

Comparison of human tonsillar mast cell localization and ultrastructural observations between IgE-mediated allergic and nonallergic donors

Hidegori Yokoi, M.D., Ph.D.,* Yoshimichi Okayama, M.D., Ph.D.,§ François Niyonsaba, M.D., Ph.D.,# Masato Fujimori, M.D., Ph.D.,* Fuyuki Enomoto, M.D., Ph.D.,* Hiroshi Yoshikawa, M.D., Ph.D.,* Katsuhisa Ikeda, M.D., Ph.D.,* and Hirohisa Saito, M.D., Ph.D.¶ (Japan)

ABSTRACT

Mast cells (MCs) are important effector cells in mucosal defense and allergic inflammation. Lymphoid tissues such as tonsils and adenoids also have modest numbers of MCs. However, the role of MCs in lymphoid tissues is not well known. In this study, we showed the local distribution of MCs in tonsils from IgE-mediated allergic or nonallergic donors, studied their ultrastructure, and specified their surrounding cell types. Tonsils were obtained from IgE-mediated allergic or nonallergic donors suffering from chronic tonsillitis or hyperplastic tonsils. The localization and distribution of MCs and IgE binding to MCs were determined by immunohistochemistry using antibodies against tryptase, c-kit, and IgE. In addition, mast cell structure was examined using electron microscopy. In both allergic and nonallergic donors, MCs were distributed mainly in the interfollicular area and the perivascular area of connective tissue. We found that the total number of MCs in tonsils was almost the same in both donor groups. However, the number of MCs in the interfollicular area was significantly higher in nonallergic versus allergic donors. Moreover, the electron microscopy revealed that MCs localized in perivascular areas of connective tissue have scroll-type granules, and MCs in the interfollicular areas contained both particle and scroll-type granules. In addition, the MCs that were surrounded by CD4⁺ lymphocytes in the interfollicular area showed empty granules, whereas MCs in the perivascular area, not surround by CD4⁺, were intact. This implies that these MCs were degranulated, and this probably was caused by CD4⁺ cells. Taken together, these findings suggest that tonsillar MCs distribute differently in allergic and nonallergic donors, and that MCs in the interfollicular area might be activated by direct contact with CD4⁺ T cells.

(Allergy Asthma Proc 27:415–421, 2006; doi: 10.2500/aap.2006.27.2891)

The potential of human mast cells (MCs) to act as important effector cells in mucosal defense and allergic diseases is now established. Human MCs release histamine, prostaglandin D₂ and other potent mediators including pro-inflammatory cytokines in response to a range of agonists.^{1,2} MCs locate mainly around small blood vessels and mucosa,^{1–3} and they are also found in lymphoid tissues such as tonsil and adenoid. However, their presence and role in lymph nodes has not been well understood.

Tonsils are one of the lymphatic organs. Although lymph nodes process antigens *via* afferent lymph vessels, tonsils are known to be in direct contact with the antigens *via* mucoepithelium. Thus, tonsils are considered to be mucosa-associated lymphatic tissue.⁴ Be-

cause of its anatomic location, tonsils are an immunologic tissue responsible for the biological defense against bacteria and viruses, as well as oral and respiratory exogenous proteins such as dietary antigens. Furthermore, tonsils have been reported to produce specific antibodies against pathogens.⁵

The wide distribution of MCs throughout the human body in general and at mucosal surfaces in particular, in allergic and nonallergic individuals alike, suggests that these cells may be involved in nonallergic conditions. Previous studies have shown that MCs are located in lymphatic tissues, where they interact with lymphatic cells in functional terms as well as cell–cell interaction.⁶ Although the presence of MCs in human tonsils and IgE-mediated activation of these cells has been investigated, little is known so far about their specific distribution and phenotype.^{7,8} Thus, this study was designed to investigate the localization and/or distribution and ultrastructural observations of MCs in different areas of human tonsils from allergic and nonallergic donors.

MATERIALS AND METHODS

Patients and Tissues

The tonsil tissues were obtained from patients who presented to Juntendo University Hospital, Otolaryn-

From the Departments of *Otorhinolaryngology and #Atopy (Allergy) Research Center, Juntendo University School of Medicine, Tokyo, Japan, §Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Yokohama, Japan, and ¶Department of Allergy and Immunology, National Research Institute for Child Health and Development, Tokyo, Japan

Address correspondence and reprints request to Hidegori Yokoi, M.D., Ph.D., Department of Otorhinolaryngology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

E-mail address: hyokoi@med.juntendo.ac.jp

Copyright © 2006, OceanSide Publications, Inc., U.S.A.

Table 1 Staining methods and cell type detected

	Single Staining		Double Staining
	Frozen Sections	Paraffin-Embedded Sections	Paraffin-Embedded Sections
Fixation	Cold acetone	Formalin	Formalin
Antigen retrieval	No	Yes	Yes
Method		LSAB2-kit	
Visualization		3,3'-Diaminobenzidine	FITC-conjugated tryptase Alexa Fluor 598 anti-mouse IgG Ab for CD4 Alexa Fluor 598 anti-rabbit IgG Ab for IgE, c-kit
MC characteristics	Tryptase (+) Chymase (+)	Tryptase (+), IgE (+), c-kit (+), von Willebrand factor (+)	Tryptase (+), IgE (+), c-kit (+), von Willebrand factor (+)

FITC = fluorescein isothiocyanate.

gology Clinic, suffering from chronic or recurrent tonsillitis and tonsillar hypertrophy for tonsillectomy after informed consent was given. Before operation, the patients who presented over 2+ scores either for house dust or vermin with a history of food or inhalant allergy or gastrointestinal symptoms with high IgE (RIST) levels (mean, 351.6; range, 27.2–705.2) belonged to IgE-mediated allergic donors with a mean age of 22.2 years (range, 5–32 years; $n = 15$). The nonallergic subjects with a mean age of 26.5 years (range, 5–38 years; $n = 28$) included those who were negative for all antigens tested and whose nonspecific IgE was in a normal range (mean, 52.9; range, 5.0–227.3) and without a history of food or inhalant allergy or gastrointestinal symptoms. After operation, tissues were snap-frozen or 10% formalin-fixed and paraffin-embedded before use.

Antibodies and Reagents

Anti-human tryptase monoclonal antibody (mAb) and anti-human chymase mAb were obtained from CHEMIKON (Temecula, CA). Anti-human IgE polyclonal Ab (pAb), anti-human c-kit pAb and anti-human von Willebrand factor (factor VIII) pAb were purchased from DAKO (Copenhagen, Denmark). Anti-human CD4 (mAb), 1F6 was obtained from Novocastra (Newcastle upon Tyne, UK).

Immunohistochemical Analysis

Formalin-fixed and paraffin-embedded samples of 4- μ m sections were deparaffinized, rehydrated, and then incubated for 10 minutes in a 3% H₂O₂/methanol solution. In some experiments, antigen retrieval was performed by autoclaving sections in 0.01 M of citrate buffer (pH 7.0) for 10 minutes before further incubation with normal goat serum for 20 minutes to reduce non-specific binding. Sections were incubated overnight at

4°C with primary Ab's including anti-human tryptase mAb (1:2000), anti-human IgE pAb (1:5000), anti-human c-kit pAb (1:500), and anti-human factor VIII pAb (1:1000). After appropriate washes, the LSAB2-kit (DAKO) was used for the secondary Ab and streptavidin staining. Visualization was performed using the 3,3'-diaminobenzidine method.

The snap-frozen specimens were stored at -80°C until use. Then, 4- μ m sections were used for evaluation of anti-human chymase mAb staining. Double staining experiments were performed between fluorescein isothiocyanate (FITC)-tryptase and anti-c-kit or anti-IgE pAb's and CD4 mAb. Then, sections were incubated for 30 minutes with Alexa Fluor 598 anti-rabbit IgG Ab (Molecular Probes, Eugene, OR) for anti-c-kit and anti-IgE, or Alexa Fluor 598 anti-mouse IgG Ab for CD4. Subsequently, the slides were mounted and observed on fluorescence microscope (ZEISS, Hallberg, Germany). The summary of staining methods and detected cells is shown in Table 1.

Electron Microscopy Analysis

Fresh tonsil tissues for electron microscopy were fixed in 2% glutaraldehyde in 0.1 M of sodium phosphate buffer (pH 7.4) at 4°C for 2 hours. After rinsing in sodium phosphate buffer overnight, the samples were postfixed in 2% osmium tetroxide in 0.1 M of sodium phosphate buffer at 4°C for 2 hours and dehydrated with ethanol and propylene oxide. Samples were then embedded in Epok 812 (Shell Chemical, San Francisco, CA). Thin sections of 0.5 μ m were stained with toluidine blue and were observed in interfollicular and perivascular areas. Ultrathin sections were double stained with uranyl acetate and lead citrate and were prepared from five to seven blocks of each sample; three to six grids were prepared from each block. At least 20 randomly selected MCs were examined and photographed with electron microscope

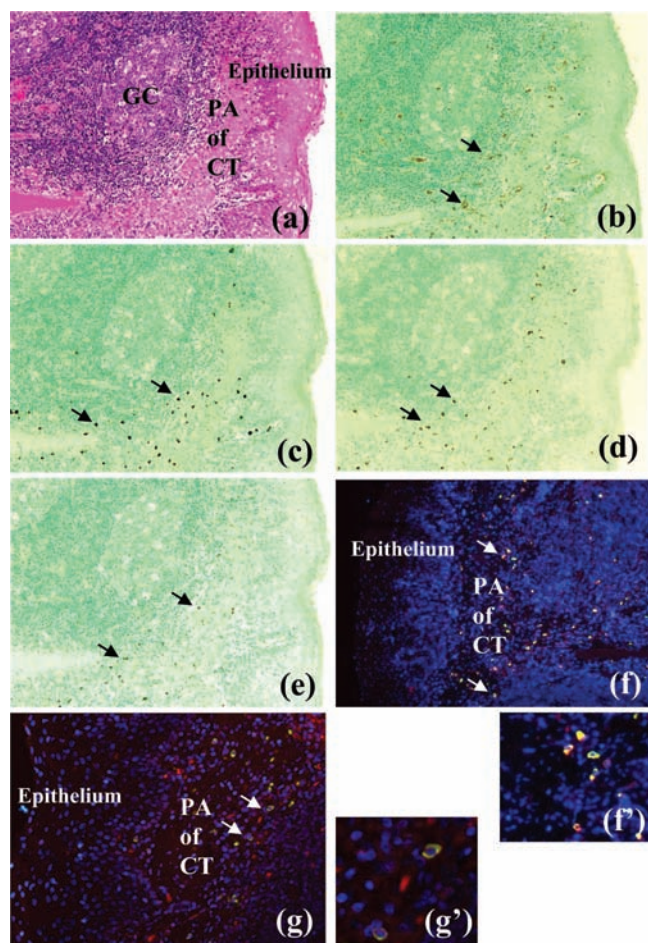


Figure 1. Distribution and localization of MCs in tonsils from allergic donors. Sections from allergic donors were stained with (a) hematoxylin-eosin staining, (b) anti-factor VIII, (c) antitryptase, (d) anti-c-kit, and (e) anti-IgE. Shown are representative data from 15 donors. (f and g) Double stained with c-kit (red)/tryptase (green) and IgE (red)/tryptase (green) in the perivascular area of connective tissue of tonsils from allergic donors. The merged cells are yellow. (f' and g') An enlargement of a part of panels f and g, respectively. (f and g) Representative data from three donors. GC, germinal center; PA in CT, perivascular area of connective tissue.

(JOEL, Tokyo, Japan) with an acceleration potential of 80 kV.

Cell Count

MCs in subepithelium, interfollicular area, mantle zone, germinal center, perivascular area and crypt were counted using a light microscope at 200 magnification with a Micrometer Eye Piece.

Statistical Analysis

Statistical differences were analyzed by Wilcoxon signed-rank test, and $p < 0.05$ was considered significant. The results are shown as mean $\pm$ SD.

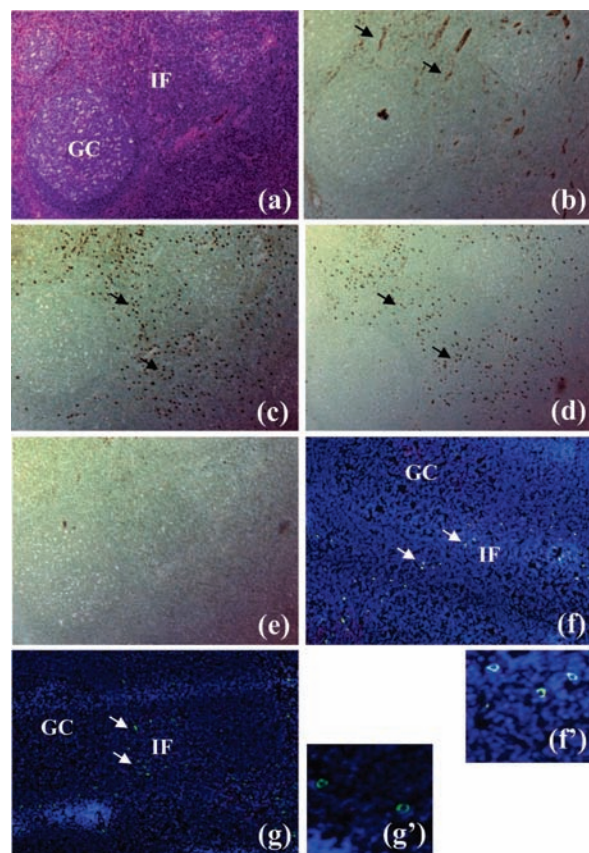


Figure 2. Distribution and localization of MCs in tonsils from nonallergic donors. Sections from nonallergic donors were stained with (a) hematoxylin-eosin staining, (b) anti-factor VIII, (c) antitryptase, (d) anti-c-kit, and (e) anti-IgE. Panels a–e are representative data from 28 donors. (f and g) Double-stained with c-kit (red)/tryptase (green) and IgE (red)/tryptase (green) in interfollicular area of tonsils from donors. The merged cells are yellow. (f' and g') An enlargement of a part of panels f and g, respectively. (f and g) Representative data from three donors. GC, germinal center; IF, interfollicular area, respectively.

RESULTS

Distribution and Localization of MCs in Tonsils

To investigate the localization of MCs in allergic donors in different areas of the tonsils, sequential serial sections were stained with hematoxylin-eosin (Fig. 1 a), anti-factor VIII (Fig. 1 b), antitryptase (Fig. 1 c), anti-c-kit (Fig. 1 d), and anti-IgE (Fig. 1 e). The staining of MCs with antitryptase and anti-c-kit Ab's revealed that in allergic donors, MCs localized mainly in the perivascular area of connective tissue and stained with factor VIII, which specifically recognizes endothelium of vessels (Fig. 1 b). We further found that 92.9% of tryptase (+) MCs were c-kit (+) (Fig. 1 f), whereas 56.3% of tryptase (+) MCs were IgE (+; Fig. 1 g) in the perivascular area of allergic donors.

To compare the localization of MCs between nonallergic and allergic donors, we performed an immuno-

histochemical study by staining serial sequential sections from nonallergic donors with hematoxylin-eosin (Fig. 2 *a*), anti-factor VIII (Fig. 2 *b*), antitryptase (Fig. 2 *c*), anti-c-kit (Fig. 2 *d*), and anti-IgE (Fig. 2 *e*). The results showed that in nonallergic donors, MCs were present in both the interfollicular area and the perivascular area of connective tissue. Furthermore, the double staining of anti-c-kit or anti-IgE and antitryptase Ab's revealed that 95% of tryptase (+) cells were c-kit (+) (Fig. 2 *f*). As expected, low amounts of IgE (+) MCs were observed in both interfollicular and perivascular areas, 9.3 and 3.4%, respectively (Fig. 2 *g*).

Next, we stained tonsillar tissues with MC markers antitryptase and antichymase Ab's to examine the phenotypes of MCs observed in tonsils. The results revealed that ~95% of MCs were double stained with both tryptase and chymase in interfollicular and perivascular areas, suggesting that tonsillar MCs are mainly MC_{TC} (MC containing both tryptase and chymase) and at low extent MC_T (MC that contain substantial amounts of tryptase and not chymase; data not shown). A summary of different tonsillar areas and MC characteristics in both allergic and nonallergic donors is found in Table 2.

The Statistical Analysis of the Distribution of MCs in Each Area

To clarify the localization and distribution of MCs in several areas of tonsils from allergic and nonallergic donors, number of tryptase (+) cells (Fig. 3 *a*) or IgE (+) cells (Fig. 3 *b*) in subepithelium, interfollicular area, mantle zone, germinal center, perivascular area of connective tissue, and crypts were counted. The results showed that in each area of the tonsils, MCs were abundant in interfollicular and perivascular areas in both allergic and nonallergic donors. The total number of MCs from allergic and nonallergic donors were $11.1 \pm 7.8/\text{mm}^2$ and $11.5 \pm 8.1/\text{mm}^2$, respectively. However, the number of MCs in the interfollicular area from nonallergic donors was significantly higher than that from allergic donors ($p < 0.05$; Fig. 3 *a*). Furthermore, IgE expression in allergic donors was statistically higher than that in nonallergic donors in subepithelium, perivascular area, and crypts ($p < 0.05$ or $p < 0.01$; Fig. 3 *b*). In contrast, tryptase (Fig. 3 *a*), IgE (Fig. 3 *b*) and c-kit (data not shown)-positive cells could not be detected in the germinal center area in both allergic and nonallergic donors.

Electron Microscopic Analysis of Tonsillar MCs and Identification of Cells Surrounding MCs in the Interfollicular Area

The investigation of the morphology of tonsillar MCs using electron microscopy showed that MCs distributed in the perivascular area of connective tissue (Fig.

Table 2 Tonsillar areas and the MC characteristics between allergic and nonallergic donors

	Sub-epithelium	Interfollicular Area	Mantle Zone	Germinal Center	Perivascular Area of Connective Tissue	Crypt
Non-MC cell types MC morphology (electron microscopy) Relation to T cells		CD4 ⁺ T cells Particles, scroll type and empty granules MCs surrounded by CD4 ⁺ cells		B cells	Mainly scroll type granules No CD4 ⁺ cells	
Number of MCs Allergic donors (AD) Nonallergic donors (NAD)	Low Low	High High	Low Low	Very low Very low	High High	Low Low
Differences between allergic and nonallergic donors	IgE: AD > NAD	Tryptase: AD < NAD NAD: tryptase-c kit double positive 95% NAD: tryptase-IgE double positive 9.3%	Not detected	Not detected	IgE: AD > NAD AD: tryptase-c kit double positive 92.9% AD: tryptase-IgE double positive 56.3% NAD: tryptase-IgE double positive 3.4%	IgE: AD > NAD

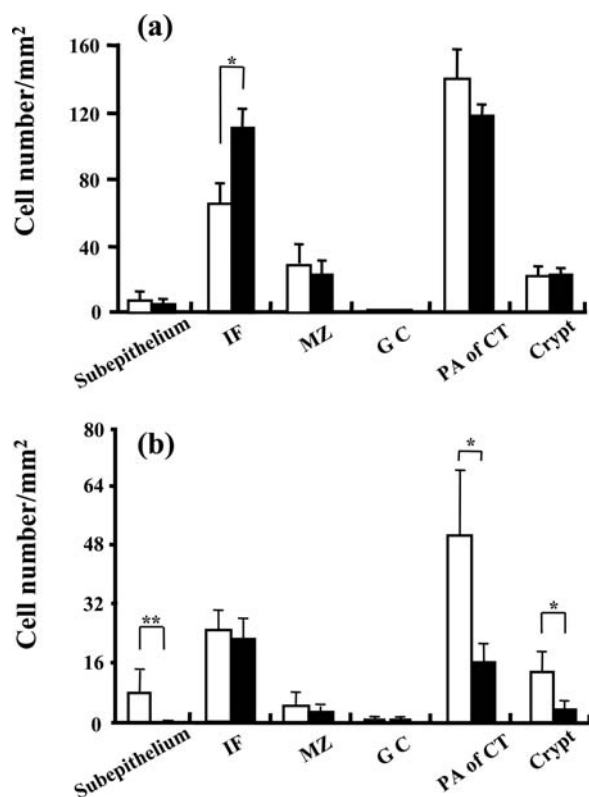


Figure 3. The statistical analysis of the distribution of MCs in each area. Tonsils from allergic and nonallergic donors were stained with (a) tryptase and (b) IgE. Number of positively stained cells per square millimeter is compared between allergic (open bars) and nonallergic donors (filled bars). Each bar represents the mean $\pm$ SD. * $p < 0.05$; ** $p < 0.01$. IF, interfollicular area; MZ, mantle zone; GC, germinal center; PA, perivascular area; CT, connective tissue.

4a) contain mainly scroll-type granules (Fig. 4b) and central, dense nucleoids condensing within granules (Fig. 4c). In contrast, MCs of the interfollicular area (Fig. 4d) possess both particle and scroll-type granules (Fig. 4e). Interestingly, we also observed that MCs in the interfollicular area were surrounded by lymphocytes (Fig. 4d), and many of these cells presented empty granules (Fig. 4d), suggesting that these MCs were degranulated, probably because of some molecules derived from lymphocytes.

In the course of identifying the origin of cells surrounding MCs in the interfollicular area, we performed immunohistochemistry on the tonsillar sections with lymphocyte markers. As expected, by double staining MCs with tryptase and CD4, we revealed that MCs in the interfollicular area were surrounded by CD4⁺ cells (Fig. 5a). Contrasting the interfollicular area, the perivascular area of connective tissue did not contain CD4⁺ T cells (Fig. 5b). Additional studies are necessary to determine whether CD4⁺ cells around MCs in the interfollicular area are activated or not.

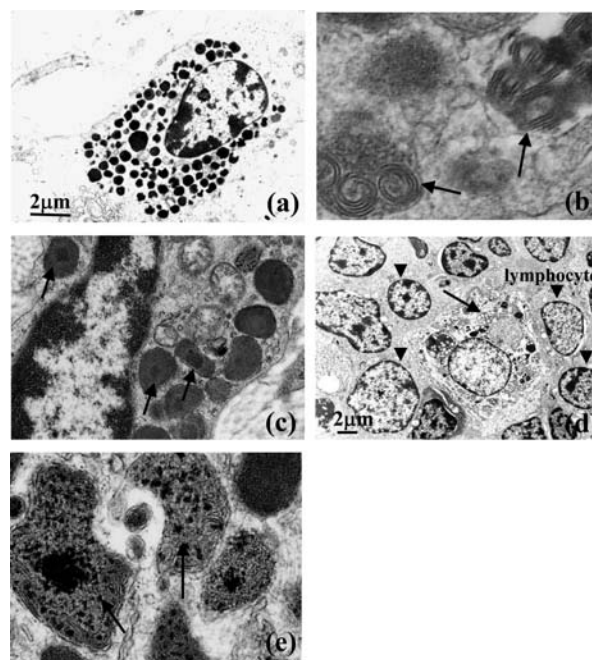


Figure 4. Electron microscopic analysis of tonsil MCs. (a) MCs distributed in perivascular area of connective tissue have mainly (b, arrow) scroll-type granules and (c, arrow) central, dense nucleoids condensing within granules. In contrast, (d) MCs in interfollicular area have (e, arrow) particle and scroll-type granules. (d, arrow) Note that MCs in interfollicular area are surrounded by lymphocytes (arrow head) and they have many empty granules.

DISCUSSION

Although the presence of MCs in tonsils has been reported,^{7,8} the detailed distribution of these cells in each tonsil area has not. This is the first report of the different existence of MCs in diverse areas of the tonsils. Our results showed that MCs were mainly present in the interfollicular area and perivascular area of connective tissue. The number of tryptase (+) and c-kit (+) cells in the interfollicular area was significantly higher in nonallergic donors than in allergic donors, and IgE was highly expressed in allergic donors versus nonallergic donors. In contrast, only 10% of MCs in the interfollicular area expressed IgE in nonallergic donors. Moreover, electron microscopic analysis of tonsil MCs revealed that MC phenotypes appeared to be different in the perivascular of connective tissue and the interfollicular areas. Interestingly, MCs of the interfollicular area were surrounded by CD4⁺ cells. These findings suggest that tonsillar MCs distribute differently in allergic and nonallergic donors, and that the role of MCs in the interfollicular area and perivascular area of connective tissue might be different.

It is well known that MCs are concentrated around small blood vessels, a finding that is consistent with the view that MCs are involved in regulation of vascular permeability and blood flow.⁹ We have confirmed this

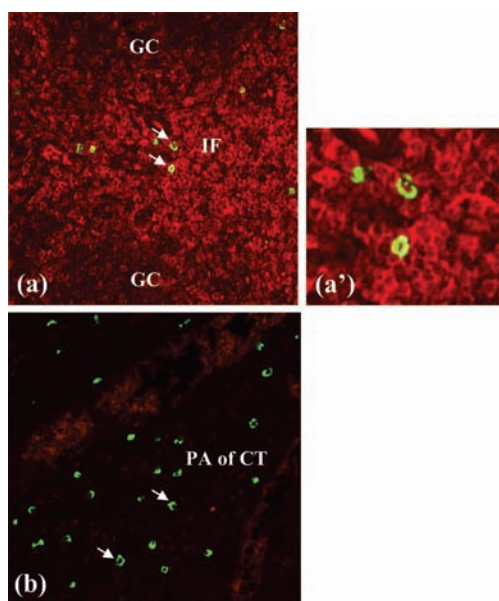


Figure 5. Identification of cells that surround MCs in interfollicular of tonsils. (a) Interfollicular area and (b) perivascular area in connective tissue of tonsils were double stained with antitryptase (green) and anti-CD4 (red). (a') An enlargement of a part of panel a. Shown is representative data from three donors. IF, interfollicular area; GC, germinal center; PA, perivascular area; CT, connective tissue.

issue by finding that tonsillar MCs were localized in the perivascular area of connective tissue, and these cells were positively stained with factor VIII. We also observed that MCs in the perivascular area have mainly scroll-filled granules, in contrast to MCs detected in the interfollicular area that have many particle-type granules and a low number of scroll granules.¹⁰ Although the scroll granule has been initially considered a characteristic feature of human MC_T,¹¹ later studies have shown that MC_{TC} also have, to some extent, scroll-type granules.¹² This is consistent with our results that tonsillar MCs, which mainly are MC_{TC}, possess scroll-type granules.

It has been reported that lymphoid tissue such as tonsils contains modest numbers of MCs^{7,8}; however, the roles of these cell populations in lymphoid tissue has not been well clarified. In this study, we found that in the interfollicular area, degranulated MCs were surrounded by CD4⁺ T cells, whereas intact MCs present in the perivascular area were not. This observation prompted us to hypothesize that the degranulation of MCs probably was caused by some molecules derived from CD4⁺ T cells, which in return activate MCs. Indeed, it has been reported that transforming growth factor β and interleukin-3 derived from T cells are able to induce the MC degranulation.¹³

The reason why the number of MCs of the interfollicular area from allergic donors was significantly

lower than that from nonallergic donors is not clear. One speculation is that MCs of the interfollicular area might relocate to the perivascular area of connective tissue *via* chemotactic migration toward antigens.¹⁴ Although MCs are thought to participate in the lymphocyte-recruiting process as has been reported, little has been known until recently about the specific molecules released by MCs that are chemotactic for lymphocytes.

It is known that CD4⁺ cells accumulate in tissues at sites of MC activation, where they are believed to play a critical role in the genesis of subsequent cellular events, such as those observed in the late-phase allergic response¹⁵ or in delayed-type hypersensitivity response.¹⁶ Our results that MCs were surrounded by CD4⁺ cells in the interfollicular area led us to speculate that MCs possibly interact with CD4⁺ cells in this area. Indeed, we have recently established the interaction between MCs and T cells by showing that tonsillar MCs express molecules that are able to recruit and induce the proliferation of T cells.¹⁷

Although MCs in different organs exhibit closely related phenotypes, their subtypes have been divided into MC_{TC} and MC_T based on the neutral protease composition of their secretory granules. The MC_T cell contains substantial amounts of tryptase but not chymase, whereas the MC_{TC} cell contains tryptase, chymase, carboxypeptidase, and a cathepsin G-like enzymes.² MC_T is a major type generally present in the mucosa of bowel and interalveolar septa of the lung, and MC_{TC} cell is found in bowel submucosa and skin. In this study, we found that tonsillar MCs were mainly MC_{TC} and, to some extent, MC_T, showing that tonsillar MCs contain a mixture of MC_T and MC_{TC} cells. In addition, the ultrastructure composition of MCs also has been reported to be a crucial criterion for identification and classification of MCs.^{11,12} The existence of two subtypes of MCs in tonsils suggests they might phenotypically express different roles in health and diseases. In conclusion, this study suggests that tonsillar MCs distribute differently in allergic and nonallergic humans and that MCs and CD4⁺ T cells interact, resulting in the activation of tonsillar MCs.

ACKNOWLEDGMENTS

We thank Noritoshi Sueyoshi, Katsumi Miyahara, and Katsuhiro Sato for their excellent technical assistance and Kozo Ueno for his helpful discussion.

REFERENCES

1. Galli SJ. Mast cells and basophils. *Curr Opin Hematol* 7:32–39, 2000.
2. Metcalfe DD, Baram D, and Mekori YA. Mast cells. *Physiol Rev* 77:1033–1079, 1997.
3. Galli SJ, Wedemeyer J, and Tsai M. Analyzing the roles of mast cells and basophils in host defense and other biological responses. *Int J Hematol*. 75:363–369, 2002.

4. Brandzaeg P. Immune functions of human nasal mucosa and tonsils in health and disease. In *Immunology of the Lung and Upper Respiratory Tract*. Bienenstock J (Ed). New York: McGraw-Hill Book Co., 28–95, 1984.
5. Harabuchi Y, Hamamoto N, Shirasaki H, et al. Specific immune response of the adenoids to a respiratory antigen. *Am J Otolaryngol* 10:138–142, 1989.
6. Wang HW, Tedla N, Lloyd AR, et al. Mast cell activation and migration to lymph nodes during induction of an immune response in mice. *J Clin Invest* 102:1617–1626, 1998.
7. Okayama Y, Benyon RC, Rees PH, et al. Inhibition profiles of sodium cromoglycate and nedocromil sodium on mediator release from mast cells of human skin, lung, tonsil, adenoid, and intestine. *Clin Exp Allergy* 22:401–409, 1992.
8. Fureder W, Bankl HC, Toth J, et al. Immunophenotypic and functional characterization of human tonsillar mast cells. *J Leukoc Biol* 61:592–599, 1997.
9. Kraneveld AD, van der Kleij HP, et al. Key role for mast cells in nonatopic asthma. *J Immunol* 15:2044–2053, 2002.
10. Dvorak AM. Human mast cells. *Adv Anat Embryol Cell Biol* 114:1–107, 1989.
11. Craig SS, Schechter NM, and Schwartz LB. Ultrastructural analysis of human T and TC mast cells identified by immunoelectron microscopy. *Lab Invest* 58:682–691, 1988.
12. Craig SS, and Schwartz LB. Human MCTC type of mast cell granule: The uncommon occurrence of discrete scrolls associated with focal absence of chymase. *Lab Invest* 63:581–585, 1990.
13. Mekori YA, and Metcalfe DD. Mast cell-T cell interactions. *J Allergy Clin Immunol* 104:517–523, 1999.
14. Ishizuka T, Okajima F, Ishiwara M, et al. Sensitized mast cells migrate toward the antigen: A response regulated by p38 mitogen-activated protein kinase and Rho-associated coiled-coil-forming protein kinase. *J Immunol* 167:2298–2304, 2001.
15. Tsicopoulos A, Hamid Q, Haczku A, et al. Kinetics of cell infiltration and cytokine messenger RNA expression after intradermal challenge with allergen and tuberculin in the same atopic individuals. *J Allergy Clin Immunol* 94:764–772, 1994.
16. Geba GP, Ptak W, Anderson GM, et al. Delayed-type hypersensitivity in mast cell-deficient mice: Dependence on platelets for expression of contact sensitivity. *J Immunol* 157:557–565, 1996.
17. Kashiwakura J, Yokoi H, Saito H, and Okayama Y. T cell proliferation by direct cross-talk between OX40 ligand on human mast cells and OX40 on human T cells: Comparison of gene expression profiles between human tonsillar and lung-cultured mast cells. *J Immunol* 173:5247–5257, 2004. □

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.