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Surface CD88 functionally distinguishes the MC_{TC} from the MC_T type of human lung mast cell

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Abstract

Background: MC_T and MC_{TC} types of human mast cells (MCs) are distinguished from one another on the basis of the protease compositions of their secretory granules, but their functional and developmental relationships have been uncertain.

Objective: These studies better define the functional properties and developmental relationship of MC_T and MC_{TC} cells.

Methods: Mast cells were dispersed from human skin and lung, purified with anti-Kit antibody, and separated into $CD88^+$ and $CD88^-$ populations by cell sorting. These cells were evaluated by immunocytochemistry with antitryptase and antichymase mAbs; for chymase and tryptase mRNA by real-time RT-PCR; for conversion of MC_T to MC_{TC} cells during cell culture with recombinant human stem cell factor and recombinant human IL-6; and for degranulation and leukotriene C_4 (LTC_4) secretion when stimulated with anti-Fc∈RI, substance P, C5a, and compound 48/80.

Results: Mature MC_T and MC_{TC} cells were separated from one another on the basis of selective expression of CD88, the C5aR, on MC_{TC} cells. Lung MC_T cells had negligible levels of chymase mRNA and retained their MC_T phenotype in culture. Mature MC_{TC} cells from skin and lung degranulated in response to Fc∈RI cross-linking, C5a, compound 48/80, and substance P. Lung MC_{TC} cells released LTC_4 on activation, but no LTC_4 was detected when skin-derived MC_{TC} cells were activated. MC_T cells from lung degranulated and released LTC_4 in response to anti-Fc∈RI and substance P, but not to C5a and compound 48/80.

Conclusion: These observations functionally distinguish MC_T from MC_{TC} types of human mast cells and suggest important differences that may affect their participation in diseases such as asthma and urticaria.

Keywords

Mast cells; human; IL-6; complement; tryptase; chymase; C5a; substance P; CD88

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The MC_{TC} and MC_T types of human mast cells (MCs) were initially recognized on the basis of the protease compositions of their secretory granules, with tryptase, chymase, carboxypeptidase A3, and cathepsin G in the former and only tryptase in the latter.[1-3] MC_{TC} cells are essentially the exclusive type of mast cell in normal skin but account for the minority of mast cells in normal lung. MC_T cells predominate in the alveolar wall and epithelium of the lung, whereas MC_{TC} cells favor the bronchial smooth muscle and glandular regions. Markedly elevated levels of the MC_{TC} cell in the bronchial smooth muscle of patients with asthma correlate with their bronchial hyperreactivity.[4] MC_T cell numbers in respiratory epithelium increase during the pollen season in sensitive subjects.[5-7] These distinct tissue distributions and disease associations suggest a purposeful presence for each type of mast cell, but little is known of their functional differences.

Skin-derived human mast cells (Sk-MCs) can be activated by C5a, whereas lung-derived human mast cells (Lu-MCs) do not respond, presumably because C5aR (CD88) is expressed on >80% of juvenile foreskin-derived mast cells but on, 10% of Lu-MC, tonsillar mast cells, and uterine mast cells.[8] Compound 48/80 also stimulates Sk-MC but not Lu-MC. Various neuropeptides such as substance P, vasoactive intestinal peptide, somatostatin, and calcitonin gene-related protein fail to activate Lu-MC in some studies[9,10] but stimulate such mast cells in others.[11,12] One possibility to account for such discrepancies is the relative portions of MC_T and MC_{TC} cells in the tested preparations, a factor that typically is not considered in most studies. Whether these tissue-aligned functional differences in mast cell activation profiles reflect differences between MC_{TC} or MC_T types of mast cells or instead reflect the different microenvironments of these tissues cannot be precisely determined unless the 2 types of mast cell present in the lung-derived preparations are first purified and separated from one another.

Another unresolved issue is the developmental relationship between MC_T and MC_{TC} types of mast cells. This protease phenotype appears *in vivo* soon after granule formation can be detected by electron microscopy,[13] suggesting parallel rather than sequential development. In contrast, observations with cord blood-derived human mast cells or bone marrow-derived mast cells suggest that tryptase$^+$/chymase$^-$ cells become tryptase$^+$/chymase$^+$ over time,[14-17] implying sequential development. Another interpretation is that MC_{TC} cells expand whereas MC_T cells expire under the culture conditions used. Indeed, Sk-MC_{TC} cells proliferate in stem cell factor (SCF)-containing serum free medium more readily than MC_T-enriched Lu-MC,[18] and MC_T but not MC_{TC} cells undergo IL-4-mediated apoptosis.[19] Alternatively, immature MC_{TC} cells that produce little if any chymase protein, and thereby appear to be of the MC_T type will become chymase$^+$ as they mature. Again, purification and separation of MC_T and MC_{TC} cells will enable a more precise delineation of their developmental relationship.

The current study shows that mature MC_{TC} cells from Lu-MC and Sk-MC express CD88 and can be separated from MC_T cells by sorting with anti-CD88 antibody. Cultured MC_T cells from lung remain deficient in CD88 and chymase protein and chymase mRNA. MC_{TC} cells obtained from skin and from lung degranulate in response to anti-Fc∈RI, compound 48/80, substance P, and C5a. Although Lu-MC_{TC} cells produce LTC_4 in response to these agonists, Sk-MC_{TC} cells do not. Lu-MC_T cells only respond to anti-Fc∈RI and substance P by degranulating and secreting LTC_4. Thus, MC_T and MC_{TC} cells appear to be functionally distinct.

METHODS

Culture of Lu-MC and Sk-MC

All experimental protocols involving human tissues were approved by the Human Studies Committee at Virginia Commonwealth University. Surgical lung or skin tissue samples were obtained from consented patients through the Cooperative Human Tissue Network (Columbus,

Ohio), the National Disease Research Interchange Center (Philadelphia, Pa), or the Departments of Surgery or Pathology at Virginia Commonwealth University. Lung specimens were typically lobectomies from patients with lung cancer, whereas skin specimens were from either breast reductions or abdominoplasties. Lu-MC[20] and Sk-MC[18] were prepared as described. Lu-MC, after enrichment by Percol (Amersham-Pharmacia Biotec, Uppsala, Sweden)-dependent sedimentation, ranged in purity from 5% to 45% (mean ± SD, 21% ± 12%; n = 9), and these mast cells consisted of, 5% (n = 2) to 13% MC_{TC} cells (6% ± 3%; n = 9). They were cultured in RPMI 1640 supplemented with 10% heat-inactivated controlled process serum replacement medium 3 (Sigma-Aldrich, St Louis, Mo), 2 mmol/L L-glutamine, 0.1 mmol/L nonessential amino acids, 10 mmol/L HEPES, 50 mmol/L 2-β-mercaptoethanol, 200 U/mL penicillin, 100 mg/mL streptomycin, and recombinant human (rh) SCF (100 ng/mL; a gift from Amgen, Thousand Oaks, Calif) alone or with rhIL-6 (50 ng/mL; R & D Systems, Minneapolis, Minn), whereas Sk-MC was cultured in AIM-V medium (Life Technologies, Rockville, Md) containing rhSCF (100 ng/mL). All cells were cultured in a Nuaire incubator (Plymouth, Minn) at 37°C in 6% CO_2, harvested and counted (viability by trypan blue dye exclusion), and then subjected to cytocentrifugation for immunocytochemistry, apoptosis determination, or cell sorting.

Immunocytochemistry

Cytocentrifuge preparations of cells were fixed in methanol containing 0.6% H_2O_2 for 30 minutes at room temperature, rinsed with H_2O, and stored at 4°C until used. Slides were labeled with biotin-conjugated B7, a mouse IgG1 antichymase mAb, alkaline phosphatase-conjugated G3, a mouse IgG1 antitryptase, or nonimmune negative controls to identify chymase$^+$ MC_{TC} cells and tryptase$^+$ mast cells as described.[21]

Flow cytometry, enrichment of mast cells, and cell sorting

Cells were analyzed for expression of surface Kit by using a phycoerythrin-labeled purified mouse antihuman CD117 mAb or a phycoerythrin-labeled isotype-matched mAb as the negative control (5 mg/mL for each mAb; BD Biosciences Pharmingen, San Diego, Calif). In the case of lung cell preparations, alveolar macrophages (about 30% of initially collected cells) were depleted with antihuman CD14 mAb bound to Dynabeads (4 beads per target cell) during a 1 hour of incubation at 4°C (Dynal, Oslo, Norway). A rabbit antihuman CD88 mAb (10 mg/mL) or a nonimmune rabbit IgG (10 mg/mL) as the negative control was used with an Alexa Fluor 488-labeled goat antirabbit IgG (5 mg/10^6 cells; Molecular Probes, Eugene, Ore) to identify CD88$^+$ cells.

In some cases, mast cells were further purified with mouse IgG anti-Kit mAb (BD Biosciences Pharmingen) and magnetic beads coupled with sheep IgG antimouse IgG (Dynal, Oslo, Norway) as described.[22] Purities of Lu-MC typically exceeded 95%. In other cases, a MoFlo high-performance cell sorter (Cytomation, Fort Collins, Colo) was used to purify Lu-MC and separate them into 2 groups, Kit$^+$/CD88$^-$ and Kit$^+$/CD88$^+$, by using the antibodies described. Each mast cell group consisted of >99% mast cells on the basis of staining with acidic toluidine blue (0.5% in 0.5 moles/L HCl) and were further characterized for the MC_T and MC_{TC} phenotype by immunocytochemistry.

Quantitative real-time PCR

Total RNA was isolated by guanidinium-phenol extraction, and RT was performed with 1 mgto5 mg total RNA, 200 U of Moloneymurine leukemia virus RT, a 3′-primer mix (containing the 3′ primers for tryptase, chymase, and glyceraldehyde phosphate dehydrogenase (GAPDH) at 25 nmol/L each), 10 mmol/L dithiothreitol, 13 first strand buffer, and 1 mmol/L deoxynucleotide triphosphate mix at 37°C for 2 hours, as recommended by Invitrogen Corp (Carlsbad, Calif). Real-time PCR was performed by the VCU Nucleic Acids Research Facility

with an ABI Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, Calif) by using the TaqMan One Step PCR Mast Mix Reagents Kit (Applied Biosystems, P/N: 4309169). All of the samples were tested in triplicate under the conditions recommended by the fabricant. Cycling conditions were 48°C for 30 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The cycle threshold was determined to provide the optimal standard curve values (0.98-1.0). The probes and primers were designed by using the Primer Express 2.0 version. Probes were labeled on the 5′ end with 6-carboxyfluoresceine and on the 3′ end with 6-carboxytetramethylrhodamine. Ribosomal RNA (18S) from the Pre-Developed TaqMan Assay Reagents (P/N: 4310893E) was used as endogenous control. The following primers and probes were used:

Tryptase. Sense (nucleotides 498-517) 5′-GGCGATGTGGACAATGATGA-3′, antisense (nucleotides 610-591) 5′-GTGTAGGCGCCAAGGTGGTA-3′, probe (nucleotides 556-584) 5′-TCCCCATAATGGAAAACCACATTTGTGAC-3′ for which the sense primer spans exons 4 and 5, whereas the antisense primer and probe recognizes sequences in exon 5.

Chymase. Sense (nucleotides 198-216) 5′-GCTGACGGCTGCTCATTGT-3′, antisense (nucleotides 290-269) 5′-TCAAGCTTCTGCCATGTGTCTT-3′, probe (nucleotides 223-254) 5′-AGGTCTATAACAGTCACCCTTGGAGCCCATAA-3′ for which the sense primer recognizes an exon 2 sequence, the probe recognizes a sequence that spans exons 2 and 3, and the antisense primer recognizes an exon 3 sequence.

18S rRNA. Sense and antisense primers and probes were obtained from the Predeveloped TaqManAssay Reagents (P/N:4310893E) and used as directed (Applied Biosystems).

Quantification of chymase and tryptase staining intensities of Lu-MC

Photomicrographs of immunocytochemically stained cytocentrifuged mast cells were taken with a digital camera by using a 40× objective (Olympus MagnaFire; Olympus, Melville, NY). A fixed area was drawn within the cytoplasm of each cell to be analyzed. Gray value intensities were displayed as pixels on a scale of 0 to 255. Staining intensities were quantified within each area, and data were analyzed by using the analySIS software package (Soft Imaging System, Lakewood, Colo). Each region of interest provided a given intensity profile, with a mean intensity calculated by the software. Background values were determined with isotype-matched negative control mAbs. Mast cells from 3 separate lung cell preparations were analyzed by a blinded observer.

Human mast cell activation and mediator assays

Mast cells were adjusted to a concentration of 10^6 cells/mL, washed twice in Tyrodes buffer (without magnesium and calcium) containing 0.05% gelatin, and resuspended in Tyrodes-gelatin buffer supplemented with 2.5 mmol/L $CaCl_2$ and 1 mmol/L $MgCl_2$. After cells were preincubated for 5 minutes at 37°C in microcentrifuge tubes, activation experiments were performed for 2 hours at 37°C with 3 mg/mL anti-FcεRIa (22E7) mAb, which was generously provided by Jarema P. Kochan, PhD (Hoffman-LaRoche, Inc, Nutley, NJ),[23,24] C5a (1 mg/mL), compound 48/80 (1 μg/mL), and substance P (1 mmol/L; Sigma-Aldrich). These doses were determined previously to be optimal for degranulation without toxicity. Ice-cold Tyrodes-gelatin buffer without calcium and magnesium was used to stop the reaction. After centrifugation, cell releasates in the supernatants were collected in separate tubes and brought to a final NaCl concentration of 1 mol/L. Cell pellets were resuspended in Tyrodes-gelatin buffer containing 1 mol/L NaCl and lysed by sonication on ice. Cell releasates and lysates were kept at 280°C until assayed. β-Hexosaminidase was measured in cell releasates and lysates and used to calculate percent degranulation: releasate/(lysate + releasate) × 100. Net percent release values were calculated by subtracting the percent release values for unstimulated cells from

those of the stimulated cells. Cysteinyl-leukotriene (LT) levels in releasates were measured with an ELISA for $LTC_4/D_4/E_4$ (Amersham Pharmacia Biotech, Piscataway, NJ). For this ELISA, the cross-reactivity between LTC_4 and LTD_4 is 100%; LTC_4 and LTE_4 is 70%; and LTC_4 and LTB_4 is 0.3%. Concentrations were determined according to the manufacturer's instructions, with a lower limit of detection of 10 pg/mL.

RESULTS

CD88-dependent sorting of MC_T and MC_{TC} cells from lung

Because MC_{TC} cells from skin express the C5aR, CD88, experiments were conducted to see whether CD88 also was expressed on MC_{TC} cells from lung and could enable MC_{TC} cells to be separated from MC_T cells by cell sorting. As shown in Fig 1, after sorting Lu-MC, 4% of the cells in the $Kit^+/CD88^-$ gate were $chymase^+$, whereas greater than 98% of the cells in the $Kit^+/CD88^+$ gate were $chymase^+$. This result was representative of 3 independent experiments. By real-time/RT-PCR, the 18S-rRNA-normalized ratios of mRNAs from $chymase^-$ Lu-MC to those of Sk-MC are shown in Table I. Chymase mRNA levels in Lu-MC_T cells were negligible (undetected in 2 of 3 preparations). In contrast, tryptase mRNA levels, although not as high as in Sk-MC_{TC} cells, were still substantial in all 3 preparations of Lu-MC_T cells.

Activation profiles for MC_T and MC_{TC} cells from skin and lung for degranulation and LTC_4 release

To define further the functional phenotypes of MC_T and MC_{TC} cells purified from Lu-MC and of MC_{TC} cells from skin, each was challenged with anti-Fc∈RI mAb, C5a, compound 48/80, and substance P. MC_T and MC_{TC} cells from lung were purified and sorted on the basis of surface Kit and CD88 as in Fig 1, whereas Sk-MC_{TC} cells were used after 4 to 8 weeks of culture. As shown in Fig 2 (*left panels*), both types of mast cell from lung as well as MC_{TC} cells from skin degranulated (β-hexosaminidase release) when exposed to anti-Fc∈RI mAb and substance P. MC_{TC} cells from each source degranulated in response to C5a and compound 48/80, with those from lung showing similar levels of β-hexosaminidase release compared with those from skin. In contrast, MC_T cells from lung failed to degranulate when challenged with C5a and compound 48/80. Secretion of the newly generated lipid mediator, LTC_4, also was assessed. As shown in Fig 2 (*right panels*), the activation profiles for Lu-MC_T and Lu-MC_{TC} cells to produce LTC_4 were similar to those for degranulation. In contrast, Sk-MC_{TC} cells failed to secrete LTC_4 in response to all stimuli.

Lu-MC_T cells fail to convert to MC_{TC} cells in culture

Kit-purified lung-derived mast cells were cultured for 1 week with rhSCF (100 ng/mL) in the presence or the absence of rhIL-6 (50 ng/mL; n = 3 lungs). rhIL-6 was used because it enhances chymase and CD88 expression by cord blood-derived human mast cells (Oskeritzian CA and Schwartz LB, unpublished data, 2004). Cells were counted, cytocentrifuged, stained separately with antichymase and antitryptase mAbs, and analyzed for staining intensity as described in Methods. The mean percentages of $chymase^+$ mast cells in the group treated with rhIL-6 for 6 days (8.7% ± 1.2%) were not significantly different from those for the group not treated with rhIL-6 (9.7% ± 2.9%; $P = .67$; 2-tailed *t* test; n = 3). Thus, new chymase-expressing cells were not evident in these mixed cultures. Nevertheless, Fig 3 shows that rhIL-6 significantly increased the mean staining intensity for chymase, presumably among those cells that already had declared themselves to be of the MC_{TC} type by day 0. In contrast, no significant difference in the mean intensities of staining for tryptase between rhIL-6-treated and rhIL-6-untreated cells was observed.

When $Kit^+/CD88^-$ mast cells were placed into culture for 6 days with and without rhIL-6, the percentages of $chymase^+$ cells among the untreated (1.8 ± 1.1) and treated (2.0 ± 2.0) groups

were not significantly different from one another ($P = .9$; n = 3), nor from the starting percentages. This result indicates that mature Lu-MC_T cells do not convert to MC_{TC} cells when placed in culture with rhSCF alone or together with rhIL-6 under the experimental conditions used. In none of the lung mast cell experiments was an appreciable difference detected in cell numbers between the treated and untreated groups after the 6-day cultures. Thus, Lu-MC_T cells do not express chymase mRNA and are not converted into MC_{TC} cells under our culture conditions.

DISCUSSION

The current study makes 3 important observations. First, the mature MC_{TC} type of mast cell in lung and skin expresses CD88, the receptor for C5a, whereas mature MC_T cells in lung do not. Accordingly, cell sorting of Kit^{hi} lung-derived mast cells with anti-CD88 antibody separates $CD88^-$ MC_T from $CD88^+$ MC_{TC} cells. Thus, surface expression of CD88 corresponds to the MC_{TC} phenotype, regardless whether the MC_{TC} cell originates from the skin or lung. Lu-MC_T cells express negligible levels, if any, of chymase mRNA, similar to a previous report.[20] This extends the definition of MC_T cells to include the absence of chymase mRNA as well as protein. How these criteria will apply to immature, primed, mastocytosis, or leukemic mast cells and to mast cells at other sites remains to be determined. For example, HMC1 leukemic mast cells express small amounts of chymase mRNA in the absence of detectable chymase protein,[20] but also exhibit functional CD88 on their surface.[8,25,26]

Second, Lu-MC_T cells do not convert to MC_{TC} cells under the culture conditions used, which include rhSCF in the presence and absence of rhIL-6 for 6 days. RhIL-6 enhances chymase and CD88 expression by cord blood-derived human mast cells (Oskeritzian CA and Schwartz LB, unpublished data, 2004). Indeed, Lu-MC_{TC} cells cultured with rhIL-6 express somewhat higher levels of chymase based on immunocytochemical staining intensity, whereas tryptase staining intensities do not change. In contrast, when Lu-MC_T cells are treated with rhIL-6, they become resistant to the apoptosis-mediating effect of IL-4.[19] MC_{TC} cells at baseline are not susceptible to IL-4-mediated apoptosis.

A third important finding is that lung MC_{TC} cells, like those from skin, are activated by C5a and compound 48/80. Previous reports that Lu-MC fail to degranulate in response to C5a or compound 48/80 were probably a result of MC_{TC} cells accounting for a minor portion of the total mast cells,[8] their activation obscured by the spontaneous release of mediators from the more numerous MC_T cells. Previously, the mean percentage of MC_T cells among the mast cells dispersed from lung were reported as 90,[21] although rarely a preparation appears with almost all MC_T cells.[20] In certain patients with chronic urticaria, activation of MC_{TC} cells in skin by anti-Fc∈RI autoantibody and locally generated C5a has been proposed.[27] In patients with asthma, C′ anaphylatoxin levels are elevated in bronchoalveolar lavage fluid[28] and MC_{TC} cell numbers are elevated in bronchial smooth muscle in relation to bronchial hyperreactivity,[4] raising the possibility of C5a-facilitated activation of these mast cells.

Mast cells dispersed from synovium of patients with rheumatoid arthritis, when examined by flow cytometry, were almost all $CD88^+$, even though only about half were $chymase^+$ by immunocytochemistry; and they released histamine in response to C5a.[29] In contrast, mast cells obtained from osteoarthritis synovium, which also were about 50% $chymase^+$, showed negligible release of histamine to C5a. Whether only $CD88^+$ mast cells expressed chymase in this study was not directly examined. Another study reported that CD88 was expressed on mast cells dispersed from juvenile foreskin, but not from adult mammary skin.[30] This latter finding contrasts with the current study in which essentially all Sk-MCs, most of which were from breast skin, were $CD88^+$. Nevertheless, these older reports raise the possibility that not all MC_{TC} cells are $CD88^+$.

In contrast with C5a, the neuropeptide substance P, like FcεRI cross-linking, activates MC_T and MC_{TC} cells to degranulate, regardless of whether their source was skin or lung. Modest variations in the magnitude of degranulation among the different types of mast cells was observed. Whether this reflects differences in the surface expression of FcεRI or the substance P receptor or in the activation status of signal transduction pathways inside these cells is uncertain. Activation of Sk-MC and Lu-MC by substance P has been previously reported,[11,12,31,32] whereas mast cells from the intestine[33] and heart[34] fail to respond to substance P. These data suggest that the tissue source or local microenvironment of mast cells might be an important determinant of substance P responsiveness. Whether neuropeptides such as substance P can be released in sufficient quantity in the lung or skin to activate mast cells remains to be clarified but poses a potentially important connection of the nervous system to bronchospastic and urticarial reactions associated with stress or neurogenic stimulation.

The activation profiles of MC_T and MC_{TC} cells leading to secretion of LTC_4 follow a pattern similar to those for degranulation with the exception that Sk-MC_{TC} cells failed to secrete LTC_4 in response to any of the 4 stimuli tested, in spite of their ability to degranulate. Low to negligible levels of LTC_4 production by Sk-MC activated by FcεRI cross-linking have been reported previously.[35,36] This may explain why chronic urticaria typically fails to respond to LT antagonists.[37] However, the current study shows that MC_{TC} cells from lung produce amounts of LTC_4 that are comparable with MC_T cells from lung, suggesting the possibility that MC_{TC} cells can acquire the ability to produce this lipid mediator. In fact, mast cells derived from intestinal mucosa[38] and those from cord blood under the influence of both SCF and IL-6,[39] when challenged with antigen to cross-link FcεRI, release small quantities of LTC_4 unless first primed with IL-4, in which case LTC synthase is induced and LTC_4 production is substantially amplified. Whether LTC_4 production by activated Sk-MC can be induced was not addressed by the current study. Our results provide compelling evidence that MC_T and MC_{TC} cells are functionally distinct types of human mast cells.

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Abbreviations used

LT, Leukotriene; Lu-MC, Lung-derived human mast cell; MC, Mast cell; rh, Recombinant human; SCF, Stem cell factor; Sk-MC, Skin-derived human mast cell.

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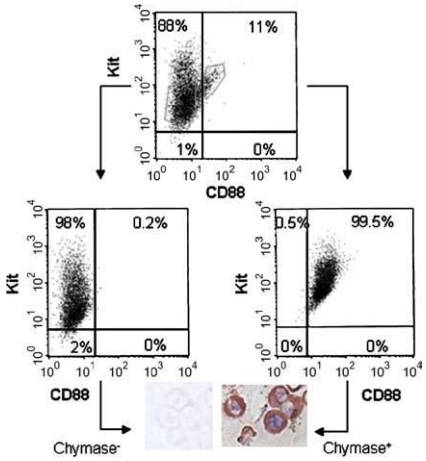

FIG 1.

Purification and separation of MC_{TC} and MC_T cells from Lu-MC. Mast cells were labeled with rabbit anti-CD88/Alexa 488-goat antirabbit IgG and phycoerythrin-mouse anti-Kit mAb and sorted by flow cytometry. Sorting gates are shown by the *gray polygons*. Sorted cells were cultured overnight and subjected to analytical flow cytometry (*lower panels*) or cytocentrifugation and immunocytochemistry with antichymase mAb.

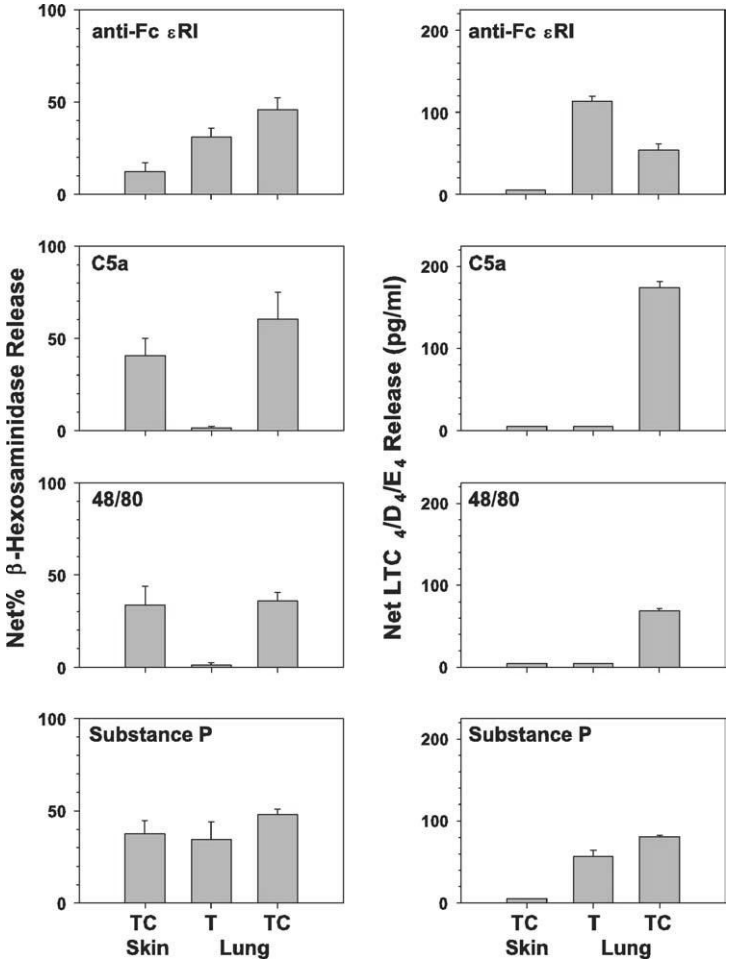

FIG 2.

Activation profiles of purified MC_T and MC_{TC} cells from different sources. Cells (10^6/mL) were exposed to anti-Fcε∈RI, C5a, compound 48/80, and substance P for 2 hours at 37°C. Net percent release of β-hexosaminidase (*left panels*) and net release of sulfidopeptide LTs (*right panels*) were determined. Overall, spontaneous release values for β-hexosaminidase were ≤6.5% and for $LTC_4/D_4/E_4$ were undetectable.

FIG 3.

Effect of IL-6 on chymase protein expression in Lu-MC. Cytospins of purified lung-derived mast cells were stained with antichymase or antitryptase mAbs. The staining intensities of positive cells from 3 different lung mast cell preparations were then measured by image analysis. For each preparation, 10 to 15 chymase^{+} and 12 to 20 tryptase^{+} mast cells were examined. $^{*}P < .05$ compared to no IL-6.

TABLE I.

Chymase and tryptase mRNA levels in MC_T cells from cord blood and lung

Cell source	18S rRNA-normalized ratio to that in Sk-MC	
	Tryptase	Chymase
Lung MC_T cells	0.48 ± 0.44	$4.0 \times 10^{-5} \pm 1.8 \times 10^{-5}$

The lower limit of mRNA detection after 40 cycles of PCR ranged from a ratio of 2 to 20×10^{-7}. Values shown are means ± SEs for 3 preparations of lung mast cells with 1.3% ± 0.3% MC_{TC} cells, each performed in triplicate.