Published in final edited form as:
Otol Neurotol. 2010 January ; 31(1): 42–47. doi:10.1097/MAO.0b013e3181b4e3e3.

Reconstitution of the Mast Cell Population in W/W^v Mice

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Abstract

Hypothesis—Intravenous injection of cultured mast cells can reconstitute the mast cell population in mast cell deficient mice. We hypothesize that injected culture-derived mast cells do not repopulate all tissues equally.

Background—Mast cells are central elements not only in anaphylaxis and allergy but also in immune reactions to bacteria and other pathogens. Their broad involvement in innate immunity requires extensive research in the future. Studies of mast cell function often utilize mast cell deficient mice to compare to wild type animals. A very elegant method to prove that the observed changes are due to the lack of mast cells is to compare results in wild type mice, mast cell deficient mice and mast cell deficient mice that have been reconstituted with cultured mast cells. Reconstitution of the mast cell population can be achieved by intravenous injection of mast cells into mast cell deficient mice. Whether the injected mast cells repopulate the desired tissues has to be proven before this model is used. Also, the time frame of the reconstitution has to be demonstrated.

Methods—Mast cell deficient mice were injected with bone marrow derived cultured mast cells and the mucosa of middle ears, nose and tracheo-bronchial system was analyzed for mast cells 4, 6, 8, 10 and 20 weeks after injection.

Results—Reconstitution of the middle ear mucosa was complete and persistent over 20 weeks. Reconstitution failed in nasal mucosa. In bronchial mucosa, reconstitution was incomplete and transient.

Conclusion—This model can be used to investigate effects of mast cells in various immune reactions in the middle ear. Studies should use the time frame 6-8 weeks after reconstitution of the mast cell population. However, the model has limitations for investigations in the respiratory tract.

Keywords

W/W^v mice; mast cell; mast cell reconstitution; allergy; immunology

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Hypothesis

We hypothesize that intravenous injection of cultured mast cells (MC) can reconstitute the MC population in genetically MC deficient mice. However, not all tissues are reconstituted equally and reconstitution is time-sensitive.

Background

Since the discovery of a broader involvement of MC in immunity than Th2-Lymphocyte/IgE-driven immune reactions (1-[5]), interest in MC research is growing. Especially investigation of the role of MC in innate immune reactions promises new aspects in treatment of infectious diseases. Therefore, models to study the effects of MC on infection and allergy in different cellular environments are needed.

Pure MC cultures can be derived in vitro from bone marrow cell cultures (6). These cells (bone marrow derived mast cells, BMMC) can be injected into MC deficient mice (WBB6F1/J-*Kit*^{W}/*Kit*$^{W\text{-}v}$; called W/W^{v} mice in the following) either locally or systemically to repopulate tissues (7). This reconstitution model is convenient to study the effects of MC comparing the reactions in wild-type, MC deficient and reconstituted mice. Moreover, this model offers the opportunity to study the effects of MC even more thoroughly: MC deficient mice can be reconstituted with MC derived from mutant or knockout mice lacking different receptors, mediators or molecules.

However, this experimental setting requires that injected cell culture derived MC repopulate the designated tissues. Besides, the time course of the reconstitution is critical. If reconstituted MC repopulate the tissues only briefly, experiments have to be designed accordingly. Also, the functionality of reconstituted MC should be demonstrated. Previous studies suggest that transplantation of BMMC does not result in a complete repopulation of all tissues (7,[8]).

In the present study we are investigating the reconstitution of middle ear (ME) mucosa, nasal mucosa and tracheo-bronchial mucosa with BMMC.

Methods

Mice

6 week old female MC-deficient mice (WBB6F1/J-*Kit*^{W}/*Kit*$^{W\text{-}v}$) were obtained from The Jackson Laboratory. Due to a spontaneous mutation, these mice lack *c-Kit*, a transmembrane receptor required for differentiation of MC precursors into MC (9). The animals are therefore MC deficient. The mutation also affects pigment-forming cells, germ cells and red blood cells causing severe macrocytic anaemia. Age-matched female congenic littermates (WBB6F1-+/+) served as bone marrow donors and wild-type controls. All protocols were approved by the Institutional Animal Care and Use Committee of the VA San Diego Healthcare System.

MC cultures

Female wild-type littermates of W/W^{v} mice were sacrificed and femurs and tibias isolated. The bones were flushed with media to remove bone marrow, which was cultured for four weeks in RAZIN'S media with 20% WEHI supernatant as a source of MC growth factors to stimulate BMMC formation. Media was changed once a week.

Media

Media was prepared by adding 50 ml of FCS, 5 ml of Nonessential Amino Acid solution, 5 ml of L-Glutamine, 6 ml of Penicillin/Streptomycin and 0.5 ml of β-mercapto-ethanol solution to

500 ml of RPMI 1640 media. As a source of IL-3 and other MC growth factors, 20% WEHI supernatant was added.

Identification and preparation of MC cultures

After four weeks in culture, 50 μl of fluid were removed from each cell culture, plated and stained with Toluidine blue to identify and enumerate MC. Purity of the colonies was determined as >98% at this time point. The MC cultures were washed three times with PBS before injection via tail vein into W/W^v mice.

Reconstitution of the MC population

A total of twenty W/W^v mice received tail vein injections of 5×10^6 BMMC each in a volume of 200 μl. Groups of four mice were sacrificed four, six, eight, ten and twenty weeks after reconstitution and nose, middle ears and lungs with trachea were obtained.

Tissue preparation

Mice were sacrificed by intracardial perfusion with PBS followed by paraformaldehyde under general anesthesia. All tissues were postfixed in 4% paraformaldehyde for at least 24 hours. The lung specimens were then embedded in paraffin as were the nose specimens after decalcification in EDTA. Sections 7μm thick were cut from different planes of each specimen at least 100μm apart from each other. The sections were deparaffinized and stained with toluidine blue.

The ME mucosa was analyzed as a whole mount: After perfusing the mice, the ME bullae were dissected. The tympanic membranes were preserved and the bullae were opened. Using fine tip forceps and a pointed scalpel, the ME mucosa was dissected as a whole and transferred into Toluidine blue stain for five minutes. The specimens were then rinsed and mounted on slides for light-microscopic evaluation and photo documentation.

Toluidine blue staining

Toluidine blue stain was prepared by adding 0.1g Toluidine blue (Toluidine Blue O, Sigma-Aldrich) and 0.1g Borax to 100ml of distilled water. This stain was used on the paraffin sections of noses and lungs as well as the whole mounts of ME mucosa.

Evaluation of results

Lungs—According to previous studies, in wild-type mice, MC distribution is only sporadic in lung tissue. In tracheal and bronchial mucosa however, MC can be found in larger numbers (8).

In the present study, paraffin sections of lungs were examined. The numbers of tracheobronchial MC were counted in three randomly chosen 40× fields of view with longitudinal sections of tracheal and bronchial mucosa respectively in each specimen. The magnification was normalized and the number of MC per millimeter mucosa was calculated from these counts.

Noses—Two types of epithelia can be distinguished in the nasal mucosa: respiratory and olfactory epithelium. With respect to MC function, respiratory epithelium seems more interesting. In the present study, three randomly chosen 40× fields of view within the respiratory epithelium of the septum and the inferior turbinate were examined in each paraffin section. The number of MC was counted as described above and the number of MC per millimeter nasal mucosa was calculated.

Ears—Mast cells in the ME mucosa were analyzed in 120× photomicrographs of whole mount specimens after Toluidine blue staining. Using Image Pro™ image analysis software, the number of MC was counted in a standard size area of interest at five randomly chosen locations in each specimen. The average number of MC per square millimeter of mucosa was calculated from these counts.

Statistics

To document statistical significance, ANOVA was performed on all data using StatView 5.0 software. Differences between groups were considered significant at $p < 0.05$.

Results

As expected, MC were not detected in any of the exmained tissues in W/W^v mice (Figure 1 A, B).

Lungs

Wildtype mice—In congenic controls, an average of 2.5 MC per millimeter was counted in tracheal mucosa and 5.3 MC per millimeter in bronchial mucosa (Figure 1 A; Figure 2 A). MC were distributed within the epithelium as well as in the subepithelium. Less frequently, MC were also found in the connective tissue adjacent to bronchi or trachea. Within lung tissue, MC were distributed sparsely.

Reconstituted mice—In tracheal mucosa, MC were detected between four and ten weeks after reconstitution. In bronchial mucosa, MC were detected only at six and eight weeks after reconstitution. At all time points however, in tracheal as well as bronchial mucosa, the number of MC was significantly lower than in wild type mice (Figure 1 A; Figure 2 b).

Nasal mucosa

Wildtype mice—An average of 7.8 MC per millimeter respiratory epithelium was detected in the nasal mucosa of wild type mice (Figure 1 A; Figure 2 C). MC were typically located in the subepithelial space.

In all sections, MC could also be detected in the subcutaneous connective tissue. Sections of bone marrow displayed no MC.

Reconstituted mice—The only time point where MC were detected sporadically within the nasal mucosa was six weeks after reconstitution. The numbers were by far smaller than in wild type mice (Figure 1 A; Figure 2 D).

However, from six to twenty weeks after reconstitution, MC appeared in the subcutaneous tissue and also in sections of bone marrow (Figure 3).

Middle ear mucosa

Wildtype mice—An average of 93 MC per square millimeter ME mucosa were detected in wild type mice (Figure 1 B; Figure 2 E). MC were distributed within the mucosa as described by Albiin et al. (10) with the difference that, in our experiments, we did not detect significant numbers of MC in the pars tensa of the tympanic membrane. However, MC were abundant in the pars flaccida of the tympanic membrane.

Reconstituted mice—MC were found in the ME mucosa of rereconstituted mice as early as four weeks after reconstitution. However, the number of MC per square millimeter at this time point was significantly lower than in wild-type mice. Six and eight weeks after injection,

the number of MC found in the ME mucosa of reconstituted mice was the same as in wild-type mice. Ten and twenty weeks after reconstitution, large numbers of MC were still observed in the ME mucosa (55 and 56 per square millimeter) but the numbers were significantly lower than in wild-type mice (Figure 1 B; Figure 2 F).

Discussion

MC are central elements not only in anaphylaxis and allergy but also in immune reactions to bacteria and other pathogens (12). Their broad involvement in innate immunity requires extensive research in the future. Especially the correlation of allergic and infectious immune reactions might involve MC (12). They are important players in ME inflammatory reactions and of course in asthma and other tracheo-bronchial pathologies. The role of MC in immunity is not yet completely elucidated but might yield novel therapeutic options in various infectious diseases. Therefore, models to study the various immunologic effects of MC are needed.

Tracheo-bronchial system

In wild type mice, high numbers of MC were found in both, tracheal and bronchial mucosa. MC were located primarily in the subepithelial stroma but also in peribronchial and peritracheal connective tissue. MC density was higher in bronchial mucosa than in tracheal mucosa (Figure 1). In contrast to previous studies (8), MC repopulation of lung parenchyma by BMMC was not observed in our specimens. Reconstitution of the tracheo-bronchial MC population in W/W^v mice was only transient (weeks six to eight). Additionally, the density of MC in reconstituted mice was significantly lower compared to wild type mice (Figure 1). The lungs were obtained from the same animals in which the reconstitution was successful in the ME mucosa which rules out failure of injection or (partial) rejection of the injected BMMC.

Nasal mucosa / bone marrow / subcutaneous tissue

In wild type mice, high numbers of MC were detected in the respiratory epithelium of the nasal mucosa. This was expected since MC mediated reactions play an important role in the nasal mucosa.

Reconstitution of this MC population was not successful at any of the observed time points. Failure of injection and rejection of the injected BMMC are ruled out also in this setting since the examined noses were obtained from the same mice as the ME and lung specimens. Also, in the nasal specimens MC were detected within the subcutaneous tissue and bone marrow spaces starting six weeks after injection and lasting until week twenty. MC population of bone marrow spaces is not observed in wild type animals and might be a sign of a clonal expansion of the population, as previously observed (11).

Middle ear mucosa

Reconstituted MC repopulate the ME mucosa well. Complete replenishment takes about six weeks of time and lasts for approximately two to three weeks. After this time span, the number of MC slightly decreases but significant numbers of MC remain within the mucosa for at least twenty weeks. The functionality of the reconstituted MC within the ME mucosa has been demonstrated before (12).

BMMC are capable of a complete and functional reconstitution of the MC population in the ME. In the tracho-bronchial mucosa, reconstitution is only transient and not complete. Reconstitution of the MC population in the nasal mucosa with BMMC failed. An explanation for the differences in reconstitution of different tissues could be that through in vitro culture, subpopulations of MC are created that have a limited ability to establish stable populations in some tissues (8). As described earlier, the problems of restoring certain tissue types do not

occur after transplantation of whole bone marrow (8). Following whole bone marrow transplantation, MC precursor cells are able to develop tissue-specific migration factors in vivo. However, transplantation of whole bone marrow corrects all hematological disorders in W/W^v mice complicating the detection of effects exerted by MC in isolation. Thus, the transplantation of BMMC is desirable in MC research. Reconstitution of the desired tissues has to be demonstrated if this model is used.

Conclusion

The mouse model of reconstituting the MC population in W/W^v mice with BMMC is elegant and very convenient. It allows comparison of findings in wild type and MC deficient mice and provides the opportunity to confirm the results in MC repopulated tissues. Effects measured can be accredited to MC almost unmistakably. However, the model is not applicable for all tissues. If the model is used, repopulation of the desired tissues has to be tested. From the desired tissues in the present study, only the ME mucosa and subcutaneous tissue were repopulated completely and persistently with BMMC. Reconstitution of the tracheo-bronchial mucosa was transient and incomplete. Thus, there seem to be limitations for studies of MC in the respiratory tract with this model. Reconstitution of the nasal mucosa with BMMC failed completely. The model of BMMC reconstitution is not suitable for studies of the nasal mucosa. Whole bone marrow transplantation could be an option in this case accepting the uncertainty of the broader correction of hematological defects of W/W^v mice in this model.

Studies of MC function in the ME should be designed using the time span six to eight weeks after reconstitution for most accurate results.

Acknowledgments

The authors would like to thank Marina Miller, Jae Y. Cho, Kirsti McElwain (Department of Medicine/Rheumatology, Allergy and Immunology, UCSD School of Medicine) and Sharon Okamoto (Department of Infectious Diseases, VA Medical Center, La Jolla, CA) for their support and advice and Eduardo Chavez (Department of Surgery/Otolaryngology, UCSD School of Medicine) and Susanne Kanabey (Department of Otorhinolaryngology, Ruhr-University of Bochum, Germany) for excellent technical assistance.

This work was supported by NIH/NIDCD grants DC00129 (to A.F.R.) & DC006279 (to S.W.), the VA Research Service and by the German DFG (EB 260/1-1, to J.E.).

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Abbreviations

BMMC	bone marrow derived cultured mast cells
EDTA	ethylenediaminetetraacetic acid
FCS	fetal calf serum = fetal bovine serum
IL-3	Interleukin-3
MC	mast cell
ME	middle ear
PBS	Phosphate buffered saline
RPMI	Roswell Park Memorial Institute medium
WEHI supernatant	supernatant from a murine myelomonocytic leukemia cell line (WEHI-3 cells), rich in Interleukin-3
WT	wild-type
W/W^{v} mice	mast cell deficient WBB6F1/J-$Kit^{W}/Kit^{W\text{-}v}$ mice

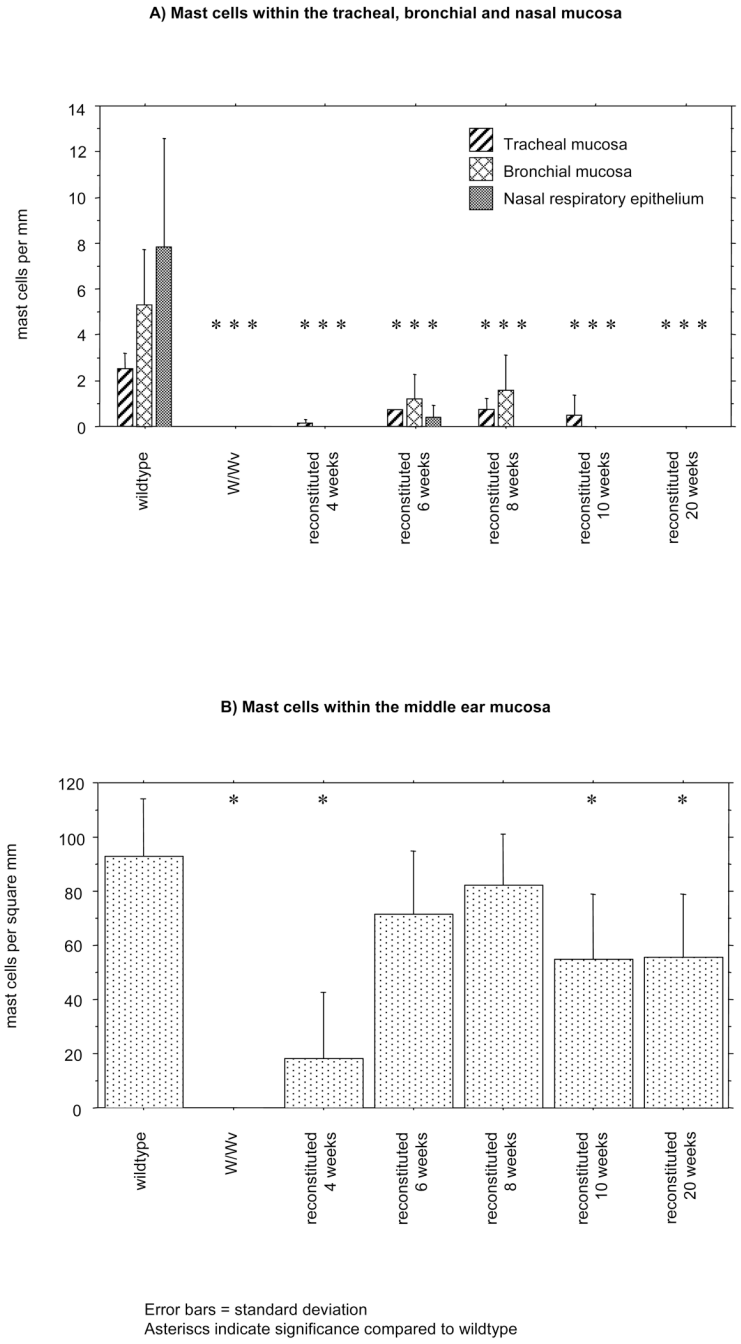

Figure 1.

A) Number of Mast cells per millimeter tracheal, bronchial and nasal mucosa (respiratory epithelium) in wild type, mast cell deficient (W/W^v) and reconstituted mice between 4 and 20 weeks after injection.

B) Number of Mast cells per square millimeter in whole mounts of middle ear mucosa of wild type, mast cell deficient (W/W^v) and reconstituted mice between 4 and 20 weeks after injection. Error bars equal one standard deviation, asterisks indicate significance compared to wild type ($p<0.05$).

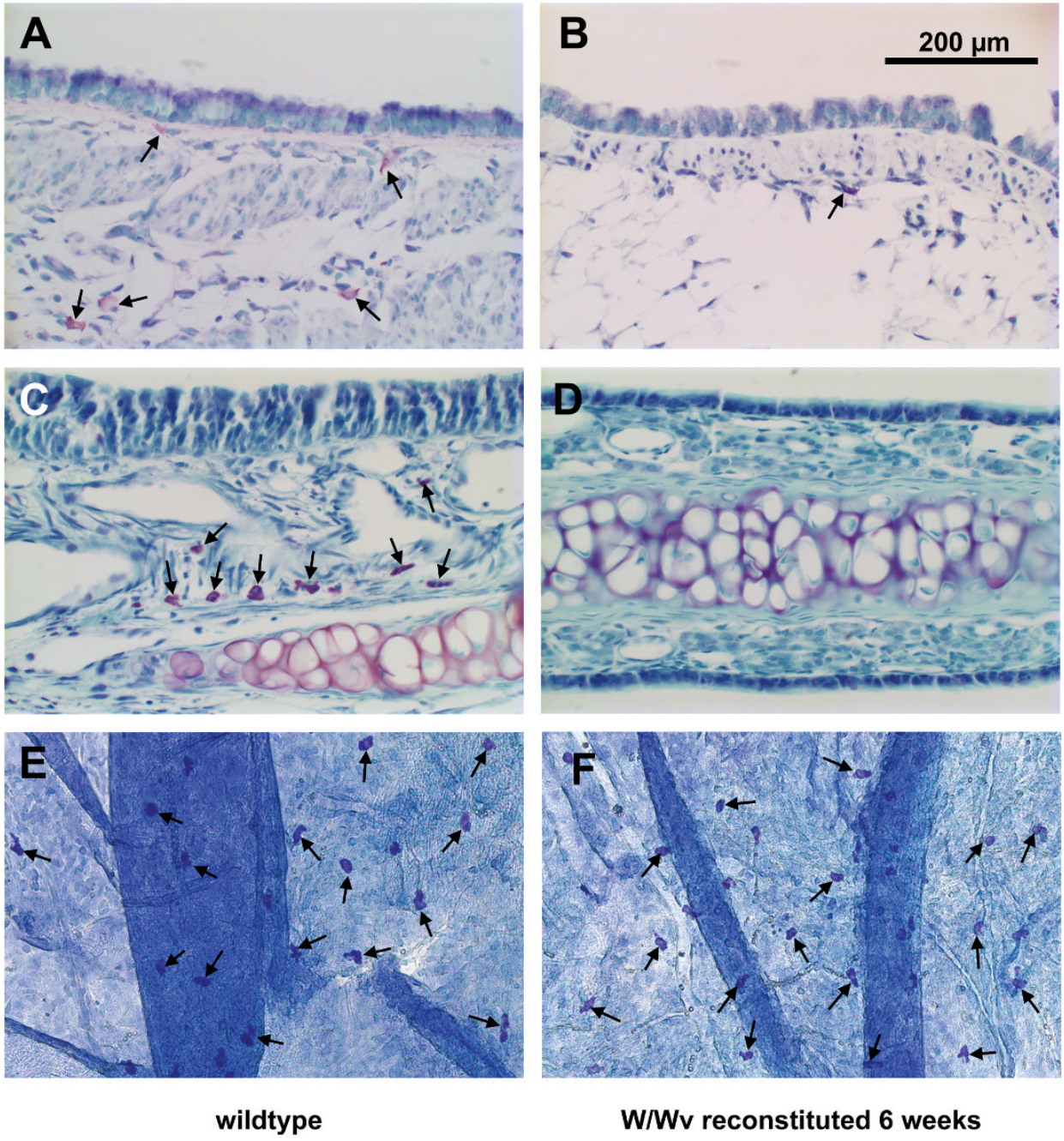

Figure 2.

Photomicrographs of different tissues, Toluidine blue staining to highlight mast cells. Left side: wild type tissues, right side: tissues of W/W^v mice 6 weeks after reconstitution with bone marrow derived cultured mast cells.

Magnification 120× in each picture. Arrows indicate mast cells.

(A) Wild type bronchial mucosa; several mast cells are visible in the subepithelium

(B) Bronchial mucosa in mast cell deficient mouse 6 weeks after reconstitution; a single mast cell is visible in the subepithelium

(C) Wild type respiratory nasal mucosa; mast cells are abundant

(D) Respiratory nasal mucosa in mast cell deficient mouse 6 weeks after reconstitution; no mast cells are visible
(E) Photomicrograph of wild type middle ear mucosa; whole mount specimen; mast cells are abundant
(F) Photomicrograph of middle ear mucosa in mast cell deficient mouse 6 weeks after reconstitution; whole mount specimen; mast cells are abundant

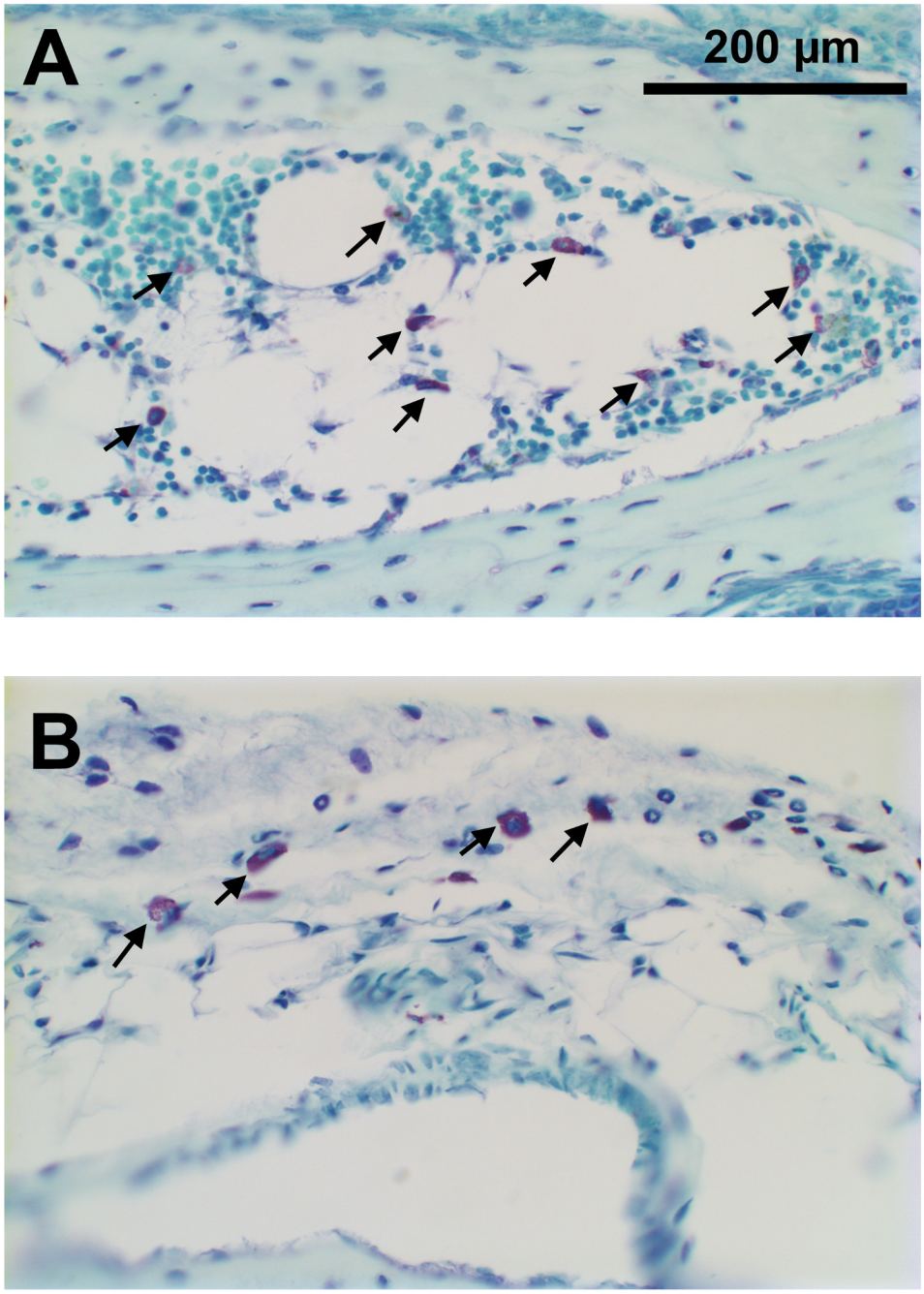

Figure 3.
Photomicrograph of (A) bone marrow and (B) subcutaneous tissue in mast cell deficient mouse 6 weeks after reconstitution. Toluidine blue staining to highlight mast cells, Magnification 120×, arrows indicate mast cells