Monoclonal antibody (Y1/82A) with specificity towards peripheral blood monocytes and tissue macrophages

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SUMMARY A new monoclonal antibody, Y1/82A, was raised against phytohaemagglutinin activated peripheral blood mononuclear cells. Using an immunohistochemical technique it was shown that Y1/82A reacts against peripheral blood and bone marrow monocytes and resident macrophages from essentially all human tissues. Y1/82A bound to determinants present in leukaemic cells from patients with acute myelomonocytic leukaemia and acute monocytic leukaemia, but not to neoplastic cells from patients with malignant lymphoproliferative disorders or malignant epithelial tumours. Y1/82A failed to react with other cell types, with the exception of osteoclasts and megakaryocytes. Analysis by Western blotting showed that the antigen detected by antibody Y1/82A was associated with intracellular granules in macrophages.

Monoclonal antibody Y1/82A may be useful in the diagnosis of monocytic leukaemias and histiocytic neoplasms and in the identification of macrophages in tissues from various inflammatory and neoplastic conditions.

The monocyte-macrophage system is regarded as a network of specialised phagocytic cells which are widely scattered throughout the body.1 These cells include peripheral blood monocytes, Kupffer cells of the liver, microglial cells of the brain, macrophages of lymph node and spleen, and fixed tissue macrophages in various other human tissues. Cells of the monocyte-macrophage system, however, do not include non-lymphoid mononuclear cells known as dendritic cells.2

There is evidence that peripheral blood monocytes originate from bone marrow precursor cells3 and that tissue macrophages are derived from circulating monocytes.4 Bone marrow precursor cells therefore serve as the cells of origin for peripheral blood monocytes and tissue macrophages.

Cells of the monocyte-macrophage system are heterogeneous in terms of their morphology, cytochemistry, function and surface determinants.5-6 As promonocytes mature into monocytes and then into tissue macrophages they increase in size. The ratio of the nucleus to that of the cytoplasm decreases and the number of lysosomes and IgG receptors increases. Functionally, mature macrophages have greater ability to undergo phagocytosis and lymphocyte interaction than the less mature monocyte.1 Furthermore, populations of resident macrophages in lymphoid tissues appear to differ from each other: resident macrophages thought to be accessory cells of the T cell immune response can be differentiated from accessory cells of the B cell immune response on the basis of different cellular determinants.5

Various morphological, cytochemical, and immunological techniques have been used to identify cells of the monocyte-macrophage system.7-9 Among these techniques immunocytological labelling with monoclonal antibodies is one of the most sensitive and specific for identifying monocytes and tissue macrophages in non-neoplastic and neoplastic disorders.10 Numerous monoclonal antibodies have been raised against cells of the monocyte-macrophage system6-15 but most of these react only with subpopulations of cells in the monocyte-macrophage system. Furthermore, many monoclonal antibodies raised against cells of the monocyte-macrophage system also react with determinants found on cells belonging to other cellular systems.10

In this respect we describe our experience with a new monoclonal antibody (Y1/28A) raised against phyto-
hemagglutinin activated peripheral blood mononuclear cells. Using an immunohistochemical technique, we showed that this monoclonal antibody reacts with peripheral blood monocytes and a range of tissue macrophages from patients with non-neoplastic and neoplastic disorders.

Material and methods

Blocks of fresh tissue were obtained from the histopathology department of the John Radcliffe Hospital at necropsy or at the time of surgical resection and were immediately snap frozen in liquid nitrogen and stored at −70°C until use. Tissues sampled from patients without neoplastic disorders included three lymph nodes with reactive hyperplasia, two sections of liver, and one section each of thymus, tonsil, spleen, liver, colon, kidney, heart, brain, striated muscle, thyroid, lung and skin. Tissues sampled from patients with neoplastic diseases included eight cases of non-Hodgkin’s lymphoma, five cases of Hodgkin’s lymphoma, six cases of squamous cell carcinoma of the bronchus, and one case of adenocarcinoma of the breast.

Cryostat sections (6 μm) were prepared from these frozen tissues and stored (wrapped in aluminium foil) at −20°C until staining. Samples of peripheral blood and bone marrow were obtained from patients attending the haematology department of the John Radcliffe Hospital. The samples were drawn into ethylene diamine tetra-acetic acid or heparin anticoagulant and either push films or cytocentrifuge films of mononuclear cells separated on Triosil-Ficoll were prepared. Ten peripheral blood and four bone marrow samples were obtained from subjects without a haematological malignancy, and peripheral blood smears, as well as one of bone marrow film were received from those with either acute myelomonocytic leukaemia or acute monocytic leukaemia.

Preparation of antigen

Peripheral blood from a healthy subject was collected in heparinised tubes. The mononuclear cells were collected from other blood elements by centrifugation on Triosil-Ficoll (Lymphoprep-Nyegaard and Company, Oslo). Mononuclear cells were removed from the interphase, washed, and resuspended in RPMI-1640 tissue culture medium at a concentration of 1 × 10^6/μl. Mononuclear cells were then incubated with a 1/200 dilution (one phial reconstituted with 5 ml of distilled water) of phytohaemagglutinin (PHA) (Flow Laboratories). After three days PHA stimulated blasts were washed once in phosphate buffered saline (PBS) and then resuspended in PBS at appropriate concentrations.

Production of monoclonal antibody

A BALB/c mouse was immunised with an intraperitoneal injection of 1 × 10^6 PHA stimulated blasts. This was followed by an intraperitoneal injection of 2 × 10^6 and 5 × 10^6 PHA stimulated blasts after intervals of 10 days and four weeks, respectively. Three days after an intravenous injection of 1.5 × 10^6 PHA stimulated blasts spleen cells from the mouse (1.8 × 10^6) were fused with 1.8 × 10^6 cells from the NS-1 myeloma cell line. After 14 days the cultures were inspected for growth and the supernatant fluid was screened for specific reactivity on cryostat sections of tonsil using the APAAP immunoalkaline phosphatase technique. The clone Y1/28A, which selectively stained germinal centre macrophages, was then isolated by standard cloning techniques and established in long term tissue culture. In all experiments antibody Y1/82A was used in the form of spent tissue culture supernatant.

Immunochemochemical labelling

Frozen tissue sections (6 μm) and blood and bone marrow films were labelled using the APAAP immunohistochemical technique. Slides were fixed before APAAP staining in acetone (sections and cytocentrifuged preparations) or acetone:methanol.

Immunofluorescence analysis

Peripheral blood monocytes, granulocytes, and lymphocytes were analysed for expression of the Y1/82A determinant by indirect immunofluorescence using flow cytometry. Aliquots of normal peripheral blood mononuclear cells were incubated for 30 minutes with antibody Y1/82A at doubling dilutions in PBS from 1/5 through 1/80. Lymphocytes, monocytes, and granulocytes were analysed separately by gating on forward and right (90°) angle light scatter.

Biochemical analysis of the determinant recognised by Y1/82A

Normal human spleen tissue was solubilised in a solution of 10% Brij (2 parts Brij 99:1 part 96, Sigma Chemical Co) as described by Dalchau and Fabre. The lysate was then centrifuged at 70 000 × g for 30 minutes at 4°C. Samples of both the supernatant and the pellet were dotted on to nitrocellulose paper and allowed to dry. Aliquots of the supernatant were then run under both reducing and non-reducing conditions in a 10% polyacrylamide slab gel in the presence of sodium dodecyl sulphate. Proteins were blotted overnight onto to nitrocellulose membranes as previously described. After blocking with 3% bovine serum albumin (BSA) both the dot blots and the Western blots were immunostained using the APAAP technique. The molecular weights of the immunostained bands were established by comparison with molecular weight standards stained with fast green.
Results

**SAMPLES FROM PATIENTS WITHOUT NEOPLASTIC DISORDERS**

Examination of lymphoid tissue showed a positive reaction in many types of tissue macrophages (table 1). In lymph nodes this included macrophages in the subcapsular and medullary sinuses, macrophages in germinal centres, mantle zones, and interfollicular areas (fig 1). Lymphocytes, endothelial cells, and dendritic reticulum cells were unreactive. In the thymus, cortical and medullary macrophages were positive, but cortical and medullary thymocytes and thymic epithelial cells were negative. There was an intense staining reaction of the macrophages of the splenic cords (fig 2). A few macrophages were present in germinal centres as well as around small arteries and capillaries.

Resident tissue macrophages in many non-lymphoid tissues were also reactive with monoclonal antibody Y1/82A. Kupffer cells and periportal macrophages of the liver (fig 3), interstitial macrophages, and rare glomerular macrophages of the kidney, microglia cells, and perivascular macrophages in the brain, as well as fixed macrophages of colon, lung, heart, skin, striated muscle and thyroid were also all positive for monoclonal antibody Y1/82A. No other cells from these tissues were positive. In the lung, macrophages in the alveolar spaces (but not the alveolar walls) were positive (fig 4).

In peripheral blood smears monocytes showed strong granular cytoplasmic labelling (table 1, fig 5) with monoclonal antibody Y1/82A. The number of positive granules varied considerably between individual monocytes; no evidence of surface membrane labelling was seen. Lymphocytes, neutrophils, and eosinophils were negative, with the exception of occasional blood smears in which some neutrophils were weakly labelled. In the bone marrow smears strong staining of macrophages and monocytes was observed. Osteoclasts and megakaryocytes exhibited an intense staining reaction. All other cells were negative.

**SAMPLES FROM PATIENTS WITH NEOPLASTIC DISORDERS**

The reactions of Y1/82A on samples from patients with neoplastic disorders are summarised in table 2. Leukaemic cells from patients with acute myelomonocytic leukaemia and acute monocytic leukaemia were clearly positive (fig 6). In contrast, neoplastic cells from patients with non-Hodgkin's lymphoma, Hodgkin's disease, squamous cell carcinoma of the bronchus and adenocarcinoma of the breast were entirely negative. Groups of neoplastic cells, however, were often associated with large numbers of positively stained macrophages. Focal areas of acute and chronic inflammation often accompanied the presence of the tumour cells and in these areas macrophages were intensely stained.

**IMMUNOFLUORESCENCE ANALYSIS**

Flow cytofluorographic analysis showed no membrane staining of granulocytes and lymphocytes with Y1/82A at any dilution. Membrane labelling of small numbers of monocytes (<3%) was observed but only at the lowest dilutions tested (1/5 and 1/10).

**BIOCHEMICAL ANALYSIS OF THE Y1/82A DETERMINANT**

Although the dot blotting experiments showed strongly immunoreactive Y1/82A antigen remaining in the high speed pellet, a weaker reaction was also observed with the supernatant.

Numerous attempts were made with the Western blotting technique to determine the molecular weight of the molecule(s) detected by Y1/82A. In some experiments a weak reaction was obtained against a reduced band, migrating with a molecular weight of about 45 000 Kd but this was not a consistently reproducible result.

**Discussion**

This study has shown that the monoclonal antibody Y1/82A raised against activated mononuclear cells reacts with a cytoplasmic determinant present in a very wide spectrum of cells from the monocyte-macrophage system. These include peripheral blood and bone marrow monocytes as well as specialised tissue macrophages of the liver, spleen, lung, brain and skin. As some of these macrophages are in areas of inflammation or juxtaposed to tumour cells we can assume that many are activated. It seems that the Y1/82A antigen is therefore present in cells of the monocyte-macrophage system, both at different stages of maturation and also in various stages of activation. Y1/82A clearly differentiates between cells of the...
Fig 1  APAAP staining of reactive lymph node (cryostat section) with antibody Y1/82A. (a) Low power view shows B cell follicles (arrowed) containing Y1/82A positive germinal centre macrophages. (b) Higher power view of B cell follicle. Note scattered Y1/82A positive cells in extrafollicular tissue (T cell area). (c) High power view showing Y1/82A positive germinal centre macrophages.

Fig 2  APAAP staining of human spleen (cryostat section) showing strong Y1/82A positive staining of macrophages in splenic cords (contrasting with unstained splenic sinusoids).

Fig 3  APAAP staining of human liver (cryostat section) with antibody Y1/82A showing intense staining of Kupffer cells, but not of hepatocytes.

Fig 4  APAAP staining of human lung (cryostat section) showing Y1/82A positive alveolar macrophages.

Fig 5  APAAP stained peripheral blood smear showing two Y1/82A positive monocytes, contrasting with unstained neutrophils and red cells.

Fig 6  APAAP staining of peripheral blood from patient with acute myelomonocytic leukaemia showing strong positive labelling of leukaemic cells with antibody Y1/82A.
Monocyte monoclonal antibody

Table 2  Distribution of Y1/82A antigen in peripheral blood and tissues from patients with neoplastic disorders

<table>
<thead>
<tr>
<th>Type of neoplasm</th>
<th>Labelling with monoclonal antibody Y1/82A</th>
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<tbody>
<tr>
<td>Acute myelomonocytic leukaemia</td>
<td>Leukaemic blasts</td>
</tr>
<tr>
<td>Acute monocytic leukaemia</td>
<td>Leukaemic blasts</td>
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<tr>
<td>Non-Hodgkin's lymphoma</td>
<td>Tissue macrophages with groups of neoplastic lymphoid cells</td>
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<tr>
<td>Hodgkin's lymphoma</td>
<td>Sinus macrophages, germinal centre macrophages, T zone macrophages</td>
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<tr>
<td>Squamous cell carcinoma of bronchus</td>
<td>Tissue macrophages near groups of neoplastic epithelial cells</td>
</tr>
<tr>
<td>Adenocarcinoma of breast</td>
<td>Tissue macrophages near groups of neoplastic epithelial cells</td>
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Monocyte-macrophage system and those of the lymphoid and granulocytic series. Lymphocytes in the peripheral blood and in a range of lymphoid tissues showed no reactivity with Y1/82A. The antibody was also unreactive with any epithelial, endothelial, or other mesenchymal cells with the exception of osteoclasts and megakaryocytes. Cells of the granulocytic series were usually unlabelled, although the inconsistent staining of some neutrophils, occasionally observed in blood films, requires further investigation (it may conceivably represent the presence of the antigen in a masked form).

Interestingly, Y1/82A reacts with osteoclasts as there is evidence that these cells may be developmentally related to cells of the monocyte-macrophage system. Studies in mice and in man have shown that osteopetrosis, a disorder secondary to a failure of osteoclastic function, is cured by an infusion of stem cells through bone marrow transplantation. Some macrophage monoclonal antibodies fail to bind to human osteoclasts, others give strong reactions of this sort. Further studies are therefore required to establish the clinical importance of these observations to the hypothesis of a developmental association between osteoclasts and cells of the monocyte-macrophage system.

This study shows that the Y1/82A antigen is also present on megakaryocytes. Previous studies have indicated that monocytes and megakaryocytes arise from multipotential stem cells capable of differentiation into cells of the erythroidic, granulocytic, macrophage and megakaryocytic series. There are probably many constituents which are shared among cells from the megakaryocytic and mononuclear-macrophage systems, and the Y1/82A determinant is probably one such shared antigen.

Y1/82A reacts with cells of the monocyte-macrophage system in a pattern of distribution similar to a number of other previously described monoclonal antibodies: EMB11, EMB24, EMB39, EMB90, and Ki-M6. These five monoclonal antibodies react with peripheral blood monocytes and tissue macrophages in most organs of the body, but major differences exist between them and Y1/82A. Monoclonal antibody 24 binds to a 174 000 dalton determinant on the surface of monocytes and tissue macrophages, the other five monoclonal antibodies react predominantly with cytoplasmic antigens. Monoclonal antibodies 3-9 and KB90 recognise the p150/95 (CD11c) leucocyte differentiation antigen present in peripheral blood monocytes, tissue macrophages and a subpopulation of neutrophils. Monoclonal antibody EBM11 reacts against a cytoplasmic determinant of undefined molecular weight and binds to Langerhans' cells as well as proximal tubular epithelial cells. This labelling pattern of monoclonal antibody EBM11 separates it from monoclonal antibodies Ki-M6 and Y1/82A which do not label Langerhans' cells nor proximal tubular epithelial cells.

The monoclonal antibody that most closely resembles Y1/82A is Ki-M6, which binds to a cytoplasmic constituent with a molecular weight of 60 000 Kd and which was detected by Western blotting. Both of these monoclonal antibodies label similar cells in the peripheral blood, bone marrow, and other tissues of the body. Conclusions regarding the relation between monoclonal antibodies Ki-M6 and Y1/82A, however, require further studies entailing metabolic labelling and immunoprecipitation.

Y1/82A reacts intensely with leukaemic cells from patients with acute myelomonocytic leukaemia and acute monocytic leukaemia and could probably help identify subtypes of acute myeloid leukaemia (FAB-M4 and M5 as described by the French-American-British international study group), which tend to respond less well to treatment and have a poor prognosis.

Y1/82A vividly shows the large number of tissue macrophages that are associated with neoplastic cells. This is particularly evident in the non-Hodgkin's and Hodgkin's lymphomas. It is possible that these tissue macrophages are acting against the tumour cells in some way on behalf of the host, but the mechanisms are as yet poorly understood. Although some recent studies with lectins and monoclonal antibodies have shown antigenic similarities between Reed-Sternberg cells and cells of the monocyte-macrophage system, our study suggests that the Y1/82A antigens are not present on Reed-Sternberg cells. This supports the view of other workers who suggest that Reed-Sternberg cells are derived from cells not part of the monocyte-macrophage system.

In conclusion, we have described a new monoclonal antibody, Y1/82A, which reacts with peripheral blood and bone marrow monocytes and resident macrophages of essentially all tissues of the body. Y1/82A does not seem to bind to either lymphocytes or to dendritic reticulum cells. It reacts with osteoclasts and megakaryocytes suggestive of a common lineage with these various cell types. Furthermore, Y1/82A reacts with leukaemic cells from patients with acute myelomonocytic leukaemia and acute monocytic leukaemia. It is evident that Y1/82A may be of considerable practical importance in the delineation of...
monocytic leukaemias and histiocytic malignancies.

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References


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