The Journal of Immunology

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J Immunol 2009; 183:6861-6862; ;
doi: 10.4049/jimmunol.0990101
http://www.jimmunol.org/content/183/11/6861

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Pillars of Immunology

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A Crucial Door to the Mast Cell Mystery Knocked In

Toshiaki Kawakami[1]

The mast cell is a type of myeloid lineage hematopoietic cell. These cells originate from progenitor cells in the bone marrow, enter the circulation, and become mature mast cells after entering destination tissues under the influence of the local microenvironment (1). The traditional wisdom about mast cells has been limited for a long time to their functions in allergy (2). However, as reviewed by S. J. Galli and colleagues (3, 4), recent progress in the field has broadened their roles in many immune responses, ranging from innate defense against the venom of bees and snakes to multiple aspects of adaptive immune responses such as antigen presentation and lymphocyte recruitment to draining lymph nodes, as well as down-modulation of immune responses. The pathogenic roles of mast cells have been extended to include not only allergic diseases and helminth infection, but also autoimmune diseases such as experimental allergic encephalomyelitis (5) and rheumatoid arthritis (6), allograft tolerance (7), angiogenesis in tissue repair (8), and carcinogenesis (9). The most critical tool that has driven the high-paced findings is mast cell engraftment into mast cell-deficient animals, by which one can confirm that the "defects" or abnormal findings observed in mast cell-deficient animals can be partially reversed or cured by providing exogenous mast cells. The article by Nakano et al. (10) opened this era of "mast cell knock-in" techniques in mast cell research.

Two major advances in mast cell research had set the stage for this study. One was the discovery of genetically mast cell-deficient animals such as *W/W^v* and *Sl/Sl^d* mice (11, 12). The other was the establishment of culture conditions to obtain non-neoplastic populations of immature mast cells from murine bone marrow, spleen cells, fetal liver, etc. (13–18). Nakano et al. (10) demonstrated that i.p. injection of WBB6F$_1$-*Kit*$^{+/+}$ mast cells derived from bone marrow cell cultures or WBB6F$_1$-*Kit*$^{+/+}$ mast cells partially purified from the peritoneal cavity could give rise to mast cells in mast cell-deficient WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ (*Kit*$^{W/W\text{-}v}$) mice not only in the peritoneal cavity, but also in spleen, skin, and glandular stomach. Basically similar observations were made when cultured or peritoneal mast cells were injected i.v. into *Kit*$^{W/W\text{-}v}$ mice, although i.v. injection resulted in more frequent development of mast cells in the spleen than in the peritoneal cavity or stomach. This distribution pattern of mast cells was the opposite of that obtained when mast cells were injected i.p. Using ultrastructure, staining properties, and cell histamine content as criteria, this study also showed that injected bone marrow-derived cultured mast cells, which share phenotypic characteristics with mucosal mast cells as well as connective tissue-type mast cells (i.e., partially purified peritoneal mast cells), can give rise to different types of mast cells depending on the site of development, and bone marrow-derived cultured mast cells become progressively more mature in their phenotypic characteristics with increasing time after adoptive transfer into the *Kit*$^{W/W\text{-}v}$ mice.

The authors of this article made three other points that helped to guide future uses of this approach in mast cell research. First, they provided evidence that repair of the mast cell deficiency of the *Kit*$^{W/W\text{-}v}$ mice was selective for that lineage (in contrast to engraftment of whole bone marrow cells from wild-type mice, which resulted in the appearance of mast cells in the recipient mice but also repaired the recipients' anemia and eventually replaced many of the animals' other hematopoietic lineages with cells of wild-type origin; Refs. 12 and 22). Second, by demonstrating that mast cells derived in vitro from the bone marrow cells of *Kit*$^{W/W\text{-}v}$ mice could not survive in vivo after injection into *Kit*$^{W/W\text{-}v}$ mice, they showed that this mast cell engraftment approach could be used to study in vivo the biology of mast cells that had mutations affecting genes thought to be involved in important characteristics of this cell type. Finally, the authors suggested that the approach used in this article could be helpful in attempts to understand mast cell function in biological responses in vivo, as well as in efforts to investigate how different anatomic microenvironments influence mast cell survival or phenotype.

Subsequent studies showed that stem cell factor (also known as Steel factor, Kit ligand, or mast cell growth factor) is a critical growth and differentiation factor for mast cell development (19); c-Kit is its receptor tyrosine kinase. Loss-of-function mutations in gene loci for this ligand (Sl)/receptor (W) pair can lead to profound reductions of mast cells. *Kit*$^{W/W\text{-}v}$ mice virtually lack mast cells and have been the nearly exclusive choice for mast cell engraftment studies for more than two decades. In addition to mast cell deficiency, these mice have other hematopoietic abnormalities such as macrocytic anemia, neutropenia, γδ T cell deficiency, and nonhematopoietic abnormalities such as deficiencies of melanocytes, germ cells, and interstitial cells of Cajal. Also, these mice are cumbersome to breed because *Kit*$^{W/W\text{-}v}$ mice are infertile. By contrast, another strain of mast cell-deficient mice, C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ (*Kit*$^{W\text{-}sh/W\text{-}sh}$), does not have anemia or neutropenia and is fertile; therefore, *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice recently have become a favorite animal for mast cell engraftment (20). This trend may continue despite the other phenotypic abnormalities of these mice, as a recent study showed that *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice exhibit splenomegaly with expanded myeloid and megakaryocyte populations, as well as neutrophilia, thrombocytosis, and cardiomegaly (21).

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.0990101

Because of the multiple abnormalities in $Kit^{W\text{-}sh/W\text{-}sh}$ and $Kit^{W/W\text{-}v}$ mice, it is essential to demonstrate that findings of abnormalities in these mice can be rectified by "mast cell knock-in" experiments before one can conclude that such abnormalities are due to mast cell deficiency. A major issue in such knock-in experiments is whether this technique can reconstitute mast cells qualitatively as well as quantitatively. This point was addressed to a limited extent in the article by Nakano et al. (10) and was more recently readdressed by M. Brown and colleagues (22). They found that engraftment with bone marrow-derived mast cells (BMMCs)[2] does not restore detectable mast cell populations to all organs, notably the brain, spinal cord, lymph nodes, and heart in $Kit^{W/W\text{-}v}$ mice. The work in the original article by Nakano et al. (10) and work by Brown and colleagues (22) and other groups now clearly show that the extent and kinetics of mast cell engraftment depend on the route of the administration of mast cells and the number of mast cells transferred. However, there is no known way to make certain that the engrafted mast cells can restore all of the normal in situ functions of the corresponding populations of mast cells in wild-type mice. This limitation restricts interpretation of the experimental results (20); one can draw the strongest conclusion about a particular role of mast cells when the engraftment of mast cells converts the phenotype of mast cell-deficient mice closer to that of wild-type mice. Negative results, i.e., no significant changes in phenotype, cannot exclude the role of mast cells in the abnormal phenotype in mast cell-deficient mice, as negative results could simply be a failure of appropriate qualitative or quantitative mast cell engraftment. However, successful examples indicate that numerically perfect reconstitution is not necessary for the "normalization" of certain immune responses in which mast cells play a significant role (23). Another issue is potential complications caused by impurities of engrafted mast cells. Usually, BMMCs are used for knock-in experiments. High purity of ~95–98%, as judged by expression of c-Kit and FcεRI, is consistently attained in most BMMC cultures; thus, the issue of impurities in the donor cell population is not considered a major problem. However, a small percentage of other cell types might influence the response being examined (the reader should be reminded of the crucial role of basophils, which represent only a small percentage of blood leukocytes, in B cell memory and IgG-mediated anaphylaxis). Moreover, the purity of BMMCs could be lower, depending on mouse strain and genotype. Therefore, this issue deserves careful attention.

This *Pillars of Immunology* article was a result of collaboration between two groups, one of Yukihiko Kitamura, who recently retired from Osaka University, and the other of Stephen J. Galli at Harvard Medical School (now at Stanford University). Kitamura's group found mast cell-deficient animals (11, 12), and Galli belonged to one of several groups that established the culture methods for deriving mast cells from mouse hematopoietic cells (14) and showed that such in vitro-derived mast cells, which had features of immature mast cells (14, 24), could undergo further maturation if induced to do so in vitro (24). This paper by Nakano et al. illustrates a nice example of fruitful collaboration between two prominent groups of mast cell researchers, which was initiated during Galli's visit to Kitamura's laboratory.

[2] Abbreviation used in this paper: BMMC, bone marrow-derived mast cell.

Disclosures

The author has no financial conflict of interest.

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