Immunology 1984 **53** 753

Monoclonal antibodies specific for human monocytes, granulocytes and endothelium

NANCY HOGG*, S. MACDONALD†‡, MARGARET SLUSARENKO†§ & P. C. L. BEVERLEY†
**Imperial Cancer Research Fund, London, and †ICRF Human Tumour Immunology Group, University College Hospital, London*

Accepted for publication 15 August 1984

Summary. Four monoclonal antibodies against antigens of human myeloid cells have been produced and thoroughly characterized in terms of their reactions with peripheral blood cells, cell lines, nine lymphoid and non-lymphoid tissues and the polypeptides with which they react. UCHM1 and SmØ identify antigens present on the majority of blood monocytes and a variable, but lower, proportion of tissue macrophages. From their morphology and location in tissues, these cells appear to be recirculating monocytes. SMØ antigen is also present on platelets. In addition, both antibodies stained endothelial cells, SMØ in all tissues examined and UCHM1 variably. Biochemical investigation indicated that the UCHM1 antigen is a protein of 52,000 MW while the SMØ antigen could not be indentified.

The antibodies TG1 and 28 identify antigens mainly present on granulocytes. While mAb 28 reacted with neutrophils, TG1 also stained eosinophils and stained strongly a proportion of monocytes. TG1 also reacted variably with some non-haemopoietic cell lines. Both antibodies reacted predominantly with granulocytes in tissue sections. MAb TG1 precipitated a single polypeptide of 156,000 MW from monocytes and granuloyctes, while mAb 28 precipitated non-convalently associated polypeptides of 83,000 and 155,000 MW from granulocytes but only a single molecule from monocytes, corresponding to the lower MW chain of 83,000. The epitope with which mAb 28 reacts appears not to be exposed on the surface of intact monocytes. This suggests that a similar or identical 83,000 MW molecule is made by both neutrophils and monocytes, but that its expression differs according to cell type.

‡ Present address: S. MacDonald, Dept. Experimental Pathology, John Radcliffe Hospital, Oxford.

§ Present address: M. Slusarenko, Amersham International, Amersham, Bucks HP7 9LL.

Abbreviations: CAE, chloroacetate esterase; E, sheep red cell rosettes; MAb, monoclonal antibody; Mph, macrophages; NSE, α-napthylacetate non-specific esterase; PBL, peripheral blood leucocytes; PML, polymorphonuclear leucocytes.

Correspondence: Dr N. Hogg, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX.

INTRODUCTION

Recently, there have been many reports of monoclonal antibodies (mAbs) reacting with human monocytes and macrophages (for review see Todd & Schlossman, 1984). When tested on peripheral blood mononuclear cells, 16 of these mAbs appear to be specific for monocytes and another 24 mAbs detect specificities shared between monocytes and other haemopoetically derived cells, frequently neutrophils. The exact specificity of these antibodies and their similarity one to another cannot, however, be determined without examination of the tissue distribution of reactivity beyond peripheral blood and characterization of the molecules with which they react. It is to be expected that the use of a panel of well-characterized monoclonal antibodies to myloid cells will be extremely useful in elucidating the lineage relationship and functional

heterogeneity of myeloid cells as has been the case for T lymphocytes (for review see Möller, 1983). In this report, we characterize four mAbs selected because of their differing specificities for myeloid cells. mAb UCHMI reacts with monocytes but also binds to vascular endothelium in some tissues; mAb SMØ reacts with monocytes, platelets and the majority of tissue vascular endothelium; mAb TG1 reacts with neutrophils, eosinophils and a subpopulation of monocytes, and mAb 28 is specific for neutrophils alone.

MATERIALS AND METHODS

Preparation of monoclonal antibodies

The immunization protocols which produced the four different monoclonal antibodies were as follows:

(i) UCHM1 (clone C-7)–BALB/c mouse received intraperitoneally 10^7 thymocytes on day 0, 10^6 peripheral blood leucocytes (PBL) from a remission AML patient with 90% OKT8 + T cells on day 24, fusion on day 27;

(ii) TG1–CBA mouse received subcutaneously 50 μg in complete Freund's adjuvant of thymocyte glycoprotein fraction adsorbed to Con A and eluted with 0·1 M glucose on day 0, 25 μg of the same extract intravenously on day 30, fusion on day 33;

(iii) 28–BALB/c mouse received $2{\cdot}4 \times 10^7$ monocytes, separated from other PBL on fibronectin plates (Ackerman & Douglas, 1978) by a combination of intravenous and intraperitoneal routes on day 0, $9{\cdot}2 \times 10^6$ monocytes on day 57, $0{\cdot}6 \times 10^7$ monocytes on day 64, 2×10^8 monocytes on day 72, fusion on day 75;

(iv) SMØ–BALB/c mouse received 5×10^7 tonsil cells subcutaneously on day 0. Fifty-six days later, the mouse was given a subcutaneous injection of 2×10^6 PBM pooled from three donors, followed on day 70 by a final injection of 3×10^6 pooled PBM intravenously and 1×10^6 cells intraperitoneally. Fusion was carried out 4 days later. The fusions using NSI-Ag4/1 (NS-1) cells for mAbs UCHM1, SMØ and TG1 and the Sp2/0-Ag14 cell line for mAb 28 were carried out as previously described (Hogg *et al.*, 1981). The hybridomas which bound to monocytes (approximately 90% pure) using indirect immunofluorescence were cloned three times by plating at limiting dilution on BALB/c peritoneal cell feeder layers in RPMI 1640 containing 10% fetal calf serum (FCS) and ascitic fluid was obtained from pristane (2, 6, 10, 14-tetra methyl pentadecane)-primed mice after inoculation with 1×10^6 cloned hybridoma cells. MAb UCHM1 was found to be an IgG2a immunoglobulin and mAbs SMG, TG1 and 28 to be IgM immunoglobulins by Ouchterlony analysis using subclass-specific antisera (Miles Laboratory, Slough, Berks).

Cells, cell lines and tissue sections

Human peripheral blood mononuclear cells were obtained from normal donors by Ficoll-Hypaque density ($d = 1{\cdot}08$) centrifugation at room temperature. Monocytes were fractionated by adherence for 30 min at 37° to petri-dishes coated with microexudate from BHK cells (fibronectin plates) (Ackerman & Douglas, 1978). The purity of monocytes prepared in this way was >95% as assessed by the presence of the cytoplasmic non-specific esterase enzyme (Yam, Li & Crosby, 1971). When monocytes free of detectable platelets were desired, this procedure was carried out using defibrinated blood (Perussia, Jankiewicz & Trinchieri, 1982). Granulocytes and red cells were prepared from the Ficoll-Hypaque pellet by two further steps. They were partially separated by incubating five volumes of reconstituted cells with one volume of 6% Macrodex (Macrodex-dextran 70, Pharmacia, Uppsala, Sweden) for 30 min at 37°. Complete fractionation was achieved with a gradient composed of 55, 70, 81% Percoll centrifuged at 3750 *g* for 20 min (Dooley, Simpson and Meryman, 1982). The neutrophils are found at and above the interface between 55 and 70% Percoll and red cells at the bottom of the gradient. Granulocytes were assessed as >95% pure by their positive staining for chloracetate esterase enzyme (Yam *et al.*, 1971). Reactions with eosinophils were examined using preparations of polymorphonuclear leucocytes (PML) from two volunteers with high eosinophil counts (20% of PML) and from one patient with an eosinophilia (50% of PML) provided by Dr P.C. Tai, Royal Postgraduate Medical School, London. Platelets were separated from other cells in citrated blood by centrifugation of blood at 160 *g* for 10 min at RT. The resulting platelet rich plasma was then spun at 1300 *g* to wash and isolate the platelets.

T cells were separated from B cells and monocytes by the standard fractionation method of rosetting with sheep red blood cells (SRC). Sheep red blood cell rosettes (E) were formed with red cells treated with AET (S-2-aminoethylisothiouronium bromide hydrobromide; Aldrich Chemical Co., Milwaukee, WI) according to the method of Kaplan & Clark (1974). Two volumes of AET-SRC were mixed with one

volume of PBM at 10^7 cells/ml in RPMI and 0·5 volume of FCS. The mixture was centrifuged at 250 *g* for 10 min and held on ice for 1 hr. The cell pellet was then resuspended by gentle rotation of the tube and rosette forming cells separated by centrigugation at 1500 *g* over Percoll (Pharmacia) at a density of 1·080 g/cm^3. E−cells were recovered from the interface and E+ cells from the pellet by lysis of the SRC using haemolytic Gey's solution (Dresser, 1978).

Bone marrow aspirates were obtained from the Haematology Department, University College Hospital, London. Samples were depleted of mature granulocytes and red cells by centrifugation over Ficoll-Hypaque. Thymus tissue removed during cardiac surgery was teased apart using 19-gauge needles and the cell suspension was washed once before staining. Samples of breast milk were also obtained from University College Hospital. These samples were centrifuged four times in RPMI 1640 and the macrophages (Mph) selected by adherence to fibronectin plates as previously described. Bronchioalveolar lavage cells from univolved lungs of non-smoking patients were obtained from Dr Terry Tetley (Charing Cross Hospital, London) and peritoneal cells from patients undergoing laparoscopy were obtained from Dr Susanne Becker (University of North Carolina, Chapel Hill, NC).

Blocks of human tissues were obtained from the following sources: post-operative tonsil, thymus, spleen, lymph node, lung and liver from University College Hospital, London; liver and kidney from Dr David Jones, Southampton General Hospital, Southampton; skin biopsies from Dr Irene Leigh, St John's Hospital for Diseases of the Skin, London, and thymus tissue from patients undergoing cardiac surgery from Dr Roland Levinsky, The Institute of Child Health, London. Tissue sections (6 μm) were prepared from the tissue blocks which had been snap frozen in isopentane and stored in liquid nitrogen. The sections were fixed in acetone at room temperature for 10 min before use.

The following human cell lines listed in Table 2 were all obtained from the authors' laboratories or from colleagues within the ICRF and were maintained in either RPMI 1640 or Dulbecco's modification of Eagle's medium (E4) in 10% FCS. Thus, human embryo lung fibroblasts, melanocytes, TR138, Hep-2, MCF-7, L132 cells were obtained from Dr Joyce Taylor; MOLT4, HSB-2 and HPB-ALL (T-ALL lines), NALM1 and NALM6 (pre–B lines), THP1–0 (myeloid) (Tsuchiya *et al.*, 1980) from Dr Mel Greaves; SK007 (human myeloma line) and EBV-infected B-cell lines from eight normal volunteers (PCLB); U937 (histiocyte), HL60 (Promyelocytic), K562 (erythroid) (NH).

Long-term cell culture of monoctyes

Peripheral blood mononuclear cells were plated onto fibronectin-coated glass coverslips in RPMI 1640 containing 5% FCS. The coverslips were washed free of non-adherent cells after 1·5 hr incubation at 37°. The adherent cells were initially > 85% NSE-positive. Subsequently, coverslips were tested for reaction with the mAbs for periods of up to 15 days.

Indirect immunofluorescence and Immunoperoxidase reactions

Cells in suspension were washed twice in RMPI 1640 medium containing 1% FCS, then incubated (10 μl of 2×10^7 cells/ml) with 50 μl of mAb containing tissue culture supernatant on ice for 30 min. In most experiments, the cells were also also tested with an IgG1 mAb, DA2, specific for HLA-DR (Brodsky *et al.*, 1979). The most frequently included controls were an IgG1 mAb 5·5 which reacts with an internal molecule in myeloid cells (N. Hogg, unpublished observations) and an IgG2 mAb which reacts with mouse red cells (I. Todd, unpublished observations). After the cells were washed three times in medium, they were incubated for a further 30 min at 4° with 40 μl of fluorescein isothiocyanate (FITC) conjugated goat or sheep anti-mouse IgG (Cappel) diluted 1:100. After washing a further three times, samples were analysed by epifluorescence microscopy or by use of a FACS cell sorter (Becton-Dickinson, Mountain View, CA). Monocytes or granulocytes adhering to coverslips were treated in a similar manner.

In 'double-labelling' experiments, the mAbs were added consecutively followed by the appropriate second layer antibody which was either fluorescein-conjugated goat anti-mouse IgM (Fc fragment) used at 1:50 dilution (Nordic, Maidenhead, Berks) or rhodemine-conjugated goat anti-mouse IgG2a used at 1:50 dilution (Nordic). Each incubation was 20 min in length and controls were included to make certain that neither 'second layer' antibody reacted with the inappropriate monoclonal antibody.

Alternatively, tissue sections and cytocentrifuged preparations were tested using immunohistochemical techniques. Samples were incubated with mAb-containing supernatant as above, but were incubated with peroxidase conjugated goat anti-mouse Ig (1:30

DAKO) as a second layer. A positive reaction was revealed by a 7 min exposure of the sample to hydrogen peroxide (0·012%) and diamino-benzidine (0·6 mg/ml). Tissues tested with rabbit anti-Factor VIII antisera (1:50 DAKO) received a second incubation with peroxidase conjugated goat anti-rabbit Ig (1:30 DAKO).

Cell labelling and immunoprecipitation

Isolated monocytes and granulocytes were surface-labelled with ^{125}I using the lactoperoxidase method (Hubbard & Cohn, 1972). Briefly, 5×10^7 cells in 0·5 ml of PBS-A containing 5 mM glucose were incubated for 15 min at room temperature with 20 μl of a mixture of lactoperoxidase (1 mg/ml, Sigma, Poole, Dorset) and glucose oxidase (5 units/ml, Sigma) and 1 mCi of ^{125}I. After two washes in PBS-A, the cells were lysed in 2·0 ml lysis buffer (1% NP40, 0·5% Na-deoxycholate in 50 mM Tris-HCl buffer, pH 8·3, which contained 1 mM EDTA, 0·15 M NaCl, 0·1% BSA, 1 mM PMSF and 0·02% NaN_3) at 4° for 30 min. The lysate was spun at 13,000 *g* for 5 min to remove insoluble material, and absorbed with 100 μl of 10% suspension of washed *Staphylococcus aureus* for 30 min in order to remove material which would non-specifically bind to *S. aureus*. Immunoprecipitation was performed by adding 10 μl of ascitic fluid from experimental mAbs or control mAb 5·5 to 0·5 ml of the lysate. After overnight incubation, 5 μl of rabbit anti-mouse immunoglobulin (DAKO) was added for 1 hr, followed by 50 μl of a 10% suspension of washed *S. aureus* for a further hour. The suspension was pelleted by centrifugation and subsequently washed, first with NET buffer (0·15 M NaCl, 50 mM Tris, 5 mM EDTA, 0·02% NaN_3, pH 7·4) supplemented with 0·5% NP40 and 0·1% SDS, and finally with 10 mM Tris, pH 8·0 containing 0·1% NP40. The bound ^{125}I-labelled surface antigens were eluted by boiling with SDS-gel sample buffer (pH 6·8) for 5 min and were subsequently analysed by SDS-gel electrophoresis and autoradiography.

RESULTS

The purpose of this study has been to characterize four monoclonal antibodies, UCHM1, SMØ, TG1 and 28, in terms of their reactions with circulating peripheral blood cells and other cell types, their reactions in lymphoid and other tissues, and to determine the biochemical properties of the molecules with which they react.

Reaction with haemopoietic cells

The reactions of the four mAbs with human peripheral blood cells and subfractionated cell types are listed in Table 1. The principal reaction of UCHM1 (Fig. 1a) and SMØ is with monocytes, of which approximately 80% react with the mAbs. SMØ also reacts with platelets (Fig. 1b) and, as platelets from heparinized blood will adhere to myeloid cells, particularly monocytes, it was necessary to prepare monocytes and granulocytes from defibrinated blood using EDTA in order to distinguish accurately antiplatelet reactivity from anti myeloid cell reactivity (Perussia *et al.*, 1982). When this was done, it was apparent that SMØ reacted with monocytes but not with granulocytes. The property of platelet adherence to both monocytes and granulocytes is undoubtedly the reason for the unexpectedly high percentage of E rosette negative cells reacting with SMØ (Payne, 1981). This may also be the reason for the high proportion of $SM\emptyset^+$ cells in bone marrow, although it is possible that cells related to the megakaryocyte are $SM\emptyset^+$. However, cells with the appearance of megakarocytes appeared not to react with SMØ.

TG1 strongly labelled an average population of 20% of monocytes, with the remainder being either negative or very weakly reactive (Fig. 1c, d). Double labelling experiments showed the TG1-positive monocytes to be contained within the UCHM1-positive cell population. Incubation with neither granulocytes nor granulocyte supernatants altered the expression of TG1 on monocytes, indicating that the molecule was not passively acquired for granulocytes. MAb 28 appeared not to have specificity for monocytes (Fig. 1e, f). In contrast, TG1 and 28 reacted with the majority of neutrophils (Table 1) and, in addition, TG1 but not 28 reacted with eosinophils. In Fig. 1, they are shown reacting with isolated neutrophils in monocyte preparations (Fig. 1c, d, e, f). MAb 28 labelled granulocytes in a pattern of uniform speckles, but the TG1 antigen became polarized to the trailing aspect of motile granulocytes.

Reactivity of mAbs with other cultured primary cells and cell lines

Table 2 lists the reactions of the four mAbs with a wide variety of cells. UCHM1 and SMØ both reacted with U937, the histiocytic lymphoma cell line. UCHM1 also reacted with THP1-0, a cell line of possible monocytic type (Tsuchiya *et al.*, 1980) and weakly

Table 1. Reactivity of four monoclonal antibodies with various haemopoietic cell populations (%)

Haemopoietic cells*	*n*	NSE	CAE	UCHM1	SMØ	TG1	28	DA2	Control mAb
PBM	9	10·3±406 (0–24)	–†	6·6±6·0 (0–20)‡	9·6±6·6 (0–20)	5·3±4·6 (0–12)	1·6±2·4 (0–6)	12·4±10 (1–32)	0·7±1·4 (0–2)
Monocytes —defibrinated	8	>95	<2	79·1±8·2 (68–90)	76·3±12·4 (60–100)	20·0±13·8 (8–39)	0	85·7±5·0 (80–92)	0·5±1·0 (0–3)
Neutrophils —heparinized	5	<1	95	4·5±8·4 (0–17)	23·3±20·1 (0–40)	80·5±14·2 (73–95)	89·3±11·2 (64–100)	0·8±1·5 (0–4)	1·0±2·0 (0–4)
—defibrinated	3	–	90	4·5±0·5	2·0±0	73·9±3	84·3±15	0	0
Eosinophils	3	–	–	0	0	>90	0	0	0
Platelets	3	0	–	0	>95	0	0	0	0
Erythrocytes	5	–	–	0	0	0	0	0	0
Non-T−E$^-$	7	35 $n=2$	–	17·6±7·1 (1·0–30)	43·4±22·9 (5–77)	20·0±9·7 (6–36)	5·4±6·1 (0–8)	42·6±8·7 (28–48)	3·0±4·7 (0–12)
T cells−E$^+$	6	–	–	0·7±1·6 (0–4)	0·3±0·8 (0–3)	0 (0)	1·8±4·02 (0–9)	2·8±4·2 (0–10)	0·5±1·2 (0–3)
Bone marrow	2	–	–	5·4 (2–9)	29·5 (16–43)	41 (39–43)	29·5 (29–30)	–	0
Thymocytes	2	–	–	0	0	0	0	0	0

* All cells from heparinized blood except where indicated.
† Not done.
‡ Range of positive immunofluorescence.

with 15% of cells from the promyelocytic cell line HL60. SMØ reacted weakly with K562, the erythroleukaemia cell line. All other cell lines and primary cell preparations of embryo lung fibroblasts and keratinocytes were negative. MAbs 28 and TG1 reacted with all four myeloid cell lines U937, THP1-0, K562 and HL60. MAb 28 reacted with none of the other cell lines tested. However, mAb TG1 reacted with a percentage, usually low, of many different cell lines. There was no discernable pattern to these reactions. Thus, in general, the reactions of the mAbs with cell lines corresponded to their expected specificity determined by their reactions with monocytes and granulocytes.

Stability of antigens on cultured monocytes

Monocytes were isolated on fibronectin-coated coverslips and were kept in culture for period up to 15 days. Many of the cells were in small clumped formations until days 9–12 when larger Mph-like forms were seen in cultures. The starting cultures were 85% NSE-positive. MAb UCHM1 and mAb DA2, which is specific for HLA-DR (Brodsky *et al.*, 1979) reacted with the monocytes for the entire culture period. On the larger Mph-like forms, there was a diminution of intensity in UCHM1- but not DA2-staining in the larger Mph-like forms. The SMØ antigen was less stable, in that it disappeared after two days of culture. The TG1 antigen was absent from monocytes ranging from 1 to 4 days in culture, although there remained the occasional positive cell in the cell aggregations. Thus, this antigen is not generally induced by the activating conditions of tissue culture. MAb 28, as expected, was not detactable in these cultures.

Reaction of mAbs with isolated tissue Mph

The percentages of isolated tissue Mph reacting with the four mAbs and with DA2 are listed in Table 3. UCHM1, SMØ and DA2 react with the majority of the Mph isolated from breast milk, peritoneal wash, tonsil and thymus. Alveolar Mph were less reactive, with approximately 15% staining positively with either UCHM1 or SMØ, compared with the 100% reactivity with DA2. However, the principle observation was that the intensity of reaction with these tissue Mph was much lower when compared to the staining of peripheral blood monocytes isolated in the same manner. The mAb SMØ reactions were barely scored as being positive. Neither mAbs TG1 nor 28 reacted with tissue Mph.

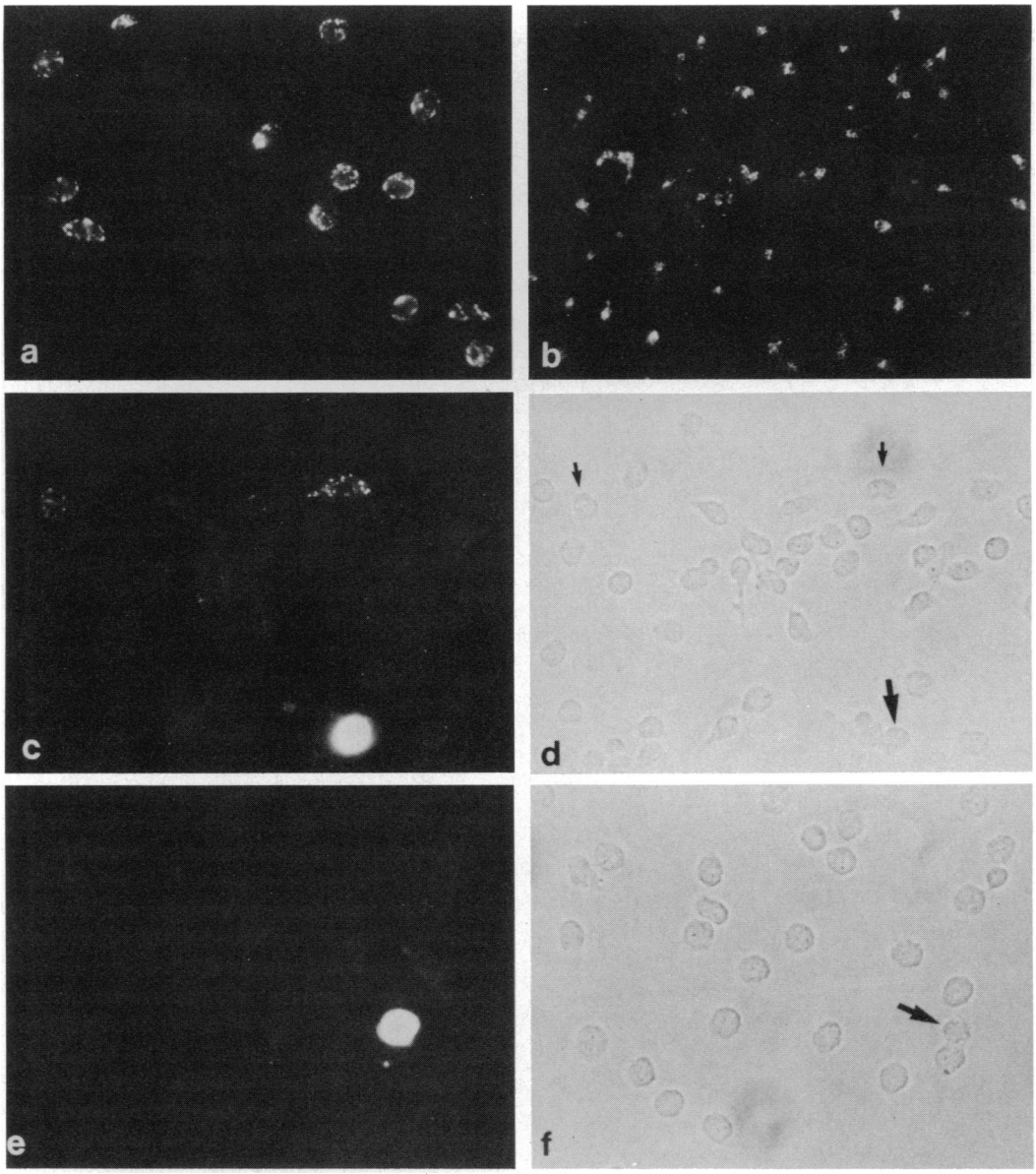

Figure 1. Reaction of mAbs UCHM1, TG1 and 28 with purified human monocytes and mAb SMØ with purified human platelets assessed by indirect immunofluorescence. (a) UCHM1-positive adherent monocytes; (b) SMØ-positive platelets; (c) two TG1-positive monocytes and one TG1-positive neturophil contaminating the monocytes preparation; (d) monocyte exposed to TG1 shown by phase microscopy—two TG1-positive monocytes (small arrows) and a single neutrophil (large arrow); (e) one 28-positive neutrophil in the monocyte preparation; (f) monocytes exposed to 28 shown by phase microscopy with one 28-positive neturophil (large arrow). Magnification × 1275.

Reaction of the mAbs with lymphoid and other tissues

In order to investigate the tissue distribution of the mAbs, four lymphoid tissues, (thymus, spleen, tonsil, reactive lymph node) and five other tissues (liver, lung, kidney, brain and skin) were tested immunohistochemically. UCHM1 reacted in lymphoid tissue with cells which, in size and morphology, resembled monocytes or small Mph (Fig. 2). Thus, in tonsil, UCHM1

Table 2. Reactivity of four monoclonal antibodies with cultured human cell lines (%)

Cell line	Percentage of cells positive after staining with: UCHM1	SMØ	TG1	28
Histiocytic cell lines				
U937	38±8	28±7	53±21	39±16 (WK)
THP1–0	45±48 (WK)*	0	57±17	55±28
Promyelocyte—HL60	15±4 (WK)	0	67±8	48±10
Erythroleuk—K562	0	19±9 (WK)	58±21	37±14
B-cell lines				
Raji	0	–†	0	0
SK 007	0	–	–	0
EBV-infected B-cell lines	0	0	10 – 50 (6/8) lines)	0
NALM-1	0	0	3	0
NALM-6	0	0	0	0
BRI 17	0	0	8	0
B85	0	–	0	–
T-cell lines				
MOLT 4, HSB-2	0	–	–	0
HPB-ALL	0	0	0–3	0
Carcinomas				
Laryngeal hep 2	0	0	0	0
Mammary MCF-7	0	0	100	
MDA-157	0	0	0	0
Keratinocytes TR138	0	0	0	0
Fetal lung L132	0	0	10	0
Unclassified leukaemia				
REH	0	0	0 – 10	0
Other cell types				
Embryo lung fibroblasts	0	0	0	0
Melanocytes	0	0	0	0

* WK, weak labelling.
† Not done.

Table 3. Reaction of mAbs with isolated tissue macrophages (%)

	n	NSE	CAE	UCHM1	SMØ	TG1	28	DA2
Breast milk	2	73	–*	75	52 (weak)	0	0	70
Alveolar	3	>90	2·0	13·6±0·6	15·1±0·4 (weak)	1·4±3·0	3·5±2·0	100
Peritoneal	6	>95	3·0	90·5±5·3	68·3±25 (weak)	2·9±1·5	2·1±0·8	86·5±2·5
Tonsil	2	60	25	52	33·5 (weak)	19 (N)†	23 (N)	60
Thymus	1‡	–	–	65 (weak)	50 (weak)	7·6	1·6	94 (48)§

* Not done.
† N, neutrophil.
‡ Observations confirmed in two additional experiments without quantitation.
§ Number in parentheses represents very brightly stained cells.

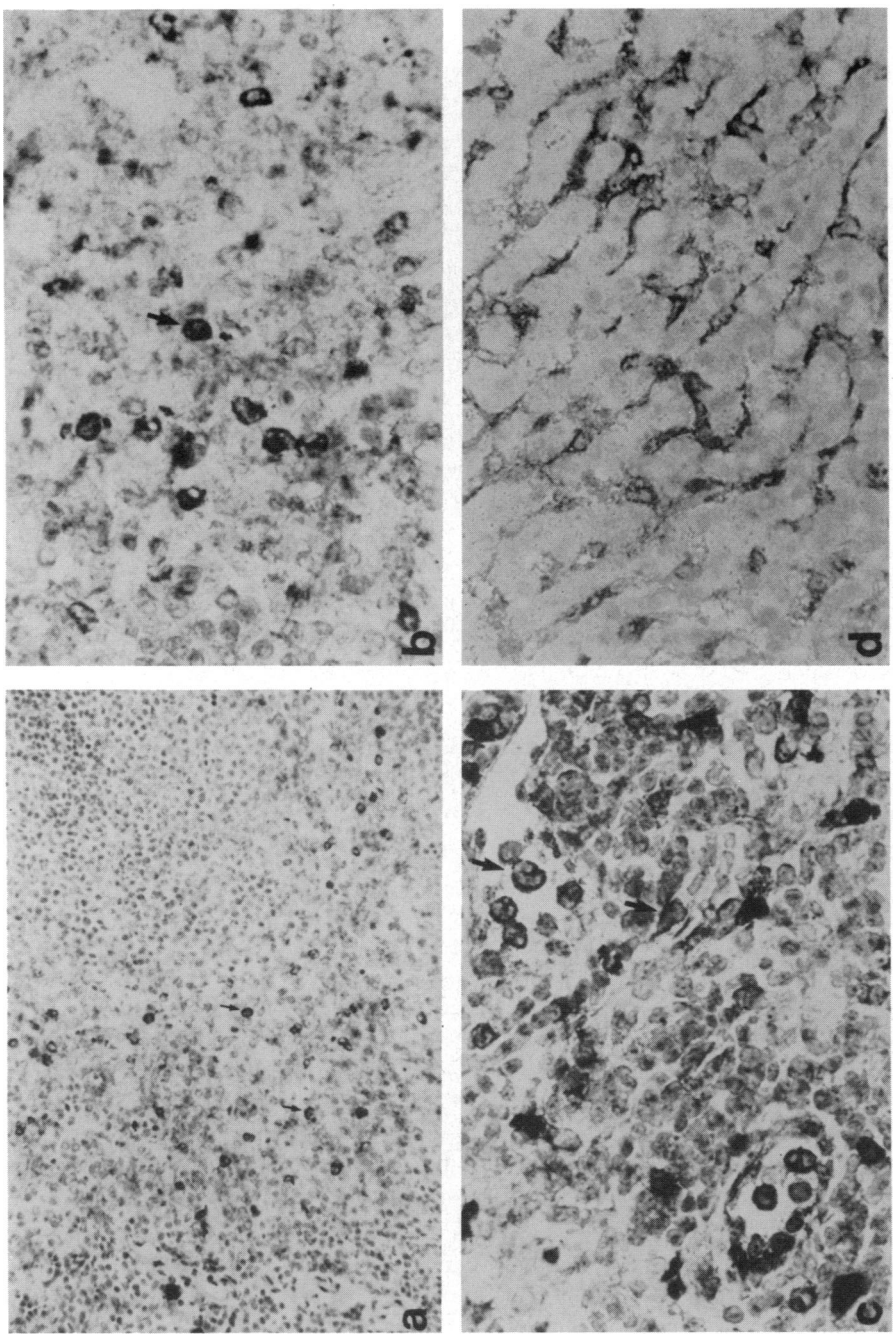

Figure 2. Indirect immunoperoxidase staining by the mAb UCHM1 of 6 μm sections of (a) spleen: arrows indicate selected UCHM1-positive cells in red pulp (magnification $\times$ 470); (b) spleen: UCHM1-positive cells in red pulp (magnification $\times$ 750); (c) lymph node: UCHM1-positive cells within vessels and scattered nearby—arrows indicate selected UCHM1-positive cells (magnification $\times$ 750); (d) liver: UCHM1-positive staining of endothelium (magnification $\times$ 750). Sections are counterstained with haematoxylin.

reacted with small cells in the subcapsular sinus and trabeculae leading from it, and scattered in the zone surrounding the follicles with their germinal centres. UCHM1 did not react with the tingible body Mph within the germinal centres, nor with the larger interfollicular Mph, nor with the interdigitating reticulum cells of the T-cell areas. In other reactive lymph nodes, UCHM1 Mph were seen within and near blood vessels and in the paracortical sinus areas (Fig. 2c). There was staining of monocyte-like cells in the red pulp of spleen (Fig. 2a, b). The reaction in the thymus was extensive, in that reactive cells were located in the subcapsular region and along the septae extending between the thymic lobules. Elsewhere in the thymus, in tonsil germinal centres and in the periarteriolar sheath of lymph nodes, there was a pattern of diffuse staining, the detail of which could not be discerned by light microscopy. UCHM1 did not react positively with tissue Mph in the other tissues examined (lung, kidney, brain and skin), although a number of UCHM1 cells of monocytic morphology were scattered through the dermis of skin. In addition to the reaction with these small tissue Mph, UCHM1 reacted variably with vascular endothelium in these tissues. The location of endothelium was determined by the reaction with a rabbit antibody to Factor VIII Rag or von Willebrand's factor, thought to be present generally on vascular endothelium (Hoyer, de los Santos & Hoyer, 1973; Jaffe, Hoyer & Nachman, 1973). When consecutive sections of liver were labelled with antibody to von Willebrand's factor and mAb UCHM1, it appeared that the reactivity of both antibodies was concentrated on endothelium, and that Kupffer cells were not labelled. However, because of the close opposition of Kupffer cells to hepatic endothelium, a double labelling experiment would be necessary to completely ascertain the extent of overlap between the two labelling patterns. Isolated monocytes did not express von Willebrand's factor. Thus, UCHM1 reaction with endothelium in lymph nodes and liver (Fig. 3d) was strong, detectable in thymus, spleen and tonsil, and absent in lung, brain, skin and kidney.

MAb SMØ, which reacted with monocytes and platelets and rather poorly with isolated tissue Mph, failed to give any detectable staining of Mph in tissue sections. Instead, a very strong reaction with vascular endothelium was obtained in every tissue tested. This was determined by observations of morphology and, as above, by a comparable pattern of reaction with rabbit antibody to von Willebrand's factor. For example, in Fig. 3 the reaction of SMØ with cells lining the venous sinusoids of spleen red pulp is shown (Fig. 3a, b). These cells may be the stave cells which have previously been thought to be endothelial in origin (Williams & Warwick, 1980). In Fig. 3c, d the reaction with thymic endothelium is shown. What appears to be single cell staining, are cleaved cross- and tangential-sections of capillaries. In the kidney, extensive SMØ reactions were seen in the cortex where much vasculature is present, and much less reaction in the medulla where kidney capillaries are more sparsely represented.

The reactions of TG1 and 28 were, in general, less complicated as they were found in locations where infiltrating polymorphonuclear leucocytes were expected. Thus, both mAbs reacted with scattered cells with neutrophil morphology in liver and lung. In spleen, cells resembling neutrophils were present throughout the red pulp (Fig. 4a) and in the thymus reactive cells were present in the trabeculae, often in groups and scattered in the medullary region. In both tonsil and lymph nodes, $TG1^+$ and 28^+ cells were located outside reactive follicles, being paracortical in distribution (Fig. 4b). In the thymus, both mAbs reacted with Hassals corpuscles and TG1 reacted weakly with endothelium in liver (Fig. 4c) and kidney. The major deviation from granulocyte specificity was represented by the reaction of TG1 and 28 with kidney (Fig. 4d). TG1 reacted strongly in the cortical area with the luminal borders of the distal convoluted tubules and more weakly with the proximal convoluted tubules. MAb 28 reactivity had a different pattern, in that only the cortical distal convoluted tubules were reactive. In addition, 28 reacted with a proportion of the collecting tubules of the medulla.

Molecular weight determination of UCHM1, TG1 and 28 reactive polypeptides

UCHM1 precipitated a $52{,}000 \pm 2000$ MW polypeptide from ^{125}I-lactoperoxidase labelled monocyte membranes prepared under reducing conditions (Fig. 5). Under non-reducing conditions, the mobility of this polypeptide was altered by approximately 4000, giving a electrophoretic mobility of 48,000. MAb TG1 precipitated a polypeptide of $156{,}000 \pm 8000$ MW from both monocytes and granulocytes. MAb 28 precipitated two polypeptides of $83{,}500 \pm 500$ and $155{,}000 \pm 9000$ MW from granulocytes, and from monocytes a single polypeptide corresponding in electrophoretic mobility to the 83,500 molecule. The

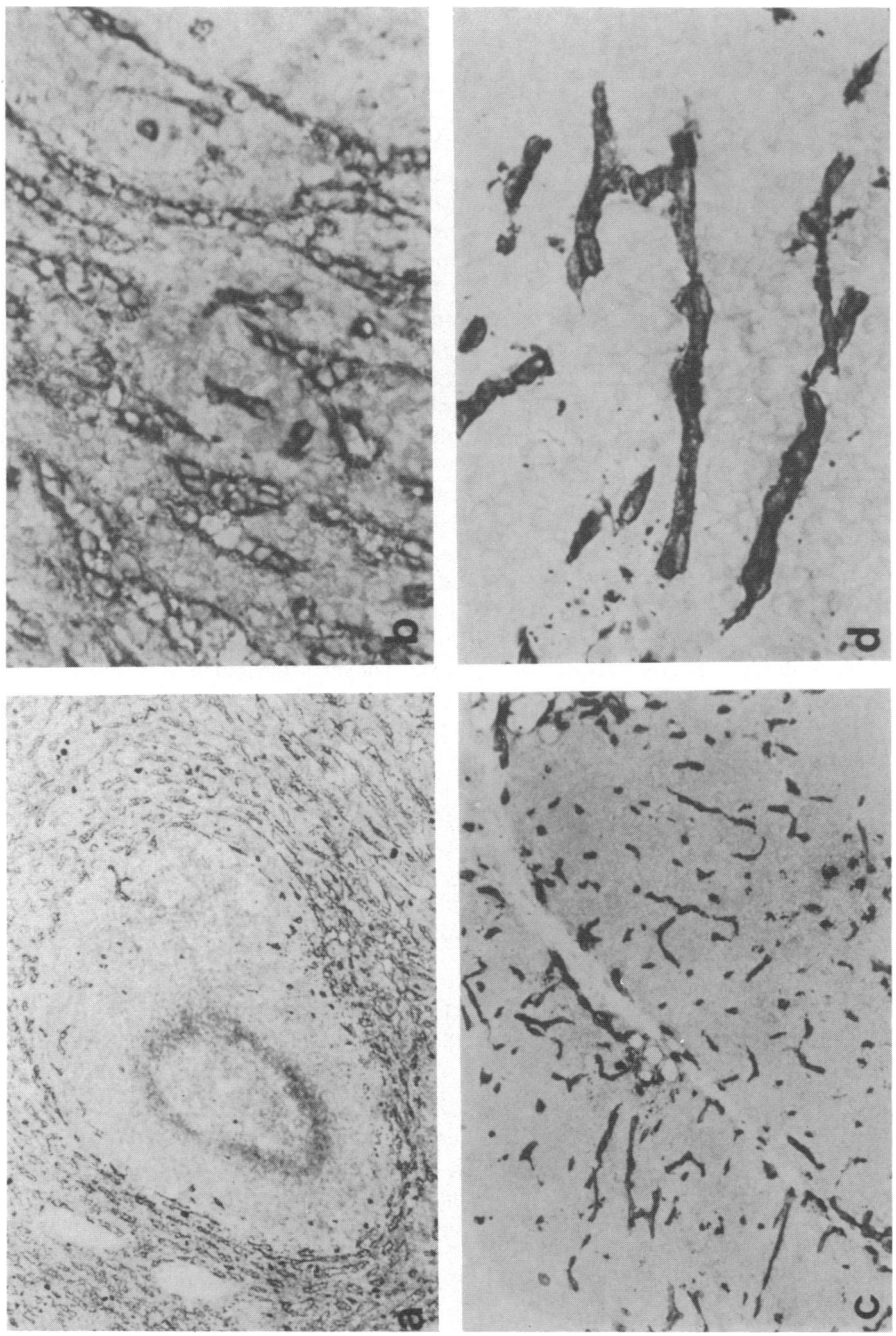

Figure 3. Indirect immunoperoxidase staining with mAb SMØ of 6 μm sections of (a) spleen: note positive reaction of red pulp sinusoids (magnification ×300); (b) spleen; higher power view of red pulp (magnification ×1180); (c) thymus: SMØ-positive vascular endothelium (Magnification ×300); (d) thymus; higher power view (magnification ×1180). Sections are counterstained with haematoxylin.

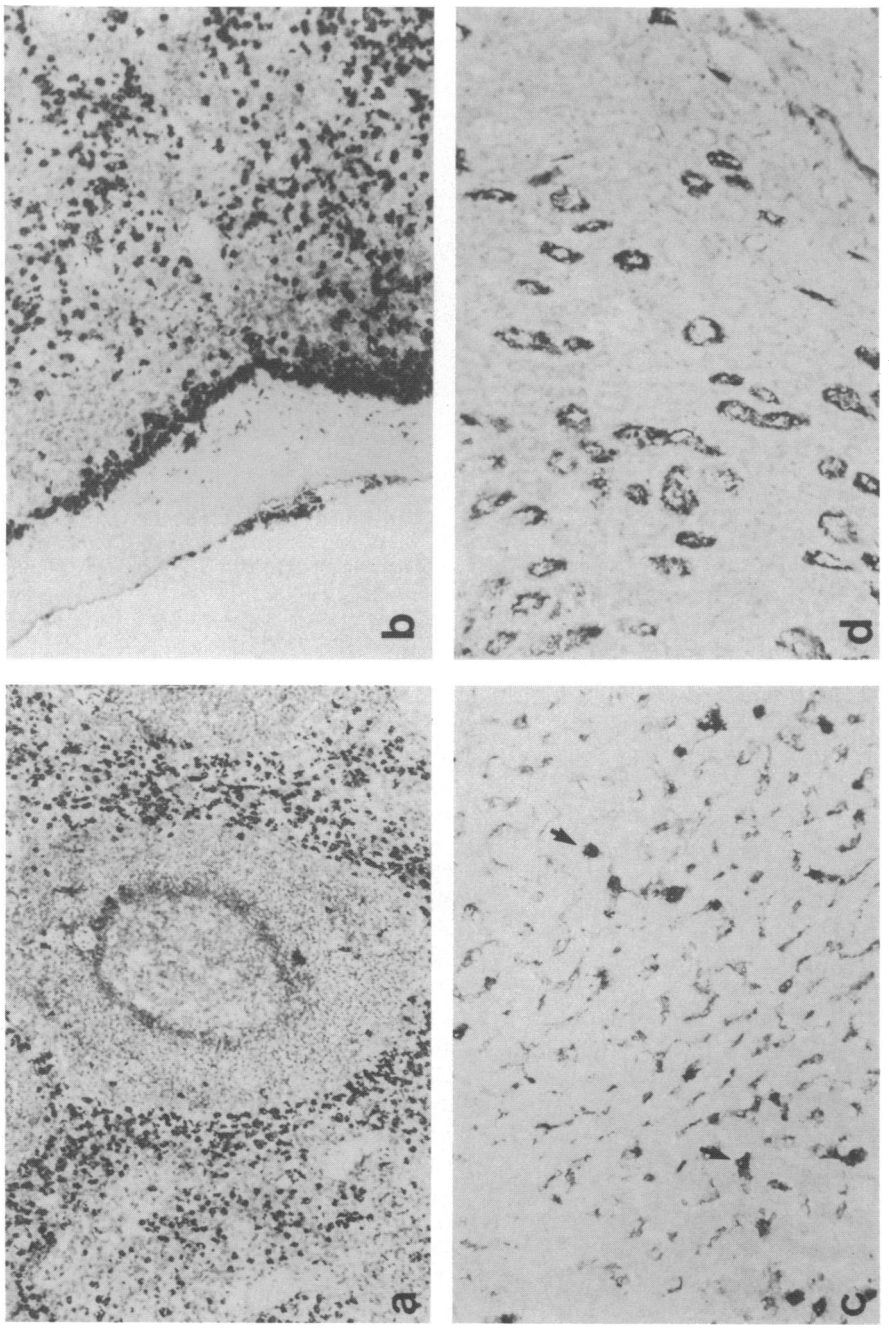

Figure 4. Indirect immunoperoxidase staining with mAbs TG1 and 28. (a) spleen: TG1-positive cells in red pulp; (b) tonsil: 28-positive cells in T-cell areas and concentrated at interface with capsule; (c) liver: TG1-positive staining of scattered granulocytes (see arrows) and vascular endothelium; (d) kidney: 28-positive staining of distal convoluted tubules (magnification ×470). Sections are counterstained with haematoxylin.

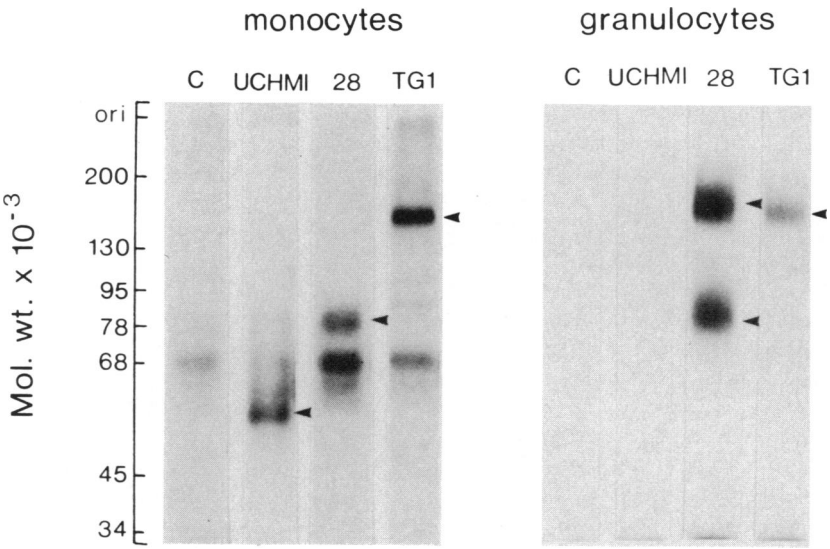

Figure 5. Monoclonal antibody immunoprecipitates from ^{125}I-labelled monocyte and granulocyte lysates identified by 7·5% SDS-polyacrylamide gel electrophoresis after sample reduction. Positive reactions are indicated by arrows. Control preparations were incubated with mAb 5·5. Molecular weight markers were myosin, 200,000; β-galactosidase, 130,000; phosphorylase *b*, 95,000; transferrin, 78,000; bovine albumin, 68,000; ovalbumin 45,000; glyceraldehyde 3-phosphate dehydrogenase 34,000; light chain, 25,000 cytochrome *c*, 12,500.

electrophoretic mobility of the TG1 and 28 precipitated molecules were not affected by reduction conditions. It was not possible to identify a polypeptide reactive with mAb SMØ. A molecule of approximately 68,000 MW was non-specifically precipitated with varying intensity from all monocyte preparations. This electrophoretic mobility is similar to that of the Fc receptor found on myeloid cells (Fleit, Wright & Unkeless, 1982). The fact that no similar molecule was seen in the granulocyte preparations could be accounted for by the much lower avidity of the granulocyte Fc receptor for some types of immunoglobulin (Kurlander & Batter, 1982). MAb 5·5 was used as in control immunoprecipitations (see Materials and Methods).

DISCUSSION

Four monoclonal antibodies have been characterized, which cover a range of myeloid reactivity. The first mAb, UCHM1, reacts with monocytes and is directed against an epitope present on a molecule with a molecular weight of 52,000 under reducing conditions and 48,000 under non-reducing conditions. Ease of extraction of the molecule with organic solvents suggests that the UCHM1 molecule might be a lipoprotein (data not presented). The antigen is easily detectable on freshly isolated monocytes and remains stable on monocytes kept in culture, but is decreased to varying degrees on isolated tissue Mph. When tissue sections were examined using immunoperoxidase techniques, rather than immunofluorescence as on isolated cells, none of the large mature tissue Mph were UCHM1$^+$. However, UCHM1$^+$ cells which were monocytic in size and morphology were found in the traffic areas of lymphoid tissue. In addition, there was a high background of reactivity which was UCHM1-specific. For example, in the thymus, spaces between the tightly packed thymocytes throughout both cortex and medulla of the thymus were UCHM1$^+$. When thymocytes were isolated, they found to be UCHM1$^-$, suggesting that UCHM1 product or cell processes from monocytes were responsible for the positive reaction.

The fact that the UCHM1 antigen is represented most strongly on circulating monocytes, and that the UCHM1$^+$ cells detected on these tissue compartments morphologically resembled monocytes, suggests that UCHM1$^+$ cells seen in tissues represent cells most

recently derived from the blood. In the activated lymph nodes, this migration may be due to an inflammatory stimulus. Alternatively, the UCHM1 antigen might be retained on these smaller tissue monocyte-like cells for longer periods than on monocytes stimulated to mature to tissue Mph. UCMH1 resembles mAbs MO2 (Todd, Nadler & Schlossman, 1981; Todd *et al.*,1982) and 3C10 (van Voorhis *et al.*, 1983) in molecular weight characteristics, and in exclusive specificity for monocytes amongst circulating cells. However, neither MO2 nor 3C10 has been extensively tested for tissue reactivity, and so a complete comparison cannot be made.

The second mAb, SMØ, reacted with circulating monocytes and platelets, but was even weaker than UCHM1 in its reaction with isolated tissue Mph. Both UCHM1 and SMØ react with the same 80% of circulating monocytes. The 'null' set of approximately 20% have monocytic characteristics, in that they give a positive NSE reaction, bear fibronectin receptors, possess HLA-DR antigens and react with mAbs, broadly specific for both monocytes and granulocytes (N. Hogg, unpublished observations). The mAb TG1 which reacts with a polypeptide of 156,000 binds to the majority of granulocytes and strongly to an average of 20% of monocytes. It was possible that these cells might represent the 'null' population of monocytes. However, double labelling experiments clearly demonstrated that the TG^+ monocytes were contained within the 80% set of cells. This might suggest that the 'null' set is either a separate subset of monocytes, or possibly at a different stage of maturation. Future cell-sorting experiments will help to resolve this matter. The proportion of $TG1^+$ monocytes was highly variable between sample of monocytes (range 8–39%). The expression of this antigen could not be simply manipulated in tissue culture and it was gradually lost from the cells.

Monocytes are known to carry antigens which are also expressed by cells of other lineages, and this has sometimes been advanced as an argument in favour of a common origin for monocytes and natural killer (NK) cells. We have not examined large granular lymphocytes (LGL) directly, but two observations suggest that none of these mAbs reacted with a significant proportion of LGL. The first is that none of these cells stained more than 10% of PBM and the majority of this can be accounted for by adherent (monocytic) cells. Secondly, none of the mAbs reacted with a significant proportion of E + lymphocytes, and these normally contain 5–10% of LGL (Beverley & Callard, 1981). The absence of antigens from NK cells, which are present on monocytes, does not of course prove that these are distinct cell types.

Both TG1 and 28 reacted with the majority of neutrophils; however, TG1, but not 28, reacted with eosinophils. Considering the reactions of these mAbs with circulating polymorphonuclear leucocytes, with the HL60 cell line and with bone marrow cells (C. Allen, unpublished observations), the first myeloid cell type identified as being $TG1^+$ is the promyelocyte, with the first cell to bear detectable 28 antigen being the neturophil specific myelocyte. The TG1 antigen becomes polarized to the trailing aspect of motile granulocytes. However, the TG1 molecule does not appear to be necessary for either adherence or motility of granulocytes, as neither activity was inhibited when granulocytes were suspended in TG1-containing tissue culture medium (P.C.L. Beverley and P. Donovan, unpublished observations).

On neutrophils, mAb 28 reacts with two polypeptides of 84,000 and 155,000, with the molecular weight unaltered by conditions of reduction. This might represent a non-convalently linked dimer, two molecules bearing the 28 epitope, or possibly a monomer-dimer relationship, or finally proteolytic action. The last possibility seems unlikely as the two polypeptides were present with equal intensity in 12 experiments. The molecular weight characteristics of the 28 molecule resemble those of the recently described human leucocyte differentiation antigen family (LFA-1, OKM1/Mac-1, P150,95) (Sanchez–Madrid *et al.*, 1983). We are, at present, testing this possibility, although the pattern of cell labelling makes this possibility seem unlikely. Although mAb 28 did not react with intact monocytes, a single polypeptide of 84,000 MW was detected when monocytes were ^{125}I-surface labelled with lactoperoxidase, detergent extracted and immunoprecipitated. This polypeptide corresponded electrophorectically to the lower molecular weight molecule precipitated by 28 from granulocytes. It would appear that mAb 28 is directed against an epitope on the 84,000 molecule, which is accessible in intact granulocytes but not monocytes, although the polypeptide must be exposed on the membrane surface to allow iodination. In a series of acute monocytic leukaemias which were investigated with the 4 mAbs, 16/16 were $UCHM1^+$ and $TG1^+$, 12/13 were $SMØ^+$ and, surprisingly, 12/16 or 75% were 28^+ (Linch *et al.*, 1984). Thus, under certain conditions or perhaps stage of monocytic maturation, the 28 epitope may be expressed by monocytic cells.

When tissue sections were examined, none of the antibodies showed strict lineage specificity. Although TG1$^+$ and 28$^+$ labelling was chiefly associated with cells of granulocyte morphology, TG1 was less lineage-specific and reacted weakly with endothelium in the liver, and the epithelium of skin and Hassel's corpuscles of the thymus. Both TG1 and 28 reacted strongly with renal convoluted tubules, and 28 reacted with a proportion of collecting tubules. Similar unexplained reactivity of other mAbs with components of the kidney have been described, but it is not known whether these molecules are synthesized or adsorbed by the kidney.

When SMØ and UCHM1 were used to stain tissue sections, SMØ consistently, and UCHM1 variably, reacted with vascular endothelial cells. In particular, long flat SMØ$^+$ cells were highly represented in the venous sinusoids of the spleen red pulp. These may be the stave cells which line the sinusoids and have been thought to be endothelial in origin (Williams & Warwick, 1980). This endothelium was identified both morphologically and by the presence of von Willebrands factor, or Factor VIII Rag, which is reported to be present on all endothelium (Hoyer *et al.*, 1973; Jaffe, Hoyer & Nachman, 1973) as well as platelets (Howard, Montgomery & Hardisty, 1974). Although we have not been able to identify the SMØ$^+$ molecule by immunoprecipitation, the fact that the SMØ epitope was present on platelets and endothelium suggested that SMØ might be specific for von Willebrands factor. This is not so, as we have shown that monocytes do not express von Willebrands factor on their membranes. Neither normal serum nor serum from von Willebrands patients, which lack Factor VIII Rag, showed any blocking of mAb SMØ.

Thus, the two mAbs UCHM1 and SMØ, which both react with the majority of monocytes, also react with endothelium. Other molecules shared between monocytes and endothelium are Class II or HLA.DR antigens (Ng *et al.*, 1982; Moen, Moen & Thorsby, 1980) and fibronectin (Jaffe & Mosher, 1978; Alitalo, Hori & Vaheri, 1980). In addition, a subset of kidney transplant patients which do badly, have circulating antibody with specificity for monocytes and endothelial cells (Moraes & Stastny, 1977; Cerilli *et al.*, 1981). Clearly, these two cell types have a number of cell membrane molecules in common. Historically, they have grouped together as the reticulo-endothelial system because of the facility with which both cell types phagocytose diffusible colloidal dyes such as trypan blue (Williams & Warwick, 1980). However, it remains to be demonstrated whether the molecular entities detected by UCHM1 and SMØ on endothelial cells are identical to those on monocytes or are only cross-reactions. It is intriguing, nevertheless, that endothelial cells, as well as sharing antigenic properties with monocytes, have been shown to be able to mediate at least the assessory cell function of monocytes (Hirschberg, Bergh & Thorsby, 1980).

ACKNOWLEDGMENTS

We wish to acknowledge the expert assistance of Yogi Selvendran and Donna Boyle. We thank Professor E. Huehns for permission to use the FACS IV in the Department of Clinical Haematology, University College, Patrick Baker for carrying out the FACS analysis there, and Laura Davies and Grace Lam for their FACS II analyses at ICRF. We are grateful for the help given by Dr Lin Bobrow and Professor P. Isaacson, Department of Morbid Anatomy, University College, for their guidance in the interpretation of stained tissue sections.

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