

Mast Cells and IgE: From History to Today

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ABSTRACT

Role of mast cells in allergy had remained undetermined until the discovery of IgE in 1966. Then, IgE purified from many Liters of plasma, which had been donated from a patient with fatal myeloma, was distributed to researchers all over the world, and thus accelerated exploring the mechanisms involved in allergic reactions, particularly about the role of mast cells and basophils in the IgE-mediated reactions. Identification of mast cells as a progeny of a bone marrow hematopoietic stem cell in 1977 led us to successful *in vitro* culture of human mast cells. Along with the development of molecular biological techniques, the structure of the high affinity IgE receptor (FcεRI) was determined in 1989. These findings and subsequent investigations brought deeper understanding of IgE-mediated allergic diseases in the past half century, especially where mast cells are involved. We have now even obtained the information about whole genome expression of FcεRI-dependently activated mast cells. In sharp contrast to our comprehension of allergic diseases where IgE and mast cells are involved, the mechanisms involved in non-IgE-mediated allergic diseases or non-IgE-mediated phase of IgE-mediated diseases are almost left unsolved and are waiting for devoted investigators to reveal it.

KEY WORDS

basophils, FcεRI, hematopoietic stem cells, histamine, mast cells

INTRODUCTION

Mast cells, presently known as immediate hypersensitivity effector cells, are plentifully found in barrier tissues connected to the external environment, like the skin or the mucosa of respiratory and gastrointestinal tracts. Another type of immediate hypersensitivity effector cells, basophils are circulating in peripheral blood as the rarest type of leukocytes. These cells were first reported by Paul Ehrlich in 1878¹ and in 1879,² respectively. He also observed a phenomenon that their granules were stained in purple when blue basic aniline dye was used, and called it metachromasia. Later it was found that highly sulfated heparin proteoglycan present in the granules binds strongly to basic dyes, polymerizes the basic dye molecules, and changes the color of dye from blue to crimson.³ However, the functional properties of these cells remained enigmatic for a long time.

It took almost a century after discovery of these cells until we perceived the important role of mast cells and basophils in allergy. Although this percep-

tion came gradually from many findings, discoveries of IgE^{4,5} and its high affinity Fc receptors (FcεRI) on mast cells⁶ and basophils⁷ seem to have affected it decisively. Since then, our knowledge about the role of mast cells and basophils in allergic diseases has rapidly progressed. IgE-sensitized mast cells/basophils have been recognized as effector cells which can immediately release a variety of biological mediators such as histamine or leukotrienes in response to allergen stimulation. We have now identified the whole mRNA transcripts including all cytokine transcripts whose levels are changed when the mast cells are activated via crosslinking of FcεRI.⁸ Here, we review the historical papers regarding the role of mast cells and basophils in allergy and discuss about the future perspective of mast cell research.

INVESTIGATION OF MAST CELLS' ROLE IN ALLERGIC REACTIONS

Histamine had been suspected to cause anaphylaxis since 1910,⁹ although anaphylactic shock was recognized to be associated with a decrease in the hista-

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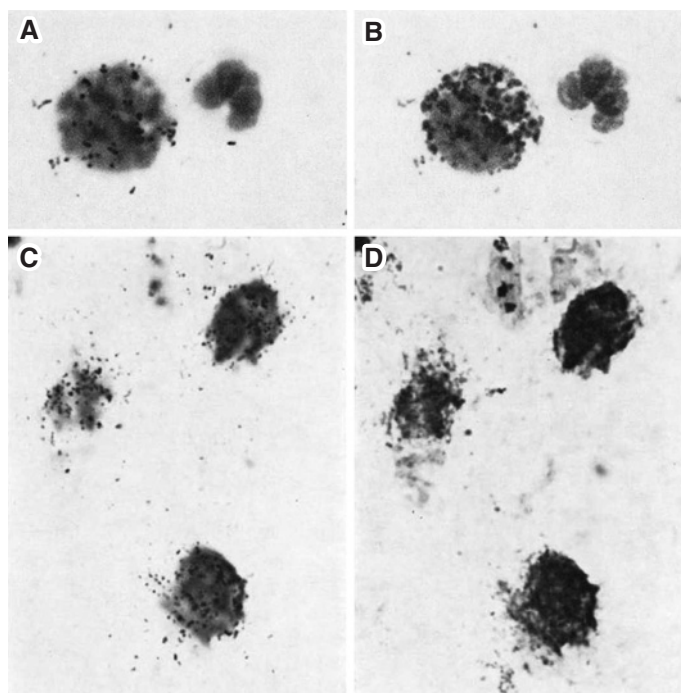


Fig. 1 First identification of IgE molecules bound to basophils and mast cells. Autoradiography of leukocytes (**A, B**) and sensitized monkey skin (**C, D**) treated with ^{125}I -anti-IgE. Microscope was focused to grains made by ^{125}I -anti-IgE in (**A, C**), or to the metachromatic granules stained with toluidine blue (**B, D**). ^{125}I -anti-IgE was found to bind only to basophils of the leukocytes and mast cells of the skin (**A** and **B** were adapted from Fig. 1A, B of the reference 7, and **C** and **D** were adapted from Fig. 1A, B of the reference 6).

mine content of the blood at first. This controversy is, of course, interpreted by the later discovery that histamine is stored in basophils,¹⁰ rapidly degenerated when it is released into blood *in vivo*, and that basophils release histamine also during serum separation *in vitro* as well as allergic reactions *in vivo*. Soon after the discovery of histamine in mast cells in 1953,¹¹ rat mast cells have been reported to release histamine when stimulated with Compound 48/80,¹² which has been known as a histamine liberator.¹³ Regarding the role of histamine release in allergic reaction, Lichtenstein and Osler first demonstrated in 1964 that histamine could be released from human leukocytes obtained from hay fever patients when challenged with specific antigen *in vitro*.¹⁴

Identification of IgE had been gradually achieved in 1960s. We (K.I. and T.I.) visualized human IgE for the first time by precipitation of the protein with the rabbit antibody specific for human "reagin" (γE) in agarose gel, and labeled the precipitin band with radio-labeled ragweed antigen, to which the donors of the serum was sensitive⁴ (The method is based on the same principle to Western blotting, currently employed). The use of antibodies against unknown pro-

tein seemed the only logical and available method in 1960s, to identify such protein as IgE, of which concentration is less than 1 microgram per mL of the serum. Independently, an atypical myeloma protein, which did not belong to any of the four known immunoglobulin isotypes (IgG, IgA, IgM and IgD), was reported by Johansson and Bennich in 1967.⁵ As the results of collaboration study, the myeloma protein was identified as IgE myeloma, as it reacted with the aforementioned rabbit antibody specific for "regain", and IgE was proven to represent a distinct isotype of immunoglobulins.¹⁵ After such discoveries of human IgE,^{4,5,15} the receptor site of human IgE ($\text{Fc}\epsilon\text{RI}$) was soon found on human basophils^{7,16} and on monkey mast cells⁶ (Fig. 1). Also, human leukocytes,¹⁷ and later, basophils¹⁸ among leukocytes were found to release histamine in an IgE-dependent manner. Accumulated knowledge regarding molecular characterization of human IgE let other investigators to discover IgE of the other species. Then, IgE-sensitized rat peritoneal mast cells were demonstrated to release histamine after challenge with allergen.¹⁹

Among numerous contributions following IgE discovery, Mr. Peter Shackford's contribution should be

remembered. He was admitted to Dartmouth College Hospital for an unknown type of myeloma in 1968. The disease turned out to be the second case of IgE myeloma in the world.²⁰ Then, more than 30 Liters of his plasma were provided by him by weekly plasmapheresis for one year until his death. The myeloma protein in his plasma was purified by us and distributed to researchers all over the world. His IgE (coded as PS-IgE) had been the only human IgE reagent available for research, and for the preparation of anti-IgE in the next 10 years. Even though we know that plasmapheresis was the only therapeutic method at that time, we should recognize that Mr. Shackford gladly consented to our solicitation for using his plasma to understand the mechanisms involved in “enigmatic” allergic diseases, and that he knew his life would be soon ended. In this way, he accelerated exploring the mechanisms involved in allergic reactions, especially regarding the role of mast cells and basophils in the IgE-mediated reactions.^{6,7,18,21}

By 1970, it was generally anticipated that an initial step of immediate hypersensitivity is a bridging of mast cell-bound antibody molecules by multivalent antigen. Requirement of cross-linking of cell-bound IgE for histamine release was concluded from the following experiments. First, bridging of two cell-bound IgE molecules by F(ab)₂ fragments of anti-IgE antibody was found to induce full reactions such as histamine release.²² Then artificially prepared dimers of IgE molecules alone were found to induce IgE-mediated exocytosis.²³ However, subsequent studies revealed that divalent F(ab)₂ fragment of the antibodies against the FcεRI on rat basophilic leukemia (RBL) cells, but not the monovalent Fab' fragment of the antibody, induced histamine release from normal rat mast cells, indicating that dimerization of the receptor molecules, rather than polymerization of IgE, is responsible for the initiation of enzymatic sequences in mast cells, leading to the histamine release.²⁴

Then, Metzger's group started to identify the structural features of FcεR by using RBL cells.²⁵ Along with rapid development of molecular biological techniques, the structure of the FcεRI was finally determined in 1989 by Metzger and his colleagues.²⁶ Now, we understand that FcεRI, which is expressed more than 10⁵ per mast cell or basophil, is a tetrameric structure comprised of an α chain (FcεRIα) which binds IgE, a β chain signaling subunit (FcεRIβ), and two γ subunits existing as a homodimer signaling subunit (FcεRIγ).²⁷ The molecular cloning of FcεRI as well as the development of techniques to generate genetically modified mice accelerated our understanding of signal transduction pathway in IgE dependently activated mast cells. Briefly, the crosslinking of FcεRI molecules initiates the association of the receptor with lipid rafts containing Lyn, a membrane

anchored member of the Src family of protein tyrosine kinases. Lyn kinase trans-phosphorylates tyrosine residues in the immunoreceptor tyrosine-based activation motif (ITAM) of FcεRI through the Src homology 2 (SH2) domain. Syk protein tyrosine kinases are recruited to the rafts and bind the doubly phosphorylated ITAMs. This ITAM phosphorylation induces fully activation of Syk, which initiates a series of events such as generation of inositol triphosphate (IP₃). Generated IP₃ induces Ca²⁺ mobilization from its intracellular storage, which results in the influx of extracellular Ca²⁺. For FcεRI-dependent activation of mast cells and basophils, extracellular Ca²⁺ is absolutely required for the release of both pre-formed (such as histamine) and newly generated (such as cysteinyl leukotriene; cys-LT) mediators.²⁸⁻³⁰ On the other hand, G-protein-dependent exocytosis of mast cells or basophils, which can be initiated with compound 48/80 or thrombin or formyl peptide, is independent from extracellular Ca²⁺ and the generation of IP₃.^{31,32}

As such, various intracellular signals produced via aggregation of FcεRI tetramer activate mast cells and basophils when these cells have been sensitized with IgE antibody and then are challenged with the allergen. Subsequently, activated mast cells and basophils can evoke immediate type reaction by releasing their granules in which histamine, neutral proteases and heparin had been stored. Then, lipid mediators such as cys-LT or prostaglandin D₂ (PGD₂) are synthesized on their membranes and are released into microenvironment within several minutes.³³ Released histamine and lipid mediators trigger various acute allergic symptoms such as nasal discharge, bronchospasms or urticaria. Histamine plays an essential role in acute skin allergic reactions, whereas cys-LT plays a pivotal role in bronchoconstriction. Mast cells exclusively express PGD₂ synthase compared to other cell types. Although the role of PGD₂ in immediate type reaction is unclear, it has been reported to act as a chemoattractant for eosinophils, basophils and Th2 cells.³³

Human mast cells also exclusively express tryptase, one of the neutral proteases, among all human cell types. Tryptase constitutes 10% of the mast cell by protein weight.³³ Proteoglycan (human mast cells use “eosinophil” major basic protein instead of proteoglycan molecules) acts as a core protein in the crystalloid structure of the mast cell granules by binding to heparin and neutral proteases.⁸ The mast cell tryptase serves as trypsin-like enzyme and thereby causes tissue remodeling such as abnormal proliferation of airway smooth muscles.³⁴

IN PURSUIT OF MAST CELLS' ORIGIN AND CULTURE METHODS

To investigate the mechanisms involved in the IgE-mediated allergic reactions, a large number of mast

cells or basophils should be desirably obtained. However, it came with difficulty for us, especially to obtain human materials, since basophils are the rarest cell type among peripheral blood and mast cells dwell only in the tissue. Thus we, mast cell researchers, had been struggling to establish the methods of obtaining a substantial number of mast cells or basophils.

Ginsburg *et al.*³⁵ first reported the development of mast cells *in vitro* in early 1960s. They found, from mouse lymph node cells, but not from bone marrow cells, mast cells grew on fibroblast layers. Since the identification of mast cells was based only on morphology, controversy occurred as to whether these cells are really mast cells. Ishizaka *et al.*³⁶ confirmed mast cell growth by using a similar system, in long-term culture of rat thymus cells on fibroblast layers. They demonstrated the presence of FcεRI on these cultured “mast” cells in 1976.

These observations can be interpreted by the later discovery that a very small number of hematopoietic cells, which should be present even in lymphoid tissues, can develop into mast cells when stimulated with a lymphocyte-derived mast cell growth factor, probably interleukin (IL)-3. Fibroblast layers would have presumably provided growth factors such as kit ligand (KITLG; formerly called as stem cell factor) to support the full maturation of mast cells.

As the origin of mast cells, Ehrlich suggested fibroblasts present in the connective tissue.¹ He could not imagine, of course, that these cells might derive from precursors of the hematopoietic lineage. Then after 99 years, Kitamura *et al.*³⁷ demonstrated hematopoietic stem cells as the origin of mast cells in 1977. They transplanted bone marrow cells of beige (C57BL-*bgl/bgl*) mice having giant granules in mast cells and granulocytes into wild type C57BL mice whose bone marrow precursors had been eliminated by irradiation. They could observe mast cells having giant granules in the C57BL mice after bone marrow transplantation.³⁷ Kitamura *et al.* also discovered the two different mice strains genetically lacking mast cells. One is *Sl/Sl^d* mice³⁸ which are later found to lack KITLG, and another is *W/W^v* mice³⁹ lacking the receptor for KITLG, called c-Kit or kit. By using these “natural” mast cell deficient mice, it was established that mast cells originate from a single hematopoietic progenitor shared also with granulocytes (Fig. 2). Then, it was revealed that immature mast cell progenitors migrate from bone marrow into the tissue through blood circulation, unlike immature granulocytes which are kept in bone marrow.⁴⁰

Identification of mast cells as a progeny of a hematopoietic stem cell enabled us to utilize hematopoietic tissues for obtaining mast cells. Denburg *et al.*⁴¹ succeeded to obtain guinea pig basophils (basophils are a dominant cell type compared to mast cells in guinea pig) *in vitro* by culturing bone marrow cells in the

presence of supernatant derived from antigen-activated lymphocytes in 1980. Then, culture of mouse mast cells was almost simultaneously reported by several groups who employed similar methods. These investigators cultured bone marrow or spleen hematopoietic cells in the presence of supernatants derived from antigen-activated lymphocytes.⁴²⁻⁴⁴ The factor that can support murine mast cell growth *in vitro* was then cloned and termed as IL-3.⁴⁵

Unlike murine mast cell development, however, only basophil growth was observed when human hematopoietic cells were cultured in the supernatant of activated lymphocytes.⁴⁶ Thus the discovery and cloning of human IL-3 was eagerly expected for culturing human mast cells. Human IL-3 was cloned based on the sequence of cDNA having a significant homology to that encoding murine IL-3. However, even by culturing hematopoietic progenitors with recombinant human IL-3, human mast cells did not grow.⁴⁷ Thus, successive failures regarding development of human mast cells disappointed us.

Although human IL-3 has a significant sequence homology with murine IL-3, the extent of homology between human and murine IL-3 (approximately 26-28% at amino acid sequence) is similarly low compared to that between human IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF). Furthermore, receptor structure for human IL-3 is somewhat different from mouse IL-3. The human has a common β subunit that does not bind any cytokine by itself but forms high-affinity receptors for GM-CSF, IL-3, IL-5 with the respective α subunit. In contrast, the mouse IL-3 has two distinct β subunits; one is the counterpart of human common β subunit, and the other exists only on murine mast cells and binds only to the murine IL-3 receptor α subunit.⁴⁸

As has been suggested in the earlier studies,^{35,36} fibroblasts were found to support development of IL-3-dependently cultured murine mast cells into the mature connective-type mast cell phenotype by Levi-Schaffer *et al.*⁴⁹ These authors also reported that mature human lung mast cells were maintained *in vitro* when cocultured with fibroblasts.⁵⁰ Then, Furitsu *et al.*⁵¹ succeeded to identify development of human mature mast cells from hematopoietic cells when cocultured with mouse 3T3 fibroblast cell line in 1989.

In 1990, a fibroblast-derived growth factor for mast cells was cloned, termed as stem cell factor (=KITLG), and the results were published almost simultaneously in more than 10 original papers by different researchers. While one of these articles was regarding cloning of human KITLG,⁵² mouse or rat KITLG was found to similarly support human mast cell development. Even though KITLG supports the development of human mast cells, a substantial number of functionally mature mast cells were not always obtained. In fact, KITLG was found to expand more hematopoietic stem cells rather than mast cells. It

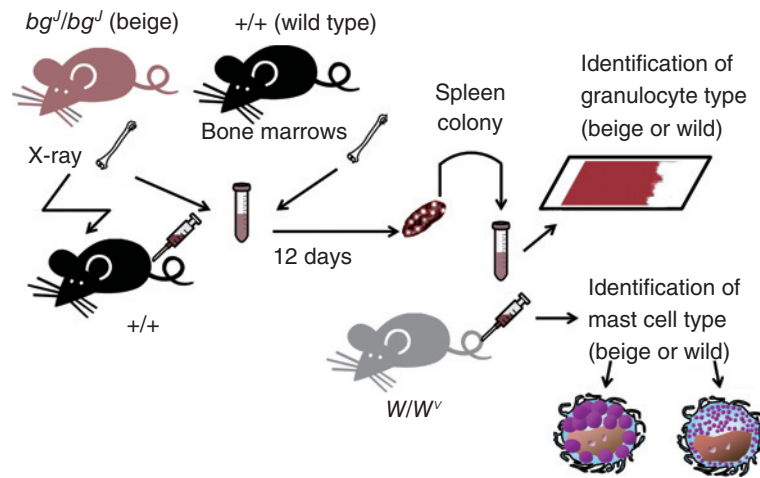


Fig. 2 Identification of mast cells as a single hematopoietic cell origin. A mixture of bone marrow cells from a beige (bg^{-}/bg^{-}) mouse and a wild type mouse ($+/+$) were injected (transplanted) to a wild type mouse who had received irradiation to accept donor's hematopoietic stem cells, which are capable of forming colonies in spleen. Cells from each hematopoietic cell-derived colony, which grew in the spleen after 12 days, were further transplanted into a mast cell-deficient (W/W^v) mouse. Each colony was found to have either mast cells having giant granules (beige) or those having normal granules (wild-type) after 180 days. Similar results were obtained about granulocyte colonies grown in 8 days of culture; i.e., they were either beige or wild-type, and that granule types were always identical between granulocytes and mast cells. Thus mast cells were demonstrated to be derived from a single hematopoietic stem cell capable of producing granulocytes.⁴⁰

was also found that IL-6, another fibroblast-derived factor,⁵³ is capable of supporting the self-renewing of hematopoietic stem cells as well as KITLG, while most other cytokines such as GM-CSF suppress it. Therefore, KITLG and IL-6 were employed for human mast cell culture.⁵⁴ IL-6 was later found to stimulate human mast cell maturation. As such, for preventing production of stem cell self-renewal-suppressive cytokines such as GM-CSF by macrophages, PGE₂,⁵⁴ methylcellulose culture system (Fig. 3)⁵⁵ or purification of mast cell progenitors⁵⁶ were used together with KITLG and IL-6. When highly purified mast cell progenitors are used, even IL-3 or IL-5 but not GM-CSF increases the number of cultured mast cells when cultured together with KITLG and IL-6.⁵⁶

DISCOVERY OF MAST CELL SUBSETS

Phenotypically distinct subsets of mast cells are present in rodents, based on their staining characteristics of their granules, T cell-dependency, and functions, namely connective tissue mast cells, and mucosal mast cells.^{57,58} In humans, two types of mast cells have been identified based on the neutral proteases they express. TC-type mast cells (MCTC) contain tryptase together with chymase and other neutral

proteases, whereas T-type mast cells (MCT) contain only tryptase, but lack the other neutral proteases present in MCTC.⁵⁹ MCTC are preferentially found in the connective tissue such as skin, while MCT are often found in mucosa such as airway epithelium. MCTC can respond to various non-immunological stimuli such as C5a or substance P, while MCT do not.

Dougherty *et al.*⁶⁰ have recently claimed that asthma can be divided into 2 subgroups based on characteristic gene expression signatures of epithelial cells; a subgroup (Th2 high asthma) has abundant expression of transcripts for Th2 cytokines such as IL-13, while the other (Th2 low asthma) almost lacks it. The patients with Th2 high asthma have more infiltration of mast cells into the airway epithelium. These intraepithelial mast cells express both tryptase and carboxypeptidaseA3 (CPA3) but not chymase. According to classical definition,⁵⁹ MCT were not supposed to express CPA3. However, based on our public microarray data (<http://www.nch.go.jp/imai/GeneChip/public.htm>), all types of mast cells and basophils express the CPA3 transcript. Therefore, these epithelial mast cells may be equivalent to MCT. Nevertheless, mast cells exposed to conditioned me-

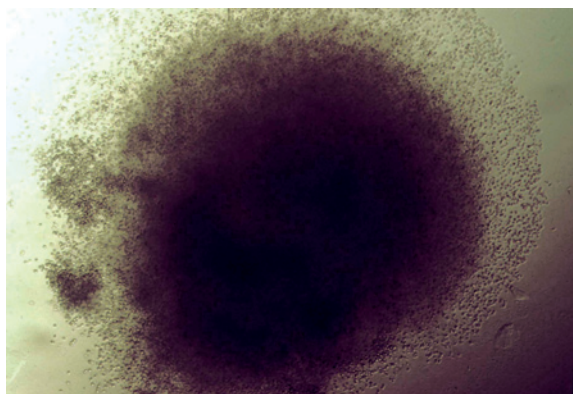


Fig. 3. Giant human mast cell colony formed in methylcellulose culture. This picture shows a giant human mast cell colony formed in methylcellulose culture, consisting of 250,000 mast cells. This colony was derived from a single CD34⁺ umbilical cord blood hematopoietic progenitor, which is capable of producing 100-fold higher number of mast cells compared to adult hematopoietic progenitor in the identical culture condition.⁹⁰

dia from IL-13-activated epithelial cells showed down-regulation of chymase but no change in tryptase.⁶⁰ This may relate to the reason why MCT are preferentially found in the mucosa and are deficient in immunodeficiency patients.⁵⁹ This is contrasting to the results demonstrating that human mast cell phenotypes such as chymase expression are retained over weeks even when these mast cells are cultured in the same standard methods (supplemented with KITLG and IL-6).⁶¹

DISCOVERY OF MAST CELLS AS SOURCES OF MULTIPLE CYTOKINES

Mast cells were first reported in 1987 to release some of cytokines such as tumor necrosis factor (TNF)- α ⁶² or IL-4.⁶³ The discovery of mast cells as sources of multiple cytokines urged us to investigate the role of mast cells and basophils in the whole immune system (Fig. 4). For example, mast cell-derived TNF- α , later turned out to play significant roles in airway hyperreactivity, T cell development (enhancement)⁶⁴ and bacterial infection (protection).⁶⁵ Regarding IL-4, a representative Th2 cytokine production, it seems reproducibly observed when mouse mast cells are used. Regarding human mast cells, so far, a few groups succeeded to immunohistochemically demonstrate the presence of IL-4 protein in the cells.^{66,67} At least in most of human studies, however, basophils are more potent producers of IL-4, while mast cells often fail to express IL-4 even upon fierce IgE-dependent stimulation.⁶⁸ Interestingly, IL-4 potentially activates human mast cell function and maturation. By incubating with IL-4 in advance for more than 48 hr, human mast cells become capable of producing

various cytokines including IL-13, another Th2 cytokine, at far more abundant levels in response to IgE-mediated stimuli.⁶⁸

Mast cells can secrete a variety of cytokines and chemokines several hours after IgE-mediated activation, via transcription of these genes. The representative cytokines/chemokines which are produced by activated human mast cells are Th2 cytokines such as IL-5, IL-13 and GM-CSF and CC-chemokines such as CCL1/I-309, CCL2/monocyte chemoattractant protein-1, CCL3/macrophage inflammatory protein (MIP)-1 α , CCL4/MIP-1 β . Activated human mast cells also secrete a substantial amount of CXCL8/IL-8.^{8,68} By the progress of biological techniques such as microarray and the mast cell culture method, now we can discern the big picture of the molecules including cytokines and chemokines which mast cells can express in response to IgE-⁸ and non-IgE-mediated stimuli.⁶⁹

However, these cytokines and chemokines are not unique to mast cells and are produced by other cell types. During antigen stimulation, more Th2 cytokines may be produced by proliferating T cells. It will be necessary to consider the relative role of mast cells and basophils in the allergic or innate-type inflammation by understanding cytokines/chemokines produced by other immune cell types such as Th2 cells and epithelial-mesenchymal tissues such as bronchial epithelium. Epithelial-mesenchymal cells are now considered as a key component of players in allergic diseases such as asthma, since the structural change that involves all of the elements of the tissue should be associated with activation of the epithelial-mesenchymal trophic unit.⁷⁰ Activated epithelial-mesenchymal trophic unit generates a variety of growth factors related to airway remodeling and cytokines manipulating the immune response such as IL-33, thymic stromal lymphopoietin (TSLP) and IL-25. Human mast cells do not normally produce aforementioned Th2 cytokines by only incubating with other cytokines such as IL-4, unless the cells are activated via Fc ϵ RI crosslinking after the incubation. However, it should be noted that IL-33, released during necrosis of epithelial-mesenchymal tissue, alone can stimulate mast cells to release a variety of cytokines such as IL-13. It was only IL-33 that can do so among 26 cytokines tested.⁷¹ It is inferred that mast cells may initiate inflammation characterized by eosinophil infiltration and polyclonal IgE production, by producing cytokines such as IL-13 even where allergens are absent, if IL-33 was released in the tissue. This may be true for basophils as well.⁷²

UNEXPECTED IDENTIFICATION OF IL33 IN RELATION TO ALLERGIC DISEASES BY GWAS

The completion of the Human Genome Project, the HapMap project,⁷³ and technological advances al-

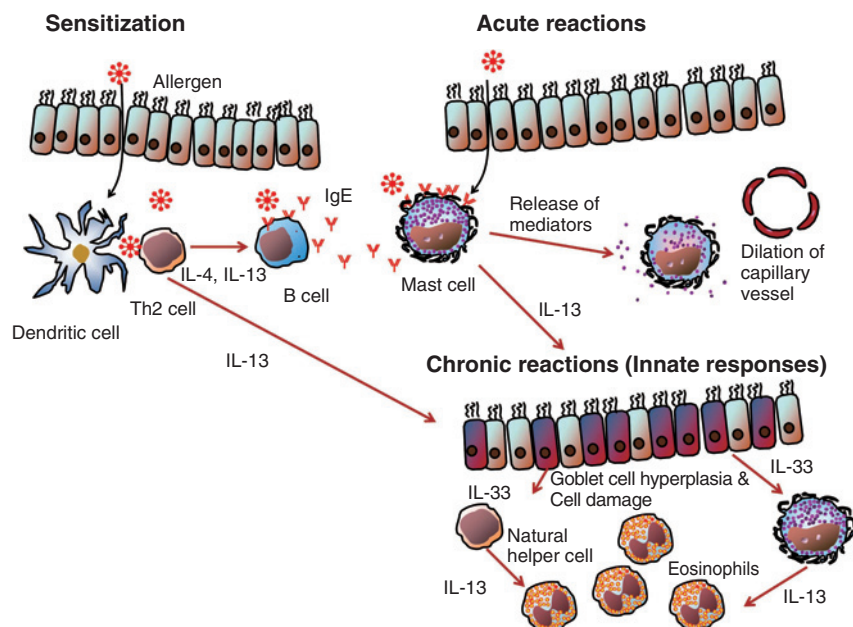


Fig. 4. Influences of Th2 cytokines on acute and chronic allergic reactions. In the step of allergen sensitization, by the influence of environment surrounding the epithelial barrier tissue (such as the presence of TSLP), dendritic cells changes their nature to selectively activate Th2 cells. Only in the presence of IL-4 or IL-13, which can be produced by Th2 cells, B cells can produce IgE antibody against the allergen. During acute reactions, urticaria, bronchoconstriction and/or hypovolemic shock are often induced via release of a variety of mediators from mast cells. During chronic reactions, a various symptoms such as grossly-bloody stool (in gastrointestinal allergy) or chronic productive cough (asthma) are caused by inflammation characterized by accumulation of eosinophils. IL-13 seems to play a pivotal role both in IgE production (at least in human) and chronic inflammation. IL-33 may enhance this chronic reactions or innate inflammatory responses independently of IgE by enhancing IL-13 production. A recent report indicated that in non-IgE-mediated gastrointestinal allergy during infancy, allergen-specific Th2 cell activation was confirmed.⁹¹ Since neonatal B cells almost lack IL-4/IL-13 receptors, chronic but not acute reactions seem to be observed in their symptoms of food allergy.

lowed genome-wide association studies (GWAS) to more comprehensively identify the susceptibility genes for asthma. Although asthma is now recognized as a syndrome consisting of heterogeneous disease entities,⁷⁴ only a few genetic polymorphisms are constantly found to be susceptible to the general type of adult asthma in recent large-scale GWAS, i.e., polymorphisms in the genes for an epithelial-mesenchymal tissue-derived cytokine IL-33 (*IL33*) and/or its receptor, ST2 (*IL1RL1*),⁷⁵⁻⁷⁷ while susceptible genes for atopy (IgE production) were different from those for asthma.⁷⁵ Also, using Japanese population, GWAS identified a polymorphism in ST2 (*IL1RL1*) as the most significantly associated with the onset of atopic dermatitis.⁷⁸ Thus, IL-33 now attracts much attention among scientists for allergic diseases.

IL-33, a member of the IL-1 cytokine family, is considered to be crucial for the induction of Th2 cell-

dominant immune responses such as host defense against nematodes and allergic diseases.⁷⁹ IL-33 was originally identified as "DVS27", a gene up-regulated in vasospastic cerebral arteries after subarachnoid hemorrhage⁸⁰ and as a nuclear factor, "nuclear factor from high endothelial venules (NF-HEV)", which is expressed in endothelial cell nuclei.⁸¹ IL-33 receptor was first identified as an IL-1 receptor-like molecule and termed as ST2 (the gene symbol was termed as *IL1RL1*) by Tominaga in 1989.⁸² In 2005, the molecule which had been provisionally termed as DVS27 or NF-HEV, was rediscovered as the 11th member in the IL-1 family of cytokines, by computationally searching, and termed as IL-33.⁸³ Human ST2 gene is preferentially expressed by mast cells and basophils compared to other leukocytes.⁸⁴

Using the gene-deficient mice, IL-33 was found to be more important for innate-type airway inflamma-

tion rather than IgE production in an asthma model.⁸⁵ Regarding the IL-33-mediated innate-type airway inflammation, Lin⁻ c-Kit⁺ Sca-1⁺ natural helper cells dwelling in the gut adipose tissue may play an important role.⁸⁶ Natural helper cells constitutively express ST2 and can produce a far larger amount of IL-5 and IL-13 even compared to basophils and mast cells in response to IL-33, at least in mice.⁸⁷ The topics regarding IL-33 are described in detail elsewhere in this issue.⁸⁸

Regarding another topic of non-IgE-mediated phase of IgE-mediated mast cell activation, histamine releasing factor present at the site of allergic inflammation has been reported capable of releasing histamine from mast cells by crosslinking IgE-bound FcεRI on mast cells, via interactions of its N-terminal and internal regions with the Fab region of IgE.⁸⁹ This finding is worthy of further investigation.

CONCLUSION - PROBLEMS TO BE SOLVED IN FUTURE

We may already have comprehended the large part of IgE-mediated allergic reactions or even IgE-mediated allergic diseases, particularly regarding mast cell activation upon allergen challenge. However, the pathophysiological role of mast cells or other innate inflammatory cell types, especially where IgE is not involved, is not yet fully investigated. From the recent discoveries based on GWAS, it is inferred that “non-IgE-mediated” innate inflammation plays far more important role in inflammatory allergic diseases such as asthma than we previously imagined. It is now required to investigate the mechanisms involved in “non-IgE-mediated” phase in mast cell activation and in allergic diseases.

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