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Immunology

Two types of human mast cells that have distinct neutral protease compositions

(tryptase/chymotryptic proteinase)

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ABSTRACT **Two human mast cell types were identified by immunohistochemical techniques in skin, lung, and small intestine. One type contains the neutral proteases, tryptase and chymotryptic proteinase, and is termed the TC mast cell. The second type contains only tryptase and is termed the T mast cell. Both types are fixed better by Carnoy's fluid than by formalin. The percentage of mast cells accounted for by the T type was 12 in skin; 98 in mucosa and 13 in submucosa of small intestine; and 77 in bronchial/bronchiolar subepithelium, about 97 in bronchial/bronchiolar epithelium, and 93 in alveoli of lung. Dispersed lung cells contained 90% T mast cells. The mean area of TC mast cells (76 μm^2) was slightly larger than that of T mast cells (66 μm^2); however, there was such extensive overlap that individual mast cells belonging to different types could not be distinguished on the basis of size. The recognition of human mast cell types with distinct protease compositions suggests a higher level of complexity of human mast cell-mediated reactions than heretofore appreciated.**

Two types of mast cells, termed connective tissue and mucosal, have been defined in rodents (1–5) based on different histologic, functional, compositional, and pharmacologic regulatory properties. Both types have high-affinity plasma membrane receptors for IgE and show coupled activation–secretion on cross-linkage of these receptors. An analogous heterogeneity has been suspected in humans based on the presence of formalin nonfixable and fixable mast cells in human gastrointestinal mucosa and submucosa, respectively (6–8), even though convincing histochemical, functional, or compositional differences between such mast cells is lacking (4, 5, 9–11).

The presence of distinct chymotryptic neutral proteases in rat mucosal and connective tissue mast cells is a useful criterion for designation of a rat mast cell to a particular type (12, 13). For example, the finding of rat mucosal mast cell chymotryptic protease in a tumor cell line previously considered to be a rat basophil leukemia tumor (RBL-1) indicated that this tumor may be a rat mucosal mast cell leukemia tumor (14). In dispersed human lung mast cells, the major neutral protease is tryptase (15–17). Tryptase is a specific marker for all mast cells in human lung, small bowel, and skin (17–19), including formalin-fixable and -nonfixable mast cells. A second neutral protease, termed human skin chymotryptic proteinase, has been purified from skin (20) and localized to mast cells in skin and lung (21, 22). The present study uses murine monoclonal anti-tryptase and affinity-purified rabbit polyclonal anti-skin chymotryptic proteinase antibodies in a double indirect immunoenzymatic technique to demonstrate two mast cell types in human skin, lung, and small bowel, based on distinct protease compositions.

MATERIALS AND METHODS

Materials. Peroxidase-conjugated goat anti-rabbit IgG (Cooper Biomedical, Malvern, PA), alkaline phosphatase-conjugated goat anti-mouse IgG (Boehringer Mannheim), peroxidase-conjugated goat anti-mouse IgG (Bio-Rad), 3,3-diaminobenzidine tetrahydrochloride (Electron Microscopy Sciences, Fort Washington, PA), 30% H_2O_2 (Fisher), and naphthol AS-MX phosphoric acid and fast blue RR (Sigma) were obtained as indicated. Murine monoclonal IgG2b,κ anti-tryptase, termed G5 (anti-T), and a negative control IgG2b,κ, termed MPC-11 (17), as well as polyclonal rabbit IgG anti-human skin chymotryptic proteinase, termed C8 (anti-C), and IgG from nonimmunized rabbits (21) were purified as described previously. Fresh surgical or autopsy tissues were fixed in Carnoy's fluid (60% methanol/30% chloroform/10% glacial acetic acid) or in neutral buffered formalin (10% formalin in 0.08 M sodium phosphate, pH 7.4) and 5-μm-thick sections were prepared. Macroscopically normal appearing areas of skin, lung, and small bowel were used. In addition, mast cells were enzymatically dispersed from human lung and cytocentrifuge preparations were obtained as described (17).

Single Indirect Immunohistochemistry. Fixed sections were dewaxed in xylene over three 5-min periods. Endogenous peroxidase was blocked with 0.6% H_2O_2 in methanol for 30 min at 22°C. Rehydration was performed with graded ethanol solutions (100%, 95%, 80%, 70%, 50%) and H_2O, each for a 3-min period. Nonspecific staining was reduced by incubation with 10% heat-inactivated normal goat serum (NGS) for 1 hr at 22°C (lung, skin, and dispersed lung cells) or with undiluted NGS for 3 hr at 22°C (small bowel). Specimens were washed with 0.05 M Tris·HCl, pH 7.6/0.15 M NaCl/0.5% Tween 20 (TTBS) for 5 min at 22°C.

Anti-C (9 μg/ml) and anti-T (7.0 μg/ml) in phosphate-buffered saline/10% normal goat serum were incubated separately on adjacent sections of tissue overnight at 4°C. Samples were washed with TTBS for three 5-min periods and incubated with a 1:50 dilution of peroxidase-conjugated goat anti-rabbit IgG and peroxidase-conjugated goat anti-mouse IgG, respectively, for 1 hr at 22°C. Samples were again washed with TTBS for three 5-min intervals, then a freshly prepared solution of 0.5 mg of diaminobenzidine per ml of 0.05 M Tris·HCl, pH 7.6/0.01% H_2O_2 was applied to each sample for 8 min at 22°C, and the slides were sequentially washed with tap water and TTBS. Positively stained cells developed a brown color.

Double Indirect Immunohistochemistry. Double-labeling experiments with anti-C and anti-T were conducted by two methods. First, simultaneous double-labeling experiments

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Abbreviation: TTBS, 0.05 M Tris·HCl, pH 7.6/0.15 M NaCl/0.5% Tween 20.

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were performed by incubating slides overnight at 4°C with anti-C (9 μg/ml) together with anti-T (7 μg/ml). Then, as above, the slides were washed, incubated with peroxidase-conjugated goat anti-rabbit IgG, and developed with diaminobenzidine. Anti-C^+ cells were brown. Sections were then incubated with a 1:50 dilution of alkaline phosphatase-conjugated goat anti-mouse IgG for 1 hr at 22°C, washed with TTBS as above, and incubated with freshly filtered fast blue RR (1 mg/ml) in 0.1 M Tris·HCl, pH 8.2, containing naphthol AS-MX phosphate (0.2 mg/ml) (10 mg of naphthol AS-MX phosphate per ml of dimethylformamide stock) for about 15 min until blue anti-T^+ mast cells were clearly seen by light microscopy. Reactions were terminated by sequential rinsing with tap water, distilled water, and TTBS.

Second, sequential double-labeling experiments were performed. Anti-C (9 μg/ml) was applied and developed by the immunoperoxidase technique as above. Anti-T (0.5 μg/ml) was then applied and developed by the immunoalkaline phosphatase technique described above.

Single-labeled specimens were mounted in Permount; double-labeled specimens were mounted in 90% glycerol in phosphate-buffered saline. Negative controls for these experiments included replacement of anti-C by nonimmune rabbit IgG and replacement of anti-T by the IgG2b,κ murine myeloma MPC-11. Photomicroscopy was performed with a Zeiss Ultraphot microscope.

Mast cells were counted under light microscopy at 312× magnification by two independent observers. Only visibly nucleated cells in double-labeled sections were counted and sized. Mean cell areas were measured by bright-field microscopy at 1500× magnification with a Zeiss videoplan apparatus and mean cell diameters were estimated based on a spherical shape for mast cells. The intra-assay coefficient of variation for area measurements was 4%.

RESULTS

Mast Cells with Tryptase Versus Those with Chymotryptic Proteinase in Adjacent Tissue Sections. Fields from adjacent tissue sections that had been single labeled with anti-C (9 μg/ml) or anti-T (7 μg/ml) are shown in Fig. 1. At higher magnifications than that shown in Fig. 1, positively stained cells had the typical appearance of mast cells, with a rounded nucleus lacking deeply divided lobes, granular cytoplasmic staining, and a round or elongated shape (illustrations not shown). Tissue sections incubated with normal rabbit IgG or MPC-11 showed no staining (not shown). In the skin, there was an abundance of both anti-T^+ (Fig. 1*A*) and anti-C^+ (Fig. 1*B*) mast cells. No staining of the epidermis above background was detected with either anti-chymotryptic proteinase or anti-tryptase antibodies. In the small intestine (Fig. 1 *C* and *D*), anti-T^+ mast cells predominated in the mucosa, while cells in the submucosa were both anti-T^+ and anti-C^+. Regions around bronchi and bronchioles contained both anti-T^+ and anti-C^+ mast cells (Fig. 1 *E* and *F*), although anti-T^+ cells predominated. The predominance of anti-T^+ mast cells was even more striking in alveoli (Fig. 1 *G* and *H*). Although tryptase, as shown previously, is present in all mast cells (19), these experiments suggest that the chymotryptic proteinase is present only in a subgroup of mast cells.

Mast Cells with Both Tryptase and Chymotryptic Proteinase Versus Those with Tryptase Alone. To further verify that tryptase is present in mast cells with or without the chymotryptic proteinase, simultaneous double labeling experiments were conducted. Under the conditions used, all mast cells reacting with anti-C (brown) also reacted with anti-T and became blue or purple, indicating the presence of tryptase in all mast cells containing chymotryptic proteinase. These cells are referred to as TC mast cells. In addition, blue anti-T^+ cells were observed that were not initially brown, demonstrating the presence of tryptase but not chymotryptic proteinase. These cells are referred to as T mast cells.

To clearly distinguish between T and TC mast cells, a sequential double indirect immunoenzymatic technique was developed. This technique involves using a lower concentration of anti-T (0.5 μg/ml) and developing the anti-C-dependent peroxidase reaction before application of anti-T. Under these conditions, anti-T binding to TC mast cells was almost completely blocked, and anti-T binding to T mast cells occurred without appreciable attenuation. After all staining was completed, TC mast cells were brown and T mast cells were blue. The distribution of TC and T mast cells in tissue sections of human skin, lung, and small intestine that had been fixed with Carnoy's fluid and subjected to the above double-staining procedure is summarized in Table 1 and shown in Fig. 2.

In two abdominal and two lower extremity specimens of normal skin, mast cells were found predominantly around adnexal structures such as sweat glands, hair follicles, and blood vessels. Mast cells were more concentrated in the papillary superficial dermis than in the reticular deep dermis and 88% were of the TC type (Fig. 2*A*). Isolated congregations of predominantly T mast cells were occasionally observed.

In the small intestine, mast cells were more concentrated in the mucosa than in the submucosa. In the mucosa, 98% of the mast cells were T mast cells and they were concentrated at the base of the villi (Fig. 2*B*). Brown TC mast cells were rarely seen in the mucosa but accounted for 87% of the mast cells in the submucosa. Mast cells were not detected in the epithelium.

In the lung, mast cells found in the epithelium and in the lumen of bronchioles and bronchi were almost exclusively of the T type. In three specimens, there were 1, 4, and 72 epithelial mast cells found and these were, respectively, 100%, 100%, and 97% T mast cells. The subepithelium contained both types in a T/TC ratio of 3.4/1 (Fig. 2*C*). The thin alveolar walls contained 93% T mast cells (Fig. 2*D*); the few TC mast cells in alveolar regions were found almost exclusively in the respiratory bronchioles and in the adventitia of arterioles and venules. Negative control sections of skin, bowel, bronchi, and alveoli (Fig. 2 *E–H*) showed no reactivity with the antibodies.

The percentages of T and TC mast cells in cytocentrifuged preparations of dispersed human lung cells (5–15% mast cells) were 90 and 10, respectively. Light (Fig. 2*I*) and phase-contrast (Fig. 2*J*) micrographs of an anti-C/anti-T double-stained section show both T and TC mast cells.

Mean cell areas were determined for T and TC mast cells in all tissues. The mean cell area (±SD) and the calculated diameter of T mast cells were 66 ± 22 μm^2 and 9.2 μm (n = 584); for TC mast cells, these values were 76 ± 25 μm^2 and 9.9 μm (n = 453). The difference in population means between T and TC mast cells is statistically significant ($P <$ 0.05), but the large SD values indicate considerable overlap. This was confirmed by analysis of histograms that showed an overlap of mast cell areas for greater than 95% of the T and TC mast cells.

Tissues fixed with neutral buffered formalin were also examined by the double-labeling procedure. In each type of tissue, both T and TC mast cells appeared to be fewer in number and to be stained less intensely than in sections fixed with Carnoy's fluid. This effect was most marked for T mast cells. In addition, background staining after formalin fixation was higher, causing greater difficulty in discerning lightly stained cells. Thus, formalin fixation alone was not a reliable criterion to distinguish between the two types of mast cells.

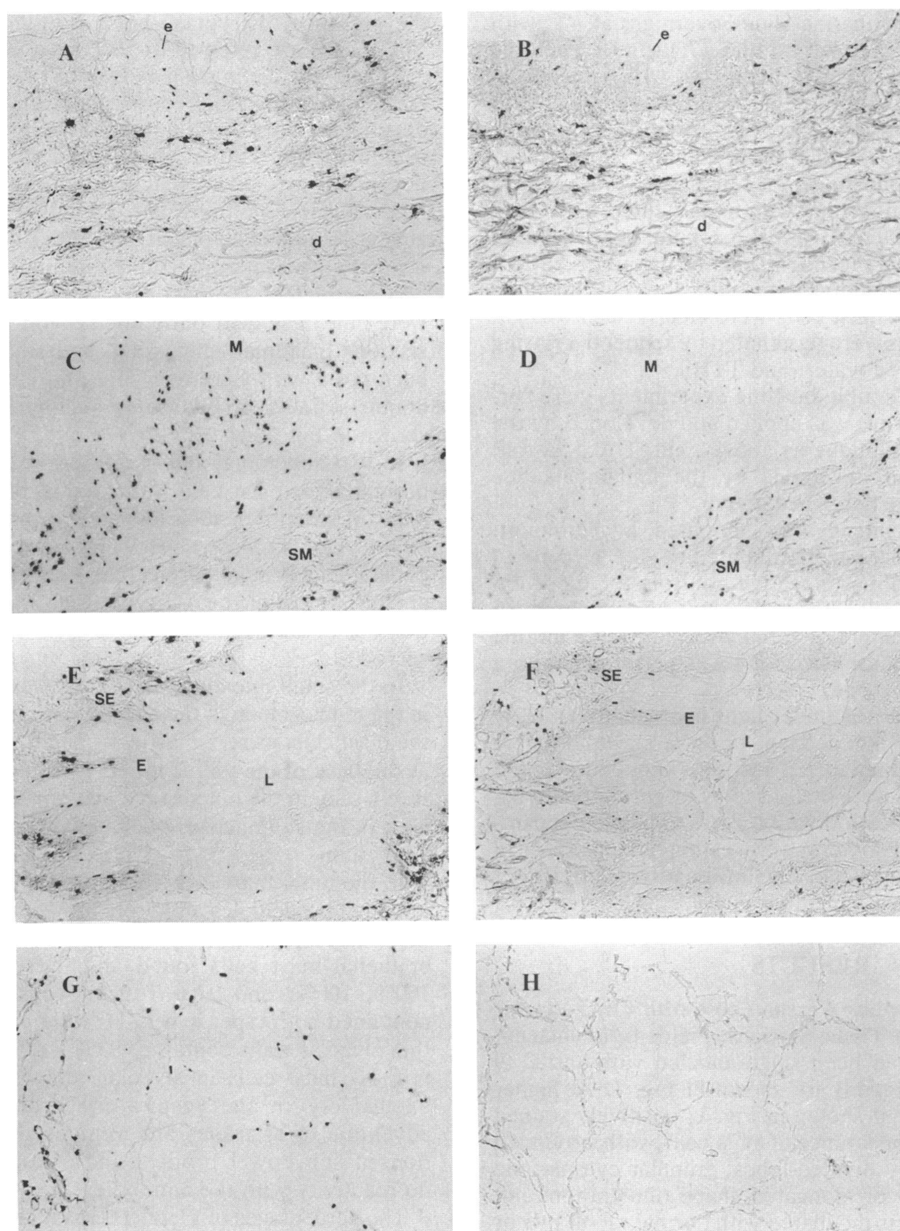

FIG. 1. Adjacent sections of skin (*A* and *B*), small bowel (*C* and *D*), bronchus (*E* and *F*), and alveoli (*G* and *H*) were stained with anti-T (*A*, *C*, *E*, and *G*) or anti-C (*B*, *D*, *F*, and *H*). e, Epidermis; d, dermis; M, bowel mucosa; SM, bowel submucosa; L, bronchial lumen; E, bronchial epithelium; SE, bronchial subepithelium. (×60.)

DISCUSSION

Two types of human mast cells have been identified based on their content of tryptase together with skin chymotryptic proteinase (TC mast cells) or of tryptase alone (T mast cells). The sequential staining procedure with anti-chymotryptic proteinase and anti-tryptase primary antibodies, using very small amounts of the latter, suppresses the appearance of anti-T reactivity in anti-C^+ cells and ensures a straightforward distinction of TC and T mast cell types. Occasionally,

Table 1. Tissue distribution of T and TC human mast cells

Tissue	Samples	Cells counted per specimen	Mast cells % T	Mast cells % TC	SEM for % T and % TC
Skin	4	90–450	12	88	±4
Small intestine					
Mucosa	5	100–300	98	2	±1
Submucosa	5	45–210	13	87	±5
Lung					
Bronchi/bronchiole subepithelium	5	25–270	77	23	±4
Alveoli	5	90–350	93	7	±2
Dispersed cells	7	150–400	90	10	±2

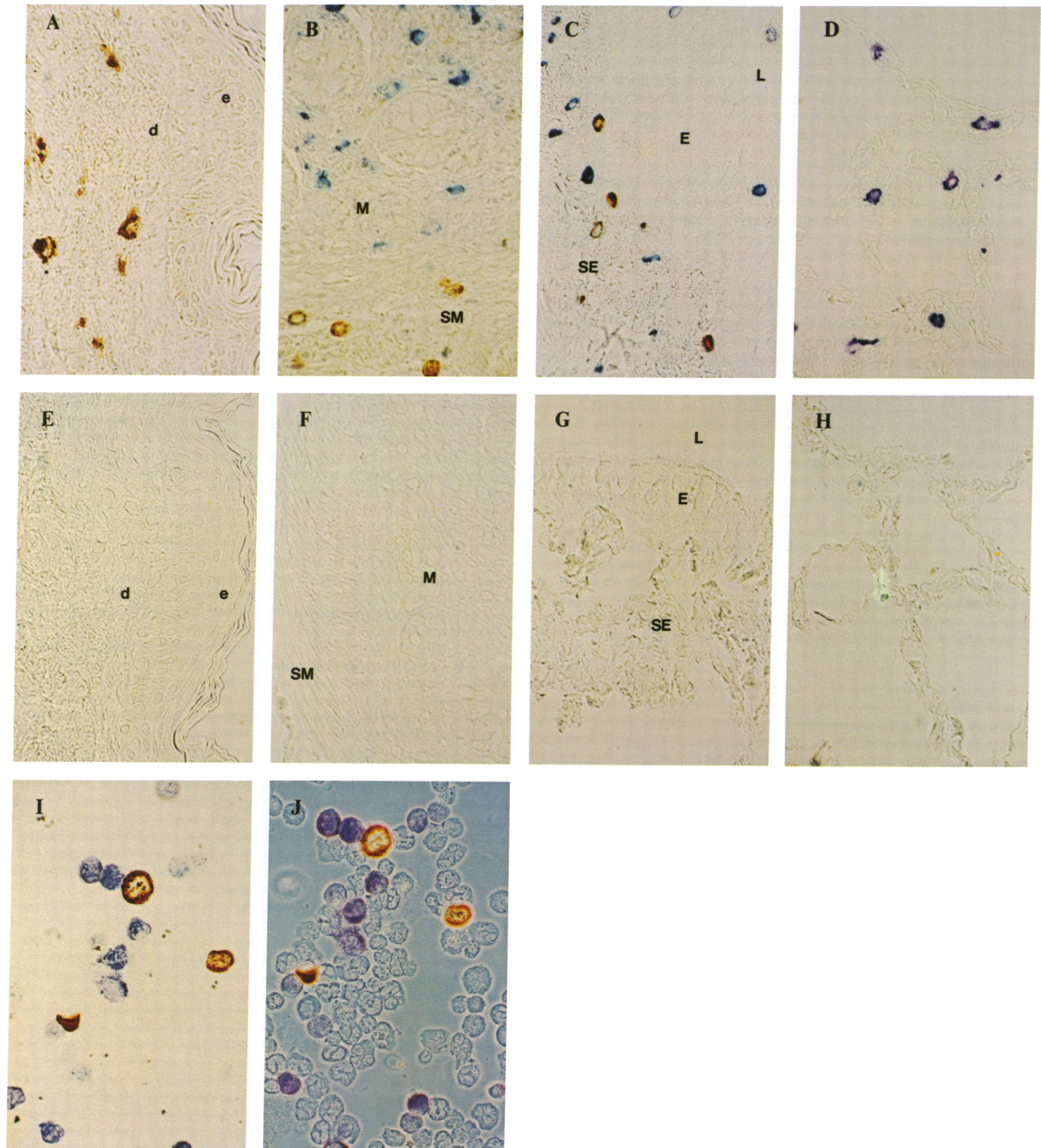

FIG. 2. Comparable sections of skin (*A* and *E*), small bowel (*B* and *F*), bronchus (*C* and *G*), alveoli (*D* and *H*), and dispersed lung cells (*I* and *J*) were stained sequentially with anti-C and anti-T (*A–D* and *I–J*) or with nonimmune rabbit IgG and MPC-11 (negative control) (*E–H*). e, d, M, SM, L, E, and SE are defined as in Fig. 1. (J) Phase-contrast micrograph of the field shown in *I*. (×520.)

a brown TC mast cell will darken, develop a blue/brown color or contain a few scattered blue granules (usually outside of the cell), but such cells are clearly distinguishable from cells that are tryptase-positive only.

TC mast cells were the predominant type found in normal skin and small bowel submucosa, whereas T mast cells were almost the exclusive type in small bowel mucosa and alveoli and also predominated in bronchial/bronchiolar regions (Table 1). Enzymatically dispersed preparations of lung mast cells contained predominantly T mast cells (90%), suggesting either a preferential dispersion of this type during the procedure used or, more likely, a substantial contribution of those in the alveolar wall (19, 23). The predominance of a particular mast cell type in specific tissue locations suggests that both types are mature cells with a purposeful presence at their respective sites.

Because TC mast cells are found predominantly in the skin and intestinal submucosa, they correspond more closely to the connective tissue type of rodent mast cells. In contrast, T mast cells are the major type in intestinal mucosa and are therefore more analogous to rodent mucosal mast cells (1-5). The failure of dispersed lung mast cells to be activated by compound 48/80 (24) is consistent with a mucosal-like mast cell designation by analogy to the behavior of rodent mucosal and connective tissue mast cells to this secretagogue (25, 26).

There is little information available to further extend these similarities to other histochemical (2, 6-9), compositional [proteoglycan class (27, 28)], or functional [products of arachidonic acid (4, 29) or dependence on T-lymphocyte mediators (3, 4)] properties of rodent and human mast cell types. One previous study showed a size variation in dispersed lung mast cells that did not correspond to heterogeneity in terms of pharmacologic regulation of mast cell mediator release (10). The size analysis performed in the present study showed that TC mast cells are slightly larger than T mast cells. However, the overlap is extensive, and accurate separation of these mast cells on the basis of size alone is unlikely. Thus, at present, the most discriminating evidence for human mast cell heterogeneity appears to be based on distinct protease composition.

Whether human skin chymotryptic proteinase is the only chymotryptic proteinase of human mast cells will need further clarification. It is possible that the T type of mast cell contains an antigenically distinct chymotryptic enzyme [analogous to the situation in rat mast cell types (12, 13)] or contains chymotryptic proteinase with blocked antigenic sites. Alternatively, chymotryptic proteinase may not be fixed in T mast cells by the current procedure. However, the relatively low level of chymotryptic activity in dispersed human lung mast cells compared to skin mast cells (16, 20, 22) suggests a profound deficiency of chymotryptic proteinase in the T type and supports the immunohistochemical data indicating absence of the chymotryptic enzyme. It is also of note that a tryptic enzyme resides in rat connective tissue mast cells (30) together with chymase; its presence in mucosal mast cells has yet to be assessed.

The physiologic and pathobiologic implications of two or more human mast cell types having distinct mediators with distinct functions will need further clarification. Tryptase, the neutral protease that is present in both T and TC mast cell types, has been shown to generate the anaphylatoxin C3a from the third component (C3) of complement (31) and to act as an anticoagulant by inactivating high molecular weight kininogen (32) and fibrinogen (33), but it has no effect on plasma prekallikrein (34), low molecular weight kininogen (35), or plasminogen (33). Chymotryptic proteinase from skin and lung mast cells is a potent activator of angiotensin I to angiotensin II (22, 36, 37). Of further importance are unresolved questions concerning the differential activation, differentiation, and proliferation of human mast cell types in different tissues and under diverse clinical conditions. The recognition of human mast cell types with distinct neutral protease compositions is a crucial step toward better understanding human mast cell biology.

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1. Enerback, L. (1966) *Acta Pathol. Microbiol. Scand.* **66**, 289-302.
2. Enerback, L. (1966) *Acta Pathol. Microbiol. Scand.* **66**, 303-312.
3. Barrett, K. E. & Metcalfe, D. D. (1984) *J. Clin. Immunol.* **4**, 253-261.
4. Katz, H. R., Stevens, R. L. & Austen, K. F. (1985) *J. Allergy Clin. Immunol.* **76**, 250-259.
5. Bienenstock, J., Befus, A. D., Denburg, J., Goodacre, R., Pearce, F. & Shanahan, F. (1983) *Monogr. Allergy* **18**, 122-128.
6. Befus, D., Goodacre, R., Dyck, N. & Bienenstock, J. (1985) *Int. Arch. Allergy Appl. Immunol.* **76**, 232-236.
7. Ruitenburg, S. J., Gustowska, L., Elgersma, A. & Ruitenburg, H. M. (1982) *Int. Arch. Allergy Appl. Immunol.* **67**, 233-238.
8. Strobel, S., Miller, H. R. P. & Ferguson, A. (1981) *J. Clin. Pathol.* **34**, 851-858.
9. Holgate, S. T., Benyon, R. C., Howarth, P. H., Agius, R., Hardy, C., Robinson, C., Durham, S. R., Kay, A. B. & Church, M. R. (1985) *Int. Arch. Allergy Appl. Immunol.* **77**, 47-56.
10. Schulman, E. S., Kagey-Sabotka, A., MacGlashan, D. W., Jr., Adkinson, H. F., Jr., Peters, S. P., Schleimer, R. P. & Lichtenstein, L. M. (1983) *J. Immunol.* **131**, 1936-1941.
11. Fox, C. C., Dvorak, A. M., Peters, S. P., Kagey-Sabotka, A. & Lichtenstein, L. M. (1985) *J. Immunol.* **135**, 483-491.
12. Haig, D. M., McKee, T. A., Jarrett, E. E. R., Woodbury, R. & Miller, H. R. P. (1982) *Nature (London)* **300**, 188-190.
13. Woodbury, R. G., Gruzenski, G. M. & Lagunoff, H. D. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2785-2789.
14. Seldin, D. C., Adelman, S., Austen, K. F., Stevens, R. L., Hein, A., Caulfield, J. P. & Woodbury, R. G. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3871-3875.
15. Schwartz, L. B., Lewis, R. A., Seldin, D. C. & Austen, K. F. (1981) *J. Immunol.* **126**, 1290-1294.
16. Schwartz, L. B., Lewis, R. A. & Austen, K. F. (1981) *J. Biol. Chem.* **256**, 11939-11943.
17. Schwartz, L. B. (1985) *J. Immunol.* **134**, 526-531.
18. Schwartz, L. B., Foley, J. V., Austen, K. F., Soter, N. A., Shepard, R. & Murphy, G. F. (1985) *J. Allergy Clin. Immunol.* **76**, 182-188.
19. Craig, S. S., Moncure, C. W., Levine, E. & Schwartz, L. B. (1985) *Ann. Allergy* **55**, 272 (abstr.).
20. Schechter, N. M., Fraki, J. E., Geesin, J. C. & Lazarus, G. S. (1983) *J. Biol. Chem.* **258**, 2973-2978.
21. Schechter, N. M., Choi, J. K. & Lazarus, G. S. (1985) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**, 984 (abstr.).
22. Wintroub, B. U., Kaempfer, C. E., Schechter, N. H. & Proud, D. (1986) *J. Clin. Invest.* **77**, 196-201.
23. Fox, B., Bull, T. B. & Guz, A. (1981) *J. Clin. Pathol.* **34**, 1333-1342.
24. Church, M. K., Pao, G. J.-K. & Holgate, S. T. (1982) *J. Immunol.* **129**, 2116-2121.
25. Befus, A. D., Pearce, F. L., Gauldie, J., Horsewood, P. & Bienenstock, J. (1982) *J. Immunol.* **128**, 2475-2480.
26. Sredni, B., Friedman, N. M., Bland, C. E. & Metcalfe, D. D. (1983) *J. Immunol.* **131**, 915-922.
27. Metcalfe, D. D., Lewis, R. A., Silbert, J. E., Rosenberg, R. D., Wasserman, S. I. & Austen, K. F. (1979) *J. Clin. Invest.* **64**, 1537-1543.
28. Yurt, R. W., Leid, R. W., Austen, K. F. & Silbert, J. E. (1977) *J. Biol. Chem.* **252**, 518-521.
29. Peters, S. P., MacGlashan, D. W., Jr., Schulman, E. S., Schleimer, R. P., Hayes, E. C., Rokach, J., Adkinson, N. F., Jr., & Lichtenstein, L. M. (1984) *J. Immunol.* **132**, 1972-1979.
30. Kido, H., Fukusen, N. & Katunuma, N. (1985) *Arch. Biochem. Biophys.* **239**, 436-443.
31. Schwartz, L. B., Kawahara, M. S., Hugli, T. E., Vik, D., Fearon, D. T. & Austen, K. F. (1983) *J. Immunol.* **130**, 1891-1895.
32. Maier, M., Spragg, J. & Schwartz, L. B. (1983) *J. Immunol.* **130**, 2352-2356.
33. Schwartz, L. B., Bradford, T. M., Littman, B. L. & Wintroub, B. U. (1985) *J. Immunol.* **135**, 2762-2767.
34. Schwartz, L. B., Bradford, T. M. & Griffin, J. H. (1985) *Biochem. Biophys. Res. Commun.* **129**, 76-81.
35. Schwartz, L. B., Maier, M. & Spragg, J. (1986) *Adv. Exp. Med. Biol.*, in press.
36. Wintroub, B. U., Schechter, N. B., Lazarus, G. S., Kaempfer, C. E. & Schwartz, L. B. (1984) *J. Invest. Dermatol.* **83**, 336-339.
37. Reilly, C. F., Tewksbury, D. A., Schechter, N. M. & Travis, J. (1982) *J. Biol. Chem.* **257**, 8619-8622.