TNF can contribute to multiple features of ovalbumin-induced allergic inflammation of the airways in mice

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Background: TNF is thought to contribute to airway hyperreactivity (AHR) and airway inflammation in asthma. However, studies with TNF-deficient or TNF receptor–deficient mice have not produced a clear picture of the role of TNF in the AHR associated with allergic inflammation in the mouse.
Objective: We used a genetic approach to investigate the contributions of TNF to antigen-induced AHR and airway inflammation in mice on the C57BL/6 background.
Methods: We analyzed features of airway allergic inflammation, including antigen-induced AHR, in C57BL/6 wild-type and TNF$^{-/-}$ mice, using 2 different methods for sensitizing the mice to ovalbumin (OVA).
Results: In mice sensitized to OVA administered with the adjuvant aluminum hydroxide (alum), which develop IgE-independent and mast cell–independent allergic inflammation and AHR, we found no significant differences in OVA-induced AHR in C57BL/6-TNF$^{-/-}$ versus wild-type mice. By contrast, in mice sensitized to OVA without alum, which develop allergic inflammation that is significantly mast cell–dependent, C57BL/6-TNF$^{-/-}$ mice exhibited significant reductions versus wild-type mice in OVA-induced AHR to methacholine; numbers of lymphocytes, neutrophils, and eosinophils in bronchoalveolar lavage fluid; levels of myeloperoxidase, eosinophil peroxidase, and the cytokines IL-4, IL-5, and IL-17 in lung tissue; and histologic evidence of pulmonary inflammation.
Conclusion: In pulmonary allergic inflammation induced in mice immunized with OVA without alum, TNF significantly contributes to several features of the response, including antigen-induced inflammation and AHR.
Clinical implications: Our findings in mice support the hypothesis that TNF can promote the allergic inflammation and AHR associated with asthma. (J Allergy Clin Immunol 2007;119:680-6.)

Key words: *Airway hyperreactivity, asthma, cytokines, mast cells, neutrophils*

Abbreviations used

AHR:	Airway hyperreactivity
alum:	Aluminum hydroxide
BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
C_{dyn}:	Dynamic compliance
EPO:	Eosinophil peroxidase
R_L:	Lung resistance
memTNF$^{\Delta/\Delta}$:	Mice that can express membrane-associated TNF but that cannot generate the secreted form of TNF
MPO:	Myeloperoxidase
OVA:	Ovalbumin
Penh:	Enhanced pause
TCR:	T-cell receptor
TNFR:	TNF receptor

It has been hypothesized that TNF may contribute to the pathogenesis of asthma.[1,2] In human beings, TNF can induce lung neutrophil infiltration and increase airway hyperreactivity (AHR) to methacholine in normal individuals[3]; some patients with asthma exhibit elevated serum TNF[4]; TNF immunoreactive cells (mostly mast cells) were ~6-fold increased in airway submucosa biopsies of patients with asthma versus normal controls[5]; and anti-TNF therapy reduced asthma severity in some subjects.[6-10]

However, experiments in TNF-deficient or TNF receptor (TNFR)–deficient mice have not fully clarified the role of TNF in pulmonary allergic inflammation, or the associated AHR, in mice. In BALB/c mice sensitized with ovalbumin (OVA) without aluminum hydroxide (alum), treatment with anti-TNF neutralizing antibodies did not significantly reduce either AHR or numbers of bronchoalveolar lavage fluid (BALF) eosinophils after OVA challenge.[11] Moreover, 129xB6-TNFR1/2$^{-/-}$ mice sensitized with OVA and alum exhibited increased lung eosinophils and mucus production and exacerbated antigen-induced AHR.[12] Such findings suggested that TNF either has no effect or can negatively regulate antigen-induced AHR.

By contrast, in mice sensitized with OVA and alum, Broide et al[13] showed that eosinophils were significantly reduced in BALF of OVA-challenged 129xB6-TNFR1$^{-/-}$ and -TNFR1/2$^{-/-}$ mice, whereas 129xB6-TNFR1$^{-/-}$ mice exhibited no significant abnormality in antigen-induced

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AHR after OVA challenge. Like Rudmann et al,[12] Kanehiro et al[14] showed that OVA/alum-induced AHR was exacerbated in 129xB6-TNF$^{-/-}$ mice. However, in contrast with Rudmann et al,[12] Kanehiro et al[14] observed that eosinophils in BALF, but not in lung tissue, were reduced after antigen challenge in 129xB6-TNF$^{-/-}$ versus TNF$^{+/+}$ mice. Kanehiro et al[15] also showed that OVA/alum-induced AHR, and eosinophil numbers in BALF and lung tissue, were reduced in C57BL/6J-TNFR1$^{-/-}$ versus TNF$^{+/+}$ mice. Finally, Choi et al,[16] using a TNF neutralizing antibody in BALB/c mice, and comparing B6x129SF2-TNF$^{-/-}$ versus -TNF$^{+/+}$ mice, reported that TNF can contribute to the late-phase component of OVA-induced AHR and to eosinophil infiltration in their model system.

The apparent differences in the roles of TNF revealed by these studies may have reflected, at least in part, the different genetic backgrounds of the mice examined.[12,17,18] Moreover, most of these previous studies were performed in mice sensitized to OVA using alum as an adjuvant. However, B cells, IgE, and mast cells are not essential for the development of antigen-induced AHR after sensitization of mice with certain protocols using OVA with alum.[19-22] By contrast, mast cells[21] and IgE[22] are necessary for optimal expression of antigen-induced AHR in mice immunized in certain protocols using OVA without alum. Mast cells are key effector cells in many IgE-associated immune responses.[23-26] Mast cells also represent a potentially significant source of TNF.[27,28] In the current study, we used TNF$^{-/-}$ mice generated on the C57BL/6 background to examine the role of TNF in the expression of both mast cell–independent and mast cell–dependent models of OVA-induced pulmonary allergic inflammation associated with AHR.

METHODS

Mice

C57BL/6-TNF$^{-/-}$ mice, and mice expressing membrane TNF but lacking the ability to secrete TNF (memTNF$^{\Delta/\Delta}$ mice), were generated from C57BL/6 ES cells.[29,30] C57BL/6J-wild-type, C57BL/6-TNFR1$^{-/-}$, and C57BL/6-TNFR2$^{-/-}$ mice were obtained from Jackson Laboratories, Bar Harbor, Maine. We used male and female mice that were 6 to 8 weeks old at the start of the experiments. Mice were kept at the Animal Care Facilities at Stanford University Medical Center (Stanford, Calif) under standard temperature, humidity, and timed lighting conditions, and were provided mouse chow and water *ad libitum*. All experiments were performed in compliance with the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, National Research Council, published by the National Academy Press (revised 1996), and with the approval of the Stanford University Committee on Animal Welfare. We previously reported that the 5 different mouse strains used in this study do not differ significantly in the numbers of mast cells present in the lungs at baseline, as assessed by counting mast cells in disaggregated lung tissue.[31]

Other methods

Please see this article's Online Repository at www.jacionline.org for the methods for sensitization with OVA with or without alum, histology, bronchoalveolar lavage (BAL), measurement of OVA-specific antibodies in sera, measurement of cytokine levels in lung homogenates, and eosinophil peroxidase (EPO) and myeloperoxidase (MPO) assays.

Statistical analyses

Data show the averages ± or + SEMs. ANOVA was used for statistical evaluation of airway responses over time; all other data were evaluated for statistical significance using the 2-tailed Student *t* test.

RESULTS

Normal development of OVA-induced AHR in C57BL/6-TNF$^{-/-}$ mice after sensitization with OVA/alum and intranasal OVA challenge

In mice sensitized with OVA/alum (a mast cell–independent model of airway allergic inflammation[21]), AHR to methacholine at 24 or 48 hours after the last OVA challenge was not significantly different in C57BL/6J-TNF$^{-/-}$ versus wild-type (WT) mice (see this article's Fig E1 in the Online Repository at www.jacionline.org, and data not shown for 48 hours). We also found that the numbers of total cells, macrophages, lymphocytes, neutrophils, and eosinophils, as well as IL-4 and IL-5 levels, in BALFs were comparable between C57BL/6J-TNF$^{-/-}$ and -WT mice at 48 hours after the last OVA or PBS inhalation (see this article's Fig E2, *A and B,* in the Online Repository at www.jacionline.org).

These observations indicate that TNF is not essential for the development of the AHR, or features of the lung inflammation, induced in C57BL/6 mice in this model.

Reduced airway responses to methacholine in C57BL/6J-TNF$^{-/-}$ versus WT mice after sensitization with OVA without alum and intranasal OVA challenge

We next examined the role of TNF in the development of antigen-induced AHR after sensitization with OVA without alum, which is a form of antigen-induced AHR that is dependent, for optimal expression, on mast cells and IgE.[21,22]

In OVA-sensitized mice, AHR to methacholine was significantly increased in OVA-challenged versus PBS-challenged WT mice (Fig 1). In OVA-sensitized TNF$^{-/-}$ mice, enhanced pause (Penh) and lung resistance (R_L) responses, but not dynamic compliance (C_{dyn}) responses, to OVA were also significantly enhanced versus the corresponding responses in PBS-challenged mice (Fig 1). However, AHR to methacholine was significantly reduced in OVA-challenged TNF$^{-/-}$ mice versus the corresponding responses in the TNF$^{+/+}$ (WT) mice (Fig 1).

These observations indicate that TNF contributes significantly to the development of OVA-induced AHR in mice on the C57BL/6J background that have been sensitized in the model using OVA in the absence of alum.

Reduced airway inflammation in C57BL/6J-TNF$^{-/-}$ vs WT mice

Although antigen-dependent AHR was significantly decreased in C57BL/6-TNF$^{-/-}$ mice versus WT mice

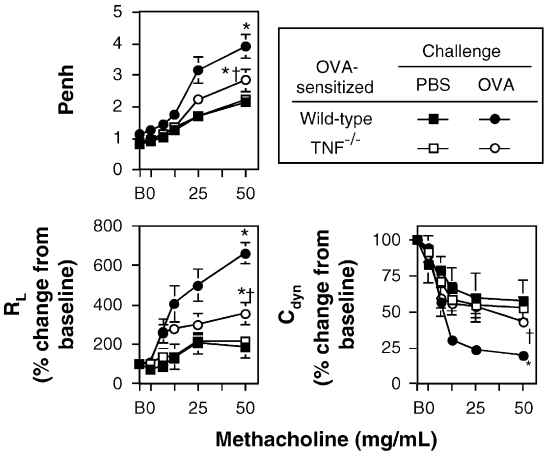


FIG 1. Reduced expression of antigen induced-AHR after OVA immunization without alum in C57BL/6J-TNF$^{-/-}$ mice. Airway responses to methacholine were measured 24 hours after the last intranasal OVA or PBS challenge. *$P < .01$ for PBS-challenged vs OVA-challenged mice; †$P < .01$ for OVA-challenged TNF$^{-/-}$ vs wild-type mice. Data were pooled from at least 2 independent experiments, each of which gave similar results. PBS-treated mice, n= 5-8/group; OVA-challenged mice, n = 12-16/group. *B,* Baseline.

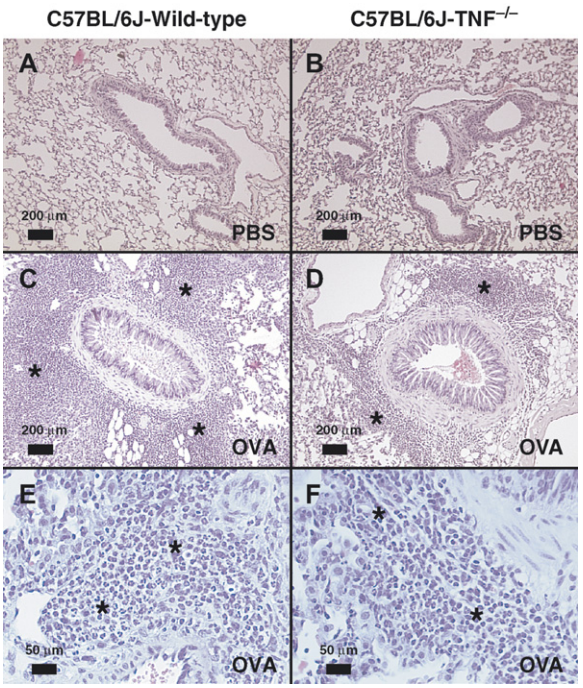


FIG 2. Histologic analysis of lungs from C57BL/6J-TNF$^{-/-}$ vs wild-type mice. Lung sections (hematoxylin and eosin–stained) obtained 24 hours after the last intranasal OVA or PBS challenge in mice immunized with OVA without alum. Striking infiltrates of inflammatory cells (*) are present near the airways in OVA-sensitized, OVA-challenged mice **(C-F)** but not in PBS-challenged mice **(A and B)**. *A-D,* ×100, scale bars = 200 μm; *E and F,* ×400, scale bars = 50 μm.

after OVA sensitization without alum and OVA challenge, the TNF$^{-/-}$ mice did develop pathological changes in the lungs after OVA challenge. However, the inflammatory infiltrates appeared to be more extensive in the WT versus the TNF$^{-/-}$ mice (Fig 2).

Consistent with the histologic findings, the numbers of neutrophils and eosinophils were increased in BALFs from OVA-challenged versus PBS-challenged WT mice (Fig 3, *A*). PBS-treated WT or TNF$^{-/-}$ mice did not differ significantly in numbers of BALF leukocytes (Fig 3, *A*). However, OVA-challenged TNF$^{-/-}$ mice exhibited significantly fewer BALF lymphocytes, neutrophils, and eosinophils than did the WT mice (Fig 3, *A*).

These observations indicate that, in C57BL/6J mice, TNF contributes significantly to the events that promote the recruitment and/or retention of neutrophils, eosinophils, and lymphocytes in the lungs in this model of allergic inflammation.

Reduced MPO and EPO levels and cytokine production in lungs from C57BL/6J-TNF$^{-/-}$ vs WT mice

In OVA/alum-sensitized mice, OVA challenge induced increases in BALF levels of MPO and EPO activities, and concentrations of T_H2 cytokines, that were roughly correlated with the numbers of inflammatory cells in the BALF (data not shown). However, in the IgE-dependent and mast cell–dependent model of allergic inflammation, BALF levels of MPO and EPO activities and concentrations of cytokines were below the limits of detection (data not shown). Therefore, we examined MPO and EPO activities and levels of T_H2 cytokines in whole lung homogenates from such mice after challenge with OVA or PBS.

In accord with the numbers of neutrophils and eosinophils in BALFs of such mice (Fig 3, *A and B*), MPO and EPO levels were markedly increased in whole lung homogenates from WT mice after OVA challenge (Fig 3, *B*), but the levels were significantly lower in the corresponding OVA-challenged TNF$^{-/-}$ mice (Fig 3, *B*).

T_H2-type cytokines such as IL-4 and IL-13 have been implicated in the induction of AHR associated with allergic inflammation in the lungs, whereas T cell–derived IL-5 can contribute to eosinophilia and T cell–derived IL-17 can contribute to neutrophilia. Consistent with our findings concerning the numbers of eosinophils and neutrophils and levels of EPO and MPO activities in the lungs, we found that levels of IL-4, IL-5, and IL-17 in lung homogenates were significantly reduced in TNF$^{-/-}$ mice compared with WT mice, whereas levels of the T_H1-type cytokine IFN-γ were not different between WT and TNF$^{-/-}$ mice (Fig 3, *C*).

These observations indicate that, in the IgE-dependent and mast cell–dependent model of OVA-induced allergic inflammation in C57BL/6 mice, TNF is required for optimal lung infiltration of neutrophils and eosinophils, and optimal expression of IL-4, IL-5, and IL-17 in the lungs.

Reduced OVA-specific IgG_1 and IgE in C57BL/6-TNF$^{-/-}$ vs WT mice

Optimal development of antigen-induced AHR by repeated OVA immunization without alum requires IgE-FcεRI signals and mast cells,[21,22] indicating that

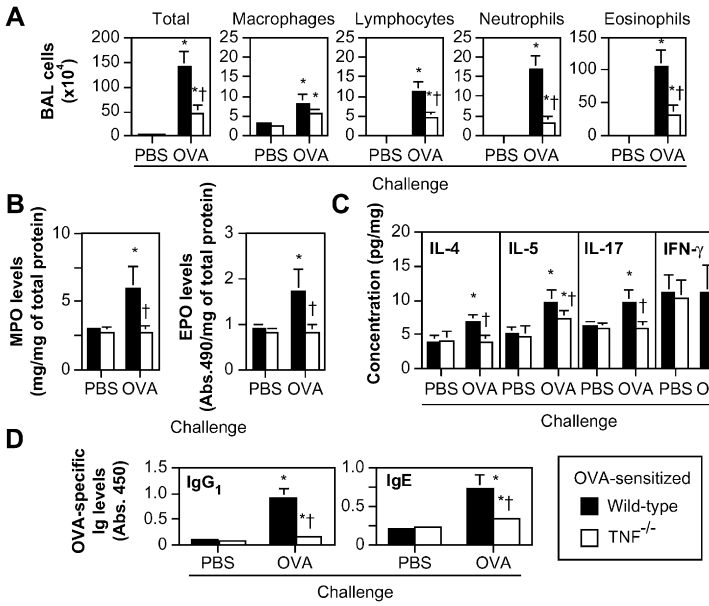


FIG 3. Reduced numbers of leukocytes in BALF; reduced lung levels of MPO, EPO, IL-4, IL-5, and IL-17; and reduced serum levels of OVA-specific IgG_1 and IgE in C57BL/6J-$TNF^{-/-}$ mice 24 hours after OVA challenge in mice immunized with OVA without alum. **A,** Cell numbers in BALFs from the same mice shown in Fig 1. **B,** MPO and EPO levels in lung homogenates. **C,** IL-4, IL-5, IL-17, and IFN-γ levels in lung homogenates. **D,** OVA-specific IgG_1 and IgE levels in sera. *$P < .01$ for comparisons shown in brackets; †$P < .05$ vs values for the corresponding PBS-treated mice. PBS-treated mice, n = 5-8/group; OVA-challenged mice, n = 12-16/group. *B,* Baseline.

IgE-dependent mast cell activation contributes to antigen-induced AHR in this model. Therefore, we assessed OVA-specific Ig levels in sera from $TNF^{-/-}$ and WT mice after OVA challenge. In OVA-challenged WT or $TNF^{-/-}$ mice, levels of OVA-specific IgE and IgG_1 were significantly elevated versus the corresponding values in OVA-sensitized, PBS-challenged mice (Fig 3, *D*). However, in $TNF^{-/-}$ mice, levels of OVA-specific IgE and IgG_1 antibodies were significantly lower than the corresponding values in the WT mice (Fig 3, *D*).

Both TNFR1 and TNFR2 as well as soluble TNF are required for optimal expression of antigen-induced AHR in C57BL/6 mice

In mice sensitized to OVA without alum, we found significant reductions in the airway responses to methacholine after OVA challenge in C57BL/6J-$TNFR2^{-/-}$ mice (Fig 4, *A*), as well as in C57BL/6J-$TNF^{-/-}$ mice (Fig 1), in comparison with the responses in C57BL/6J WT mice. C57BL/6J-$TNFR1^{-/-}$ mice exhibited partial, but not dramatic, reductions in some measures of OVA-induced AHR, as reflected in significantly reduced Penh responses to methacholine (these mice also exhibited reduced responses as assessed by C_{dyn}, but this change did not reach statistical significance; Fig 4, *A*). These findings indicate that TNFR2 is more important than TNFR1 in this model of antigen-induced AHR.

TNF consists of 2 biologically active forms, 1 secreted and 1 membrane-associated. C57BL/6-$memTNF^{\Delta/\Delta}$ mice, which can produce membrane-bound TNF but not the secreted form of TNF,[30] exhibited essentially no antigen-induced AHR to methacholine in the IgE-dependent and mast cell–dependent model of OVA-induced allergic inflammation (Fig 4, *A*). Thus, membrane-associated TNF appears to be unable to substitute for soluble TNF in the development of this form of antigen-induced AHR.

Consistent with the findings for antigen-induced AHR, in comparison with WT mice, $TNFR2^{-/-}$ and $memTNF^{\Delta/\Delta}$ mice exhibited significantly reduced numbers of total cells, eosinophils, neutrophils, and lymphocytes in BALFs after OVA challenge (Fig 4, *B*), as did $TNF^{-/-}$ mice (Fig 3, *A*). Even though a deficiency of TNFR1 resulted in only a modest reduction in some measures of antigen-induced AHR (Fig 4, *A*), $TNFR1^{-/-}$ mice exhibited significant reductions in inflammatory cell infiltration in BALFs after OVA challenge that were very similar in magnitude to those observed in the $TNFR2^{-/-}$ and $memTNF^{\Delta/\Delta}$ mice (Fig 4, *B*).

These observations indicate that, in the IgE-dependent and mast cell–dependent model of antigen-induced allergic inflammation in C57BL/6 mice, soluble TNF and both TNFR1 and TNFR2 are important for the induction of optimal levels of airway inflammation.

DISCUSSION

Much of the apparent controversy regarding the roles of TNF and TNFRs in various features of mouse models of asthma probably reflects differences in the strains of mice

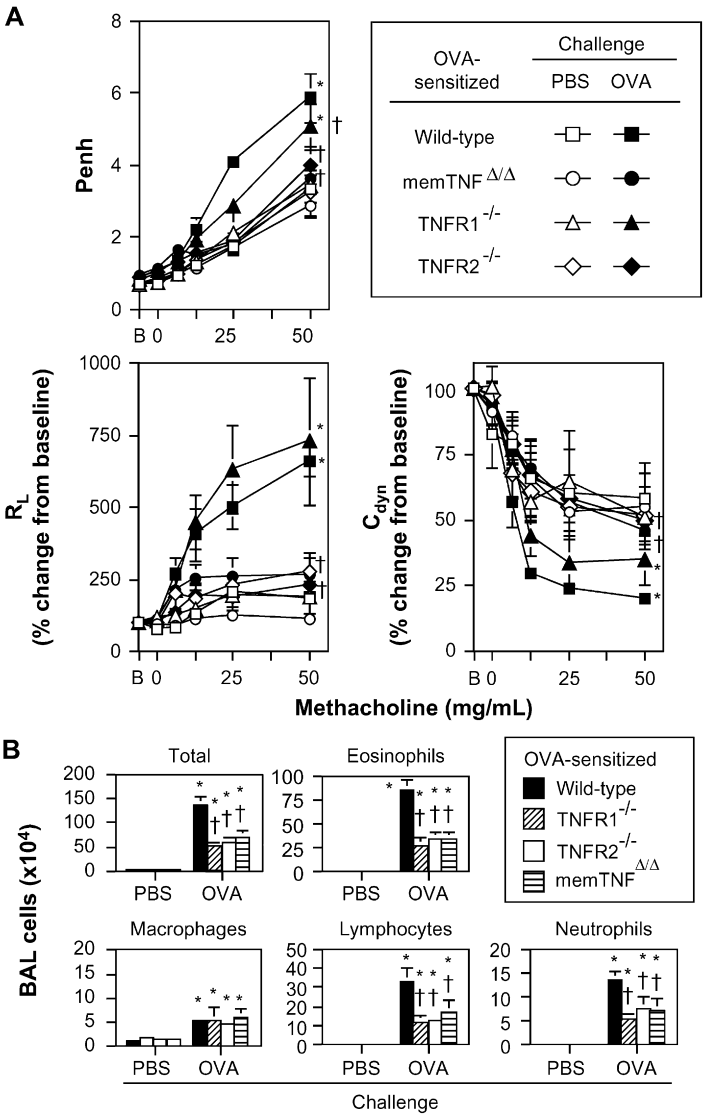


FIG 4. Airway responses to methacholine and BALF leukocytes 24 hours after the last intranasal OVA or PBS challenge in C57BL/6J wild-type, TNFR1$^{-/-}$, TNFR2$^{-/-}$, and memTNF$^{\Delta/\Delta}$ mice immunized with OVA without alum. **A,** Airway responses to methacholine. *P < .01 for PBS-treated vs the corresponding OVA-challenged mice; †P < .05 for OVA-treated wild-type vs TNFR1$^{-/-}$, TNFR2$^{-/-}$, or memTNF$^{\Delta/\Delta}$ mice. Data are pooled from the 3 independent experiments that we performed, each of which gave similar results. *B,* Baseline. **B,** Cell number in BALFs collected from the same mice shown in *A*. *P < .01 for PBS-treated vs the corresponding OVA-challenged mice; †P < .05 for OVA-treated wild-type vs TNFR1$^{-/-}$, TNFR2$^{-/-}$, or memTNF$^{\Delta/\Delta}$ mice. PBS-treated mice, n = 8-10/group; OVA-treated mice, n = 10-16/group.

examined and/or in the protocols used for antigen sensitization and challenge.[17,18] Most analyses have employed protocols for sensitization in which OVA is administered intraperitoneally with the adjuvant aluminum hydroxide.[12-15] On subsequent challenge with OVA, such mice develop eosinophil-rich pulmonary infiltrates and AHR to methacholine,[32] but such protocols can elicit antigen-induced AHR in mice that lack mast cells[20,21] or B cells/IgE.[19,22] Indeed, alum can induce a strong T_H2 response in the absence of IL-4Rα signaling.[33]

We found no significant differences in the antigen-induced AHR to methacholine at 24 or 48 hours after OVA challenge in C57BL/6-TNF$^{-/-}$ versus -WT mice sensitized with OVA/alum (see this article's Fig E1 in the Online Repository at www.jacionline.org, and data not shown for 48 hours). Nor were there any significant differences between these groups in numbers of OVA-induced leukocytes or T_H2 cytokine levels in BALF obtained 48 hours after OVA challenge.

However, analyses of C57BL/6 mice sensitized to OVA without alum revealed significant roles for TNF in this model. TNF was required for optimal antigen-induced (1) increases in lymphocytes, neutrophils, and eosinophils in BALF (Fig 3); (2) pulmonary inflammation (Fig 2); and

(3) lung levels of MPO, EPO, IL-4, IL-5, and IL-17 (Fig 3). Moreover, in C57BL/6J mice, optimal antigen-induced AHR to methacholine in this model required expression of TNF (Fig 1), TNFR1, and TNFR2, as well as expression of soluble (as opposed to membrane-associated) TNF (Fig 4).

In mice and human beings, antigen-induced AHR is regarded as one manifestation of a T_H2-dependent allergic disorder, and T_H2-associated IL-4, IL-5, IL-9, and IL-13 are believed to be the key cytokines involved in the production of IgE for the activation of mast cells, as well as for the induction of bronchial hyperresponsiveness, lung eosinophil infiltration, and mucus secretion.[34] Moreover, many patients with asthma can develop dramatic pathophysiological airway responses to challenge with low doses of allergens,[35] and IgE-dependent mast cell activation can result in the rapid development of AHR to methacholine challenge in mice.[36] Accordingly, even though no mouse model of asthma is likely to reproduce all of the features of the human disorder, one could argue that the antigen-induced pulmonary allergic inflammation and AHR observed in mice that have been immunized to antigen in the absence of alum may more closely resemble the pathology of human asthma than does that induced in mice immunized to antigen in the presence of alum.

Indeed, in contrast with the generally accepted role of IgE antibody and mast cells in human atopic asthma,[34,37-39] B-cell–deficient, mast cell–deficient, or $Fc\epsilon RI^{-/-}$ mice can exhibit unimpaired development of antigen-induced AHR after sensitization with OVA/alum.[19-22] By contrast, antigen-induced AHR was markedly reduced in mast cell–deficient or $Fc\epsilon RI^{-/-}$ mice sensitized to OVA without alum[21,22]; similar observations were also reported in mice lacking IL-1R or IL-1, a proinflammatory cytokine with pathophysiological activities similar to those of TNF.[40,41] As noted, alum can induce a strong T_H2 response without IL-4Rα signaling,[33] and this may contribute to the differences that have been observed in the mechanisms required for the expression of optimal allergic inflammation in mice that have been sensitized to OVA by using protocols that do or do not include alum.

Our findings indicate that the importance of TNF for the manifestation of various features of mouse models of allergic inflammation of the airways differs depending on the model investigated, even in a single strain of mouse. However, the mechanisms that link TNF deficiency to phenotypic abnormalities in pulmonary allergic inflammation in mice remain to be fully defined. There is evidence that increased AHR in 129xB6F1-$TNF^{-/-}$ mice can be related, at least in part, to a dysregulation of γδ T-cell receptor $(TCR)^+$ T-cell function.[15] However, the contribution of $\gamma\delta TCR^+$ T cells to AHR appears to be complex. Thus, the same group showed that C57BL/6-$\gamma\delta TCR^+$ T-cell–deficient mice exhibited increased antigen-induced AHR but reduced lung eosinophil infiltration on OVA/alum immunization,[42] a phenotype similar to that exhibited by 129xB6F1-$TNF^{-/-}$ mice.[15] On the other hand, C57BL/6- or BALB/c-$\gamma\delta TCR^+$ T-cell–deficient mice exhibit reduced antigen-induced AHR, lung eosinophil infiltration, and mucus secretion after OVA/alum immunization and antigen challenge.[43-46] Moreover, in models in which OVA sensitization was performed without alum, BALB/c-γδ TCR^+ T-cell–deficient mice exhibited normal AHR but reduced lung eosinophil infiltration, mucus secretion, and OVA-specific IgE production.[47]

Taken together, these findings indicate that the relationships among $\gamma\delta TCR^+$ T cells, TNF, and antigen-induced AHR and pulmonary inflammation may be quite complex and may be influenced substantially by the specific model of antigen-induced AHR studied. However, in studies like those shown in Fig 1, we observed no differences in the numbers of $\gamma\delta TCR^+$ T cells in the lungs of C57BL/6J-WT versus -$TNF^{-/-}$ mice at 24 hours after either PBS or OVA challenge (data not shown).

In summary, our observations in mice on the C57BL/6 background demonstrate that TNF can have important roles in the development of antigen-induced bronchial hyperreactivity to methacholine, and the associated pulmonary infiltration of lymphocytes, eosinophils, and neutrophils, in a model of OVA-induced bronchial allergic inflammation that omits the use of alum to elicit antigen sensitization. Moreover, our work indicates that the membrane-associated form of TNF cannot compensate for the important functions of soluble TNF in this setting. Finally, studies in C57BL/6 mice lacking either TNFR1 or TNFR2 indicate that both of these receptors can contribute to the development of antigen-induced AHR and, to an even greater extent, the antigen-induced allergic inflammation elicited in this model.

Because no mouse model of asthma is likely to mimic all of the features of the human disorders, one should use great caution in attempting to extrapolate to the human setting conclusions based on studies of mouse models of airway allergic inflammation. However, both an open-label, uncontrolled pilot study[8] and a placebo-controlled, double-blind, cross-over pilot study[9] provided evidence that 12 weeks[8] or 10 weeks[9] of treatment with the soluble $TNFR\text{-}IgG_1$ Fc fusion protein etanercept improved airway function, reduced AHR to methacholine, and reduced symptom scores in subjects with refractory, steroid-resistant asthma. By contrast, etanercept treatment had little or no benefit in a small number of patients with mild asthma.[7] Although it is clear that larger, placebo-controlled, multicenter studies will be needed to assess the efficiacy, safety, and cost-effectiveness of TNF-targeted therapies in various subsets of patients with asthma, this certainly seems to represent a promising area for future study.[10]

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REFERENCES

1. Kips JC, Tavernier J, Pauwels RA. Tumor necrosis factor causes bronchial hyperresponsiveness in rats. Am Rev Respir Dis 1992;145:332-6.

2. Kips JC, Tavernier JH, Joos GF, Peleman RA, Pauwels RA. The potential role of tumour necrosis factor alpha in asthma. Clin Exp Allergy 1993;23:247-50.
3. Thomas PS, Yates DH, Barnes PJ. Tumor necrosis factor-α increases airway responsiveness and sputum neutrophilia in normal human subjects. Am J Respir Crit Care Med 1995;152:76-80.
4. Halasz A, Cserhati E, Magyar R, Kovacs M, Cseh K. Role of TNF-α and its 55 and 75 kDa receptors in bronchial hyperreactivity. Respir Med 2002;96:262-7.
5. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, et al. Interleukin-4, -5, and -6 and tumor necrosis factor-α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. Am J Respir Cell Mol Biol 1994;10:471-80.
6. Babu KS, Davies DE, Holgate ST. Role of tumor necrosis factor α in asthma. Immunol Allergy Clin North Am 2004;24:583-97, v-vi.
7. Rouhani FN, Meitin CA, Kaler M, Miskinis-Hilligoss D, Stylianou M, Levine SJ. Effect of tumor necrosis factor antagonism on allergen-mediated asthmatic airway inflammation. Respir Med 2005;99:1175-82.
8. Howarth PH, Babu KS, Arshad HS, Lau L, Buckley M, McConnell W, et al. Tumour necrosis factor (TNFα) as a novel therapeutic target in symptomatic corticosteroid dependent asthma. Thorax 2005;60:1012-8.
9. Berry MA, Hargadon B, Shelley M, Parker D, Shaw DE, Green RH, et al. Evidence of a role of tumor necrosis factor α in refractory asthma. N Engl J Med 2006;354:697-708.
10. Erzurum SC. Inhibition of tumor necrosis factor α for refractory asthma. N Engl J Med 2006;354:754-8.
11. Hessel EM, Van Oosterhout AJ, Van Ark I, Van Esch B, Hofman G, Van Loveren H, et al. Development of airway hyperresponsiveness is dependent on interferon-γ and independent of eosinophil infiltration. Am J Respir Cell Mol Biol 1997;16:325-34.
12. Rudmann DG, Moore MW, Tepper JS, Aldrich MC, Pfeiffer JW, Hogenesch H, et al. Modulation of allergic inflammation in mice deficient in TNF receptors. Am J Physiol Lung Cell Mol Physiol 2000;279:L1047-57.
13. Broide DH, Stachnick G, Castaneda D, Nayar J, Sriramarao P. Inhibition of eosinophilic inflammation in allergen-challenged TNF receptor p55/p75- and TNF receptor p55-deficient mice. Am J Respir Cell Mol Biol 2001;24:304-11.
14. Kanehiro A, Lahn M, Makela MJ, Dakhama A, Fujita M, Joetham A, et al. Tumor necrosis factor-α negatively regulates airway hyperresponsiveness through γδ T cells. Am J Respir Crit Care Med 2001;164:2229-38.
15. Kanehiro A, Lahn M, Makela MJ, Dakhama A, Joetham A, Rha YH, et al. Requirement for the p75 TNF-α receptor 2 in the regulation of airway hyperresponsiveness by γδ T cells. J Immunol 2002;169:4190-7.
16. Choi IW, Sun-Kim, Kim YS, Ko HM, Im SY, Kim JH, et al. TNF-α induces the late-phase airway hyperresponsiveness and airway inflammation through cytosolic phospholipase A_2 activation. J Allergy Clin Immunol 2005;116:537-43.
17. Kips JC, Anderson GP, Fredberg JJ, Herz U, Inman MD, Jordana M, et al. Murine models of asthma. Eur Respir J 2003;22:374-82.
18. Boyce JA, Austen KF. No audible wheezing: nuggets and conundrums from mouse asthma models. J Exp Med 2005;201:1869-73.
19. Korsgren M, Erjefalt JS, Korsgren O, Sundler F, Persson CG. Allergic eosinophil-rich inflammation develops in lungs and airways of B cell-deficient mice. J Exp Med 1997;185:885-92.
20. Takeda K, Hamelmann E, Joetham A, Shultz LD, Larsen GL, Irvin CG, et al. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. J Exp Med 1997;186:449-54.
21. Williams CM, Galli SJ. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. J Exp Med 2000;192:455-62.
22. Mayr SI, Zuberi RI, Zhang M, de Sousa-Hitzler J, Ngo K, Kuwabara Y, et al. IgE-dependent mast cell activation potentiates airway responses in murine asthma models. J Immunol 2002;169:2061-8.
23. Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev 1997;77:1033-79.
24. Gurish MF, Austen KF. The diverse roles of mast cells. J Exp Med 2001;194:F1-5.
25. Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. Nat Immunol 2005;6:135-42.
26. Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. Annu Rev Immunol 2005;23:749-86.
27. Gordon JR, Galli SJ. Mast cells as a source of both preformed and immunologically inducible TNF-α/cachectin. Nature 1990;346:274-6.
28. Gordon JR, Galli SJ. Release of both preformed and newly synthesized tumor necrosis factor α (TNF-α)/cachectin by mouse mast cells stimulated via the FcεRI. A mechanism for the sustained action of mast cell-derived TNF-α during IgE-dependent biological responses. J Exp Med 1991;174:103-7.
29. Korner H, Cook M, Riminton DS, Lemckert FA, Hoek RM, Ledermann B, et al. Distinct roles for lymphotoxin-α and tumor necrosis factor in organogenesis and spatial organization of lymphoid tissue. Eur J Immunol 1997;27:2600-9.
30. Ruuls SR, Hoek RM, Ngo VN, McNeil T, Lucian LA, Janatpour MJ, et al. Membrane-bound TNF supports secondary lymphoid organ structure but is subservient to secreted TNF in driving autoimmune inflammation. Immunity 2001;15:533-43.
31. Suto H, Nakae S, Kakurai M, Sedgwick JD, Tsai M, Galli SJ. Mast cell-associated TNF promotes dendritic cell migration. J Immunol 2006;176:4102-12.
32. Lloyd CM, Gonzalo JA, Coyle AJ, Gutierrez-Ramos JC. Mouse models of allergic airway disease. Adv Immunol 2001;77:263-95.
33. Brewer JM, Conacher M, Hunter CA, Mohrs M, Brombacher F, Alexander J. Aluminium hydroxide adjuvant initiates strong antigen-specific Th2 responses in the absence of IL-4- or IL-13-mediated signaling. J Immunol 1999;163:6448-54.
34. Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. Annu Rev Immunol 1999;17:255-81.
35. Sulakvelidze I, Inman MD, Rerecich T, O'Byrne PM. Increases in airway eosinophils and interleukin-5 with minimal bronchoconstriction during repeated low-dose allergen challenge in atopic asthmatics. Eur Respir J 1998;11:821-7.
36. Martin TR, Takeishi T, Katz HR, Austen KF, Drazen JM, Galli SJ. Mast cell activation enhances airway responsiveness to methacholine in the mouse. J Clin Invest 1993;91:1176-82.
37. Shakoory B, Fitzgerald SM, Lee SA, Chi DS, Krishnaswamy G. The role of human mast cell-derived cytokines in eosinophil biology. J Interferon Cytokine Res 2004;24:271-81.
38. Robinson DS. The role of the mast cell in asthma: induction of airway hyperresponsiveness by interaction with smooth muscle? J Allergy Clin Immunol 2004;114:58-65.
39. Holgate ST, Djukanovic R, Casale T, Bousquet J. Anti-immunoglobulin E treatment with omalizumab in allergic diseases: an update on anti-inflammatory activity and clinical efficacy. Clin Exp Allergy 2005;35:408-16.
40. Nakae S, Komiyama Y, Yokoyama H, Nambu A, Umeda M, Iwase M, et al. IL-1 is required for allergen-specific Th2 cell activation and the development of airway hypersensitivity response. Int Immunol 2003;15:483-90.
41. Schmitz N, Kurrer M, Kopf M. The IL-1 receptor 1 is critical for Th2 cell type airway immune responses in a mild but not in a more severe asthma model. Eur J Immunol 2003;33:991-1000.
42. Lahn M, Kanehiro A, Takeda K, Joetham A, Schwarze J, Kohler G, et al. Negative regulation of airway responsiveness that is dependent on γδ T cells and independent of αβ T cells. Nat Med 1999;5:1150-6.
43. Korsgren M, Persson CG, Sundler F, Bjerke T, Hansson T, Chambers BJ, et al. Natural killer cells determine development of allergen-induced eosinophilic airway inflammation in mice. J Exp Med 1999;189:553-62.
44. Schramm CM, Puddington L, Yiamouyiannis CA, Lingenheld EG, Whiteley HE, Wolyniec WW, et al. Proinflammatory roles of T-cell receptor (TCR)γδ and TCRαβ lymphocytes in a murine model of asthma. Am J Respir Cell Mol Biol 2000;22:218-25.
45. Russo M, Nahori MA, Lefort J, Gomes E, de Castro Keller A, Rodriguez D, et al. Suppression of asthma-like responses in different mouse strains by oral tolerance. Am J Respir Cell Mol Biol 2001;24:518-26.
46. Crosby JR, Cieslewicz G, Borchers M, Hines E, Carrigan P, Lee JJ, et al. Early phase bronchoconstriction in the mouse requires allergen-specific IgG. J Immunol 2002;168:4050-4.
47. Zuany-Amorim C, Ruffie C, Haile S, Vargaftig BB, Pereira P, Pretolani M. Requirement for γδ T cells in allergic airway inflammation. Science 1998;280:1265-7.