The Journal of Immunology

RESEARCH ARTICLE | APRIL 01 2004

TNF Receptor-Associated Factor 5 Limits the Induction of Th2 Immune Responses[1] FREE

Takanori So; ... et. al

J Immunol (2004) 172 (7): 4292–4297.
https://doi.org/10.4049/jimmunol.172.7.4292

TNF Receptor-Associated Factor 5 Limits the Induction of Th2 Immune Responses[1]

Takanori So,* Shahram Salek-Ardakani,* Hiroyasu Nakano,† Carl F. Ware,* and Michael Croft[2]*

The TNF receptor-associated factor (TRAF) family of molecules acts as adapter proteins for signaling pathways initiated by several members of the TNF receptor (TNFR) superfamily. TRAF5$^{-/-}$ animals are viable and have normal development of the immune system despite interacting with several TNFR family members. A clear role for TRAF5 has yet to emerge. OX40 (CD134) interacts with TRAF5, suggesting that this pathway could be involved in regulating T cell differentiation into Th1 or Th2 cells. In tissue culture, OX40 stimulation of TRAF5$^{-/-}$ T cells resulted in a pronounced Th2 phenotype with elevated levels of IL-4 and IL-5. Similarly, in vivo immunization with protein in adjuvant in the presence of an agonist anti-OX40 Ab resulted in enhanced Th2 development in TRAF5$^{-/-}$ mice. Additionally, lung inflammation induced by T cells, which is critically controlled by OX40, was more pronounced in TRAF5$^{-/-}$ mice, characterized by higher levels of Th2 cytokines. These results suggest that TRAF5 can limit the induction of Th2 responses, and that TRAF5 can play a role in modulating responses driven by OX40 costimulation. ***The Journal of Immunology*, 2004, 172: 4292–4297.**

Tumor necrosis factor receptor-associated factors (TRAFs)[3] are a family of proteins that serve as adaptors directly engaging the cytoplasmic tail of several members of the TNF receptor (TNFR) superfamily. In addition, some TRAFs function as regulators of other signaling activators, such as TRAF-associated NF-κB activator and NF-κB-inducing kinase, and participate in IL-1 and Toll-like receptor signaling. To date, six members, numbered sequentially TRAF1 through -6, have been identified in the TRAF family (1–3), which is defined by a conserved β-sheet sandwich (TRAF) domain (4, 5). The TRAF domain accommodates a range of short proline-anchored peptide motifs found in the receptors or signal activators (1, 6). The N-terminal domains of TRAF2 to TRAF6 contain RING and multiple zinc fingers that are critical for their downstream effector functions (2, 6).

TRAF5 was identified as an activator of NF-κB and a potential regulator of signaling for the lymphotoxin-β receptor (7) and CD40 (8, 9). It is now known that TRAF5 can associate with several other TNFR superfamily members, including CD27 (10), CD30 (11), herpes virus entry mediator (12), and OX40 (CD134) (13). Although these studies have suggested that TRAF5 has the potential to regulate signaling through the TNFR family, there are few physiological data to indicate what that role may be. TRAF5 is highly expressed in normal tissues, such as epidermis, spleen, lung, and thymus (8, 14). Whereas TRAF2$^{-/-}$ and TRAF3$^{-/-}$ mice are runted and die prematurely (15, 16), TRAF5$^{-/-}$ mice are viable and show no obvious abnormalities (17). Modest defects were reported in the proliferation of TRAF5$^{-/-}$ thymocytes and B cells to anti-CD27 and anti-CD40 in vitro (17), but no analyses of mature T cells or in vivo studies have been performed to determine whether altered immune responses result in the absence of TRAF5.

The phenotype of TRAF5$^{-/-}$ mice does not match the developmentally impaired phenotypes of lymphotoxin-β receptor and CD40-deficient mice, suggesting that TRAF5 may be more important for signaling via molecules such as OX40. As well as TRAF5, OX40 binds TRAF2 and TRAF3 (13, 18) via a PIQEE motif (5). In vitro transfection systems have shown that after the recruitment of TRAF molecules to the cytoplasmic tail of OX40, TRAF2 and TRAF5 might modulate an early step in NF-κB activation, and dominant negative forms of TRAF2 and TRAF5 were shown to block NF-κB activation (13, 18). However, under physiological conditions, it is unclear how OX40 uses TRAF5 to support its functions. OX40 can regulate the expansion and survival of T cells and is critical for a number of Th1 and Th2 immune responses, implying a pivotal role in Th cell differentiation (reviewed in Ref. 19).

We now show that OX40 stimulation of TRAF5$^{-/-}$ CD4^{+} T cells in tissue culture resulted in a profound Th2 phenotype, with elevated levels of IL-4 and IL-5. In vivo, immunization with protein in the presence of an agonistic anti-OX40 Ab resulted in enhanced Th2 development in TRAF5$^{-/-}$ mice. Additionally, lung inflammation in a murine model of asthma was more pronounced in TRAF5$^{-/-}$ mice, characterized by eosinophilia, airway hyperreactivity, and higher levels of Th2 cytokines and plasma IgE. These results suggest that TRAF5 pathways can modulate the Th1/Th2 balance and limit the induction of Th2 responses.

*Division of Molecular Immunology, La Jolla Institute for Allergy and Immunology, San Diego, CA 92121; †Department of Immunology, Juntendo University School of Medicine, Hongo, Bunkyo-ku, Tokyo, Japan; and PRESTO, Japan Science and Technology Corporation, Honcho Kawaguchi, Saitama, Japan

Received for publication November 5, 2003. Accepted for publication January 27, 2004.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported in part by National Institutes of Health Grants AI49453 and AI50498 (to M.C.), and AI03368 (to C.F.W.). H.N. is supported by a grant from Human Frontier Science Program. This is manuscript 609 from La Jolla Institute for Allergy and Immunology.

[2] Address correspondence and reprint requests to Dr. Michael Croft, Division of Molecular Immunology, La Jolla Institute for Allergy and Immunology, 10355 Science Center Drive, San Diego, CA 92121. E-mail address: mick@liai.org

[3] Abbreviations used in this paper: TRAF, TNF receptor-associated factor; BAL, bronchoalveolar lavage; JNK, c-Jun N-terminal kinase; KLH, keyhole limpet hemocyanin; NIP45, NFAT-interacting protein 45.

0022-1767/04/$02.00

Materials and Methods

Mice

The studies reported in this study conform to the principles outlined by the Animal Welfare Act and the National Institutes of Health guidelines for the care and use of animals in biomedical research. TRAF5$^{-/-}$ mice and littermate TRAF5$^{+/+}$ mice (17) were separately maintained on a C57BL/6 background and used in this study.

CD4$^+$ T cells

CD4$^+$ T cells were purified from spleen and lymph nodes of 6- to 12-wk-old mice as previously described (20). Briefly, whole cells passed over nylon column were subjected to complement lysis using Abs to CD8 (3.155), heat-stable Ag (J11D), class II MHC (M5/114, Y17 and CA-4.A12), B cells (RA3.6B2), macrophage (M1/70), NK cells (PK136), and dendritic cells (33D1). Cells were cultured in RPMI 1640 (Invitrogen, Carlsbad, CA) with penicillin, streptomycin, glutamine, 2-ME, and 7% FCS (Omega Scientific, Tarzana, CA). CD4$^+$ T cells (5×10^5 cells/ml) were stimulated with plate-bound anti-CD3 (145-2C11) and soluble anti-CD28 (37N51) with or without soluble anti-OX40 (OX86). Cell proliferation was assessed by addition of 1 μCi of tritiated thymidine (ICN, Irvine, CA). Culture supernatants were assessed for cytokine content by standard ELISA protocols as described previously (21), using commercially available Abs or those produced in-house. JES6-1A12 and biotin-JES6-5H4 (BD PharMingen, San Diego, CA) were used for IL-2, 11B11 and biotin-BVD6-24G2 (BD PharMingen) were used for IL-4, TRFK5 and biotin-TRFK4 (eBioscience, San Diego, CA) were used for IL-5, 38213.11 and biotin-goat polyclonal Ab were used for IL-13 (R&D Systems, Minneapolis, MN), and R46A-2 and biotin-XMG1.2 (BD PharMingen) were used for IFN-γ.

Immunization

Mice were immunized s.c. in the tail base with keyhole limpet hemocyanin (KLH; Calbiochem, La Jolla, CA) emulsified in CFA (Difco, Detroit, MI). Anti-OX40 or purified control rat IgG (Chemicon, Temecula, CA) was injected i.p. in PBS 2 days after Ag. For boosting, 50 μg of KLH in PBS was injected i.p. 35 days after Ag. Spleen cells were plated in 0.2-ml volumes in 96-well plates in triplicate with increasing concentrations of KLH. Culture supernatants were harvested at 36 h for cytokine ELISA.

Airway inflammation

Allergic airway inflammation was induced as described previously (22). Briefly, groups of mice were sensitized by i.p. injection of OVA protein (grade V, A5503; Sigma-Aldrich, St. Louis, MO) and alum adjuvant (Pierce, Rockford, IL) in PBS on day 0. OVA protein (5 mg/ml) was administered through the airways for consecutive days during days 6–9 or days 25–29. One day after the last aerosol challenge, airway hyper-reactivity in response to inhaled methacholine, bronchoalveolar lavage (BAL) cytology, lung histopathology, OVA-specific and total IgE, and lung cytokine profiles obtained by ELISA were determined. Peribronchial lymph nodes were collected at the time of lung harvest, and the cells were stimulated with increasing concentrations of OVA to measure proliferative and cytokine responses, as previously described (23).

Results

Increased Th2 cytokine production in TRAF5$^{-/-}$ CD4$^+$ T cells

To investigate the role of TRAF5 in CD4$^+$ T cell proliferation and IL-2 production, purified CD4$^+$ T cells from TRAF5$^{+/+}$ or TRAF5$^{-/-}$ mice were stimulated with anti-CD3/CD28 in the presence or the absence of an anti-OX40 agonist Ab. Relative to wild-type CD4$^+$ T cells, TRAF5$^{-/-}$ CD4$^+$ T cells equivalently proliferated and produced IL-2 (Fig. 1). In addition, CD4$^+$ T cells from TRAF5$^{+/+}$ and TRAF5$^{-/-}$ mice equivalently expressed OX40 (Fig. 2).

To assess whether TRAF5 deficiency could affect T cell differentiation, CD4 T cells were stimulated for 4 days with anti-CD3, anti-CD28, and anti-OX40. Production of the Th1 cytokine IFN-γ in primary cultures of TRAF5$^{-/-}$ CD4$^+$ T cells was similar to that in wild-type CD4$^+$ T cells in response to anti-CD3/CD28 (Fig. 3*A*). In the presence of anti-OX40, the levels of IFN-γ decreased by ~50%, but were again similar in TRAF5$^{-/-}$ and $^{+/+}$ T cells. TRAF5$^{-/-}$ CD4$^+$ T cells secreted slightly enhanced amounts of the Th2 cytokines IL-4 and IL-5 in response to anti-CD3/CD28, but this was strongly elevated in OX40-stimulated cultures.

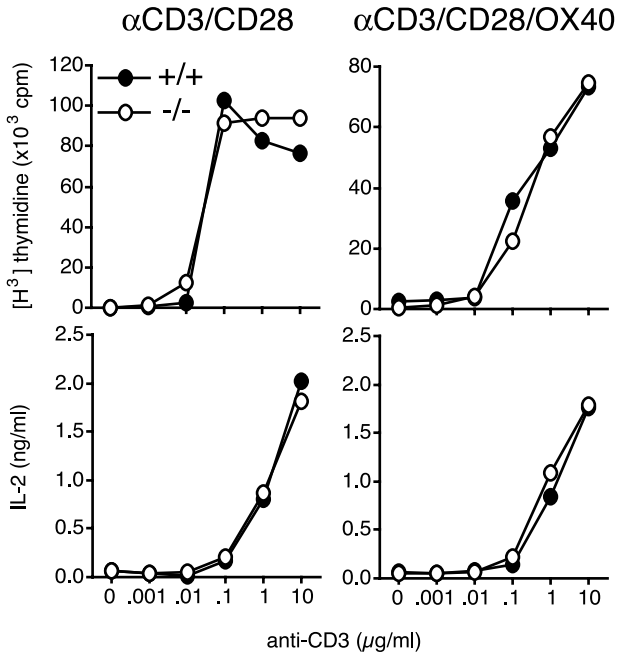

FIGURE 1. CD4$^+$ T cells from TRAF5$^{+/+}$ and TRAF$^{-/-}$ mice comparably proliferate and produce IL-2 in vitro. CD4$^+$ T cells from lymph nodes and spleen of TRAF5$^{+/+}$ (●) or TRAF5$^{-/-}$ (○) mice were stimulated with different concentrations of plate-bound anti-CD3 and soluble anti-CD28 (3 μg/ml) in the presence or the absence of soluble anti-OX40 (10 μg/ml). For cell proliferation, T cells were cultured for 40 h. For IL-2, supernatants were analyzed at 24 h. Results are representative of two experiments.

To evaluate the role of TRAF5 in OX40-mediated Th cell differentiation more precisely, CD4$^+$ T cells were primed with anti-CD3/CD28 in the presence of increasing concentrations of anti-OX40. After 7 days of culture, cells were restimulated with anti-CD3/CD28. Supernatants from secondary cultures were harvested, and levels of

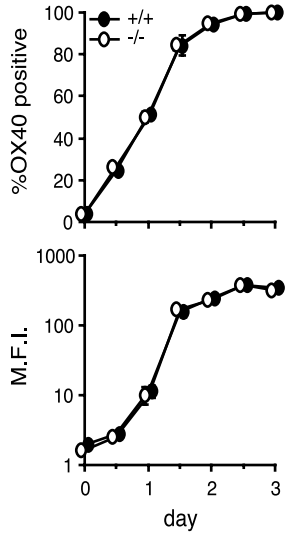

FIGURE 2. CD4$^+$ T cells from TRAF5$^{+/+}$ and TRAF$^{-/-}$ mice comparably express OX40. CD4$^+$ T cells from TRAF5$^{+/+}$ and TRAF5$^{-/-}$ mice were stimulated with plate-bound anti-CD3 and soluble anti-CD28. Cells were harvested at the indicated times and stained with anti-CD4-FITC and biotinylated anti-OX40, followed by streptavidin-PE. CD4-gated cells were analyzed. Data are presented as the percentage of OX40$^+$ CD4 cells and the median fluorescence intensity (M.F.I.) of OX40 expression. Results are representative of two experiments.

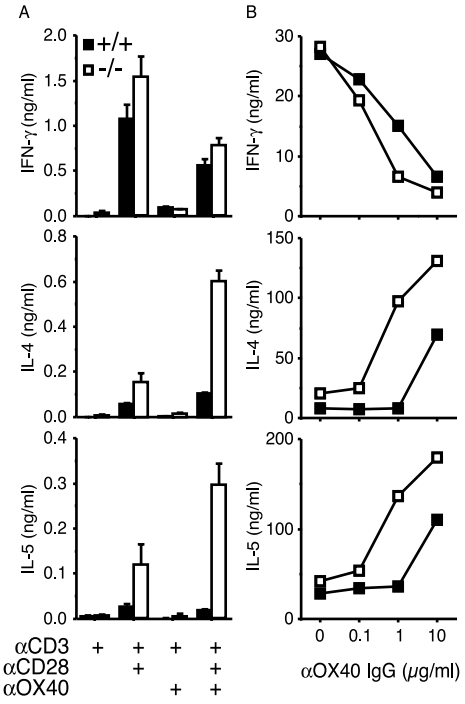

FIGURE 3. OX40 stimulation up-regulates Th2 cytokines in TRAF5$^{-/-}$ CD4$^+$ T cells in vitro. CD4$^+$ T cells from TRAF5$^{+/+}$ or TRAF5$^{-/-}$ mice were stimulated as described in Fig. 1. *A*, Primary cytokine production from cells cultured for 4 days. *B*, Recall cytokine production from cells cultured with anti-CD3 and anti-CD28 and the indicated concentrations of anti-OX40 for 7 days. Live cells were restimulated with anti-CD3 and anti-CD28, and supernatants were harvested 36 h after secondary stimulation. The results shown are representative of four experiments.

IFN-γ, IL-4, and IL-5 were quantitated. With increasing concentrations of anti-OX40, IFN-γ recall responses decreased, and this was almost equivalent between TRAF5$^{+/+}$ and TRAF5$^{-/-}$ CD4$^+$ T cells (Fig. 3*B*). In both T cell populations, the levels of IL-4 and IL-5 after secondary stimulation increased with the dose of anti-OX40 used, but in contrast to IFN-γ, these cytokines were produced in significantly higher quantities in cultures of TRAF5$^{-/-}$ T cells, i.e., fold increases of TRAF5$^{-/-}$ over TRAF5$^{+/+}$ cultures were 2.58 ± 0.89 (p = 0.18) and 2.35 ± 0.31 (p = 0.02), respectively. We also observed enhanced IL-13 production in TRAF5$^{-/-}$ CD4$^+$ T cells (data not shown). In the absence of anti-OX40, there was no significant difference between TRAF5$^{+/+}$ and TRAF5$^{-/-}$ CD4$^+$ T cells for IL-4 and IL-5 recall responses, i.e., fold increases in TRAF5$^{-/-}$ over TRAF5$^{+/+}$ cultures were 1.29 ± 0.45 (p = 0.57) and 1.52 ± 0.32 (p = 0.20), respectively. These observations indicate that the expression of TRAF5 can antagonize Th2 differentiation and that this is revealed primarily when signaling via OX40 is sustained.

Enhanced Th2 cell development in TRAF5-deficient mice

We previously reported that treatment of mice with an agonist, OX40 Ab, induced clonal expansion and survival of CD4$^+$ T cells during primary responses and up-regulated the secretion of Th1 and Th2 cytokines (24). To examine the role of TRAF5 in vivo, we immunized TRAF5$^{+/+}$ or TRAF5$^{-/-}$ mice with KLH in CFA, a situation that normally induces a weak Th2 response. Administering an agonistic Ab to OX40 on day 2 was used to boost the immune response. Spleen cells were harvested on day 7 and at >4 wk to evaluate primary and secondary T cell responses, respectively. Cultures from anti-OX40-injected mice showed much higher KLH-specific IFN-γ, IL-4, and IL-5 production in both primary and secondary responses (Fig. 4). Th2 responses in TRAF5$^{-/-}$ mice were higher than those in TRAF5$^{+/+}$ mice, whereas IFN-γ, under these predominantly Th1 conditions with CFA, was unaffected or reduced. A summary of four separate in vivo experiments is shown in Table I. When alum was used as an adjuvant, which does not favor such a robust IFN-γ response, a similar higher Th2 response was observed in TRAF5$^{-/-}$ mice injected with anti-OX40 (Table I). These data again suggest that a lack of TRAF5 leads to enhanced Th2 differentiation.

Exaggerated Th2-driven lung inflammation in TRAF5$^{-/-}$ mice

The data obtained in vitro (Fig. 3) and in vivo (Fig. 4 and Table I) suggested that TRAF5 has an attenuating role in Th2 differentiation, in particular when driven by OX40 costimulation. To investigate further the role of TRAF5 during Th cell differentiation in vivo, we induced an asthmatic-like response in the lung using OVA and alum adjuvant. In this study Th2 cells are the critical

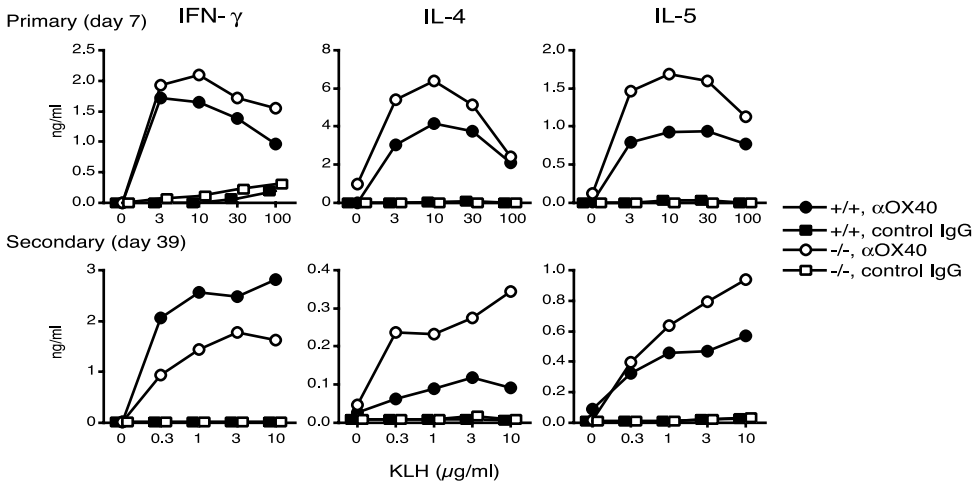

FIGURE 4. Enhanced Th2 development in TRAF5$^{-/-}$ mice. Groups of TRAF5$^{+/+}$ and TRAF5$^{-/-}$ mice (n = 3) were immunized s.c. with 50 μg of KLH emulsified in CFA. Mice were treated with 100 μg of control rat IgG or anti-OX40 given i.p. on day 2. For measuring primary T cell responses, spleen cells were harvested on day 7 (*top graphs*). For secondary responses, mice were additionally injected with 50 μg of KLH in PBS given i.p. on day 35, and spleen cells were harvested on day 39 (*bottom graphs*). Cells were cultured in vitro in triplicate with varying doses of KLH. The secretion of IFN-γ, IL-4, and IL-5 was assessed at 36 h. Results are the mean responses of three individual mice.

Table I. *Higher Th2 cytokine response in TRAF5$^{-/-}$ mice*[a]

Days after Immunization	Immunization	Booster Injection[b]	Stimulation Index[c] IFN-γ +/+	IFN-γ −/−	IL-4 +/+	IL-4 −/−	IL-5 +/+	IL-5 −/−
Day 7	CFA + KLH	−	55	18	62	181	18	52
Day 35	CFA + KLH	−	14	14	1	1	16	33
Day 39	CFA + KLH	+	83	59	4	8	13	24
Day 7	Alum + KLH	−	1	5	11	56	6	49
p value[a]				0.24		0.23		0.04

[a] Spleen cells were cultured in vitro with no Ag or KLH and secretion of IFN-γ, IL-4, and IL-5 was assessed at 36 h as shown in Fig. 4.
[b] Booster with soluble KLH at day 35.
[c] Stimulation index was determined by the following formula, $\text{cytokine}_{\text{anti-OX40, KLH stimulation}} - \text{cytokine}_{\text{anti-OX40, no Ag}}/\text{cytokine}_{\text{control I gG, KLH stimulation}} - \text{cytokine}_{\text{control I gG, no Agg}}$
[d] Statistical significance between TRAF5$^{+/+}$ and TRAF5$^{-/-}$ mice was evaluated by *p* value in the paired Student *t* test.

mediator of lung inflammation, and endogenous OX40/OX40L interactions are essential for the priming of these cells (22, 23).

After systemic sensitization with OVA, mice were challenged via the airways with aerosolized OVA from days 6–9 or from days 25–29. The lungs were lavaged, and the BAL fluid was assessed for the presence of cellular infiltrates. TRAF5$^{-/-}$ mice had 2- and 4-fold higher numbers of total cells in the BAL fluid compared with TRAF5$^{+/+}$ mice on days 10 and 30, respectively (Fig. 5*A*). The predominant infiltrate was eosinophils, with lower numbers of neutrophils, monocytes, and lymphocytes (Fig. 5*A*). We did not observe any eosinophilia in mice injected with alum alone, then challenged with OVA or immunized with OVA/alum, then exposed to PBS as a control, in either TRAF5$^{+/+}$ or TRAF5$^{-/-}$ mice (data not shown). TRAF5$^{-/-}$ mice had a 20-fold higher level of IL-5 in the BAL fluid and a 3-fold higher level of OVA-specific IgE in the plasma compared with TRAF5$^{+/+}$ mice (Fig. 5*B*). Higher levels of IL-13 in the BAL and total IgE were also seen in TRAF5$^{-/-}$ mice (data not shown). TRAF5$^{-/-}$ mice additionally showed a significantly higher airway hyper-reactivity response than TRAF5$^{+/+}$ mice (Fig. 5*C*). Lung draining lymph node cells from TRAF5$^{+/+}$ and TRAF5$^{-/-}$ mice equally proliferated in a recall response to OVA in vitro (Fig. 5*D*). In contrast, greatly enhanced OVA-specific IL-5 and IL-13 production was detected in cultures from TRAF5$^{-/-}$ mice. Similarly enhanced Th2 cytokine responses were observed in cultures of lung cells from TRAF5$^{-/-}$ mice (data not shown).

The lungs from sensitized and challenged mice were removed and examined histologically by staining for tissue architecture around the bronchioles. As shown in Fig. 6*A*, mice injected with alum and then challenged with OVA showed little inflammation in the lung sections. When immunized with OVA/alum and then challenged with OVA, TRAF5$^{+/+}$ mice had prominent cellular infiltration around bronchioles at both early and late points (Fig. 6, *B* and D). Importantly, we observed more severe inflammation in TRAF5$^{-/-}$ mice (Fig. 6, *C* and *E*), correlating with the enhanced numbers of cells, particularly eosinophils, found in BAL fluid (Fig. 5*A*). Collectively, these data demonstrate that TRAF5$^{-/-}$ mice have a higher susceptibility to develop an asthma-like phenotype and exhibit a greater ability to mount a Th2 response.

Discussion

In this report we demonstrate that TRAF5 plays a limiting role during the differentiation of Th2 cells. Ag stimulation in the presence of anti-OX40 resulted in higher Th2 cytokine responses in TRAF5$^{-/-}$ mice. Also, in an experimental OVA-induced allergic airway inflammation model, eosinophilic infiltration, airway hyper-responsiveness, and Th2-type responses were significantly enhanced in TRAF5$^{-/-}$ mice. These results define a new regulatory role for TRAF5 signaling pathways in T cell differentiation and suggest that TRAF5 can limit certain responses induced by costimulatory molecules such as OX40.

TRAF5 has been shown to have the potential to associate with several members of the TNFR family (7, 9–13). TRAF5 also binds other signal activators, such as NF-κB-inducing kinase, and has been ascribed a positive regulatory role in NF-κB and c-Jun N-terminal kinase (JNK) activation, identified in experiments in cell lines. However, these results are not compatible with the phenotypes observed in TRAF5$^{-/-}$ mice or cells in several respects. For example, CD27-mediated proliferation was impaired in TRAF5$^{-/-}$ thymocytes, but the activation of NF-κB nor JNK was not significantly altered (17). TRAF5$^{-/-}$ B cells showed defects in proliferation and up-regulation of various cell surface molecules, including CD23, CD54, CD80, CD86, and Fas, in response to CD40 stimulation, but CD40-mediated activation of NF-κB or JNK was also not impaired in TRAF5$^{-/-}$ B cells (17). Moreover, CD30-mediated induction of JNK, p38, and NF-κB was suggested to be intact in T cells from TRAF5$^{-/-}$ mice (25). These data indicate that in physiological conditions, pathways involving other TRAF molecules may dominate over TRAF5 for a number of particular functions (26).

In contrast, the results presented in this study suggest that the Th1/Th2 balance depends on the availability of TRAF5 and unknown factors that might dictate the use of TRAF5 within the cell. It has previously been established that OX40 signaling can promote both Th1- and Th2-type immune responses, and both Th1- and Th2-polarized T cells express the OX40 receptor (23, 24, 27). In contrast, it has been reported in several in vitro culture systems that OX40 costimulation can favor the development of effector Th2 cells (28, 29), and OX40 can promote Th2 cytokine secretion from already differentiated effector cells (27). In our study, enhanced Th2 responses in TRAF5$^{-/-}$ mice were primarily observed when the agonist anti-OX40 was used and in the asthma model, where it is known that OX40/OX40 ligand interactions are critical to Th2 development. Thus, one conclusion could be that TRAF5 is integral to OX40-mediated T cell differentiation, and that TRAF5 might regulate Th2 cytokine transcription only when OX40 is engaged. However, we cannot rule out a developmental defect in TRAF5$^{-/-}$ mice that favors the generation of Th2 responses under appropriate inflammatory conditions, and that normally OX40 might not, in fact, engage TRAF5. Also, it is possible that similar deregulated Th2 responses would be seen if other TNFR family members that use TRAF molecules are ligated on T cells. Additional experiments will be required to discriminate between these possibilities.

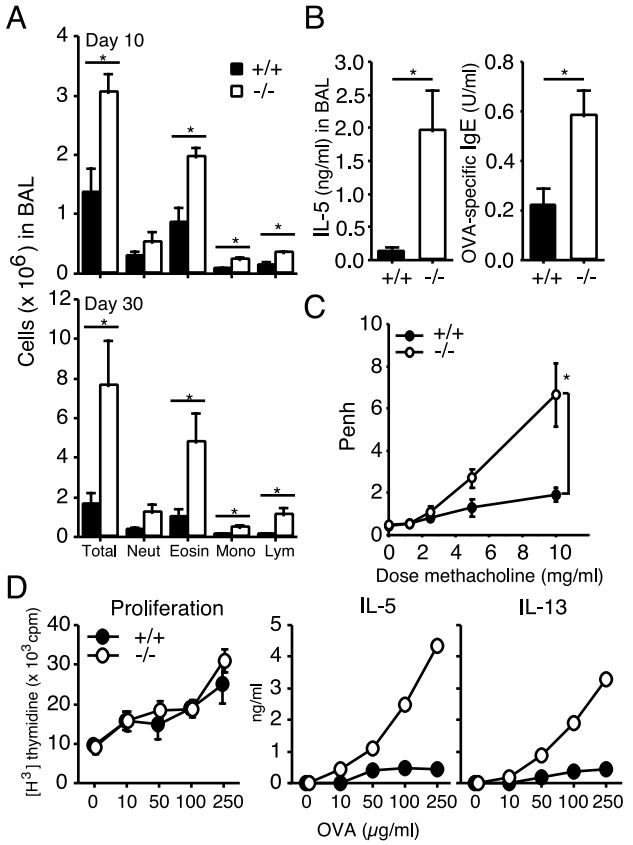

FIGURE 5. TRAF5$^{-/-}$ mice are highly susceptible to Th2-driven allergic inflammation. Groups of TRAF5$^{+/+}$ and TRAF5$^{-/-}$ mice ($n = 4$) were immunized with 5 μg of OVA and 2 mg of aluminum hydroxide, given i.p. Mice were challenged through the airways with OVA for 40 min on days 6–9 for early responses or on days 25–29 for late responses. *A*, Characterization of cellular infiltrates in the lung. On day 10 or 30, lungs were lavaged, and BAL fluid was assessed for cellular content by differential cell counting. The total cell number (total) and numbers of neutrophils (neut), eosinophils (eosin), monocytes (mono), and lymphocytes (lym) are shown. *B*, BAL were assessed for IL-5 production, and plasma was analyzed for OVA-specific IgE on day 10. *C*, Airway hyper-responsiveness in response to increasing doses of methacholine was measured by barometric plethysmography on day 30. *D*, Lymph node cells were harvested on day 30 and cultured with graded doses of OVA. Proliferation and cytokine production were measured at 72 and 96 h, respectively. Results are the mean ± SEM from four mice per group. Statistical significance was evaluated by Student's *t* test (*, $p < 0.05$).

One candidate molecule that might be regulated by TRAF5, and hence might play a role in the OX40-mediated Th2 bias seen in TRAF5$^{-/-}$ mice, is NIP45. This is an NFAT-interacting protein that has been shown to synergize with c-Maf and NFAT to promote IL-4 transcription. A recent study suggested that a number of TRAF molecules, including TRAF2 and TRAF5, can bind NIP45, and hence could potentially regulate Th2 responses through this molecule (30). Thus, one possibility is that TRAF molecules act as a sink for NIP45, and they normally restrict its use and/or access to the nucleus, where it is required for transcribing the IL-4 gene. If this is the case, two scenarios might exist in TRAF5$^{-/-}$ T cells. One is that more unbound NIP45 could be available to be recruited into a response, and this could then be used as long as factors such as OX40 are present to initiate the Th2 process. Alternatively, without TRAF5, more NIP45 could be available to bind other TRAF molecules, such as TRAF2, and then when TNFR family members that bind TRAF2 (such as OX40) are engaged, more

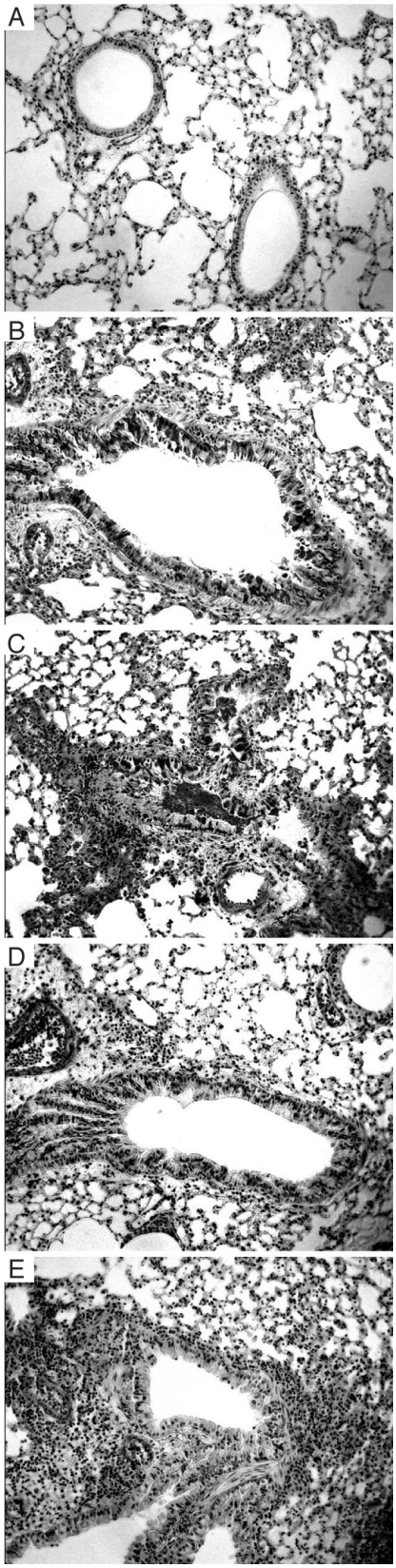

FIGURE 6. Lung inflammatory infiltrates and goblet cell hyperplasia are enhanced in TRAF5$^{-/-}$ mice. Mice were immunized with either alum only or OVA-alum, challenged with OVA, and sacrificed on day 10 or 30 as described in Fig. 5. Lung tissues were fixed and stained with H&E (magnification, ×100). *A*, TRAF5$^{+/+}$, alum/OVA, day 30. *B*, TRAF5$^{+/+}$, alum-OVA/OVA, day 10. *C*, TRAF5$^{-/-}$, alum-OVA/OVA, day 10. *D*, TRAF5$^{+/+}$, alum-OVA/OVA, day 30. *E*, TRAF5$^{-/-}$, alum-OVA/OVA, day 30.

NIP45 could be released and available to promote IL-4. Whether NIP45 does, in fact, play a role in the elevated Th2 responses in the absence of TRAF5 remains to be determined.

In conclusion, using TRAF5-deficient mice, we have defined a novel role for the TRAF5 pathway in $CD4^+$ T cell differentiation. T cells from $TRAF5^{-/-}$ mice have a greater capacity to develop into Th2 cells, suggesting that TRAF5 can function as a limiting (attenuating) step in Th2 cytokine responses. In the future it will be important to understand how TRAF5 modulates Th2 cytokine transcription as well as to define the putative effector molecules that regulate OX40-dependent Th2 responses.

Acknowledgments

We thank Beth Halteman and Mary Cheng for technical assistance.

References

1. Li, C., P. S. Norris, C. Z. Ni, M. L. Havert, E. M. Chiong, B. R. Tran, E. Cabezas, J. C. Reed, A. C. Satterthwait, C. F. Ware, et al. 2003. Structurally distinct recognition motifs in lymphotoxin-β receptor and CD40 for TRAF-mediated signaling. *J. Biol. Chem. 278:50523*.
2. Arch, R. H., R. W. Gedrich, and C. B. Thompson. 1998. Tumor necrosis factor receptor-associated factors (TRAFs): a family of adapter proteins that regulates life and death. *Genes Dev. 12:2821*.
3. Bradley, J. R., and J. S. Pober. 2001. Tumor necrosis factor receptor-associated factors (TRAFs). *Oncogene 20:6482*.
4. Ni, C. Z., K. Welsh, E. Leo, C. K. Chiou, H. Wu, J. C. Reed, and K. R. Ely. 2000. Molecular basis for CD40 signaling mediated by TRAF3. *Proc. Natl. Acad. Sci. USA 97:10395*.
5. Ye, H., Y. C. Park, M. Kreishman, E. Kieff, and H. Wu. 1999. The structural basis for the recognition of diverse receptor sequences by TRAF2. *Mol. Cell. 4:321*.
6. Chung, J. Y., Y. C. Park, H. Ye, and H. Wu. 2002. All TRAFs are not created equal: common and distinct molecular mechanisms of TRAF-mediated signal transduction. *J. Cell Sci. 115:679*.
7. Nakano, H., H. Oshima, W. Chung, A. L. Williams-Abbott, C. F. Ware, H. Yagita, and K. Okumura. 1996. TRAF5, an activator of NF-κB and putative signal transducer for the lymphotoxin-β receptor. *J. Biol. Chem. 271:14661*.
8. Ishida, T., T. Tojo, T. Aoki, N. Kobayashi, T. Ohishi, T. Watanabe, T. Yamamoto, and J. Inoue. 1996. TRAF5, a novel tumor necrosis factor receptor-associated factor family protein, mediates CD40 signaling. *Proc. Natl. Acad. Sci. USA 93:9437*.
9. Leo, E., K. Welsh, S. Matsuzawa, J. M. Zapata, S. Kitada, R. S. Mitchell, K. R. Ely, and J. C. Reed. 1999. Differential requirements for tumor necrosis factor receptor-associated factor family proteins in CD40-mediated induction of NF-κB and Jun N-terminal kinase activation. *J. Biol. Chem. 274:22414*.
10. Akiba, H., H. Nakano, S. Nishinaka, M. Shindo, T. Kobata, M. Atsuta, C. Morimoto, C. F. Ware, N. L. Malinin, D. Wallach, et al. 1998. CD27, a member of the tumor necrosis factor receptor superfamily, activates NF-κB and stress-activated protein kinase/c-Jun N-terminal kinase via TRAF2, TRAF5, and NF-κB-inducing kinase. *J. Biol. Chem. 273:13353*.
11. Aizawa, S., H. Nakano, T. Ishida, R. Horie, M. Nagai, K. Ito, H. Yagita, K. Okumura, J. Inoue, and T. Watanabe. 1997. Tumor necrosis factor receptor-associated factor (TRAF) 5 and TRAF2 are involved in CD30-mediated NF-κB activation. *J. Biol. Chem. 272:2042*.
12. Marsters, S. A., T. M. Ayres, M. Skubatch, C. L. Gray, M. Rothe, and A. Ashkenazi. 1997. Herpesvirus entry mediator, a member of the tumor necrosis factor receptor (TNFR) family, interacts with members of the TNFR-associated factor family and activates the transcription factors NF-κB and AP-1. *J. Biol. Chem. 272:14029*.
13. Kawamata, S., T. Hori, A. Imura, A. Takaori-Kondo, and T. Uchiyama. 1998. Activation of OX40 signal transduction pathways leads to tumor necrosis factor receptor-associated factor (TRAF) 2- and TRAF5-mediated NF-κB activation. *J. Biol. Chem. 273:5808*.
14. Zapata, J. M., M. Krajewska, S. Krajewski, S. Kitada, K. Welsh, A. Monks, N. McCloskey, J. Gordon, T. J. Kipps, R. D. Gascoyne, et al. 2000. TNFR-associated factor family protein expression in normal tissues and lymphoid malignancies. *J. Immunol. 165:5084*.
15. Yeh, W.-C., A. Shahinian, D. Speiser, J. Kraunus, F. Billia, A. Wakeham, J. L. de la Pompa, D. Ferrick, B. Hum, N. Iscove, et al. 1997. Early lethality, functional NF-κB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity 7:715*.
16. Xu, Y., G. Cheng, and D. Baltimore. 1996. Targeted disruption of TRAF3 leads to postnatal lethality and defective T-dependent immune responses. *Immunity 5:407*.
17. Nakano, H., S. Sakon, H. Koseki, T. Takemori, K. Tada, M. Matsumoto, E. Munechika, T. Sakai, T. Shirasawa, H. Akiba, et al. 1999. Targeted disruption of Traf5 gene causes defects in CD40- and CD27-mediated lymphocyte activation. *Proc. Natl. Acad. Sci. USA 96:9803*.
18. Arch, R. H., and C. B. Thompson. 1998. 4–1BB and Ox40 are members of a tumor necrosis factor (TNF)-nerve growth factor receptor subfamily that bind TNF receptor-associated factors and activate nuclear factor κB. *Mol. Cell. Biol. 18:558*.
19. Croft, M. 2003. Co-stimulatory members of the TNFR family: keys to effective T-cell immunity? *Nat. Rev. Immunol. 3:609*.
20. Rogers, P. R., H. M. Grey, and M. Croft. 1998. Modulation of naive CD4 T cell activation with altered peptide ligands: the nature of the peptide and presentation in the context of costimulation are critical for a sustained response. *J. Immunol. 160:3698*.
21. Rogers, P. R., and M. Croft. 2000. CD28, Ox-40, LFA-1, and CD4 modulation of Th1/Th2 differentiation is directly dependent on the dose of antigen. *J. Immunol. 164:2955*.
22. Jember, A. G.-H., R. Zuberi, F.-T. Liu, and M. Croft. 2001. Development of allergic inflammation in a murine model of asthma is dependent on the costimulatory receptor OX40. *J. Exp. Med. 193:387*.
23. Salek-Ardakani, S., J. Song, B. S. Halteman, A. G.-H. Jember, H. Akiba, H. Yagita, and M. Croft. 2003. OX40 (CD134) controls memory T helper 2 cells that drive lung inflammation. *J. Exp. Med. 198:315*.
24. Gramaglia, I., A. Jember, S. D. Pippig, A. D. Weinberg, N. Killeen, and M. Croft. 2000. The OX40 costimulatory receptor determines the development of CD4 memory by regulating primary clonal expansion. *J. Immunol. 165:3043*.
25. Harlin, H., E. Podack, M. Boothby, and M.-L. Alegre. 2002. TCR-independent CD30 signaling selectively induces IL-13 production via a TNF receptor-associated factor/p38 mitogen-activated protein kinase-dependent mechanism. *J. Immunol. 169:2451*.
26. Tada, K., T. Okazaki, S. Sakon, T. Kobarai, K. Kurosawa, S. Yamaoka, H. Hashimoto, T. W. Mak, H. Yagita, K. Okumura, et al. 2001. Critical roles of TRAF2 and TRAF5 in tumor necrosis factor-induced NF-κB activation and protection from cell death. *J. Biol. Chem. 276:36530*.
27. Gramaglia, I., A. D. Weinberg, M. Lemon, and M. Croft. 1998. Ox-40 ligand: a potent costimulatory molecule for sustaining primary CD4 T cell responses. *J. Immunol. 161:6510*.
28. Ohshima, Y., L.-P. Yang, T. Uchiyama, Y. Tanaka, P. Baum, M. Sergerie, P. Hermann, and G. Delespesse. 1998. OX40 costimulation enhances interleukin-4 (IL-4) expression at priming and promotes the differentiation of naive human $CD4^+$ T cells into high IL-4-producing effectors. *Blood 92:3338*.
29. Flynn, S., K.-M. Toellner, C. Raykundalia, M. Goodall, and P. Lane. 1998. CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and upregulates expression of the chemokine receptor, Blr-1. *J. Exp. Med. 188:297*.
30. Lieberson, R., K. A. Mowen, K. D. McBride, V. Leautaud, X. Zhang, W. K. Suh, L. Wu, and L. H. Glimcher. 2001. Tumor necrosis factor receptor-associated factor (TRAF)2 represses the T helper cell type 2 response through interaction with NFAT-interacting protein (NIP45). *J. Exp. Med. 194:89*.