

Role of mast cells in otitis media

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Background: New pathophysiologic concepts are needed to explain the clinically observed connection between the allergic diathesis and otitis media. Although mast cells, unlike lymphocytes, are common in the normal middle ear mucosa, their potential role in innate immunity of the middle ear and in the expression of inflammatory responses in that space to bacterial challenge, as opposed to allergy, has received relatively little attention.

Objective: In the current study, we examine the contributions of mast cells to the pathogenesis of bacterially induced inflammation in the middle ear and thus to otitis media.

Methods: Wild-type mice, mast cell-deficient mice, and mast cell-deficient mice whose mast cell populations were restored by transplantation of bone marrow-derived mast cells were challenged by using models of bacterial and allergic middle ear inflammation.

Results: Our results indicate that mast cells account for a substantial proportion of the innate immune response to bacteria in the middle ear.

Conclusion: This mechanism may link responses to allergy and infection in the middle ear mucosa, and thus the mast cell may be a critical control element in the pathogenesis of otitis media. (*J Allergy Clin Immunol* 2005;116:1129-35.)

Key words: Otitis media, allergy, mast cell, NTHi, ovalbumin, mast cell-deficient mice, *Haemophilus influenzae*

Mast cells are hematopoietically derived and cells closely associated with anaphylaxis and allergy.¹⁻³ This association has often been limited to T_H2-lymphocyte/IgE-driven immune reactions. However, recent research

Abbreviations used

BMMC: Bone marrow-derived cultured mast cells

ME: Middle ear

NTHi: Nontypeable *Haemophilus influenzae*

W/W^v mice: Mast cell-deficient WBB6F1/J-*Kit*^W/*Kit*^{W-v} mice

has identified a role for mast cells in innate⁴⁻⁸ and other adaptive immune reactions,^{6,8} suggesting that they are an essential part of first-line host defence against such pathogens as viruses, parasites, and bacteria.⁸ Mast cells directly interact with bacterial products via cell surface molecules including Toll-like receptors and CD48^{6,7,9-12} and are capable of phagocytosis,⁶ thereby recruiting an array of intracellular processes. When activated, mast cells rapidly exert various biological effects by releasing preformed (histamine, tryptase) and *de novo* synthesized (leukotrienes, prostaglandins) mediators and a variety of cytokines including TNF and ILs.^{6,13} By this early response, mast cells can contribute to innate immunity and host defense and bias subsequent adaptive immune responses. For example, mast cells are important regulators of neutrophilic inflammatory responses in the peritoneal cavity¹⁴ and essential for the full expression of articular pathology in a mouse model of arthritis.¹⁵ Thus, there is support for a more versatile involvement of mast cells in immunologic responses than the expression of IgE-dependent processes.

Despite extensive research, the pathogenic mechanisms underlying otitis media remain obscure. Eustachian tube dysfunction, bacterial infection, and immune-mediated inflammation all appear to play roles (reviewed by Rovers et al¹⁶). Mast cells are common in the normal middle ear (ME), where they account for the most numerous population of resident leukocytes by far,¹⁷⁻¹⁹ a finding that has supported previous attempts to connect allergy to the expression of otitis media.²⁰⁻²² Although allergic challenge has been shown to induce ME effusion,²³⁻²⁵ a link between atopy and otitis media in human beings has been difficult to establish.

The potential for a nonallergic role of mast cells in the ME has received relatively little attention. During otitis media, mast cell populations increase dramatically,^{26,27} and high levels of mast cell-specific mediators such as histamine, tryptase, and TNF are observed in ME effusions.^{20,28-31} In addition, nonspecific mast cell degranulation or the instillation of histamine into the normal ME

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can induce effusion and leukocyte infiltration.^{25,32,33} The possibility that mast cells are a major contributor not only to allergic but also to infectious reactions in the ME could provide a new perspective in otitis media.

The current study was designed to increase our understanding of mast cell contribution to the expression of inflammatory responses, both allergic and infectious, in the ME, and thus their potential place in otitis media pathogenesis. We assessed the magnitude of the inflammatory reaction in the MEs of allergic and nonallergic mice after challenge with allergen, bacteria, or their combination. The results in wild-type mice were compared with those found in genetically mast cell-deficient (WBB6F1/J-*Kit*^W/*Kit*^{W-v}; W/W^v) mice and in mast cell-deficient mice whose complement of mast cells was replenished with cultured bone marrow-derived cultured mast cells (BMMCs). Our results indicate mast cells are a major contributor to the early inflammatory response of the ME to bacteria and may provide a link between infection and allergy in the genesis of otitis media.

METHODS

Mice

Six-week old female mast cell-deficient mice (W/W^v) were obtained from the Jackson Laboratory (Bar Harbor, Me). Because of a spontaneous mutation, these mice lack *Kit*, a receptor for mast cell growth factor, critical for differentiation of mast cell precursors in bone marrow. Age-matched female congenic littermates (WBB6F1-^{+/+}) served as controls and bone marrow donors. All protocols were approved by the Institutional Animal Care and Use Committee of the VA San Diego Healthcare System.

Immunization

To induce allergic reactivity to chicken ovalbumin (Imject Ovalbumin; Pierce Biotechnology, Rockford, Ill), mice received intraperitoneal injections of 20 µg ovalbumin emulsified in 2.25 mg aluminum hydroxide (Imject Alum; Pierce Biotechnology) in a total volume of 200 µL sterile PBS on days 1 and 15.³⁴ Surgery was performed on these animals on day 30.

Bacteria

Haemophilus influenzae strain 3655 (nontypeable, biotype II; NTHi) was used at a concentration of 10⁵ to 10⁶/mL to induce experimental otitis media. The inocula were prepared as previously described.³⁵

Surgical procedure

Anesthesia was induced by intraperitoneal injection of 0.4 mL per 100 g bodyweight of a rodent cocktail containing 13.3 mg/mL Ketamine HCl (Fort Dodge Animal Health, Fort Dodge, Iowa), 1.3 mg/mL Xylazine (ICN Biomedicals, Aurora, Ohio), and 0.25 mg/mL Acepromazine maleate (Atravet, Ayerst Labs, Montreal, Quebec, Canada). The ME bullae were exposed bilaterally through a vertical midline incision on the neck. A 25-gauge syringe needle was used to fenestrate the center of the bulla. Approximately 3 to 5 µL PBS, NTHi (10⁵-10⁶/mL), ovalbumin (100 µg/mL), or the combination of NTHi and ovalbumin was injected. Excess fluid was absorbed with a sterile cotton swab, the cervical tissue was replaced, and the skin incision was stapled closed. Twenty-four, 48, and 72 hours after inoculation, the mice were killed under general anesthesia by

intracardiac perfusion with PBS and 4% paraformaldehyde. The ME bullae were isolated and postfixed in 4% paraformaldehyde overnight.

Mast cell cultures

Female wild-type littermates of W/W^v mice were killed, and femurs and tibias were isolated. The bones were flushed with media to recover bone marrow, which was cultured for 4 weeks in RAZIN'S media with 20% WEHI supernatant as a source of mast cell growth factors to stimulate BMMC formation. Media were changed once a week.

Identification and preparation of mast cell cultures

After 4 weeks in culture, 50 µL fluid was removed from each cell culture, plated, and stained with toluidine blue to identify and enumerate mast cells. Purity of the colonies was determined as >98% at this time point. The cultures were washed 3 times with PBS before injection via tail vein into W/W^v mice.

Experimental groups

Mice were divided into 4 experimental groups (n = 36 for each group). Group 1 consisted of congenic wild-type mice not sensitized to ovalbumin. Group 2 contained congenic wild-type mice sensitized to ovalbumin. Group 3 was nonsensitized W/W^v mice, and group 4 was W/W^v mice sensitized to ovalbumin. Each of the groups was subdivided into 4 sets of 9 mice each, which received the following injections: (1) PBS, (2) bacteria (NTHi), (3) allergen (ovalbumin), or (4) the combination of NTHi and ovalbumin. This resulted in 16 experimental groups. Three mice from each set were euthanized 24, 48, and 72 hours after surgery, and the ME bullae were obtained as described.

Reconstitution of the mast cell population

W/W^v mice received tail vein injections of 5 × 10⁶ BMMCs each. Six weeks after reconstitution, 4 mice were killed, and the mucosa of each ME were stained with toluidine blue and examined microscopically as a whole mount. Mast cells were identified and the number of mast cells per square millimeter counted. An additional 12 reconstituted W/W^v mice received ME injections of NTHi. Groups of 4 mice (totaling 8 ears) were killed 24, 48, and 72 hours after surgery, and the ME bullae were preserved. Because allergic sensitization did not affect the magnitude of the mucosal reaction to bacteria in wild-type and W/W^v mice, we did not examine the reaction of sensitized reconstituted mice to NTHi.

Evaluation of results

The ME bullae were decalcified for 14 days in EDTA and embedded in paraffin. Sections 7 µm were stained with hematoxylin and eosin. Digital micrographs were taken from a standardized region of the ME of each specimen. The thickness of the epithelium and subepithelial space and the sum of the 2 were computer-averaged over approximately 500 µm in the plane of, but not directly adjacent to, the eustachian tube.

For analysis of inflammatory cells, digital micrographs were obtained from each specimen in the plane that displayed the maximum amount of cells in the ME cavity. The area covered by inflammatory cells as a percentage of a standard area in the ME was then calculated by using image analysis software. This calculation was performed independently by 2 observers with matching results.

The numbers of intact versus degranulated mast cells were counted at 400× magnification in toluidine blue-stained paraffin sections of MEs 24 hours after challenge with NTHi. Mast cells were

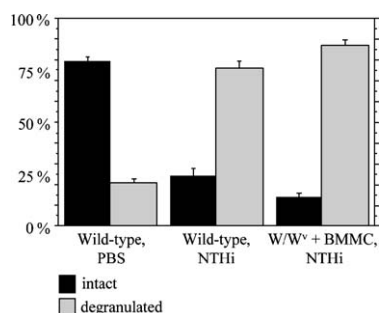


FIG 1. Differential mast cell count in the ME mucosa of wild-type and reconstituted W/W^v mice. Shown is the percentage of intact versus degranulated mast cells in wild-type mice 24 hours after injection of PBS or NTHi and in reconstituted W/W^v mice 24 hours after injection of NTHi. N = 6-8 ears per group; values are means $\pm$ SDs; all possible comparisons are significantly different in this dataset ($P \leq .05$).

counted as degranulated if granules were obviously decreased and/or expelled granules were observed adjacent to the cell.

Statistics

ANOVA was performed on all data by using StatView 5.0 software (JMP-SAS Institute Inc, Cary, NC). Differences between groups were considered significant at $P < .05$. Percentage data from differential mast cell counts were transformed by calculation of the square root of the arcsine and tested for normality before ANOVA was performed.

Both right and left ears of each subject were used for experimentation on the basis of the cost and scarcity of the mast cell-deficient mice. For purposes of statistical analysis, left and right ears were considered independent on the advice of our statistician, on the basis of the following 5 points

- (1) Individual mice were F1 crosses of 2 inbred strains, and thus there were no individual genetic differences between subjects. In the genetic sense, all ears can be considered to be from the same subject.
- (2) The coefficients of variance for the left and right ears were equivalent to the coefficients of variance for all mice in all measures, indicating that the degree of variation between the 2 ears of the mice was similar to that measured between the individual mice. This suggests that there were no systematic differences between the 2 sides.
- (3) No differences between MEs were observed on the basis of paired t tests for each variable.
- (4) There was no significant main effect for side (ie, left vs right) and no significant day/side interaction in a 2-way ANOVA for each day.
- (5) There was no main effect for side in a 3-way ANOVA across all days, and there were no significant interaction terms.

RESULTS

Reconstitution of the ME mast cell population in W/W^v mice

Six weeks after intravenous injection of BMMCs, the number of mast cells in reconstituted W/W^v mice (71 per mm² ME mucosa) was not significantly different than in wild-type mice (93 per mm² ME mucosa).

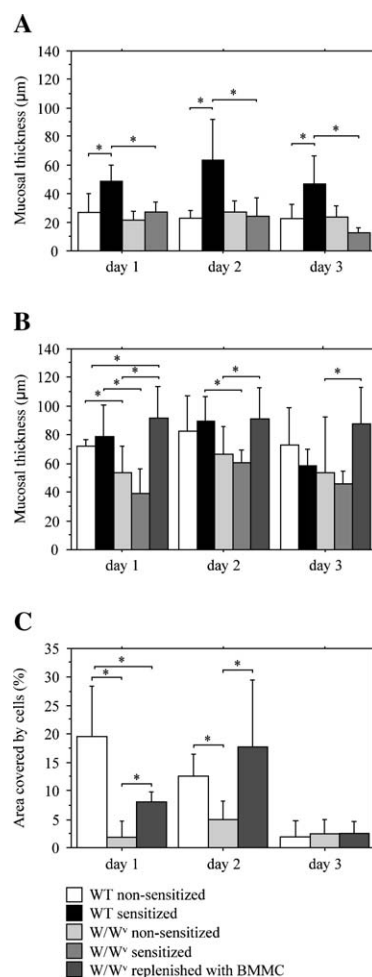


FIG 2. Middle ear reaction in wild-type and W/W^v mice to allergic and bacterial challenge. **A**, Thickness of the ME mucosa after allergic challenge in sensitized and nonsensitized wild-type and W/W^v mice (also see Fig 3). **B**, Thickness of the ME mucosa after bacterial challenge in sensitized and nonsensitized wild-type and W/W^v mice and W/W^v mice reconstituted with BMMCs (also see Fig 4). **C**, Area covered by inflammatory cells as a percentage of an area with a standard size in the ME cavity after bacterial challenge in nonsensitized mice. N = 6-8 ears per group; values are means $\pm$ SDs; asterisks indicate significance ($P \leq .05$).

Morphological differences between the mast cells in wild-type and replenished animals were not observed.

Injection of PBS

Injection of PBS did not evoke a significant response in the MEs of any group of mice, and no fluid or inflammatory cells were observed in the ME cavity. Mucosal infiltration by inflammatory cells was sparse and limited to the area directly adjacent to the surgical site. Likewise, no differences in the thickness of the ME mucosa epithelium and subepithelium were detected among the 4 study groups. Differences between wild-type and W/W^v mice were not observed, and allergic sensitization without ME challenge did not have an effect on the ME (see

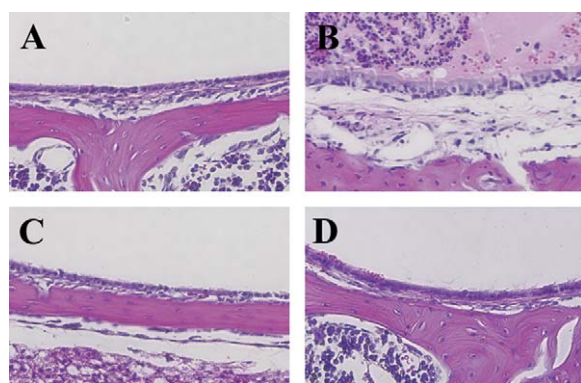


FIG 3. Microscopic sections of the ME 2 days after allergic challenge. Systemic allergic sensitization is reflected in the ME mucosa of wild-type but not W/W^v mice (also see Fig 2, A). **A**, Nonsensitized wild-type mouse; no inflammatory reaction. **B**, Sensitized wild-type mouse; otitis media with mucosal hyperplasia, effusion, and infiltration of inflammatory cells can be observed. **C**, Nonsensitized W/W^v mouse; no inflammatory reaction. **D**, Sensitized W/W^v mouse; no inflammatory reaction. Hematoxylin and eosin staining; magnification 120 $\times$.

Table E1 in the Online Repository in the online version of this article at www.jacionline.org.

Twenty-four hours after surgery, 80% of mast cells found in the ME mucosa of wild-type mice were intact, versus 20% degranulated (Fig 1).

Injection of allergen

Injection of ovalbumin caused significant mucosal thickening in sensitized compared with nonsensitized wild-type mice at all time points. This reaction was maximal at 48 hours (Fig 2, A) and was predominantly a consequence of subepithelial edema (Fig 3; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

Significant leukocyte infiltration and effusion of fluid into the ME cavity was observed 24 hours after challenge only in sensitized wild-type mice. By 48 hours, the number of cells was not significantly different from that seen in unsensitized controls, and by 72 hours, fluid and leukocytes were essentially absent from the ME (Fig 3; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

Injection of ovalbumin into the MEs of W/W^v mice, regardless of sensitization status, provoked a reaction no different from injection of PBS (Fig 2, A, and 3; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

Injection of bacteria in wild-type control mice

Inoculation of NTHi in wild-type mice consistently caused otitis media with significant mucosal hyperplasia and edema that persisted for 72 hours. Mucosal thickness peaked on day 2 after challenge. Epithelium and subepithelial stroma contributed equally to mucosal growth and edema at all time points (Figs 2, B, and 4; see Table E1

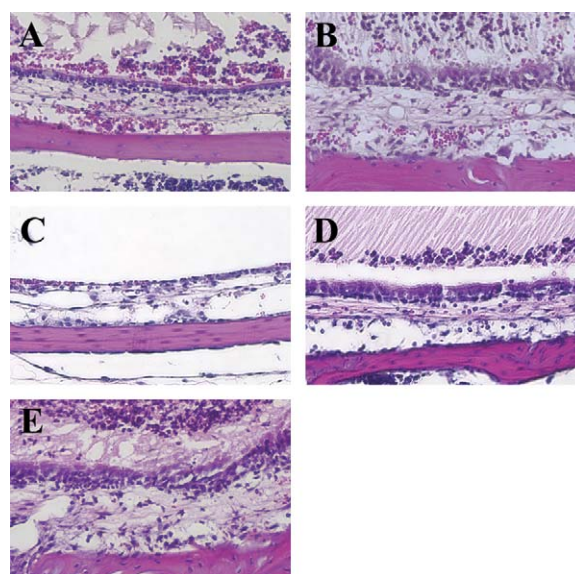


FIG 4. Microscopic sections of the ME 2 days after bacterial challenge. The inflammatory reaction is weaker in W/W^v mice; reconstitution of the mast cell population restores the reaction (also see Fig 2, B). **A**, Nonsensitized wild-type mouse. **B**, Sensitized wild-type mouse. **C**, Nonsensitized W/W^v mouse. **D**, Sensitized W/W^v mouse. **E**, W/W^v mouse replenished with BMMCs; the magnitude of the reaction is the same as in wild-type animals. Hematoxylin and eosin staining; magnification 120 $\times$.

in the Online Repository in the online version of this article at www.jacionline.org).

Significant numbers of cells and effusion in the ME lumen were detected at 24 hours and 48 hours after injection of bacteria. On day 3, effusions had cleared from all MEs (Fig 2, C; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

No significant differences in response to bacterial instillation were noted between sensitized and nonsensitized wild-type mice in the amount of mucosal hyperplasia or in the number of inflammatory cells identified in the ME cavity.

Twenty-four hours after infection, 25% of mast cells found in the ME mucosa were intact, and 75% were degranulated. This represents a significant change compared with PBS injection (Fig 1).

Injection of bacteria in W/W^v mice

In mast cell-deficient mice, NTHi injection also produced significant mucosal hyperplasia, although in sensitized animals, mucosal hyperplasia was not significant on day 1 after injection. Similar to the findings in wild-type mice, mucosal hyperplasia peaked on day 2 after challenge, and epithelium and subepithelial stroma contributed equally to mucosal growth and edema (Figs 2, B, and 4; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

A significant number of cells was found in the ME cavity only of sensitized W/W^v mice 48 hours after NTHi

injection. At all other time points and in nonsensitized W/W^v mice at all time points, no cells were observed in the ME lumen after NTHi challenge (Figs 2, C, and 4; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

As in wild-type mice, no significant differences between sensitized and nonsensitized animals were detected in mucosal hyperplasia or in the number of inflammatory cells in the ME lumen.

Comparison of the reaction to bacteria in wild-type and W/W^v mice

Significant differences were observed in mucosal hyperplasia of W/W^v mice in response to bacterial challenge compared with wild-type animals. One day after NTHi challenge, mucosal hyperplasia was significantly blunted in W/W^v animals regardless of immunization status (Fig 2, B; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org). On day 2, whereas in sensitized W/W^v mice the response was still significantly less than in wild-type mice, the difference in nonsensitized animals was not significant. Three days after infection, in both sensitized and nonsensitized mice, mucosal hyperplasia in W/W^v animals and wild-type controls was similar (Fig 2, B; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

Distinct differences in the number of cells in the ME lumen of W/W^v mice and wild-type mice were observed. Regardless of sensitization, significantly fewer cells were found in W/W^v mice 24 and 48 hours after challenge. On day 3, cells and fluid had essentially cleared from all animals, and no significant differences were observed (Fig 2, C; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

Injection of bacteria in mast cell-deficient mice reconstituted with BMDCs

Reconstitution of W/W^v mice with BMDCs restored the ME response to bacteria. Reconstituted animals exhibited significantly more mucosal hyperplasia in response to NTHi than nonreconstituted W/W^v mice at all time points. The response of both epithelium and subepithelial stroma was restored (Figs 2, B and C, and 4; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org). Hyperplasia of the ME mucosa in reconstituted mice on day 1 was in fact greater even than that of wild-type mice (Fig 2, B), although no differences between these groups were observed on days 2 and 3.

Forty-eight hours after NTHi infection, the number of inflammatory cells in the ME lumens of reconstituted mice was significantly higher than in W/W^v mice (Fig 2, C). On day 1, this difference was not significant, and by day 3, all cells and fluid had cleared.

Twenty-four hours after infection, less than 20% of the mast cells found in the ME mucosa were intact, and more than 80% were degranulated (Fig 1).

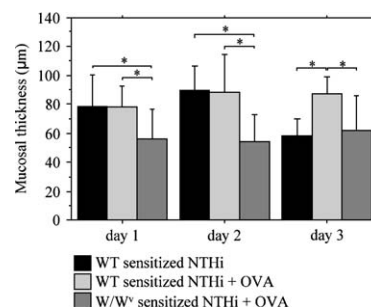


FIG 5. Thickness of the ME mucosa in sensitized wild-type and W/W^v mice after challenge with bacteria or the combination of bacteria and allergen. After combined challenge, recovery is delayed in wild-type mice. This effect is not observed in W/W^v mice. N = 6-8 ears per group; values are means ± SDs; asterisks indicate significance ($P \leq .05$).

Injection of the combination of bacteria and allergen

In contrast with our findings after single challenge with NTHi, mucosal hyperplasia after combined challenge was significant in sensitized W/W^v mice, even on day 1 (see Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

However, combined challenge did not produce significantly stronger mucosal hyperplasia than NTHi alone in either wild-type or W/W^v mice, regardless of sensitization status (Fig 5; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org). In contrast, the time course of the reaction in double-stimulated sensitized wild-type animals was different from all other NTHi-stimulated or NTHi + ovalbumin-stimulated animals: the mucosal reaction did not peak on day 2 and then decrease on day 3 but instead remained at a high level throughout the observation period. Thus, on day 3, the ME mucosa in sensitized wild-type mice challenged with NTHi + ovalbumin was significantly thicker than in nonsensitized wild-type mice or in sensitized wild-type mice challenged with NTHi alone. This enhanced inflammation in response to dual challenge was not observed in W/W^v mice (Fig 5; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

Congruent with our findings after injection of NTHi alone, mucosal hyperplasia was less in W/W^v mice compared with wild-type controls at all time points after injection of NTHi + ovalbumin. In sensitized mice, this difference was significant at all time points. In nonsensitized mice, however, it was significant only on day 1.

The ratio between the thickness of epithelium and subepithelial space was similar in all of the study groups.

On day 1 after combined challenge, significantly more cells were found in the MEs of sensitized wild-type mice compared with nonsensitized wild-type mice or W/W^v mice regardless of their sensitization status. On day 2, the response noted in sensitized wild-type mice had diminished to match that of nonsensitized wild-type mice. At this time point, the response in W/W^v mice had declined

to almost baseline, regardless of sensitization status (see Table E1 in the Online Repository in the online version of this article at www.jacionline.org). In all groups, effusion and cells had cleared after 72 hours.

DISCUSSION

ME challenge with NTHi causes otitis media

Corresponding with earlier studies,³⁵ injection of NTHi into the MEs of wild-type mice consistently caused otitis media characterized by mucosal expansion, transudation of fluid, and transmigration of inflammatory cells into the ME lumen (Figs 2, B and C, and 4; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org). Mucosal hyperplasia peaked on day 2 after infection and began to decrease on day 3 (Fig 2, B).

Allergic challenge causes otitis media

Injection of ovalbumin into the MEs of wild-type mice systemically sensitized to this antigen resulted in a significant inflammatory reaction including mucosal edema, effusion, and leukocyte infiltration (Figs 2, A, and 3). This observation confirms that systemic allergic sensitization is reflected in the ME mucosa, as in earlier studies.^{23,24,36,37} Our study and earlier studies also suggest that a local allergic reaction can contribute to otitis media by attracting inflammatory cells and inducing mucosal edema. In W/W^v mice, however, the ME response to allergic challenge did not occur (Figs 2, A, and 3). This strongly indicates that the reaction is mast cell-mediated and thus that systemic sensitization produces classic allergic reactivity in the ME mucosa. In this respect, the ME resembles other sites in the upper and lower airway.^{38,39}

Interaction between bacterial infection and allergy

In our experiments, allergen challenge of systemically sensitized mice did not have a significant effect on the magnitude of the ME response to bacteria (see Table E1 in the Online Repository in the online version of this article at www.jacionline.org). We believe this is because the dose of bacteria used (10^5 - 10^6 /mL) was sufficient to cause infection and a vigorous mucosal reaction consistently. An increase in the magnitude of this reaction as a result of allergic challenge may not have been possible.

However, the reaction to combined bacteria and allergen in sensitized wild-type mice was more persistent than the response to either alone (Fig 5). This suggests that an interaction between allergic and infectious ME reactions does occur, reflected in delayed recovery from inflammatory otitis media. Moreover, the absence of this persistent reaction in W/W^v mice indicates that mast cells are central to this interaction.

Role of mast cells in innate immunity of the ME

In the absence of mast cells, injection of bacteria still caused otitis media persistent over a period of 72 hours. This indicates that mast cells are not required for bacterial

otitis media. However, the early response to bacteria in W/W^v mice was substantially less than in wild-type animals or in W/W^v animals whose mast cells had been replenished (Figs 2, B, and 4; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org). These data support the inference that mast cells play a major role in the early innate immune reaction to bacteria. Although not required to mount an innate immune response, mast cells are important not only in its magnitude but also in the promptness of the response. At later times, mast cell-independent factors^{40,41} appear to be more dominant.

At other anatomic sites, mast cells have been shown to act as a first line of defense against bacterial antigens and inflammatory proteins (reviewed by Abraham and Malaviya⁶). Our data suggest that this concept can be extended to the ME mucosa and that mast cells play an important role in the induction of inflammation in the ME. The finding that after bacterial challenge, mast cell degranulation was observed in the ME mucosa of wild-type mice also supports this conclusion. In our experiments, mast cells accounted for approximately half of the strength of the inflammatory reaction to NTHi in the ME. Early in otitis media, they act as sentinels for innate immunity and, by releasing proinflammatory and immunoregulatory mediators and cytokines, may act as regulators of an acquired immune response.^{8,13} Our finding that reconstitution of the mast cell population returns the ME response to bacterial challenge to that seen in wild-type mice (Fig 2, B) strongly supports this conclusion.

In our experiments, mast cells successfully repopulated the MEs of W/W^v mice 6 weeks after intravenous injection. Our observation that challenge with bacteria caused mast cell degranulation in wild-type as well as reconstituted W/W^v mice (Fig 1) indicates that the reconstituted mast cells are functional.

We do not have an explanation for our observation that mucosal hyperplasia in reconstituted W/W^v mice on the first day after challenge with bacteria is even stronger than in wild-type mice (Fig 2, B). It is possible that injected BMDCs integrate into a mucosal environment in which alternative inflammatory pathways have been upregulated because of the previous lack of mast cells, and thus the early phase of the reaction is enhanced. Another explanation for this observation might lie in the differential count of intact versus degranulated mast cells. Reconstituted mast cells seem to be slightly more reactive than mast cells in wild-type mice, because the number of degranulated mast cells after bacterial challenge was higher in reconstituted than in wild-type mice.

In conclusion, our results indicate that mast cells contribute significantly to the response of the ME to both allergic and bacterial challenge and represent a pathophysiologic link between allergy and infection. Mast cells have an important effect in the early phase of either allergic or bacterial inflammatory responses, in which they may help to initiate the immunologic defense action. Thus, the concept of "mast cells as sentinels of innate immunity"⁸ applies to the ME mucosa. In addition,

in allergic mice stimulated with a combination of allergen and bacteria, responses mediated by mast cells increase the persistence of otitis media. These findings provide a new perspective regarding the treatment of otitis media and prevention of the serious sequelae thereof.

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