Human mast cells detected by monoclonal antibodies

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SUMMARY We report the establishment of seven mouse-mouse hybridoma cell lines secreting monoclonal antibodies with specificity for granule components of all human mast cells. Reactivity is directed against a molecule which is also found intracytoplasmically in human mature small intestinal enterocytes, liver parenchymal cells, and kidney proximal tubule epithelial cells. No reactivity of these antibodies was found with any other human or animal cell type examined. In particular, the antibodies did not react with basophils or other haemopoietic cell types. This study shows the potential of specific monoclonal antibodies as a tool for identifying and enumerating infiltrating mast cells in tissues. Such antibodies should be of value in investigations into the role of the mast cell in immunological reactions and hypersensitivity diseases.

The mast cell is the most important cell type participating in type I (reaginic) hypersensitivity reactions; mast cells also play a subsidiary role in the generation of hypersensitivity, and associated tissue damage, in some other immune phenomena. Local infiltration by, or proliferation of, mast cells is thus associated, for example, with parasitic infection,¹ with inflammatory diseases of skin² and gastrointestinal tract,³ and with some carcinomas.⁴ The immunological properties and clinical importance of mast cells have been reviewed extensively elsewhere.^{5,6}

Central to further understanding of the role of mast cells in disease is the ability to identify and hence to enumerate them accurately in tissue. Mast cells show a characteristic metachromatic staining reaction with dilute acidic toluidine blue and this is the classic method for their demonstration. After fixation in standard formalin based histological fixatives, however, a substantial proportion of mucosal mast cells, in particular, fail to stain metachromatically with toluidine blue⁷; this problem is compounded in the presence of large numbers of degranulated mast cells, which may be difficult to identify. It has, therefore, been suggested that these factors lead to underestimation of mast cell numbers in formalin fixed tissues and hence the contribution of mast cells to certain disease states may not have been fully appreciated. Toluidine blue staining has the further disadvantage that the pH values which are optimal for demonstrating connective tissue,

mucosal, and neoplastic mast cells differ considerably from each other⁴ and that basophils are also stained metachromatically by this technique. Other methods which have been used for demonstrating infiltrating mast cells include the histochemical demonstration of chloroacetate esterase enzyme; immunohistochemical demonstration of 5-hydroxytryptamine, heparin, fibronectin, prostaglandin, reaginic immunoglobulin⁸; and distinctive staining reactions with alcian blue and astra blue dyes.⁷ Unfortunately, none of these methods is mast cell specific. We thought that the use of monoclonal antibodies with specificity for mast cells would be a valuable alternative to such methods. We report here the establishment of seven hybridomas secreting monoclonal antibodies which distinguish mast cells from all other haemopoietic, connective tissue, and most epithelial cell types.

Material and methods

MONOCLONAL ANTIBODY PRODUCTION

BALB/c mice were immunised with approximately 5×10^6 high density (>1.077 ; erythrocyte depleted cell pellet after fractionation on Histopaque (Sigma)) bone marrow cells from a patient with systemic mastocytosis; the preparation consisted of 50% typical mast cells. Three weeks later the mice were boosted with 200 μ l of packed, washed spleen cells from the same patient, most of which were mast cells and of similar morphology to those in the bone marrow. Details of the characterisation of mast cells in systemic mastocytosis have been published else-

where.⁹ Three days after boosting, the mouse spleen cells were fused with X63.Ag8.653 myeloma cells by a standard method and growing hybridomas were selected in HAT medium.¹⁰

Supernatants from growth positive cultures were screened for antibody against acetone fixed frozen sections of the mast cell infiltrated spleen used for immunisation using the indirect immunofluorescence technique described below. Supernatants reactive with mast cells but not with residual spleen cells were further screened against acetone fixed cytocentrifuge preparations of buffy coat cells from peripheral blood of patients with chronic myeloid leukaemia and against acetone fixed cytocentrifuge preparations of normal human bone marrow. Cultures producing antibodies reactive with any other haemopoietic cell type (granulocytes, monocytes, or lymphocytes) were discarded. Antibodies with apparent specificity for mast cells gave a characteristic strong, coarse granular immunofluorescence, apparently corresponding with the distribution of mast cell granules identified by phase microscopy. Hybridomas producing such antibodies were cloned twice by limiting dilution, and their supernatants were rescreened for reactivity with mast cells in sections of infiltrated spleen.

The following hybridoma clones were selected for further characterisation: MCG 3, 8, 9, 10, 34, 35, 42.

PREPARATION OF TISSUES USED FOR SCREENING *Solid tissues*

Specimens were obtained from operative or surgical biopsy samples or from freshly killed animals, as appropriate, with the exception of human liver and kidney, which were obtained at necropsy, and fetal tissues, which were obtained from an 18 week old fetus after suction termination of pregnancy. Pieces of roughly 0.5 cm² were cut, placed in OCT medium (Tissue-Tek), and snap frozen in liquid nitrogen, where they were stored until use. Frozen sections (5–8 µm) were cut on a cryostat (Cryocut E, Reichert-Jung), air dried, fixed in acetone for 10 min, and stored at –20°C for no longer than one month.

Blood cells and cell lines

Peripheral blood leucocytes were obtained by centrifugation of the heparin anticoagulated blood of a patient with chronic myeloid leukaemia and removal of the resultant buffy coat layer. Basophils were isolated by the same method from the blood of a patient with chronic myeloid leukaemia with 50% basophilia and from long term suspension cultures of normal human bone marrow. Peripheral blood mononuclear cells were obtained from the low

density (<1.077) interface layer after Histopaque (Sigma) fractionation of heparinised normal human blood; granulocytes were obtained from the high density (>1.077) cell pellet separated from the same samples.

Established cell lines (HL-60, U937, K562, CEM, DAUDI, CHANG) were maintained in culture in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS), glutamine, and antibiotics (all from Gibco, Paisley) at 37°C in an atmosphere of 5% CO₂ in air.

Cytocentrifuge preparations (Shandon UK, Cytospin II) were made of about 5 × 10⁴ washed, live cells of each blood cell type or cell line. These were acetone fixed, air dried, and stored at –20°C for not more than one month.

SCREENING PROTOCOL

Supernatants from growth positive hybridoma clones, after screening for positive antimast cell reactivity, were further screened for reactivity (assessed by indirect immunofluorescence) against the panel of human and animal tissues detailed in Tables 1–4.

Indirect immunofluorescence technique

Frozen sections or cytopsins were allowed to attain room temperature, then rehydrated for 5 min in phosphate buffered saline (PBS)-FCS. Excess medium was then blotted from the slide (without permitting drying of the specimen) and 50 µl of test supernatant was placed on to the specimens. The slides were incubated for 30 min in a humidified box, then immersed in PBS-FCS for 30 min. Excess moisture was then blotted, 50 µl of FITC conjugated polyspecific goat antiserum to mouse immunoglobulin (Nordic or Coulter-Clone) was placed on the specimen, and the slides were incubated for 30 min in a humid box, after which they were washed in PBS-FCS for not less than 1 h. Coverslips were then mounted in a 9:1, glycerol and PBS mixture and examined by ultraviolet epillumination with a Zeiss microscope.

Demonstration of mast cells by toluidine blue

Serial sections from the same tissues as those used in the screening protocol were stained by toluidine blue to show the presence of mast cells. Air dried frozen sections or cytospin specimens were fixed in Mota's basic lead acetate for 30 s, washed in distilled water, stained with 0.1% aqueous toluidine blue (pH 4.0 or 2.0) for 5 min, washed in tap water, air dried and mounted. These sections served as reference controls, allowing the enumeration and localisation of mast cells within each specimen, thus facilitating identification of mast cells by phase con-

Table 1 *Distribution of mast cells in human tissues as defined by monoclonal antibodies*

Source of mast cells*†	Reactivity of monoclonal antibodies with mast cells‡						
	MCG3	MCG8	MCG9	MCG10	MCG34	MCG35	MCG42
Mast cell infiltrated spleen (1)	+++	+++	+++	+++	+++	+++	+++
Mast cell infiltrated bone marrow smears (4)	±/+++	±/+++	±/+++	±/+++	±/+++	±/+++	±/+++
Nasal polyp (2)	++	+	+	+	++	++	++
Nasal mucosal biopsy (3)	++	+	+	+	++	++	++
Lung (1)	++	+	+	+	++	++	++
Skin (2)	+	+	+	+	+	+	+
Tonsil (2)	++	++	++	++	++	++	++
Gastric mucosa (2)	++	+	+	+	++	++	++
Colonic mucosa (3)	++	+	+	+	++	++	++
Ileal mucosa (2)	++	+	+	+	++	++	++
Jejunal mucosa (2)	++	+	+	+	++	++	++
Rectal mucosa (2)	++	+	+	+	++	++	++
Fetal intestine (1)§	+	ND	ND	ND	ND	ND	ND

*All tissue specimens were taken from macroscopically undamaged areas of surgical specimens.

†Numbers in parentheses indicate the number of tissue samples studied.

‡Assessed by indirect immunofluorescence. Reactivity graded as: - = negative, ± = weakly positive, + to +++ = increasing degrees of positivity.

§Morphologically recognisable mast cells were not seen in the mucosal layer of fetal intestine, though they were present in small numbers in the smooth muscle layers around the gut.

ND = not done.

Table 2 *Distribution of antigens defined by mast cell granule monoclonal antibodies in non-haemopoietic tissue*

Tissue	Reactivity with mast cell granule monoclonal antibodies*
Thyroid	-
Lung parenchyma	-
Gastric epithelium	-
Jejunal epithelium	++
Ileal epithelium	++
Colonic epithelium	-
Rectal epithelium	-
Fetal gut epithelium	+/+++
Adult liver hepatocytes	+++
Fetal liver hepatocytes†	+++
CHANG liver cell line	+++
Adult kidney	-
Glomerulus	-
Glomerular capsule	+
Proximal tubule	+/+++
Distal tubule	-
Fetal kidney‡	-
S-body	-
Glomerulus	-
Proximal tubules	+/+++
Distal tubules	-
Skin	-
Epithelium	-
Dermis	-
Spleen	-
Endothelium	-

*Assessed on the same sections used for assessment of monoclonal antibody reactivity with mast cells.

†Islands of mononuclear haemopoietic cells were seen in the fetal liver, many of which were J5/BA2 positive, but which were negative with the mast cell granule monoclonal antibodies.

‡The pattern of staining with mast cell granule monoclonal antibodies on fetal kidney was similar to that with J5 (anti-CALLA), except that the former do not react with glomerular visceral epithelium.

Table 3 *Reactivity of monoclonal antibodies with haemopoietic cells other than mast cells*

Haemopoietic cell type/tissue source	Reactivity with monoclonal antibodies	
	MCG3-42	Hle-1*
Spleen	-	++
White pulp	-	++
Red pulp	-	ND
Tonsillar lymphocytes	-	+++
Peripheral blood lymphocytes	-	++
Peripheral blood monocytes	-	++
Peripheral blood granulocytes	-	++
Normal bone marrow cells	-	+/+++
Leucocytes from chronic myeloid leukaemia	-	++
Basophils from chronic myeloid leukaemia with basophilia	-	+
Basophils in long term bone marrow culture	-	+
Eosinophils from eosinophilic patient	-	ND
CEM (T lymphoid cell line)	-	+
ΓAUDI (B lymphoid cell line)	-	+
HL60 (promyelocytic leukaemia cell line)	-	++
Uninduced	-	++
Dimethyl sulphoxide induced	-	++
K562 (chronic myeloid leukaemia derived cell line with erythroid/myeloid phenotype)	-	++
U937 (myelomonocytic leukaemia cell line)	-	++

ND = not done.

*The 2D1 monoclonal antibody defining the Hle-1 panhaemopoietic antigen was a kind gift of Dr Peter Beverley.

Table 4 *Non-reactivity of monoclonal antibodies with mast cells and other cell types in a panel of animal tissues*

	<i>Species</i>		
	<i>Rat</i>	<i>Mouse</i>	<i>Dog</i>
<i>Tissues examined</i>	Kidney Skeletal Muscle Peritoneal mast cells* Tongue Colon Ileum Subcutaneous connective tissue	Colon Ileum Peritoneal mast cells* P815 (mastocytoma cell line)	Stomach Duodenum Jejunum Ileum Colon

*Obtained by peritoneal lavage.

trast microscopy in the immunostained sections.

DETERMINATION OF IMMUNOGLOBULIN

SUBCLASS OF MAST CELL GRANULE ANTIBODIES

Immunoglobulin subclasses of the monoclonal antibodies MCG3, 9, 34, 35, and 42 were determined by radial immunodiffusion. The supernatants containing mast cell granule monoclonal antibodies were concentrated by precipitation with saturated ammonium sulphate and reacted overnight with rabbit antisera to mouse IgM, IgG, IgG1, IgG2a, IgG2b, and IgG3 (Miles-Yeda, Israel). The gels were then stained with Coomassie blue to display lines of precipitation.

Results

REACTIVITY OF MONOCLONAL ANTIBODIES WITH HUMAN TISSUES

Seven cloned hybridomas (MCG 3, 8, 9, 10, 34, 35, 42) were established which secreted monoclonal antibodies reacting with granule components of human mast cells. These gave a characteristic coarse granular fluorescence pattern with mast cells in the immunising tissue (Figure (a)) and in all normal human tissues screened (see Figure (b–d) and Table 1). Both connective tissue mast cells and gastrointestinal mucosal mast cells were labelled with these antibodies. The Figure illustrates these findings—thus, dermal connective tissue mast cells (b) and jejunal and colonic gastrointestinal mucosal mast cells (c and d) and connective tissue mast cells (not illustrated) are visualised. The different monoclonal antibodies appeared to have equivalent specificity for mast cells in different tissue locations, although MCG 8, 9, and 10 consistently gave weaker reactions. In general, connective tissue mast cells were more intensely stained than gastrointestinal mucosal mast cells. This may be due to their greater granularity, as seen by staining of parallel sections with acidic toluidine blue and phase microscopy; it was also

noted that the highly granular infiltrating mast cells of systemic mastocytosis stained more intensely than normal connective tissue mast cells (Figure (a)). Furthermore, the monoclonal antibodies bound to extracellular mast cell granules and hence identified at least some degranulated mast cells, such as are seen in large numbers in nasal mucosal biopsies (data not shown).

The seven monoclonal antibodies also stained mature small intestinal enterocytes, but not small intestinal crypt, colonic, gastric, or rectal epithelial cells (Table 2 and Figure (c and d)). They also reacted with hepatocytes (Figure (f)) and with fetal and adult renal proximal tubules and parietal glomerular epithelium (Figure (e) and Table 2). In all these latter cell types reactivity was of a diffuse type, evenly distributed throughout the cytoplasm, and hence strikingly different from the granular pattern seen with mast cells. With these exceptions no reactivity was seen with any other non-haemopoietic cell type (Tables 2 and 3), although the panel of tissues tested was not exhaustive.

The monoclonal antibodies did not react with any other type of haemopoietic cell against which they were tested (Table 3). In particular, it should be noted that the granules of other myeloid cells, and of basophils especially (Table 3), are not labelled with these monoclonal antibodies.

FIXATION SENSITIVITY OF MAST CELL GRANULE ANTIGENS

None of the seven monoclonal antibodies stained mast cells, or other cell types, in tissues fixed by methods other than acetone fixation. The antigenic determinants thus were not retained in conventional formalin fixed, wax embedded histology specimens.

REACTIVITY OF MONOCLONAL ANTIBODIES WITH ANIMAL TISSUES

None of the monoclonal antibodies reacted with mast cells or other cell types in a wide range of tissues from rat, mouse, or dog (see Table 4).

Immunofluorescence micrographs of frozen sections of tissues stained with monoclonal antibody MCG3. Magnification: Figs. a–e $\times 264$; f $\times 66$.

(a) Spleen from a patient with systemic mastocytosis showing strong granular reaction pattern of monoclonal antibody MCG3 with mast cells. Residual uninvolved splenic tissue is negative.

(b) Cluster of stained mast cells (arrowed) around dermal blood vessel in section of normal adult skin.

(c) Section of adult jejunal villus showing positive granular staining pattern of mast cells (arrowed) in villus core and even cytoplasmic staining of mature enterocytes.

(d) Mast cells in adult colonic mucosa (arrowed). Note that the colonic epithelium is not stained by monoclonal antibody MCG3.

(e) 18 wk fetal kidney showing staining of proximal tubules and parietal glomerular epithelium.

(f) 18 wk fetal liver showing staining of hepatocytes. Unstained areas consisted of foci of fetal haemopoietic cells, as identified by routine histological stains.

IMMUNOGLOBULIN SUBCLASS OF MAST CELL GRANULE MONOCLONAL ANTIBODIES

All of the mast cell granule monoclonal antibodies were of the IgG1 subclass. Monoclonal antibodies MCG8 and 10 were not tested.

Discussion

Mast cells play a major part in the immediate type hypersensitivity response and participate in some other hypersensitivity reactions. Furthermore, mast cell infiltration is associated with some carcinomas and is thought to mediate tissue damage in certain inflammatory bowel and skin diseases. In many of these conditions mast cell infiltration is associated with, and thus influenced by, other cells present within the inflammatory infiltrate. An understanding of the relative contributions of these different cell types in disease pathogenesis clearly depends on their accurate identification in tissues. Standard histological techniques are not readily able to identify mast cells in tissues if they are: (a) present within the intestinal mucosa, (b) partially or completely degranulated, or (c) part of a malignant or pre-malignant proliferation of mast cells. Our previous studies on the cell membrane phenotype of mast cells suggested that they were antigenically dissimilar from other leucocytes, and especially from morphologically and functionally similar basophils,^{9,11} and we therefore reasoned that the production of monoclonal antibodies of defined specificity for mast cells would be a valid approach towards an alternative means of cell identification. Our original intention was to make monoclonal antibodies to membrane markers of mast cells and their haemopoietic precursors, which would aid in the distinction of connective tissue mast cells from gastrointestinal mucosal mast cells and thus enable their development and relative involvement in disease to be studied. However, we actually derived a series of monoclonal antibodies which reacted with granule component(s) of all mast cells. Nevertheless, the studies reported here suggest that these antibodies will be an equally useful immunohistological tool for use in the identification of mast cells in tissues.

The monoclonal antibodies possess several properties which make them a viable alternative to metachromatic dyes for the *in situ* identification of mast cells in tissues. Most importantly, they are strongly reactive with all human mast cells in both connective tissue and mucosal sites, but not with any other infiltrating leucocyte or connective tissue cell type. This is a considerable advantage over toluidine blue, which is not specific for mast cells and also largely fails to stain mucosal mast cells except in tissues fixed in non-routine fixatives such as Car-

noy's or Mota's. Furthermore, the ability to distinguish mast cells from basophils in certain situations—for example, in renal allograft rejection or cutaneous basophil hypersensitivity—is an added advantage and should allow their relative involvement to be further defined. The reaction of mast cell monoclonal antibodies seen with certain other cell types—that is, small intestinal enterocytes, hepatocytes, and proximal renal tubules—is substantially different in character to that seen with mast cells and is thus unlikely to interfere with the interpretation of immunostained tissue biopsies. In fact, these immunological “cross reactions” may aid in the eventual identification of the molecule(s) recognised by this series of monoclonal antibodies. All the tissues in question have major synthetic/secretory functions and this suggests that the mast cell granule component is likely to be one of the many enzymes released during mast cell degranulation.^{6,12} To date preliminary biochemical studies, by means of immunoprecipitation from metabolically labelled CHANG human liver cells, have as yet failed to identify the molecular nature of the antigen(s) concerned.

In summary, monoclonal antibodies specific for mast cells allow the identification and hence enumeration of mast cells compared with other cells present in inflammatory tissue infiltrates by means of immunohistological staining of either serial frozen sections or by appropriate double labelling techniques. The use of the mast cell granule monoclonal antibodies described in this paper should thus prove to be a valuable adjunct to conventional histological techniques in the investigation of the role of mast cells in the genesis of various skin, respiratory, and gastrointestinal diseases.

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