elicited	Activated
342	98–100	96–100	94–100
305	95–100	60–64	98–100
344	48–54	92–96	96–100
321	38–45	74–82	88–94
253	2–4	18–20	7–10
310	4–6	17–22	4–8
257	2–4	38–45	2–3
249	ND*	6–12	ND*
Control	2–4	2–5	1–4

PEC were stained with saturating amount of moAb by indirect immunofluorescence (see Fig. 2). Stained cells were analysed using an FACS IV. Data are expressed as percent fluorescent mph. One-hundred percent mph fluorescent corresponds to about 70% PEC-staining, reflecting the percentage of mph in the individual PEC preparation. Analysis was performed on non-stimulated (resident), oil-elicited and immunologically activated PEC.

* ND, not done.

Reaction of moAb 253, 257 and 310 with *in vitro* stimulated macrophages

Resident mph were incubated as fluid cultures with phorbol myristate acetate (PMA) or platelet activating factor (PAF) to induce mph activation *in vitro*. Subsequently, the reactivity of moAb 253, 257 and 310 with these stimulated mph was examined in the indirect RIA. As a parameter for the induction of mph activation, we examined the generation of oxidative burst products in a chemiluminescence assay. Resident mph produced high amounts of oxidative burst products when incubated with PMA (10^{-8} M) or PAF (10^{-6} M) (data not shown).

Binding of the moAb to stimulated mph was tested after various time intervals of cultivation (2, 6, 14 or 21 hr) in the presence of PMA or PAF. Cultivation for 6, 14 or 21 hr, but not for 2 hr, led to a positive reaction with moAb 253 (Fig. 4). MoAb 257 and 310 did not bind to PMA- or PAF-treated mph independent of the incubation time with the stimulus. Binding of Ab 253 to *in vitro* stimulated mph was in the same range as binding to *in vivo* oil-elicited or activated mph (Fig. 3).

In order to rule out an alteration of mph antigen expression by culturing the cells, control cultures of mph were incubated for 21 hr under identical conditions used for PMA or PAF treatment, but with the corresponding solvent only. MoAb 253 failed to bind to these control mph (Fig. 4, upper panel), demonstrating that induction of antigen only occurred in the presence of PMA or PAF. The time course of the appearance of antigen 253 differed from one experiment to the other, and also depended upon the stimulus used. Treatment of resident mph with PAF for 6 or 14 hr resulted in a positive reaction with moAb 253 (Fig. 4, lower panel). PAF treatment for 21 hr always led to a strong decrease in the expression of antigen 253.

Similar to PAF, the stimulus PMA in some experiments led to an early expression of antigen 253 on mph (6 hr, Fig. 4, upper panel). Antigen expression, however, did not decrease after prolonged cultivation with PMA.

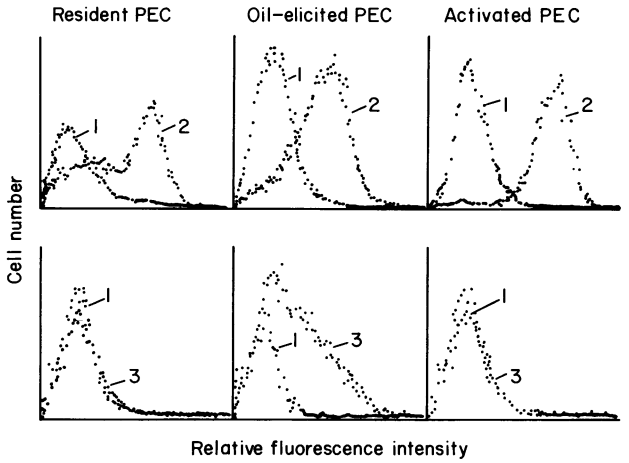

Figure 2. FACS analysis of reactivity of moAb 342 and 257 with resident, oil-elicited and activated PEC. PEC were incubated with control ascites (1), Ab 342 (2, upper panel) or Ab 257 (3, lower panel) (ascites fluid 1:200) and stained with FITC-conjugated rabbit $F(ab')_2$ anti-mouse Ig; 2×10^4 cells were analysed, the fluorescence intensity is linearly displayed. Ab 257 did not react with resident or activated PEC, and staining therefore did not exceed the background fluorescence obtained with control ascites.

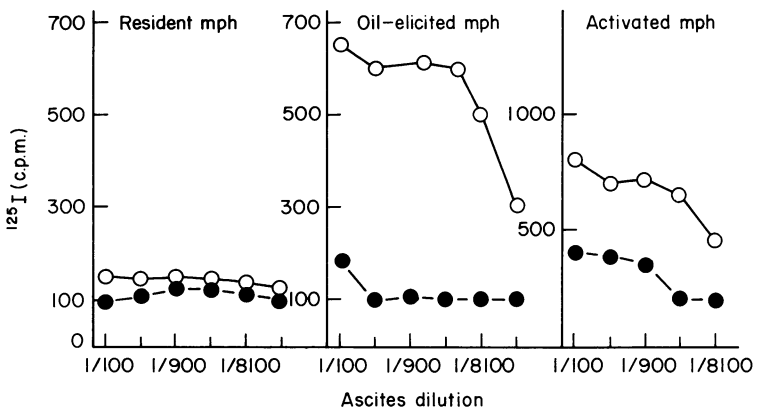

Figure 3. Reaction of moAb 253 with *in vivo* stimulated macrophages. Non-stimulated, oil-elicited or immunologically activated PEC were incubated with serial dilutions of Ab 253 (ascites fluid) (○——○) or control ascites (●——●). Bound Ab was detected using radioiodinated second-stage Ab (see Fig. 1).

Influence of the monoclonal antibodies on antigen- or alloantigen-induced T-cell proliferation

A first series of experiments was performed to test a direct involvement of the corresponding membrane determinants in antigen presentation to T cells. Mph were incubated with antigen (OVA, PPD) and syngeneic T cells or, alternatively, with allogeneic T cells (MLR) in the continuous presence of moAb. The presence of the moAb, however, had no influence on antigen- or on alloantigen-stimulated T-cell proliferation, even when Ab was added at a relatively high concentration (1:200 final dilution of ascites fluid) and was present during the total culture period (data not shown).

We then investigated the effect of depletion of antigen-bearing mph on the proliferative response of T cells. Oil-induced PEC (60–70% mph) were incubated

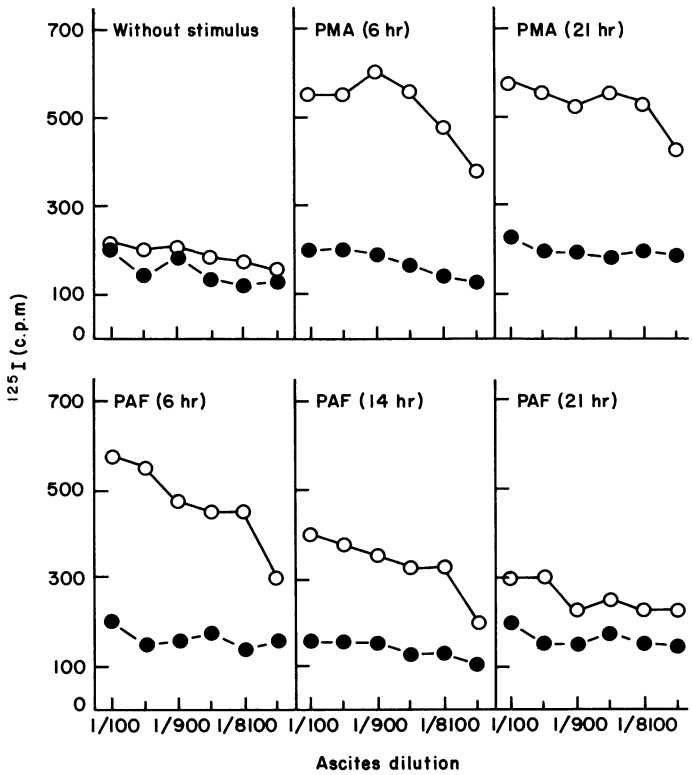

Figure 4. Binding of moAb 253 to PMA- or PAF-stimulated macrophages. Resident PEC were incubated with PMA (10^{-8} M) (upper panel) or with PAF (10^{-6} M) (lower panel) for the time intervals indicated in the figure. Control cultures were incubated without any stimulus (upper panel). Reaction of Ab 253 (o——o) or control ascites (●——●) with stimulated or non-stimulated cells was detected with a radioiodinated second-stage Ab (see Fig. 1).

with the cytotoxic moAb 342, 321 or 344 and complement. MoAb 342 lysed 61–68% of the peritoneal exudate mph, moAb 321 lysed 40–55% mph, and moAb 344 lysed 31–42% mph, as determined by trypan blue dye exclusion. The remaining viable cells were then tested for antigen presentation in antigen-, mitogen- or alloantigen-stimulated T-cell proliferation assays. Compared to control cultures incubated with control ascites and complement, treatment with cytotoxic Ab in all experiments reduced the T-cell response. Inhibition was most prominent with moAb 342 or 321, and ranged from 40 to 80% inhibition of PPD-, OVA- or Con A-stimulated T-cell proliferation (Table 3), respectively 77–83% inhibition of MLR (Table 4).

Treatment of PEC with a mixture of two different cytotoxic Ab and complement resulted in a total abolition of the MLR-inducing capacity (Table 4) when combinations of moAb 342 and 344 or 342 and 321 were employed. These Ab lysed nearly all mph (65–70%) in PEC. In contrast, the combination of moAb 321 and 344 was less effective. As evaluated by binding inhibition experiments with antibody labelled internally with [^{3}H] leucine (data not shown), moAb 321, 342 and 344 recognized different antigenic sites on the mph.

In contrast to the T-cell response to alloantigen in the MLR, the response to OVA, PPD or Con A was not totally abrogated by pretreatment of the mph. As shown in Table 3, we only once observed an almost total inhibition of T-cell proliferation of 90%.

Table 3. Inhibition of antigen- or mitogen-induced T-cell response by pretreatment of macrophages with cytotoxic antibody and complement

Stimulus	[^{3}H]TdR incorporation c.p.m. (% inhibition)			
	Ascites			
	Control	Ab 321	Ab 342	Ab 344
OVA	6·225	1·986 (70%)	1·797 (72%)	4·463 (30%)
	18·021	10·843 (40%)	8·708 (66%)	13·100 (27%)
PPD	18·274	5·241 (72%)	3·966 (80%)	10·683 (42%)
	40·200	25·500 (37%)	20·000 (50%)	24·500 (40%)
Con A	25·011	10·105 (60%)	6·950 (73%)	16·257 (35%)
	41·132	17·100 (60%)	19·953 (53%)	24·678 (40%)
	Control	Ab 342/321*	Ab 342/344	Ab 321/344
OVA	18·320	10·463 (40%)	8·451 (66%)	ND†
	12·510	1·612 (87%)	7·005 (44%)	
PPD	40·263	20·137 (50%)	21·265 (47%)	ND
	22·213	2·445 (90%)	13·772 (38%)	
Con A	41·439	15·663 (62%)	15·543 (63%)	ND
	13·233	4·011 (70%)	2·911 (78%)	

Strain 2 LNL (2×10^5) were cultured for 3 days with strain 2 PEC (1×10^5) in the presence of OVA (50 μg/ml), PPD (25 μg/ml) or Con A (1 μg/ml). PEC were pretreated with non-cytotoxic control ascites or moAb (1:200) and complement (1:10). Inhibition is expressed as percentage of control [^{3}H]TdR incorporation (PEC treated with control ascites).

* Pretreatment of PEC with a mixture of two different moAb and complement.

† ND, not done.

DISCUSSION

In this report, ten moAb directed against surface antigens of the guinea-pig monocyte/mph lineage are characterized. The established moAb selectively reacted with mph membrane antigens, in that they did not bind to B cells, T cells, or to other haematopoietic cells, including granulocytes (Fig. 1). In addition, the moAb proved to be unreactive with connective tissue and epithelial cells of the liver, whereas Kupffer cells were intensively stained G. Ramadori, U. Mauer, U. Hadding and K.-H. Meyer zum Büschenfelde, manuscript in preparation). Two of the moAb (249, 253) cross-reacted with thrombocytes, indicating a link between the myeloid and the megakaryocyte lineage. Alternatively, both cell types might bear common membrane molecules unrelated to differentiation but signifying common functional properties. A cross-reaction with platelets has been described for two other moAb reacting with human monocytes, Ab Mo4 (Todd & Schlossman, 1982) or rat alveolar mph, AbVEP6 (Rumpold *et al.*, 1982). Antigen VEP6 exhibits similar molecular properties on mph and platelets. Functional properties of the antigens have not been reported.

The various moAb yielded characteristic patterns of reactivity with monocytes and non-stimulated, inflammatory (oil-elicited) or immunologically activated peritoneal mph. MoAb 342, 322 and 249 detected obvious lineage antigens (i.e. membrane molecules) which are continuously expressed during maturation of monocytes to mph, and are present on mph independent of their state of activation. From these moAb, Ab 342 might define a major cell surface component of mph as demonstrated by quantification of its surface expression in the FACS and by complement-mediated cell lysis.

Four moAb (305, 344, 320 and 321) were directed against antigens present on mature peritoneal mph

Table 4. Inhibition of the MLR by pretreatment of macrophages with cytotoxic antibody and complement

	[^{3}H]TdR incorporation (c.p.m.)		
Ascites	13* > 2†	13 > 13	% inhibition
Control	6·523	203	
AB 342	1·510	161	77%
Ab 344	3·188	230	52%
Ab 321	1·179	126	83%
Control	20·803	210	
Ab 342/344‡	432	80	98%
Ab 321/344	7·740	156	63%
Ab 342/321	1·664	188	92%

Strain 13 LNL (3×10^5) were incubated for 5 days with strain 2 PEC (1×10^5) pretreated with control ascites or moAb and complement (see Table 3). As negative control, strain 13 PEC were incubated with strain 13 LNL. Inhibition is expressed as percentage of control [^{3}H]TdR incorporation.

* Responder.

† Stimulator.

‡ Pretreatment of PEC with a mixture of two different moAb and complement.

only. The antigens were not detectable on monocytes, but were expressed by a significant proportion of resident, stimulated or activated mph. From these Ab, Ab344 and 321 exhibited similar binding patterns and might therefore recognize the same antigen. Binding inhibition studies, however, using internally [^{3}H]-labelled Ab, indicated that the Ab detected other different determinants.

MoAb 253, 310 and 257 defined antigens which were not present on monocytes or unstimulated mph, but which were induced on the mph cell surface by inflammatory agents or—with the exception of Ab 257—during activation of the cells; Ab 257 specifically reacted with oil-induced mph. All three moAb defined discrete subpopulations of stimulated or activated mph. Support for the specific expression of the antigens on stimulated or activated mph was obtained by inducing the expression of antigen 253 on antigen-negative resident mph after *in vitro* culture with PMA or PAF. Both substances are well-known mph activators (Hartung *et al.*, 1983, Klassen, Con Kling & Sagone, 1982), and these stimulated the respiratory burst activity, representing one of the physiological changes which distinguish activated from resident mph (Nathan, 1982). In agreement with our findings, it was recently reported that mph exhibit characteristic properties of activated mph after stimulation of their respiratory burst activity and subsequent *in vitro* culture (Schnyder & Baggolini, 1978).

The failure of PMA or PAF to induce expression of antigen 257 or 310 on resident mph might be due to a selective induction of only some, but not other, membrane components, or to a selective effect of these substances on certain mph subpopulations (Karnovsky *et al.*, 1980).

It should be mentioned that, in addition to monocytes and peritoneal mph, the moAb reacted with thymic mph. Hasell's corpuscles of the thymus, whose cellular structure and possible mph contribution are still unclear, were also brightly stained by most of the moAb (F. Zepp and H. Schulte-Wissermann, manuscript in preparation).

Recently, a moAb detecting a surface component of guinea-pig mph has been described (Mathew *et al.*, 1983). The antigen is present on many cells of the mononuclear phagocyte series, including mph in peritoneal oil-induced exudates, alveolar mph and Kupffer cells, and is additionally found on epitheloid cells of the BCG granuloma. There was no indication as to whether the antigen expression varied according to mph differentiation or activation. As with the moAb defined by Mathew *et al.* (1983), the anti-guinea-pig mph moAb 342, 344 and 321 bound to a high proportion of oil-induced peritoneal mph, and one of these Ab might recognize an identical antigen. Further biochemical characterization of the antigens defined by our moAb should clarify whether there are structural similarities to differentiation antigens found on guinea-pig, mouse or human mph.

The functional significance of the antigens defined by the moAb remains unclear. A contribution of the antigens to the mph receptors for C3b (CR1), C3bi (CR3) or Fc receptors could be excluded from the reaction pattern of the Ab. They did not bind to granulocytes which express CR1, CR3 and FcRII, or to erythrocytes and B lymphocytes bearing CR1 and FcRII (Fearon, 1984; Dickler, 1976). In addition, the moAb did not recognize Ia antigens which are displayed on the mph cell surface and are crucially involved in the presentation of foreign antigen to T cells. Besides the Ia antigens, however, other mph membrane molecules might be involved in antigen presentation (Sanchez-Madrid *et al.*, 1983; Weinberg & Unanue, 1981; Raff, Picker & Stobo, 1980). We therefore examined whether the moAb detect mph

membrane molecules, contributing directly to the antigen presenting function. Mph were incubated with moAb and antigen (OVA, PPD) and their capacity to induce T-cell proliferation was subsequently tested. In several experiments, including alloantigen-stimulated T-cell proliferation (MLR), the continuous presence of moAb had no influence on the T-cell response. These data might indicate that the Ab-defined antigens did not participate directly in antigen presentation. This possibility, however, could not be totally excluded. The moAb might well bind to functional relevant antigens, but to domains which are not closely associated with the function of the antigens. In a second set of experiments, we therefore tried to eliminate the antigen-bearing mph applying cytotoxic Ab and complement.

Treatment of mph with moAb 344, 321 or 342 and complement resulted in an inhibition of antigen-, mitogen- or alloantigen-induced T-cell proliferation varying between 27–52% (Ab 344) and 37–83% (Ab 321, 342). In these experiments, a high percentage of mph was lysed by the moAb 344 (57–86%), and moAb 342 and 321 (68–98%). Treatment of mph with combinations of two moAb (342 and 321, 342 and 344) did not further reduce the T-cell response, although almost all mph (94–98%) were depleted by these Ab. After lysis by the moAb, a sufficient number of antigen-presenting mph obviously remained for the stimulation of T-cell proliferation. In fact, it has been reported that less than 1% mph are able to induce a secondary, antigen-stimulated T-cell response (Mizel & Ben-Zvi, 1980). Instead of this incomplete elimination by moAb and complement treatment, the antigens defined by the moAb might not be expressed on all antigen-presenting mph. They might be present only on a part of the mph capable of stimulating T-cell responses.

In contrast to the antigen-induced T-cell proliferation, the MLR was totally abrogated after lysis of the mph with the AB combinations mentioned above. This discrepancy in the sensitivity against mph depletion of antigen versus alloantigen-stimulated T-cell responses might be the consequence of a different requirement for mph in these systems. Additionally, the results obtained from the MLR might indicate that the moAb also reacted with dendritic cells, as these cells have been shown to be potent stimulators of the MLR (Steinman *et al.*, 1983). A possible reaction of the moAb with guinea-pig dendritic cells remains to be analysed in future studies, and depends on suitable methods for isolation of dendritic cells in this species.

Taken together, our investigations support the notion that cells committed to the monocyte/mph lineage share a family of differentiation antigens, which distinguish them from other lines. The antigens recognized by the moAb were expressed to variable degrees on members of the guinea-pig mph differentiation pathway, reflecting different developmental stages. Thus, monocytes and unstimulated, inflammatory or activated mph were phenotypically defined by the expression of the different antigens.

The functional nature of the antigens has not yet been determined, although their selective distribution on cells of the monocyte/mph lineage suggests a participation in specialized functions of these cells. Studies are in progress to characterize the molecular properties of the antigens, which might allow a comparison with membrane components of mouse mph or human monocytes known to be involved in mph functions.

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