

Mast cell–derived TNF contributes to airway hyperreactivity, inflammation, and T_H2 cytokine production in an asthma model in mice

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Background: Mast cells, IgE, and TNF, which have been implicated in human atopic asthma, contribute significantly to the allergic airway inflammation induced by ovalbumin (OVA) challenge in mice sensitized with OVA without alum. However, it is not clear to what extent mast cells represent a significant source of TNF in this mouse model.

Objective: We investigated the importance of mast cell–derived TNF in a mast cell–dependent model of OVA-induced airway hyperreactivity (AHR) and allergic airway inflammation.

Methods: Features of this model of airway inflammation were analyzed in C57BL/6J–wild-type mice, mast cell–deficient C57BL/6J-*Kit^{W-sh/W-sh}* mice, and C57BL/6J *Kit^{W-sh/W-sh}* mice that had been systemically engrafted with bone marrow–derived cultured mast cells from C57BL/6J–wild-type or C57BL/6J-TNF^{−/−} mice.

Results: Ovalbumin-induced AHR and airway inflammation were significantly reduced in mast cell–deficient *Kit^{W-sh/W-sh}* mice versus wild-type mice. By contrast, *Kit^{W-sh/W-sh}* mice that had been engrafted with wild-type but not with TNF^{−/−} bone marrow–derived cultured mast cells exhibited responses very similar to those observed in wild-type mice. Mast cells and mast cell–derived TNF were not required for induction of OVA-specific memory T cells in the sensitization phase, but significantly enhanced lymphocyte recruitment and T_H2 cytokine production in the challenge phase.

Conclusion: Mast cell–derived TNF contributes significantly to the pathogenesis of mast cell–dependent and IgE-dependent, OVA-induced allergic inflammation and AHR in mice, perhaps in part by enhancing lymphocyte recruitment and T_H2 cytokine production.

Clinical implications: Our findings in mice support the hypothesis that mast cell–derived TNF can promote allergic inflammation and AHR in asthma. (J Allergy Clin Immunol 2007;119:1016–1024.)

Key words: Airway hyperreactivity, asthma, cytokines, mast cells, TNF

Allergic asthma is a complex inflammatory disease of the lungs characterized by reversible airway obstruction, chronic airway inflammation, and airway hyperresponsiveness (AHR) to spasmogenic stimuli.^{1,2} The airway inflammation in atopic asthma, which includes a prominent component of eosinophils, is associated with a T_H2-type immune response in which T_H2 cell–derived cytokines, such as IL-4, IL-5, IL-9, IL-13, and IL-25, are thought to contribute to eosinophil recruitment, mucus hypersecretion, and AHR.^{1–3} IL-4 and IL-13 also have critical roles in antigen-specific IgE production in atopic asthma, which is a prerequisite for eliciting antigen-dependent and IgE-dependent mast cell and basophil activation at sites of antigen challenge.^{1,2,4–8}

Mast cells can be important effector cells in examples of innate or acquired immunity that contribute to host defense.^{4,9,10} Unfortunately, the inappropriate or excessive activation of mast cells can contribute to the development of allergic or autoimmune diseases, including atopic asthma.^{4–8,10–13} For example, mast cell activation by IgE and specific antigen induces the cells to release spasmogenic, vasoactive, and proinflammatory mediators, which in turn can enhance airway smooth muscle contraction, vascular permeability, and inflammatory cell recruitment at sites of antigen challenge.^{4–8,10–13}

Although of course there are important differences between the features of asthma in human beings and the findings in models of asthma in mice,^{11,14,15} gene-deficient or antigen-specific T-cell receptor–transgenic mice can be very useful in attempts to characterize the importance of specific molecular or cellular factors that contribute to various features of allergic airway inflammation in mice.¹⁶ Many mouse models of allergic airway inflammation involve sensitizing the mice with antigen admixed with aluminum hydroxide (alum).¹⁶ However, alum can enhance T_H2 responses in the absence of IL-4 receptor α , which is essential for IL-4 and IL-13 signals,¹⁷ and therefore might compensate for and thereby mask important functions of certain proinflammatory mediators in these settings.

For example, sensitization with ovalbumin (OVA) emulsified in alum (OVA/alum–induced AHR) can induce

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Abbreviations used

AHR:	Airway hyperreactivity
alum:	Aluminum hydroxide
BALF:	Bronchoalveolar lavage fluid
BMCMC:	Bone marrow–derived cultured mast cell
EPO:	Eosinophil peroxidase
FITC:	Fluorescein isothiocyanate
LN:	Lymph node
MPO:	Myeloperoxidase
OVA:	Ovalbumin

AHR in B cell–deficient,¹⁸ FcεRI–deficient,¹⁹ or WBB6F1-*Kit*^W/*Kit*^{W-v} mast cell–deficient^{20–22} mice. By contrast, B cell–deficient,²³ FcεRI–deficient,¹⁹ and WBB6F1-*Kit*^W/*Kit*^{W-v} mast cell–deficient²² mice exhibited significant impairment in the allergic airway inflammation and AHR induced by sensitization with OVA in PBS without alum (OVA/PBS-induced AHR). Mice deficient in IL-1 or IL-1 receptor 1,^{24,25} or in TNF or TNF receptor,^{26–29} also exhibited impaired OVA/PBS-induced AHR.

In this study, we found that, in comparison with wild-type mice, OVA challenge of OVA/PBS-sensitized C57BL/6-*Kit*^{W-sh/W-sh} mast cell–deficient mice resulted in reduced levels of AHR, lung inflammation, lymphocytes, eosinophils, and neutrophils in bronchoalveolar lavage fluids (BALFs) and lung IL-4, IL-5, IL-13, and IL-17. The impaired responses in C57BL/6-*Kit*^{W-sh/W-sh} mice were restored essentially to wild-type levels if the mice had been engrafted long-term with wild-type but not TNF^{-/-} bone marrow–derived cultured mast cells (BMCMCs). Our findings indicate that mast cell–derived TNF enhances the development of multiple features of allergic airway inflammation and AHR in this mouse model.

METHODS

Mice

We used mast cell–deficient *Kit*^{W-sh/W-sh} mice on the C57BL/6 background,³⁰ TNF^{-/-} mice generated from C57BL/6 embryonic stem cells,³¹ C57BL/6-OTII transgenic mice,³² and C57BL/6J (wild-type) mice. See this article's Online Repository at www.jacionline.org for additional information about the mouse experiments and for descriptions of all of the methods used in this study.

Statistical analyses

The results were evaluated for statistical significance (defined as $P < .05$) by ANOVA or Student *t* test (2-tailed), as indicated.

RESULTS

Mast cells and mast cell–derived TNF are required for optimal development of OVA-induced AHR after sensitization with OVA without alum in C57BL/6 mice

We previously showed, by using mast cell–deficient *Kit*^{W/W-v} mice on the WBxB6 F1 background, that mast

cells contributed significantly to the OVA-induced AHR that developed in mice sensitized with OVA without alum (OVA/PBS-induced AHR), but not that induced in mice sensitized with OVA emulsified with alum (OVA/alum–induced AHR).²² However, allergic airway responses and AHR in the mouse can be significantly influenced by genetic background.^{15,16} Therefore, we first assessed the importance of mast cells and/or c-Kit in the 2 models of airway allergic inflammation and AHR in normal and c-*kit* mutant mast cell–deficient mice on a different strain background—that is, C57BL/6J–wild-type and C57BL/6J-*Kit*^{W-sh/W-sh} mice.

In accord with our previous studies using WBxB6 F1-*Kit*^W/*Kit*^{W-v} mast cell–deficient mice,²² we found that AHR and inflammatory cell numbers in BALFs after OVA challenge in mice sensitized with OVA/alum were very similar between C57BL/6J–wild-type (*Kit*^{+/+}) and C57BL/6J-*Kit*^{W-sh/W-sh} mice (see this article's Fig E1, A and B, in the Online Repository at www.jacionline.org). This indicates that mast cells and/or adequate c-Kit function are not essential for the development of OVA/alum–induced AHR or increases in BALF leukocytes.

However, when mice were repeatedly sensitized with OVA in PBS without alum intraperitoneally (i.p.) and then challenged with OVA in PBS intranasally (i.n.), OVA-induced AHR was significantly lower in *Kit*^{W-sh/W-sh} mice compared with that in the corresponding wild-type (*Kit*^{+/+}) mice (Fig 1). Moreover, OVA-induced AHR in *Kit*^{W-sh/W-sh} mice that had been systemically engrafted with wild-type BMCMCs, but not with TNF^{-/-} BMCMCs, was very similar to that observed in wild-type mice (Fig 1). These findings confirm that mast cells are required for optimal development of OVA-induced AHR in this model. Moreover, although it is well known that mouse mast cells represent a potential source of TNF,^{33–35} the findings presented here show that the mast cell's ability to produce TNF contributes significantly to the cell's ability to enhance the antigen-induced AHR that is observed in this model.

Although AHR to methacholine was profoundly impaired 24 hours after OVA challenge in *Kit*^{W-sh/W-sh} mice or TNF^{-/-} BMCMC-engrafted *Kit*^{W-sh/W-sh} mice, we observed infiltration of leukocytes in histologic sections of lungs from OVA-challenged *Kit*^{W-sh/W-sh} mice or TNF^{-/-} BMCMC-engrafted *Kit*^{W-sh/W-sh} mice, as well as in OVA-challenged wild-type *Kit*^{+/+} mice or wild-type BMCMC-engrafted *Kit*^{W-sh/W-sh} mice (Fig 2). However, the numbers of lymphocytes, neutrophils, and eosinophils in BALFs from OVA-challenged *Kit*^{W-sh/W-sh} mice were significantly lower than those from OVA-challenged wild-type *Kit*^{+/+} mice (Fig 3, A).

By contrast, the numbers of leukocytes in BALFs from OVA-treated *Kit*^{W-sh/W-sh} mice engrafted with wild-type BMCMCs, but not in BALFs from OVA-treated *Kit*^{W-sh/W-sh} mice engrafted with TNF^{-/-} BMCMCs, were similar to those from OVA-treated wild-type *Kit*^{+/+} mice (Fig 3, A). Analyzing single-cell suspensions of lung cells, we confirmed that the increases in numbers of cells caused by OVA-induced inflammation were greater in

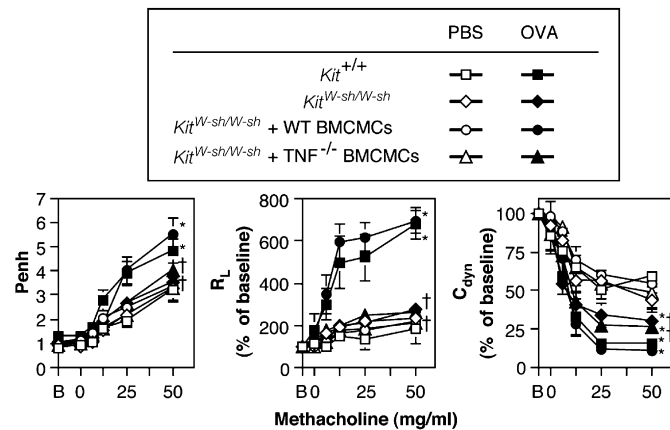


FIG 1. Mast cells and mast cell–derived TNF contribute to AHR in mice sensitized with OVA/PBS. Mice were sensitized i.p. with 10 μ g OVA in 0.1 mL PBS on days 0, 2, 4, 6, 8, 10, and 12. On days 40, 43, and 46, mice were challenged i.n. with 20 μ L PBS containing 200 μ g OVA or 20 μ L PBS alone. Airway responses to methacholine were assessed by noninvasive (enhanced pause [Penh]) and invasive (lung resistance [RL] and dynamic compliance [C_{dyn}]) systems 24 hours after the last OVA challenge or PBS inhalation. Data are the averages $\pm$ SEMs of results pooled from the 2 independent experiments that we performed, each of which gave similar results. * P < .01 vs PBS-treated mice and † P < .05 vs OVA-challenged wild-type *Kit*^{+/+} mice or wild-type BMCMC-engrafted *Kit*^{W-sh/Kit}^{W-sh} mice. For Penh studies, we examined 10 PBS-treated mice and 15 OVA-treated mice for each of the 4 groups of mice tested. For invasive studies, PBS-treated mice: n = 8-12/group; OVA-treated mice: n = 12-18/group. B, Baseline; WT, wild-type.

OVA-challenged *Kit*^{W-sh/W-sh} mice engrafted with wild-type BMCMCs than in those engrafted with TNF^{-/-} BMCMCs (Fig 3, B).

PBS-treated *Kit*^{W-sh/W-sh} mice engrafted with wild-type vs TNF^{-/-} BMCMCs had very similar numbers of mast cells per lung (Fig 3, B). However, OVA challenge resulted in modest but significant increases in the numbers of mast cells per lung in wild-type mice and in *Kit*^{W-sh/W-sh} mice engrafted with wild-type but not TNF^{-/-} BMCMCs (Fig 3, B), although the numbers of mast cells per 2×10^5 total lung cells were not different between OVA-treated *Kit*^{W-sh/W-sh} mice engrafted with wild-type versus TNF^{-/-} BMCMCs (see this article's Table E1 in the Online Repository at www.jacionline.org). Although the basal number of mast cells in the lungs of naive TNF^{-/-} mice is indistinguishable from that in naive wild-type mice,³⁶ the findings shown in Fig 3, B (right side) are consistent with the possibility that mast cell–derived TNF can directly and/or indirectly enhance numbers of lung mast cells in OVA-challenged mice in this model.^{37,38} Moreover, as reported previously,³⁹ the numbers of mast cells in the lungs of PBS-treated or OVA-challenged *Kit*^{W-sh/W-sh} mice engrafted with wild-type or TNF^{-/-} BMCMCs were significantly greater than those in PBS-treated or OVA-challenged wild-type mice (Fig 3, B; see this article's Table E1 in the Online Repository at www.jacionline.org).

Taken together, these results indicate that mast cells and mast cell–derived TNF contribute significantly to the development of both antigen-induced AHR and antigen-induced increases in BALF leukocytes in mice on the C57BL/6 background that have been sensitized with OVA without alum.

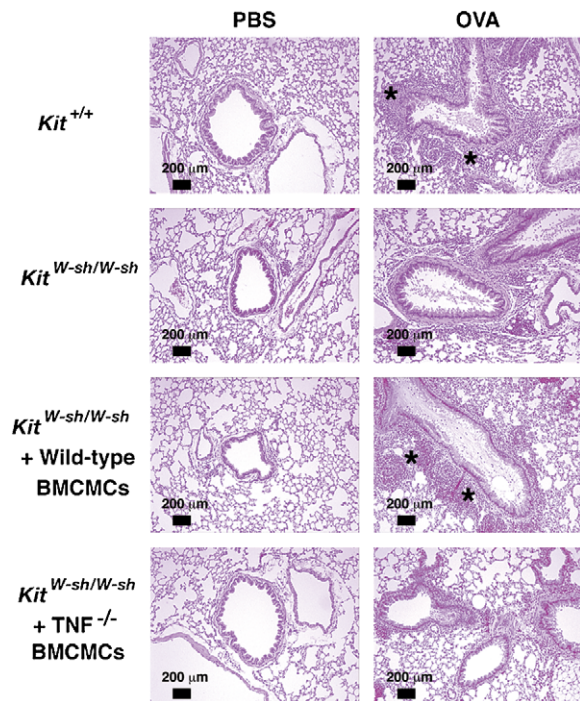


FIG 2. Pulmonary inflammation in OVA-challenged or PBS-challenged, OVA/PBS-sensitized mice. Lungs were collected from the mice used for the Penh studies shown in Fig 1 at 24 hours after last OVA challenge or PBS inhalation. Lung sections were stained with hematoxylin and eosin ($\times 200$; scale bar = 200 microns). Areas of inflammation (*) appear larger in sections of the OVA-challenged *Kit*^{+/+} or wild-type BMCMC-engrafted *Kit*^{W-sh/W-sh} mice than in the other 2 OVA-challenged groups.

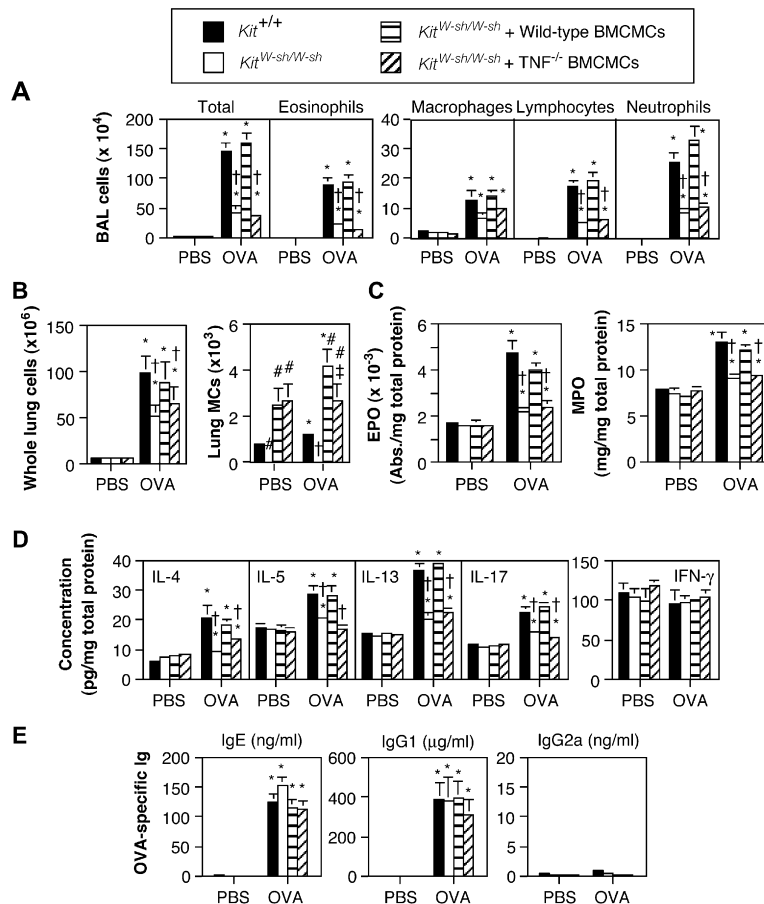


FIG 3. Mast cells and mast cell-derived TNF contribute to airway inflammation in mice sensitized with OVA/PBS. BALFs, single-cell preparations of lungs, lung homogenates, and sera were obtained from the PBS-treated ($n = 10/\text{group}$) and OVA-treated ($n = 15/\text{group}$) mice used for the Penh studies shown in Fig 1 at 24 hours after last OVA challenge or PBS inhalation. **A**, Numbers of cells in BALFs. **B**, Numbers of total cells (left) and mast cells (right) in single-cell preparations of whole lungs. **C**, EPO and MPO levels in lung homogenates. **D**, Cytokine levels in lung homogenates. **E**, OVA-specific IgE, IgG1, and IgG2a antibody levels in sera. Data show the averages $\pm$ SEMs. * $P < .05$ vs PBS-treated mice; †values for OVA-challenged mice that are $P < .05$ vs OVA-challenged wild-type $Kit^{+/+}$ mice or wild-type BMCMCs-engrafted Kit^{W-sh}/Kit^{W-sh} mice ($Kit^{W-sh}/W-sh$ + wild-type BMCMCs); in **B**, right (mast cells), # $P < .05$ vs corresponding values for PBS-treated or OVA-challenged wild-type $Kit^{+/+}$ mice, and ‡ $P < .05$ vs corresponding values for OVA-challenged wild-type BMCMCs-engrafted Kit^{W-sh}/Kit^{W-sh} mice ($Kit^{W-sh}/W-sh$ + wild-type BMCMCs). BAL, Bronchoalveolar lavage; MC, mast cell.

Importance of mast cell-derived TNF in OVA-induced changes in levels of eosinophil peroxidase, myeloperoxidase, and cytokines in the lung

Tissue levels of eosinophil peroxidase (EPO) or myeloperoxidase (MPO) correlate well with the numbers of eosinophils or neutrophils, respectively, at sites of inflammation.⁴⁰ In accord with the numbers of granulocytes measured in BALFs of OVA-challenged mice (Fig 3, A), we found that levels of EPO and MPO were more markedly elevated 24 hours after the last OVA challenge in wild-type mice or wild-type BMCMC-engrafted $Kit^{W-sh}/W-sh$ mice versus the smaller, albeit still significant, increases over those in PBS-challenged mice that were observed in $Kit^{W-sh}/W-sh$ mice or $TNF^{-/-}$ BMCMC-engrafted $Kit^{W-sh}/W-sh$ mice (Fig 3, C).

Similar results were obtained when we assessed lung levels of IL-4, IL-13, IL-5, and IL-17 (a cytokine implicated in airway neutrophil recruitment⁴¹) 24 hours after the last OVA or PBS challenge. OVA challenge resulted in increases, most of them significant, in these cytokines in $Kit^{W-sh}/W-sh$ mice or $TNF^{-/-}$ BMCMC-engrafted $Kit^{W-sh}/W-sh$ mice, but the increases were significantly greater in wild-type mice or wild-type BMCMC-engrafted $Kit^{W-sh}/W-sh$ mice (Fig 3, D). As previously reported,²⁹ levels of EPO, MPO, and cytokines were below the limit of detection by enzyme immunoassay (EIA) in the BALFs from any of the groups of mice (data not shown).

Ovalbumin-specific IgG1 and IgE antibodies measured in sera 24 hours after last OVA challenge were significantly increased to roughly the same extent in all groups of mice, whereas levels of OVA-specific IgG2a antibodies remained low (Fig 3, E). Thus, a deficiency of mast cells or

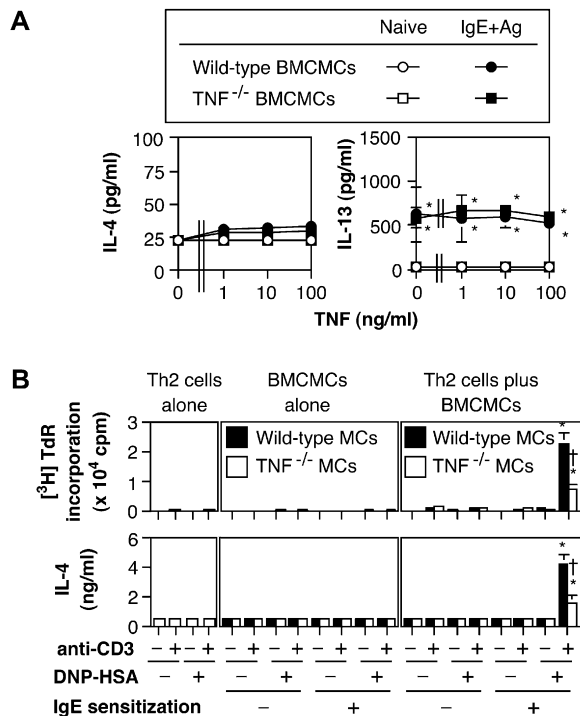


FIG 4. Mast cell–derived TNF is required for optimal T_H2 cell activation, but not for mast cell T_H2 cell cytokine production, *in vitro*. Wild-type or TNF^{-/-} BMCMCs were sensitized with 10 μ g/mL anti-DNP IgE (H1- ϵ -26) at 37°C overnight. After washing, naive (no IgE sensitization) or IgE-sensitized BMCMCs were cultured $\pm$ specific antigen (20 ng/mL 2,4-dinitrophenyl-conjugated human serum albumin [DNP-HSA]) in the presence of various concentrations of recombinant mouse TNF (*rmTNF*) at 37°C for 6 hours, and IL-4 and IL-13 levels in the culture supernatants were measured by ELISA. Data show the averages $\pm$ SDs from triplicate determinations from 1 of the 2 independent experiments we performed, each of which gave similar results. IL-4–derived T_H2 cells were co-cultured with mitomycin C-treated naive or IgE-sensitized wild-type (WT) or TNF^{-/-} BMCMCs (MCs) $\pm$ 5 ng/mL DNP-HSA at 37°C for 72 hours. Proliferation of T_H2 cells and IL-4 levels in culture supernatants were measured by [³H]-thymidine (TdR) incorporation and by ELISA, respectively. Data show the averages $\pm$ SDs from triplicate determinations in 1 of the 3 independent experiments we performed, each of which gave similar results. * P < .05 vs the corresponding values for any other culture condition and † P < .05 vs corresponding values for WT BMCMCs.

mast cell–derived TNF did not appear to influence production of OVA-specific IgG₁ or IgE in this model.

Mast cells and mast cell–derived TNF are not required for the induction of OVA-specific memory T cells

We next investigated whether mast cells or TNF influenced the induction of OVA-specific memory T cells. When pooled inguinal, axillary, and brachial lymph node (LN) cells from wild-type OTII mice (which constitutively express I-A^b–restricted OVA-specific T-cell receptor on CD4⁺ T cells³²), *Kit*^{W-sh/W-sh} OTII mice, or TNF^{-/-} OTII mice were cultured in the presence or absence of OVA peptides, a deficiency in either mast cells or TNF had no significant effect on antigen-induced OTII cell

proliferation or IFN- γ , IL-4, or IL-17 production (see this article's Fig E2, A, in the Online Repository at www.jacionline.org, and data not shown). These results indicate that such intrinsic T-cell functions can be observed independently of either TNF or the small number of mast cells that typically are present in LNs under baseline conditions.

We reported that mast cells and mast cell–derived TNF can enhance dendritic cell (DC) migration from the skin and airway to draining LNs at 24 hours after antigen exposure,³⁶ raising the possibility that mast cells also might contribute to DC-dependent T-cell priming by OVA and/or induction of OVA-specific memory T cells. We found a significant impairment in airway DC migration to thoracic LNs in *Kit*^{W-sh/W-sh}, TNF^{-/-}, and TNF^{-/-} BMCMC-engrafted *Kit*^{W-sh/W-sh} mice in comparison with that in wild-type or wild-type BMCMC-engrafted *Kit*^{W-sh/W-sh} mice at 24 hours after inhalation of fluorescein isothiocyanate (FITC)–OVA (see this article's Fig E2, B and C, in the Online Repository at www.jacionline.org). However, such defects were not observed at 48 or 72 hours after FITC–OVA inhalation, suggesting that mast cells, TNF, and mast cell–derived TNF can enhance the early events but are not required for later phases of airway DC migration to local LNs.

We then investigated whether the delay in DC migration in *Kit*^{W-sh/W-sh} and TNF^{-/-} mice influenced the induction of OVA-specific memory T cells. When we cultured mesenteric LN cells obtained 5 days after last i.p. OVA sensitization or thoracic LN cells obtained 24 hours after last OVA challenge in the presence or absence of OVA LN cell proliferation (see this article's Fig E2, D and E, in the Online Repository at www.jacionline.org) and IL-4, IL-17, and IFN- γ production (data not shown) in the culture supernatants were comparable among wild-type, *Kit*^{W-sh/W-sh}, and TNF^{-/-} mice. These observations suggest that a deficiency in mast cells or TNF does not significantly modulate the induction or activation of OVA-specific memory T cells in the draining LNs in either the sensitization or challenge phases of this model of OVA-induced allergic inflammation.

Mast cell–derived TNF is important for T_H2 cell activation

Mice deficient in mast cells,^{20–22} Fc ϵ RI,¹⁹ TNF–TNF receptor,^{26–29} IL-1^{-/-}, or IL-1 receptor 1^{-/-}^{24,25} exhibit impaired development of OVA/PBS–induced AHR,^{19,22,24,25,29} but normal development of OVA/alum–induced AHR.^{19–22,24–29} TNF and IL-1 are the potent proinflammatory cytokines that can express overlapping pathophysiological activities.⁴² Because IL-1 can enhance T_H2 cytokine production by mast cells in the presence of IgE/Ag stimulation,^{43,44} we examined the effects of exogenous or endogenous TNF on T_H2 cytokine production by mast cells. IL-13 production by wild-type BMCMCs was markedly increased at 6 hours after IgE/Ag stimulation, whereas IL-4 production was barely detectable (Fig 4, A). However, recombinant mouse TNF did not enhance IL-13 or IL-4 production by either wild-type BMCMCs or

TNF^{-/-} BMCMCs (Fig 4, A). By contrast, we confirmed that recombinant mouse IL-1 (mIL-1) can enhance IL-13 production by BMCMCs.⁴⁵ These observations indicate that neither exogenous nor endogenous TNF significantly modulates IgE-induced and antigen-induced production of IL-13 or IL-4 by mast cells under the conditions examined.

We recently showed that mast cells and mast cell–derived secreted TNF can enhance T-cell activation.^{46,47} In accord with those observations, we found that coculture of small numbers of T_H2 cells, which had been diluted below the limit of detection of proliferation in this assay, with IgE/Ag–stimulated BMCMCs significantly enhanced both T_H2-cell proliferation and IL-4 production; this effect was not observed when the T_H2 cells were cocultured with naive (no IgE sensitization) or IgE-sensitized but not Ag-pulsed BMCMCs (Fig 4, B). However, the T_H2 cell responses were markedly impaired when we substituted TNF^{-/-} BMCMCs for wild-type BMCMCs in this coculture system (Fig 4, B).

Taken together, these observations suggest that although mast cells and TNF are not required for the induction and activation of OVA-specific memory T cells in draining LNs during the sensitization or challenge phases of in this model of OVA-induced allergic inflammation, mast cells and mast cell–derived TNF might contribute to pathology in this model by enhancing T_H2 cell proliferation and/or activation in the sites of inflammation.

DISCUSSION

Mast cells can produce a wide variety of proinflammatory mediators, cytokines, growth factors, and chemokines and can be stimulated to secrete these products on stimulation by IgE and specific antigen.^{4-8,10-13} Although many of these mast cell–derived products have effects that can contribute to airway inflammation and airway AHR,^{4-8,10-13,48} in the current study, we specifically investigated the roles of mast cells and mast cell–derived TNF in a mouse model of allergic airway inflammation.

The potential importance of TNF in asthma, and in mouse models of allergic airway inflammation, is now a topic of considerable interest. Both mouse³³⁻³⁵ and human⁴⁹ mast cells can produce TNF, and TNF contributes to the ability of mouse mast cells to recruit neutrophils⁵⁰ to sites of IgE and antigen-dependent mast cell stimulation *in vivo*. TNF can induce lung neutrophil infiltration and increase AHR to methacholine in normal human subjects,⁵¹ and some patients with asthma exhibit elevated serum TNF.⁵² In addition, TNF immunoreactive cells, most of which were mast cells, were increased ~6-fold in airway submucosa biopsies of patients with asthma compared with normal controls,⁴⁹ and anti-TNF therapy reduced signs and symptoms of asthma in some subjects with severe asthma.⁵³⁻⁵⁶

We previously reported²⁹ that, compared with responses in wild-type C57BL/6 mice, multiple features of

OVA-induced airway inflammation, as well as AHR, were significantly reduced in C57BL/6–TNF^{-/-} mice that had been sensitized by using the OVA/PBS model from the current study. However, the key cellular source of TNF in that setting was not identified. In the current study, we found that mast cell–derived TNF contributes significantly to the OVA-induced lung inflammation; levels of BALF lymphocytes, macrophages, eosinophils, and neutrophils; and levels of lung EPO, MPO, IL-4, IL-5, IL-13, and IL-17, and AHR to methacholine in C57BL/6 mice that have been sensitized by immunization with OVA/PBS.

The possible direct or indirect mechanisms by which mast cells and mast cell–derived TNF influence significantly each of these features of this model remain to be fully elucidated. However, our *in vitro* and *in vivo* studies suggest that mast cells and mast cell–derived TNF are not required for induction of OVA-specific memory T cells in the sensitization phase of the OVA/PBS model, but that mast cells, and specifically mast cell–derived TNF, can significantly enhance lung lymphocyte recruitment and lung T_H2 cytokine production in response to antigen challenge in sensitized mice. Although it is possible that lung T_H2 cytokine production in this model may reflect contributions of both T_H2 cells and mast cells (and/or other cell types) activated at sites of disease, our *in vitro* evidence suggests that mast cell–derived TNF may enhance T_H2 cell proliferation and cytokine production in this setting, and might also promote increased numbers of mast cells, while having little or no effect on IgE and antigen-dependent production of IL-4 or IL-13 by mast cells.

Our findings show that mast cells and mast cell–derived TNF can enhance multiple features of a model of allergic airway inflammation and AHR in C57BL/6 mice. One of course must use great caution in attempting to extrapolate findings in such asthma models in mice to human beings. However, in light of work implicating TNF⁵¹⁻⁵⁶ and mast cells^{12,13,49} in human asthma, it seems reasonable to suggest that mast cells may also represent a clinically significant source of TNF in at least some patients with asthma.

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