Detection of MCT and MCTC Types of Human Mast Cells by Immunohistochemistry Using New Monoclonal Anti-tryptase and Anti-chymase Antibodies

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Abstract:

We developed an improved immunohistochemical technique for distinguishing human mast cells of the MCT (tryptase-positive, chymase-negative) and MCTC (tryptase-positive, chymase-positive) types utilizing a biotinylated murine anti-chymase monoclonal antibody (MAb), termed B7, and an alkaline phosphatase-conjugated murine anti-tryptase MAb, termed G3. The B7 MAb also was used to show the selective presence of chymase in mast cells. The distribution of MCT and MCTC cells in Carnoy's fluid-fixed tissue sections of human lung, skin, small intestine, and tonsils was analyzed by the new technique and the results compared to those obtained with the older method using a rabbit polyclonal antichymase antibody and a mouse anti-tryptase MAb in indirect immunoperoxidase and indirect immunoalkaline phosphatase protocols, respectively. In tissues known to contain predominantly mature mast cells, there were no quantitative differences between the two techniques, although the staining intensity achieved with the anti-chymase MAb was greater and without development of high background, compared to results achieved with the polyclonal antibody. MCT cells were the predominant type seen in the alveoli of the lung (93%) and in the small intestinal mucosa (81%). MCTC cells predominanted in the skin (99%) and in the small intestinal submucosa (77%) and, to a lesser degree, in tonsils (60%). However, in newborn foreskin tissue which contains predominantly immature forms of mast cells, 75% of all mast cells were stained uniformly and intensely with B7, whereas only 43% were stained with the polyclonal anti-chymase antibody. Therefore, the use of MAb provides for better standardization of reagents and more accurate assessment of the distribution of human MCT and MCTC cells in tissues than previously available methods.

Keywords: Mast cell | Tryptase | Chymase | Immunohistochemistry | Heterogeneity | Human | Lung | Skin | Bowel | Tonsils

Article:

Introduction

Two types of mature mast cells have been described in the human, on the basis of differences in neutral protease composition (Schwartz et al., 1987; Irani et al., 1986), ultrastructure (Craig et al., 1988), and dependency on T-lymphocyte function (Irani et al., 1987). The MC_T cell contains tryptase alone; the MC_{TC} cell contains both tryptase and chymase. Immature forms of MC_T and MC_{TC} cells have been described which also have distinct ultrastructural features and retain the protease compositional differences of their mature counterparts (Craig et al., 1989). The original double indirect immunohistochemical technique used to distinguish MC_T and and MC_{TC} cells utilized a rabbit polyclonal anti-chymase antibody (Schechter et al. , 1986) and a mouse anti-tryptase monoclonal antibody (MAb) (Schwartz, 1985). Using this technique with tissues from adult subjects and children, MC_T cells were found to be the predominant mast cell type in small intestinal mucosa (98%) and alveoli (93%), whereas MC_{TC} cells were the predominant mast cell type in skin (88%) and small intestinal submucosa (87%) (Irani et al., 1986). However, the rabbit polyclonal antibody gives a relatively high non- specific background and has the disadvantages of availability and lot-to-lot standardization inherent to polyclonal antibodies.

The present article reports the characterization of newly produced mouse anti-chymase and anti-tryptase MAb. A modification of the previously published double immunohistochemical technique for detection of human MC_T and MC_{TC} cells was developed to overcome potential cross-linking of two antibodies from the same species. The new technique shows increased sensitivity for detection of immature forms of MC_{TC} cells.

Materials and Methods

Materials

Thirty percent H_2O_2 (Fisher Scientific; Fairlawn, NJ), 50% glutaraldehyde (Electron Microscopy Sciences; Fort Washington, PA), Alcian blue, alkaline phosphatase, biotin-amidocaproate-*N*-hydroxysuccinimide ester, peroxidase-conjugated goat anti-mouse IgG, peroxidase-conjugated streptavidin, peroxidase-conjugated goat anti-rabbit IgG, alkaline phosphatase-conjugated goat anti-rabbit IgG, 3-amino-9-ethylcarbazole, fast blue RR, and naphthol AS-MX phosphoric acid (Sigma; St Louis, MO) were obtained as indicated. Polyclonal rabbit IgG anti-chymase was purified by Schechter et al. (1983). A murine myeloma IgG_{2b}, kappa with unknown antigenic specificity, termed MPC-11, was obtained from American Type Culture Collection (Rockville, MD). Fresh surgical tissues were fixed in Carnoy's fluid (60% ethanol, 30% chloroform, 10% glacial acetic acid) for 24 hr and transferred to absolute ethanol. Tissues were embedded in paraffin and 4 μm sections were prepared. Macroscopically normal-appearing areas of skin, lung, and small bowel were used. Tonsils were hypertrophied but not acutely inflamed.

Preparation of Anti-tryptase Mab

Human tryptase was purified to homogeneity from dispersed human lung mast cells as previously described (Schwartz et al., 1981) and was used to immunize BALB/c mice. Serum samples from immunized mice were tested for the presence of anti-tryptase antibodies by an indirect ELISA (Schwartz, 1985). Spleen cells from responder mice were fused with P3X63Ag8-653 (American Type Culture Collection) murine myeloma cells. Hybridomas were grown in Dulbecco's modified Eagle's medium supplemented with 2 mmoles of L-glutamine per liter, 100

units of penicillin-streptomycin per milliliter, and 10% (v/v) fetal calf serum. Cells were grown in a Lunaire model B106 1OH incubator (Lunaime Environmental; Williamsport, PA) at 37°C and 6% CO_2. Hybridoma cells secreting antitryptase antibody were cloned and subcloned by limiting dilution into 96-well microtiter plates. One stable subclone, termed G3, was selected which secreted an anti-tryptase MAb, as determined by the indirect ELISA (Table 1). The G3 MAb was characterized as an IgG_1, kappa, antibody by an ELISA subtyping procedure (Boehringer-Mannheim Biochemicals; Indianapolis, IN). G3 antibody was generated in ascites fluid of pristane-primed BALB/c mice and was purified by chromatography on protein A-agarose. The purified antibody selectively stained tryptase by Western blot analysis of crude extracts of partially purified mast cells from lung and skin and gave results identical to those obtained with a previously reported anti-tryptase MAb termed H4 (Schwartz, 1985).

Purified MAb G3 was conjugated to alkaline phosphatase by a modification of the technique described by Voller et al. (1976). In brief, 1000 units of alkaline phosphatase were added to 0.4 mg of MAb G3 in the presence of glutaraldehyde at a final concentration of 0.2% (v/v). The mixture was incubated for 2 hr at 4°C, then dialyzed against PBS, pH 7.4, and then against 0.05 M Tris buffer, pH 8.0, containing 1 mM magnesium chloride. Bovine serum albumin (1%)(w/v) and 0.02% sodium azide (w/v)were added to the final product.

Table 1. ELISA results for anti-chymase and anti-tryptase monoclonal antibodies[a]

	OD 405	
Antibody preparation	B7 anti-chymase	G3 anti-tryptase
Pre-immune mouse serum	0.07 (0.11)[b]	0.02 (0.07)
Immune mouse serum	0.65 (0.19)	0.23 (0.06)
Hybridoma medium	0.47 (0.09)	1.28 (0.04)

[a] Sera from the chymase-immunized mouse (left column) and tryptase-immunized mouse (right column) were tested at 1:100 dilution in PBS; hybridoma media from each cell line were tested undiluted.

[b] Values in parentheses represent background OD obtained in the absence of chymase or tryptase bound to the wells.

Preparation of Anti-chymase MAb

Chymase, a neutral protease with chymotryptic specificity, was purified to homogeneity from human skin by Schechter et al. (1983) and was used to produce murine hybridomas, essentially as described previously for tryptase. BALB/c mice were immunized by intraperitoneal injection of 5 μg of chymase in complete Freund's adjuvant on day 0, boosted with an intravenous injection of 5 μg of chymase in incomplete Freund's adjuvant at 4 weeks, and boosted again at 8 weeks, 3 days before fusion, with an intraperitoneal injection of 5 μg of chymase in PBS. Serum and hybridoma media were tested for the presence of anti-chymase antibody by an indirect ELISA. Chymase (20 ng/well) was bound to Immulon C microtitem plates (Dynatech Laboratories; Chantilly, VA) according to the manufacturer's instructions. Nonspecific binding sites were blocked with 0.1% bovine serum albumin in PBS at pH 7.0 for 60 min at room temperature. Serum or hybridoma medium was added and incubated for 60 min at room temperature, followed by alkaline phosphatase-conjugated goat anti-mouse IgG (Boehringer-Mannheim) for 60 min at room temperature. The wells were washed and the alkaline

phosphatase reaction was developed with p-nitrophenyl-phosphate for 90 min at room temperature as described previously (Schwartz, 1985). Results of the ELISA for the B7 anti chymase MAb are shown in Table 1. The corresponding cloned hybridoma cell line producing this IgG_1, kappa, anti-chymase antibody was grown in ascites of pristane-primed BALB/c mice. MAb B7 was purified from ascites fluid by protein A-agarose chromatography. The purified B7 antibody failed to recognize chymase by Western blot analysis of crude extracts of dispersed human skin mast cells, probably because the corresponding antigenic site on chymase is tertiary rather than linear and is lost during the procedure. Biotinylation of purified MAb B7 was performed by incubating biotin-amidocaproate N-hydroxysuccinimide ester with MAb B7 (7.3 mg/ml) in a 5:1 molar ratio, at pH 9.2 in 0.1 M sodium bicarbonate buffer for 4 hr at room temperature, followed by dialysis against PBS, pH 7.4, at 4°C overnight.

Immunohistochemistry

Single Staining. Cytocentrifuge preparations of dispersed human lung cells, dispersed human skin cells, and of purified human neutrophils, basophils, eosinophils, and mononuclear peripheral blood cells were obtained as described (Castells et al., 1987; Schwartz et al., 1987). Slides were fixed in Carnoy's fluid for 15 mm at room temperature and subjected to indirect immunoperoxidase staining as previously described (Irani et al., 1986; Craig et al., 1986), unless stated otherwise. Endogenous peroxidase was blocked by incubation with 0.6% H_2O_2 in methanol for 30 min at room temperature, followed by washing in Tris-buffered saline with 0.05% Tween 20, pH 7.4 (TTBS). Slides were incubated with G3 (1.5 μg/ml), B7 (1.5 μg/ml), or MPC-11 (1.4 μg/ml) overnight at 4°C. After washing the slides in TTBS, a 1:50 dilution of peroxidase-conjugated goat anti-mouse IgG was added for 1 hr at room temperature. Samples were washed in TTBS and a freshly prepared solution of 3-amino-9-ethyl carbazole (0.2 mg/ml of 0.1 M acetate buffer, pH 5.2) containing 0.01% H_2O_2 was applied for 8 min at room temperature. After a final wash in distilled water, the slides were mounted in a 90% glycerol solution. Positively stained cells developed a reddish-brown color. The different cell preparations were also stained with biotinylated B7 (2 μg/ml), followed by peroxidase-conjugated streptavidin (1:50) and 3-amino-9-ethyl carbazole as described above and with alkaline phosphatase-conjugated G3 (0.7 μg/ml), followed by fast blue RR (1 mg/ml) in 0.1 M Tris, pH 8.2, containing naphthol AS-MX phosphate (0.2 mg/ml), which resulted in a blue color in positively stained cells. In addition, cytocentrifuge preparations of dispersed human lung cells were stained with 0.1% (w/v) Alcian blue in 0.7 N HCI for 15 min at room temperature (Enerback et al., 1986) and mounted in 90% glycerol. Photomicroscopy of Alcian blue-positive cells was performed. The coverslips were then floated off and the cells were fixed in Carnoy's solution and subjected to indirect immunoperoxidase with G3 or MPC-11 as described above. Photomicroscopy of the same cells was then repeated.

Simultaneous Double Labeling with Anti-tryptase and Anti-chymase MAb. Carnoy's-fixed tissue sections were de-waxed in xylene and rehydrated in graded ethanol solutions. Endogenous peroxidase was inhibited as above and slides were incubated with a mixture of alkaline phosphatase-conjugated G3 (0.7 μg/ml) and biotinylated B7 (4 μg/ml) overnight at 4°C. The tryptase positive cells (G3-positive) were stained blue by the addition of fast blue RR and naphthol AS-MX phosphate. Slides were mounted in 90% glycerol and photomicroscopy was performed. The coverslips were then floated off and chymase-positive cells (B7-positive) were stained brown by indirect immunoperoxidase utilizing peroxidase-conjugated streptavidin (1:50)

as a secondary antibody. Photomicroscopy of the same fields was then repeated. Negative controls for these experiments consisted of replacing B7 by MPC-11.

Simultaneous Double Labeling with Two Anti-chymase Antibodies. Tissue sections were processed as above and incubated with a mixture of rabbit polyclonal anti-chymase (13 μg/ml) and B7 mouse anti-chymase Mab (1.5 μg/ml) overnight at 4°C. Cells reacting with the polyclonal antibody were stained blue by addition of alkaline phosphatase-conjugated goat anti rabbit IgG (1:50), followed by fast blue RR and naphthol AS-MX phosphate as described above. After photomicroscopy was performed, B7-positive cells were stained brown by the immunoperoxidase technique and photomicroscopy was repeated. Negative controls for these experiments consisted of replacing B7 by MPC-11.

Sequential Double Labeling with Anti-chymase and Anti-tryptase Antibodies. Tissue sections were incubated first with biotinylated B7 (4 μg/ml) or with the polyclonal anti-chymase antibody (13 μg/ml), overnight at 4°C, followed by indirect immunoperoxidase utilizing peroxidase-conjugated streptavidin and peroxidase-conjugated goat anti-rabbit IgG, respectively. Alkaline phosphatase-conjugated G3 (0.7 μg/ml) was then applied overnight at 4°C, followed by fast blue RR and naphthol AS-MX phosphate.

Mast cells were counted under light microscopy at x 160 magnification. Only visibly nucleated cells were counted. Photomicroscopy was performed with a Zeiss Ultraphot microscope.

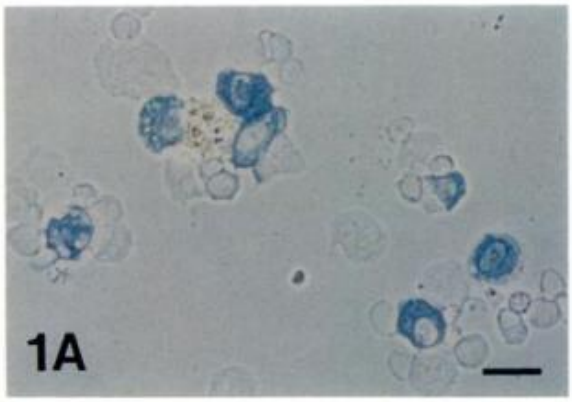
Results

Tryptase and Chymase in Dispersed Human Mast Cells and Peripheral Blood Leukocytes

In cytospin preparations of cells dispersed from mature human lung (35% mast cell purity) and human skin (64% mast cell purity), mast cells were stained intensely with the anti-tryptase MAb G3, both by an indirect immunoperoxidase technique using G3 at 1.5 μg/ml and by the direct technique using alkaline phosphatase conjugated G3 at 0.7 μg/ml. Mast cells dispersed from human skin also reacted very strongly with the anti-chymase MAb B7, at 1.5 μg/ml, and with biotinylated B7 at 2 μg/ml, consistent with previous results obtained with the polyclonal anti-chymase antibody (Schwartz et al., 1987; Irani et al., 1986). No staining was seen in the negative control experiments using MPC-11 as a primary antibody.

Neither the G3 nor the B7 antibodies stained cytocentrifuge preparations of neutrophils (95% purity), neutrophils and eosinophils (70% and 30% purity, respectively), and lymphocytes and monocytes (95% and 5% purity, respectively).

In three cytocentrifuge preparations containing 10-30% basophils, the basophils were faintly stained with MAb G3 at 1.5 μg/ml in the indirect immunoperoxidase technique, but required very high concentrations of alkaline phosphatase-conjugated G3 (≥20 μg/ml) for visualization. Basophils were not stained by B7 nor by biotinylated B7 at antibody concentrations of up to 100 μg/ml. Therefore, at the concentrations of alkaline phosphatase-conjugated anti-tryptase and of biotinylated anti-chymase antibodies used the following experiments, only mast cells are stained. Furthermore, a one-to-one correlation between Alcian blue-positive cells and G3-positive cells was observed in dispersed lung cells, as illustrated in Figures 1A and 1B, confirming the specific binding of anti-tryptase MAb G3 to mast cells.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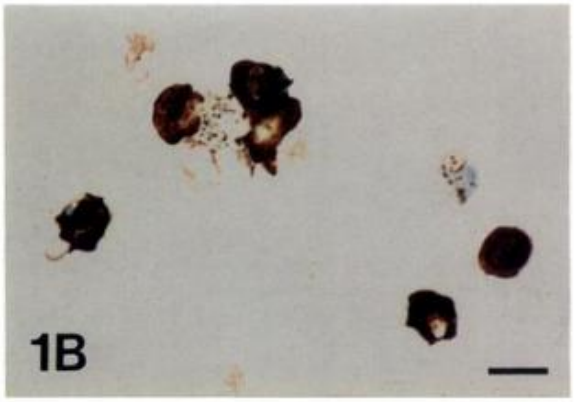
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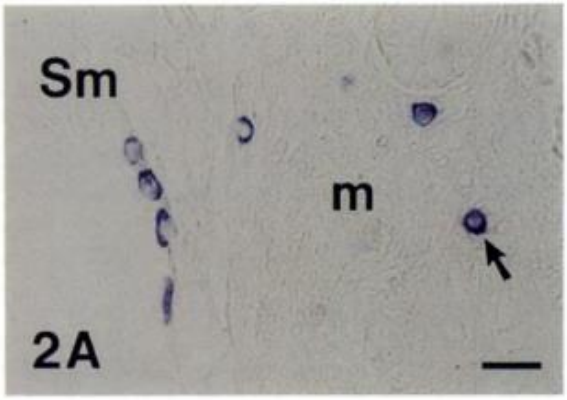
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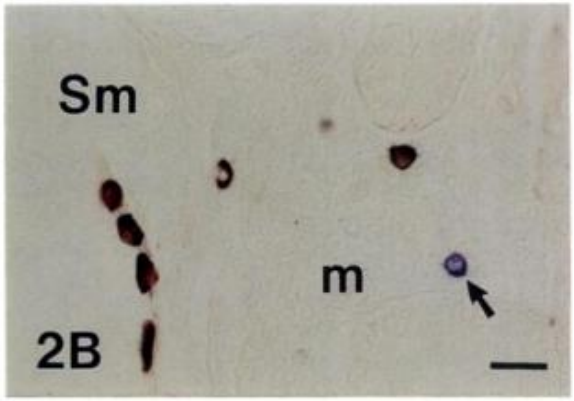
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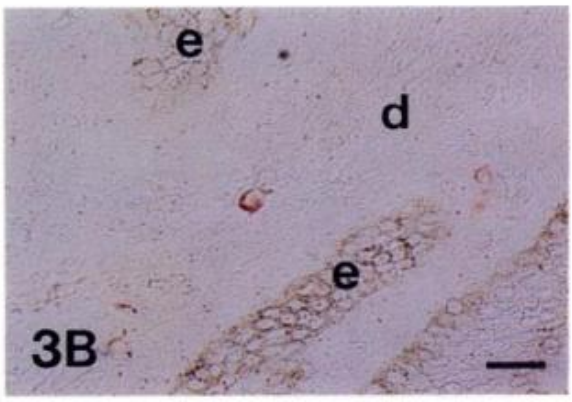
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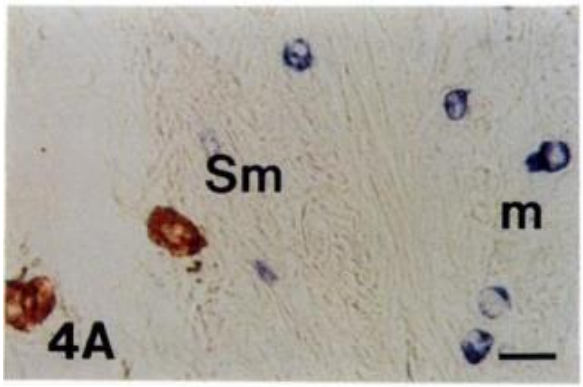
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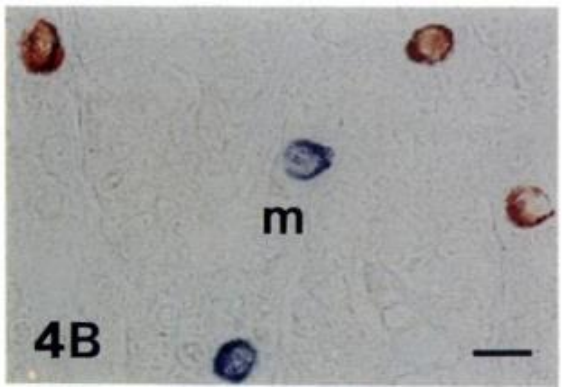
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Identification of Anti-chymase MAb B7-positive Cells as Tryptase-containing Mast Cells

Simultaneous double-labeling experiments utilizing alkaline phosphatase-conjugated anti-tryptase MAb G3, together with biotinylated anti-chymase MAb B7, were conducted on sections of human skin and small intestine fixed in Carnoy's fluid. In the first phase of staining, MAb G3 stained all mast cells blue, as shown for the intestinal submucosa and mucosa (Figure 2A). In the second phase of the experiment, chymase-positive mast cells, or MC_{TC} cells, known to predominate in the submucosa, were stained brown by MAb B7 (Figure 2B). No chymase-positive, tryptase-negative cells were found. In addition, chymase-negative mast cells, or MC_T cells, remained blue after staining with antichymase MAb B7, as demonstrated for the single mucosal MC_T cell shown in Figure 2B and for many MC-r cells in lung sections (not shown). When MAb B7 was replaced by MPC-11, all the mast cells remained blue. These findings have been reproduced in multiple sections from more than five lung, skin, and intestinal specimens.

Comparison of the Polyclonal vs the Monoclonal Anti-chymase Antibodies in Staining Mast Cells

To verify that the previously used polyclonal rabbit IgG and the monoclonal murine B7 anti-chymase antibodies recognized exactly the same mast cell subpopulation, simultaneous double-labeling experiments were performed utilizing polyclonal rabbit anti chymase antibody with the alkaline phosphatase technique, together with the murine anti-chymase MAb with the peroxidase technique. In a representative sample of adult skin, all mast cells reacting with the polyclonal antibody during the first phase of staining, visualized in blue, also reacted with MAb B7 during the second phase of staining and turned brown. When B7 was replaced by MPC-11, all the mast cells remained blue. Tissues stained with the anti-chymase MAb appeared to display less background staining and more intense specific staining of the mast cells than tissues stained with the polyclonal anti-chymase antibody.

To compare the sensitivities of the B7 and the rabbit polyclonal anti-chymase antibodies in staining mast cells, immature MC_{TC} cells in Camnoy's-fixed tissue sections of newborn foreskin were analyzed with these antibodies by the indirect immunoperoxidase technique. Mast cells dispersed from newborn foreskin have been shown to contain about 12% of the chymase and 40% of the tryptase 1evels found in mature MCTC cells (Schwartz et al., 1987). Sections stained with MAb B7 at 1.5 μg/ml showed uniformly intense staining of mast cells (Figure 3A), whereas sections stained with the polyclonal anti-chymase antibody at 26 μg/ml showed a wide range of staining intensity which on average was much weaker than with B7, and which showed a higher level of background and lower numbers of positively stained mast cells (Figure 3B).

Distribution of MC_T and MC_{TC} Cells in Normal Human Tissues

Sequential double-labeling experiments were performed on tissue sections fixed with Carnoy's fluid as described in Methods. As previously reported with the polyclonal anti-chymase antibody (Irani et al., 1986), deposition of the brown peroxidase-dependent reaction product in chymase-positive MC_{TC} cells stained with MAb B7 blocks the subsequent staining of tryptase present in these cells by alkaline phosphatase-conjugated anti-tryptase MAb G3. As a result, only chymase-negative MC_T cells bind G3 and are stained blue with the alkaline phosphatase substrate, whereas MC_{TC} cells remain brown, allowing a clear distinction between the two types of mast cells. The

increased intensity of the brown stain with MAb B7 over the polyclonal anti-chymase antibody better suppresses the staining of MC_{TC} cells by anti-tryptase antibody. This difference is crucial, as MCTC cells that are not visibly stained by the polyclonal antibody will react with the anti-tryptase antibody and be erroneously recognized as MC_T cells.

The percent distribution of MC_T and MC_{TC} cells in normal adult human skin, lung, small intestine, and tonsils was similar whether the sections were stained with MAb B7 or the polyclonal anti-chymase antibodies. Table 2 shows the results obtained with B7. As previously reported, MCTC cells were the predominant type found in the skin, where they were concentrated in the superficial dermis around blood vessels and appendigeal structures. In six of seven specimens, MC_{TC} cells were the exclusive type. In the seventh specimen, which consisted of breast skin, several MC_T cells were present around glandular structures. In the alveolar wall of the lungs, mast cells were almost exclusively of the MC_T type, with MC_{TC} cells being seen around venules and arterioles. In six of eight small intestinal specimens studied, the mucosa contained more than 85% MC_T cells (Figure 4A). However, in two specimens there was an approximately equal distribution of MC_T and MC_{TC} cells in the mucosa (Figure 4B). Each specimen was obtained from jejunum removed at surgery for a gastric bypass procedure and appeared to be normal by routine histology when reviewed by the pathologist. The small intestinal submucosa contained a majority of MC_{TC} cells in each specimen studied. In tonsil tissue mast cells were found in the connective tissue, where an approximately equal distribution of MC_T and MC_{TC} cells was demonstrated. Only MC_T cells were seen in the lymphoid follicles, although few in number. The epithelium was mostly devoid of mast cells, except in one specimen where many MC_T mast cells were seen in the epithelium just above the basement membrane.

Five different specimens of newborn foreskin were subjected to the sequential double-labeling technique utilizing MAb B7 and polyclonal anti-chymase antibody on adjacent tissue sections, respectively. The number of mast cells analyzed ranged from 200-800 per specimen. The results are shown in Table 3. In specimens incubated with B7, 25% of the mast cells stained as MC_T cells, while in those incubated with the polyclonal anti-chymase antibody 57% of the mast cells stained as MC_T cells. Since the polyclonal anti-chymase antibody failed to detect all chymase-positive cells in the newborn foreskin, as demonstrated above, the latter figure reflects falsely elevated numbers of MC_T cells.

Table 2. Distribution of MC_T and MC_{TC} cells in human tissues from adult subjects[a]

Tissue (*n*)	% MC_T	% MC_{TC}	SD
Skin (7)	0.3	99.7	0.5
Small intestine (8)			
Mucosa	81	19	23
Submucosa	23	77	30
Alveolar wall (6)	93	7	7
Tonsils (6)	40	60	11

[a] Tissue sections were stained by the sequential double-labeling technique with biotin-B7 and alkaline phosphatasc-G3.

Table 3. Distribution of MC_T and MC_{TC} cells in newborn foreskin tissue[a]

	Type of anti-chymase antibody used					
	Monoclonal anti-chymase (B7)			Polyclonal anti-chymase		
Specimen	% MC_T	(SD)	% MC_{TC}	% MC_T	(SD)	% MC_{TC}
1	32		68	51		49
2	21		79	57		43
3	39		61	66		34
4	20		80	64		36
5	12		88	49		51
Mean	25	(11)	75	57	(8)	43

[a]Tissue sections were stained by the sequential double-labeling technique using either biotin-B7 or rabbit polyclonal anti-chymase antibodies and alkaline phosphatase-G3.

Discussion

New monoclonal anti-tryptase (G3) and anti-chymase (B7) anti- bodies were produced and utilized for detection of human MC_T and MC_{TC} cells. At the concentrations used for staining, both MAb recognized only mast cells in tissue sections of human lung, skin, and bowel and in cytocentrifuge preparations of peripheral blood cells, dispersed human lung cells, and dispersed human skin cells. MAb G3 does not appear to recognize shared epitopes in other cell types, unlike the previously described anti-tryptase MAb called G5 (Craig et al., 1986; Schwartz, 1985), which stains neutrophils weakly by reacting with a crossreactive epitope on a protein distinct from tryptase. G3 binds to tryptase more effectively than the anti-tryptase MAb called H4 (Schwartz, 1985; Schwartz et al., 1985), as evidenced by ELISA and immunohistochemical staining intensity. Furthermore, MAb G3, like G5 but unlike H4, stains mast cells in thin sections by immunogold electron microscopy (unpublished data). Using these new MAb in the present study, all chymase containing mast cells were shown to contain tryptase, as previously reported (Craig et al., 1987; Irani et al., 1986). In addition, no chymase was detected by immunohistochemistry in peripheral blood neutrophils, eosinophils, lymphocytes, monocytes, and basophils, indicating its selective presence in MC_{TC} cells.

Because both MAb were from the same murine species, a new indirect immunohistochemical staining technique was developed to differentiate between MC_T and MC_{TC} cells. The procedure utilized biotinylated anti-chymase antibody and alkaline phosphatase-conjugated anti-tryptase antibody, thereby avoiding the use of secondary anti-mouse antibody that would crossreact with both primary antibodies. A similar double-labeling technique has been described by Sako et al. (1986), utilizing immunogold-silver staining in conjunction with the avidin-biotin-peroxidase complex method for simultaneous detection of T-cells and B-cells with two mouse MAb.

Percentages of MC_T and MC_{TC} cells in mature human tissues using the original and new techniques were similar. The new technique offers the advantages associated with the use of monoclonal antibodies, i.e., unlimited supply, increased staining intensity, and decreased nonspecific background staining.

The distribution of MC_T and MC_{TC} cells in skin, lung, and small intestinal submucosa was similar to what has been reported previously, with MC_{TC} cells predominating in the dermis and small intestinal submucosa and MC_T cells predominating in the alveoli. However, in the present series of small intestinal specimens, more variation was seen in the mucosal distribution of mast cell types, with six of eight specimens showing the expected predominance of MC_T cells

and the other two specimens showing equal proportions of MC_T and MC_{TC} cells. As all specimens were obtained and processed in a similar fashion and no underlying pathology was found in any, the results probably reflect normal variation. These findings stress further the importance of analyzing the distribution of MC_T and MC_{TC} cells in any individual preparation of mast cells, to unambiguously define the cell types that are present.

The distribution of mast cell types in tonsil tissue was analyzed and approximately equal numbers of MC_T and MC_{TC} cells were found in the connective tissue. Mast cells seen in lymphoid follicles and epithelium were exclusively of the MC_T type, perhaps reflecting T-lymphocyte factors in the local environment which may facilitate the appearance or development of MC_T cells. Such a phenomenon is believed to be responsible for the appearance of MC_T cells in the inflamed areas of rheumatoid synovium (Irani et al., 1987). These observations, in turn, are consistent with the apparent dependence of MC_T cells on functional T-lymphocytes, as assessed by the selective deficiency of MC_T cells in the small intestinal mucosa of subjects with acquired immunodeficiency syndrome as well as subjects with inherited combined immunodeficiency diseases (Irani et al., 1987).

A major improvement offered by the new technique is the ability to detect immature as well as mature forms of MC_{TC} cells in tissue sections. As shown by Craig et al. (1988), essentially all mast cells in the foreskin of newborn infants were immature and at various stages of development, whereas only a small proportion of mast cells in adult lung, foreskin, and small intestine (5%-15%) appeared immature. Mast cells dispersed from newborn foreskin were shown to contain decreased amounts of tryptase (two- to threefold lower) and chymase (eight- to tenfold lower) compared to mast cells from adult foreskin (Schwartz et al., 1987). Mast cells in cytocentrifuge preparations of dispersed newborn foreskin were stained much less intensely with polyclonal anti-chymase antibody than mast cells dispersed from adult foreskin (Schwartz et al., 1987). Similarly, the polyclonal anti-chymase antibody results in weak on negative staining of mast cells in tissue sections of newborn foreskin, even at the high concentration of 26 μg/ml. The polyclonal antibody probably stains the more developed forms of immature mast cells which contain higher levels of chymase, whereas the least developed forms are stained very weakly or not at all. In contrast, the staining intensity obtained in tissue sections with the new anti-chymase MAb B7 appears to be as great for mast cells from newborn foreskin as for mast cells from adult foreskin.

Immature MC_{TC} cells that are not detected with the polyclonal anti-chymase antibody are stained by the anti-tryptase MAb G3 subsequently added to the tissue sections and are erroneously detected as MC_T cells. Given that only the least mature forms of MC_{TC} cells would be mislabeled by the older immunohistochemical technique, previous reports of the distribution of mast cell types in various tissues could have underestimated the percentage of the MC_{TC} type by 5-15%, based on the percentage of immature mast cells in adult tissues (Craig et al., 1989). This may explain the higher percentage of MC_{TC} cells in adult human skin reported here (99%) as compared with the previous report of 88% MC_{TC} cells in human skin (Irani et al., 1986). On the other hand, this study demonstrated that 25% of the mast cells in tissue sections of new- born foreskin are of the MCI type. We had previously shown, in cytocentrifuge preparations of cells dispersed from newborn foreskin and stained by the older immunohistochemical technique, that 1% of the mast cells were of the MCI type (Schwartz et al., 1987). The different results obtained previously may reflect either a preferential dispersion and/or purification of MC_{TC} cells from new- born foreskin tissue or a failure of the weaker anti-tryptase MAb H4 used in that study to stain immature MC_T cells

The involvement of MC_T and MC_{TC} cells in various disease states may have pathogenic, prognostic, or therapeutic implications. The new immunohistochemical technique described in this report utilizes reagents that are better standardized, accurate, and more readily available for determination of the distribution of MC_T and MC_{TC} cells in various tissues in health and disease.

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