

NEWS AND COMMENTARIES

The search for mast cell and basophil models – are we getting closer to pathophysiological relevance?

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Mast cells (MC) and basophils (BAS) have long been recognized for their detrimental role in the elicitation of allergic diseases. In recent years, scientific results revealed both cell types as versatile effector cells that exhibit far more complex functions beyond their role in allergy. MC and BAS have been shown to be critically involved in various innate and adaptive immune responses and, thereby, providing beneficial host protecting immunity. In contrast, they also contribute to the development and maintenance of several chronic inflammatory diseases that, even at the present time, lack sufficient treatment options. The diversity of important MC and BAS functions places these cell types into promising therapeutic targets. COST is an intergovernmental framework for European Cooperation in Science and Technology, allowing the coordination of nationally funded research on a European level. The COST Action 'BM1007 Mast Cells and Basophils – Targets for Innovative Therapies' is a network of European experts to foster a multidisciplinary approach for the identification, characterization and development of such targets and their translation into novel therapeutic strategies. In addition, the Action provides a platform for young researchers to gain up-to-date insights into the field.

The 2nd COST Action BM1007 Training School was held in Jerusalem, Israel, in January 2014 as part of the COST Action working group activities to study the physiological and pathophysiological importance of MC and BAS in health and disease. Thirty-four students from 12 countries across Europe participated in two full and intensive days of training and discussion about the pathophysiological relevance, benefits and limitations of *in vitro* and *in vivo* models currently available for MC and BAS research. This report summarizes and concludes on its outcomes.

***In vitro* models for rodent and human mast cells and basophils**

MC and BAS are inflammatory effector cells for which a number of challenges exist in *in vitro* research. They are tissue bound (MC) or circulate in the blood (BAS) and are in contact with many other cells. These environmental specifications are difficult to replicate *in vitro*. Tissue-derived MC and BAS can be extracted and purified as well as outgrown from the peripheral blood, but costs, time and low yield of cells are limiting factors. The murine peritoneum also represents an easier source for MC. High cell number, reproducibility and homogeneity can be obtained from culture-derived cells, for example bone marrow-derived cultured MC or BAS (Hoxb8 immortalized) (1), or cell lines. Various murine cell

cultures are currently available, including RBL-2H3, murine peritoneal cultured MC (MPMC), peripheral CD34+ stem cell-derived MC (PSCMC) (2) and the novel MCBS1, which are suitable for IgE-mediated MC degranulation studies (3) and *in vitro* proteomics (4). MCBS1 is a novel Kit-deficient rapidly proliferating culture derived from mTOR knockin BMCMC (5) and will be most useful in studying diseases associated with Kit mutations, such as mastocytosis and gastrointestinal stromal tumours.

Current human MC lines are HMC-1 and LAD-2, which have been obtained from patients with mast cell leukaemia. The HMC-1 line virtually lacks FcεR expression and is subdivided into two cell lines. HMC-1.1 contains a juxtamembrane domain mutation (V560G) and HMC-1.2 contains both, the V560G and catalytic domain (D816V) mutations. LAD-2 cells express FcεR, but very low levels of tryptase and chymase. MC can also be isolated and purified from human tissues, for example skin, lung and gut, however, in very low numbers. Culture approaches lasting for weeks are very pricy. Another approach is to derive MC from CD34+ or CD133+ progenitors from either cord blood, peripheral blood or embryonic stem cells (6). In comparison with cell lines, primary differentiated cells compare best to mature tissue MC and conserve genetic variability (7).

***In vivo* models for mast cell and basophil research**

During the last decades, *in vivo* models enlightened the central role of MC and BAS in multiple physiological and pathophysiological pathways. However, there is still the need for improving existing and the developing novel reliable models for MC and BAS research.

The most common experimental tools are based on murine models that are considered the best approach to study the functions and mechanisms of MC and BAS *in vivo*. For this reason, models for MC deficiency have been extensively studied, but no appropriate BAS model has yet been defined. New approaches have been recently performed to establish specific BAS-deficient mouse models. There are two general strategies to generate BAS-depleted mice: (i) antibody depletion, for example Ba103, MAR-1 and CD200R3, and (ii) genetically modified strains. Two types of ablation models have been described, including constitutively depleted models, for example Mcpt8Cre, Cpa3-Cre; Mcl-1 fl/fl; and inducible ablation, that is Mcpt8DTR and Baso-DTR. Selective ablation represents a powerful tool to study BAS function, although it poses certain limitations in the availability of BAS-specific markers and methods to induce proper depletion. The

Table 1 MC-/BAS-deficient mouse models

Strain	Systemic abnormalities	Cellular abnormalities	Known or potential limitations for MC/BAS studies	Level of deficiency	Restoring phenotype	Reference
<i>Mgf^{Sc}/Mgf^{Scld}</i>	White coat, macrocytic anaemia, sterility	Lack melanocytes, age-dependent changes in intestinal intra-epithelial lymphocyte populations, variable deficiency of the interstitial cells of Cajal	SCF signalling of several subtypes, e.g. B cells	Depletion >99%	Injection of SCF	(31)
<i>Kit^W/Kit^{W-v}</i>	White coat, macrocytic anaemia, sterility	Lack melanocytes, age-dependent changes in intestinal intra-epithelial lymphocyte populations, variable deficiency of the interstitial cells of Cajal, impaired T-cell development in the thymus, shift in intra-epithelial T cells in the gut in favour of TCR $\alpha\beta$ + and against TCR $\gamma\delta$ + cells, neutropenia and 75%–90% reduced basophil numbers, idiopathic dermatitis, stomach papilloma and ulcers	Bystander effects, reduced muscular fitness, weight loss and survival	Depletion >99%	Reconstitution	(32, 33)
<i>Kit^{W-sh}/Kit^{W-sh}</i>	White coat, splenomegaly, histological aberrations of the spleen	Neutrophilia, megakaryocytosis and thrombocytosis. Effect in other genes due to the mutation, that is <i>corin</i>	Bystander effects, reduced muscular fitness, weight loss and survival	Depletion >99%	Reconstitution	(34)
<i>Mcpt5-Cre</i>	Small size, Cre instability	No report about basophil numbers in PBMC	Side-effects of DT injection	Around 97% in ear skin	Time: 3 weeks	(13, 35)
<i>Mcpt5-Cre</i>	Not reported	Not reported	Side-effects of DT injection	Around 96% in peritoneum and ear skin. 89% in the back and abdominal skin	Reconstitution	(13, 18)
<i>Mas-TRECK</i>	Not reported	Basophils are also transiently depleted	Side-effects of DT injection	Total depletion	Time: 18 days	(36)
<i>Cpa3^{Cre/+}</i>	Not reported	Basophils reduction around 40%	Other Cpa3-expressing cells may be affected, that is basophils and T cells	Total depletion under steady-state or inflammatory conditions	Reconstitution	(15)
<i>Cpa3-Cre; Mcl^{fl/fl}</i>	Mild macrocytic anaemia	Reductions in basophil numbers in spleen (58%), blood (74%) and bone marrow (75%), splenic neutrophilia	Other Cpa3-expressing cells and depending whether Mcl-1 to survive may be affected, for example basophils	Depletion 92–100%	Reconstitution	(14)
<i>Kit-Cre^{ERT2/+}</i>	Partial lack of Kit function	Minor increase in neutrophil numbers	Not yet assessed	Total depletion in skin and stomach, about 90% of peritoneal mast cells	Not before 8 weeks after tamoxifen injection	(16)
<i>BasoDTR</i>	Not reported	Partial decrease in T-cell numbers	No decrease of BAS in bone marrow	~84% in peripheral blood	Not reported	(37)

Table 1 (Continued)

Strain	Systemic abnormalities	Cellular abnormalities	Known or potential limitations for MC/BAS studies	Level of deficiency	Restoring phenotype	Reference
Mcpt8 ^{DTR}	Not reported	Mast cells not affected	Side-effects of DT injection	Depletion >90%	Start to increase 6 days after DT injection almost fully recovered by day 8	(38)
Mcpt8-Cre	Not reported	Peritoneal and mucosal mast cells were not affected	Up to ~15% of peripheral T cells, NK cells, DCs and eosinophils have transient expression of Mcpt8 gene	Depletion >90%	Not rescued by exogenous IL-3	(39)
Bas-TRECK	Not reported	No effect on MCs, neutrophils, eosinophils or T-cell populations	Side-effects of DT injection	Total depletion (>98%)	Not reported	(40)
Runx1 (P1N/P1N)	Not reported	Mast cells, neutrophils and eosinophils not affected	Severe reductions in NKT cells and other cell types in lymphoid compartment	>90% reduction in the numbers of basophils in the BM, spleen and blood	Modest rescue by exogenous IL-3 administration or parasite infection	(41)

induction of basophil haematopoiesis by IL-3 and TSLP is an interesting alternative to study BAS (and MC) function *in vivo* (8).

MC-deficient mouse models consist of Kit-dependent and Kit-independent models (Table 1). Among these, constitutive and inducible models are available that allow for detailed analysis of MC functions *in vivo*. Most of the currently existing evidence about the role of MC in health and disease has been obtained from *in vivo* models using Kit-mutant mice which, due to the loss of function mutations in Kit, lack mature MC (9, 10). These models, that is WBB6F₁ *Kit^W/Kit^{W-v}* and C57BL/6 *Kit^{W-sh}/Kit^{W-sh}* mice, exhibit additional abnormalities because of the widespread distribution of Kit and its important function in the development of other cell types including hematopoietic stem and progenitor cells, melanocytes and interstitial cells of Cajal. Therefore, adaptive transfer of the MC compartment is required in these models to overcome other Kit-mediated but MC-independent abnormalities (11). During the past years, novel transgenic approaches to generate MC-deficient mouse models independent of Kit mutations have been developed (12). Although these models exhibit less off-target changes in the immune system compared with Kit-dependent models, they mostly also affect BAS numbers, which is a limiting concern. The Mcpt-5 and the Cpa3 promoters, both of which encode for MC proteases, have been used for the insertion of Cre recombinase in combination with either loxP-controlled expression of diphtheria toxin (DT) or its receptor (13), anti-apoptotic proteins, such as Mcl-1 (14), or direct gene toxicity of high-level Cre expression in MC (15). Another model uses a fusion protein of Cre and the tamoxifen-specific oestrogen receptor, which allows for inducible gene targeting and ablation of mast cells under the control of the Kit promoter (16).

Studies conducted in these new models interestingly not only reproduced key functions of MC that have been proposed from the earlier Kit-dependent models, but also revealed discrepant results in some of the disease models that have been previously reported to be, at least in part, MC mediated (17). For example, the role of MC in the elicitation of chronic hypersensitivity reactions (18), antibody-mediated arthritis or experimental encephalomyelitis (15) showed unexpectedly different results in Kit-independent compared with those obtained from Kit-dependent models (19, 20). These controversies that have been also reported for other MC functions, including wound healing (21–24), tumour growth (21, 25) and bacterial defence (26–29), as well as possible underlying reasons for the divergent *in vivo* responses comparing the 'newer' to the 'older' models, are currently discussed (12, 17). Besides variations in the protocols that have been used for different disease models, the Cre-mediated strains may also exhibit particular limitations that could be due to cell-intrinsic defects in other cell types, constitutive or induced expression of Cre-controlling promoters in other cells than MC (particularly under chronic inflammatory conditions) or effects induced by diphtheria toxin and/or inflammatory responses induced by synchronized MC apoptosis. In constitutive deficient models, the lack of MC may

also initiate compensatory mechanisms that could influence the experimental outcome.

No doubt that new transgenic mouse models already have and will in future enable us to more critically discuss the role and the functions of MC/BAS in health and disease. The interpretation of results obtained from different mouse strains, however, requires particular consideration of potential nonspecific effects regardless of the model used. Detailed investigation of the transgenic mouse strains will definitely improve our understanding of discrepancies that have been recently observed. The use of Cre reporter genes could be a useful tool to investigate constitutive and induced levels of Cre expression not only under steady-state conditions but also in acute and chronic inflammatory settings. The Kit-mutant strains may preserve their status as established MC-deficient models because of their wide distribution, availability and cost efficacy. Results obtained from Kit-mutant models are recommended to be reproduced in a Kit-independent strain and *vice versa* to collect more detailed information about potential divergent outcomes from different mouse models and protocols. This will probably lead to the generation of robust data and better evidence about the pathophysiological functions of MC and BAS in future.

The translation of findings from mice to men remains an obstacle in biomedical research. Humanized mice are useful tools to bridge the animal model to the human system, and models are also being established for MC- and BAS-mediated diseases (30). Such models may help in future to develop and study novel therapeutic strategies for MC-/BAS-driven diseases, such as urticaria, asthma, atopic dermatitis or food allergy.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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