

# Blockade of CD70–CD27 Interaction Inhibits Induction of Allergic Lung Inflammation in Mice

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The interaction between the TNF receptor family member CD27 and its ligand CD70 provides a costimulatory signal for T-cell activation. In this study, we investigated the effects of neutralizing anti-CD70 monoclonal antibody (mAb) in a murine model of allergic lung inflammation to determine whether CD27 contributes to the development of pathogenic Th2 cells and pulmonary inflammation. BALB/c mice were immunized by an injection of ovalbumin (OVA) with alum adjuvant and challenged with aerosolized OVA in PBS. Some groups of mice were treated with anti-CD70 mAb or control rat IgG during the induction or effector phase. The administration of anti-CD70 mAb during the induction phase, but not the effector phase, reduced eosinophil infiltration in lung tissue compared with control IgG-treated mice. Treatment with anti-CD70 mAb also resulted in the decreased production of Th2 cytokines (IL-4, IL-5, and IL-13) in the bronchoalveolar lavage fluid and draining lymph node cell cultures. We further revealed that antigen-specific CD4 T cells were separated into CD27<sup>+</sup> and CD27<sup>−</sup> populations in the lymph nodes of OVA-immunized DO11.10/Rag-2<sup>−/−</sup> mice. The CD27<sup>+</sup> CD4 T cells produced a high concentration of IFN- $\gamma$ , representing Th1 cells. In contrast, CD27<sup>−</sup> CD4 T cells produced high concentrations of IL-4, IL-5, and IL-13, representing Th2 cells. Moreover, the population of CD27<sup>+</sup> Th2 cells was significantly reduced by the anti-CD70 mAb treatment. These results indicate an important role for CD27 in the development of pathogenic Th2 cells in a murine model of allergic lung inflammation.

**Keywords:** CD70; CD27; costimulation; lung inflammation; Th2 development

Allergic airway disease is characterized by chronic airway inflammation with a massive infiltration of eosinophils, increased mucus production in the bronchioles, and airway hyperreactivity to a variety of specific and nonspecific stimuli. Although a variety of cell types are involved in allergic inflammation, substantial evidence indicates that the infiltration of Th2 cells is common in the lungs of allergic subjects, as are increased concentrations of Th2 cytokines, including IL-4, IL-5, and IL-13 (1, 2). The concentration of Th2 cytokines appears to correlate with severity of disease. These cytokines are responsible for the recruitment and activation of other cell types, including eosinophils, which have been associated with lung injury. They also mediate the production of mucus by the airway epithelium, contributing to

## CLINICAL RELEVANCE

A potent inhibition of the inflammatory response in a murine model of allergic lung inflammation was achieved with anti-CD70 monoclonal antibody administration. The CD70–CD27 interaction appears to play a critical role in the development of pathogenic Th2 cells.

the airway obstruction that comprises a major component in the pathology of allergic airway inflammation (3–5). Although the critical roles of these Th2 cytokines in the pathogenesis of allergic lung inflammation have been established, the mechanisms for the overproduction of Th2 cytokines are not fully understood.

The optimal activation of antigen-specific CD4 T cells requires not only the engagement of the T-cell receptor (TCR) with antigen peptide/major histocompatibility complex Class II, but also a costimulatory signal provided by antigen-presenting cells (APCs) (6, 7). The interaction between the TNF receptor family member CD27 on T cells and its ligand CD70 on APCs provides a costimulatory signal for T-cell activation (8, 9). Signals through CD27 lead to the activation of nuclear factor- $\kappa$ B and the c-Jun kinase pathways that regulate various cellular responses including proliferation, differentiation, and survival (10, 11). Upon T-cell activation, the expression of CD27 is increased, but it is known to be down-regulated after contact with CD70 (12, 13). Murine CD70 has been detected on APCs, including activated B cells and stimulated dendritic cells (14, 15). The role of the CD70–CD27 interaction in the differentiation and survival of CD8 T cells has been well studied, whereas the role of this pathway in CD4 T-cell responses has been less fully investigated (8, 9). However, recent studies have shown that the CD70–CD27 interaction is involved in CD4 T-cell-mediated immunopathologies, including experimental autoimmune encephalomyelitis, experimental colitis, and collagen-induced arthritis (16–18). These studies indicate that the CD70–CD27 interaction enhanced the pathogenic activity of CD4 T cells, and thus was critically involved in the development of autoimmune and inflammatory diseases. In this study, we examined the contribution of the CD70–CD27 interaction to the development of allergic lung inflammation by administering the anti-CD70 monoclonal antibody (mAb).

## MATERIALS AND METHODS

### Animals

Female BALB/cByJJcl mice were purchased from CREA Japan (Tokyo, Japan). Mice transgenic for the OVA<sub>323–339</sub>-specific and I-A<sup>d</sup>-restricted DO11.10 TCR- $\alpha\beta$  on a Rag-2<sup>−/−</sup> BALB/c background (DO11.10/Rag-2<sup>−/−</sup>) were generously supplied by Dr. S. Koyasu (Keio University School of Medicine, Tokyo, Japan) (19). All mice were 6–8 weeks old at the start of experiments, and were maintained under specific pathogen-free conditions during the experiments. All animal experiments were approved by the Animal Experimental Ethics Committee

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of Juntendo University, and complied with National Institutes of Health guidelines for animal care.

### Induction of Allergic Lung Inflammation and mAb Treatment

Allergic lung inflammation was induced and assessed as previously described (20). Briefly, BALB/c mice were immunized by an intraperitoneal injection of 100  $\mu$ g of ovalbumin (OVA; Sigma-Aldrich, St. Louis, MO) with 2 mg of alum adjuvant (OVA/alum; Thermo Fisher Scientific, Yokohama, Japan) on Days 0 and 14. On Days 22, 24, 26, and 28, the mice were challenged by an inhalation of aerosolized 1% OVA in PBS. The aerosol was generated by a nebulizer (NE-U07; Omron, Kyoto, Japan). Some groups of mice were intraperitoneally injected with 300  $\mu$ g of anti-CD70 mAb (FR70) (14) or control rat IgG (Sigma-Aldrich) on Days 0, 2, 4, 7, 11, 14, 16, 18, 22, 25, and 28. Negative control animals were immunized with OVA/alum, challenged with PBS, and intraperitoneally injected with PBS in a similar manner. To examine the effects of anti-CD70 mAb during the induction phase, mice were treated with anti-CD70 mAb or control rat IgG on Days 0, 2, 4, 7, 11, 14, 16, and 18. To examine the effects during the effector phase, mice were treated with anti-CD70 mAb or control rat IgG on Days 22, 25, and 28. For adoptive transfer experiments, OVA-specific CD4 T cells were derived as described elsewhere (21, 22). Complete details are provided in the online supplement.

### Characterization of Bronchoalveolar Lavage Fluid

Two days after the last OVA challenge, bronchoalveolar lavage (BAL) fluid was collected as described in the online supplement.

### Histological Analysis of Lung Sections

For a detailed description of the protocols, please refer to the online supplement.

### Stimulation of Bronchial Lymph Node Cells *In Vitro*

Bronchial lymph node (LN) cells were cultured in RPMI-1640 medium containing 10% FCS, 10 mM HEPES, 2 mM L-glutamine, 0.1 mg/ml penicillin and streptomycin, and 50  $\mu$ M 2-mercaptoethanol in the presence or absence of the indicated doses of OVA. Additional details are provided in the online supplement.

### Flow Cytometric Analysis and Cytokine Production by CD4 T-Cell Subsets

For a detailed description of the protocols, please refer to the online supplement.

### Statistical Analysis

Statistical analyses for parametric data were performed using the unpaired Student *t* test. Nonparametric data were analyzed with the Mann-Whitney U test. The results are expressed as means  $\pm$  SEM. Values of *P* < 0.05 were considered significant.

## RESULTS

### Anti-CD70 mAb Treatment Inhibits the Development of Allergic Lung Inflammation

To explore the contribution of the CD70–CD27 interaction to the pathogenesis of allergic lung inflammation, we first administered a neutralizing anti-CD70 mAb or control rat IgG from Day 0 to Day 27. BALB/c mice were immunized by an injection of OVA with alum on Days 0 and 14, and then challenged by an inhalation of aerosolized OVA on Days 22, 24, 26, and 28. On Day 30, the control IgG-treated mice developed an accumulation of eosinophils (Figure 1A) and production Th2 cytokines (IL-4, IL-5, and IL-13) (Figure 1B) in BAL fluid. Histological

examination showed a massive infiltration of eosinophils around the bronchioles (Figures 1C and 1E) and mucus overproduction in the bronchioles (Figures 1D and 1F). In contrast, the accumulation of eosinophils and lymphocytes (Figure 1A) and production of Th2 cytokines (IL-4 and IL-13) (Figure 1B) in BAL fluid as well as eosinophilia (Figures 1C and 1E) and mucus overproduction (Figures 1D and 1F) in the lung were significantly reduced in the anti-CD70–treated mice. The amelioration of lung inflammation by anti-CD70 mAb may have resulted from the modulation of OVA-specific CD4 T-cell responses. To address this possibility, bronchial LN cells were prepared on Day 31, and the proliferative response and Th2 cytokine production (IL-4, IL-5, and IL-13) against various doses of OVA were assessed. As represented in Figure 1G, the OVA-specific proliferative response was significantly reduced in LN cells from anti-CD70–treated mice, compared with the proliferative response in control IgG-treated mice. The production of IL-4, IL-5, and IL-13 was also significantly reduced in the anti-CD70–treated mice compared with the control IgG-treated mice (Figure 1H). These results indicate that the CD70–CD27 interaction plays a critical role in the development of allergic lung inflammation and Th2 responses in this model.

### Effect of Anti-CD70 mAb Treatment during the Induction Phase of Allergic Lung Inflammation

Although the results thus far indicate a critical contribution of the CD70–CD27 interaction to the development of allergic lung inflammation, it remained unclear whether the CD70–CD27 interaction played a role during the induction phase of pathogenic Th2 cell development or during the effector phase of pathogenic Th2 cell migration and activation in the lung. We therefore examined whether the CD70–CD27 interaction played a role during the induction phase of allergic lung inflammation. OVA-immunized BALB/c mice were treated with anti-CD70 mAb or control IgG at the time of OVA immunization from Day 0 to Day 18. The administration of anti-CD70 mAb significantly inhibited the accumulation of eosinophils and lymphocytes (Figure 2A) and the production Th2 cytokines (IL-4, IL-5, and IL-13) (Figure 2B) in BAL fluid, as well as eosinophilia (Figures 2C and 2E) and mucus overproduction (Figures 2D and 2F) in the lung. In addition, anti-CD70–treated mice showed a significantly reduced OVA-specific proliferative response (Figure 2G) and production of Th2 cytokines (IL-4 and IL-5) (Figure 2H) in LN cells, compared with control IgG-treated mice.

Behrendt and Hansen recently reported that CD27-deficient C57BL/6 mice develop the complete asthma phenotype after sensitization and pulmonary challenge with OVA, comparable to the phenotype in wild-type mice (23). To determine the effect of anti-CD70 mAb on the pathogenesis of allergic lung inflammation in C57BL/6 mice, we induced allergic lung inflammation in C57BL/6 mice according to the short-term protocols of Behrendt and Hansen (23) and our long-term protocols, and treated the mice with anti-CD70 mAb or control IgG. However, anti-CD70 mAb treatment exhibited no significant effect on the development of airway hyperreactivity, the accumulation of eosinophils in the lung, or the OVA-specific proliferative response and production of Th2 cytokines (IL-5 and IL-13) in LN cells when induced by the short-term protocol (see Figure E1 in the online supplement) or the long-term protocol (Figure E2). We also examined the induction of allergic lung inflammation in BALB/c mice according to the short-term protocol of Behrendt and Hansen (23). Anti-CD70 mAb treatment significantly inhibited the development of airway hyperreactivity, the accumulation of eosinophils in the lung, and the OVA-specific proliferative response and production of Th2

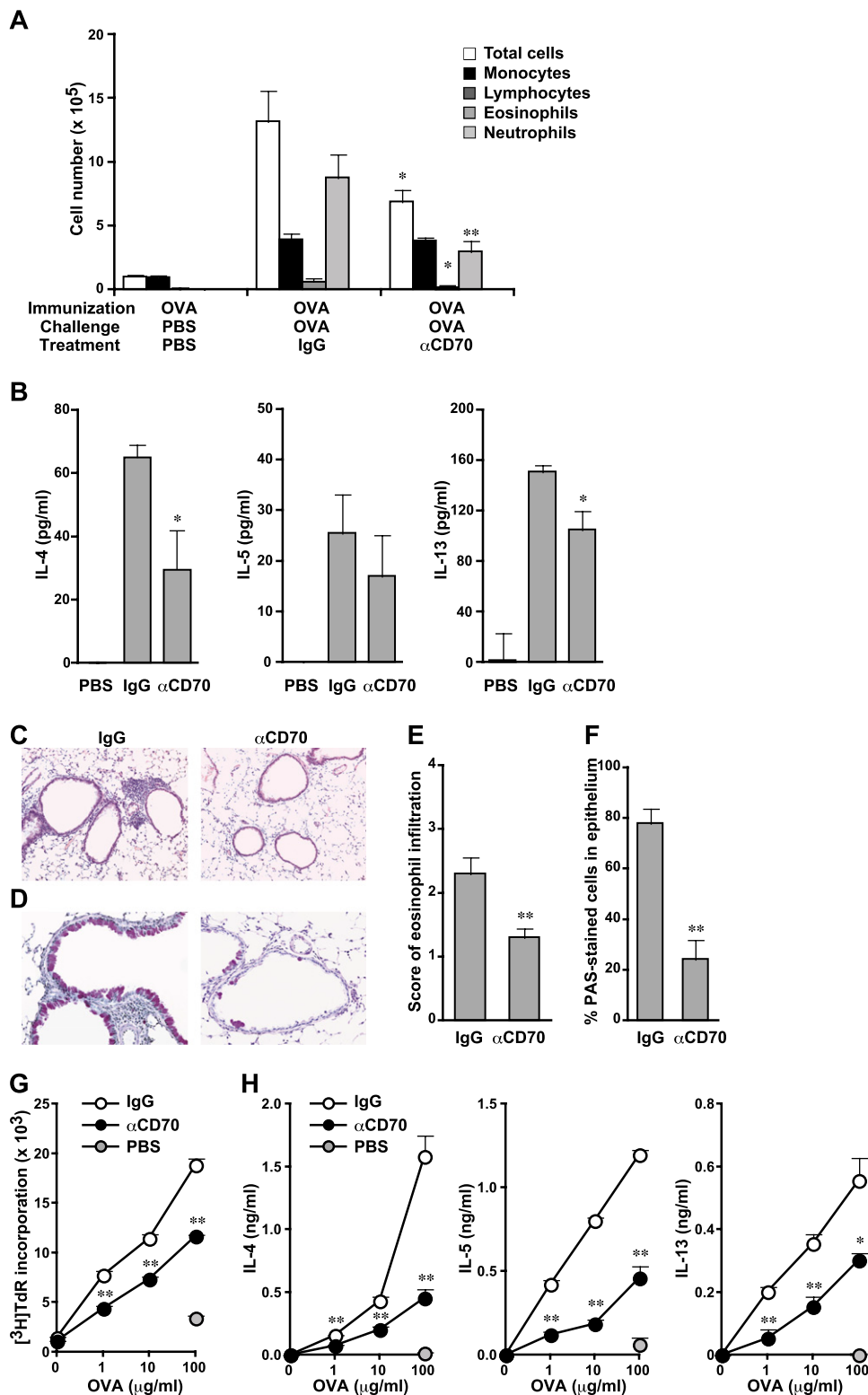


**Figure 1.** The anti-CD70 monoclonal antibody (mAb) inhibits the development of allergic lung inflammation. BALB/c mice were immunized and challenged with OVA, as described in MATERIALS AND METHODS. Some groups of mice were intraperitoneally injected with anti-CD70 mAb (αCD70), control rat IgG (IgG), or PBS from Day 0 to Day 28. (A) Bronchoalveolar lavage (BAL) fluid was collected from individual mice, and the cellular composition of airway infiltrates is shown. (B) Cytokine production (IL-4, IL-5, and IL-13) in BAL fluid was determined by ELISA. Lung tissue was stained with (C) hematoxylin and eosin (×40) and (D) periodic acid-Schiff (PAS) (×200). Representative sections are shown. (E) The infiltration of eosinophils is defined as the average of the scores. (F) Airway mucus concentrations are expressed as the percentage of PAS-positive cells in total epithelial cells. Bronchial lymph node cells were isolated and cultured with the indicated concentrations of ovalbumin (OVA). (G) For estimating proliferation, 0.5 μCi <sup>3</sup>H-thymidine ([<sup>3</sup>H]TdR) was added during the last 6 hours of a 48-hour culture. (H) Cytokine production in the culture supernatants at 72 hours was determined by ELISA. Results are expressed as means ± SEM. Similar results were obtained in three independent experiments (*n* = 7–8 mice per group). \**P* < 0.05 and \*\**P* < 0.01, compared with control IgG.

cytokines (IL-5 and IL-13) in LN cells (Figure E3), as we observed with the long-term protocol (Figure 2).

#### Effect of Anti-CD70 mAb Treatment during the Effector Phase or Adoptively Transferred Lung Inflammation

We next examined whether the CD70–CD27 interaction played a role during the effector phase of allergic lung inflammation. OVA-immunized BALB/c mice were treated with anti-CD70

mAb or control IgG from Day 21 to Day 27 during the OVA challenge. Anti-CD70 mAb treatment during the effector phase did not inhibit the accumulation of eosinophils in the lung (Figure 3A) or the OVA-specific proliferative response (Figure 3B) and production of Th2 cytokines (IL-4, IL-5, and IL-13) (Figure 3C) in LN cells. To determine more directly the effects of the anti-CD70 mAb on pathogenic T-cell migration and activation, we examined the effects of the anti-CD70 mAb on an adoptive transfer model of allergic lung inflammation (21, 22). OVA-immunized

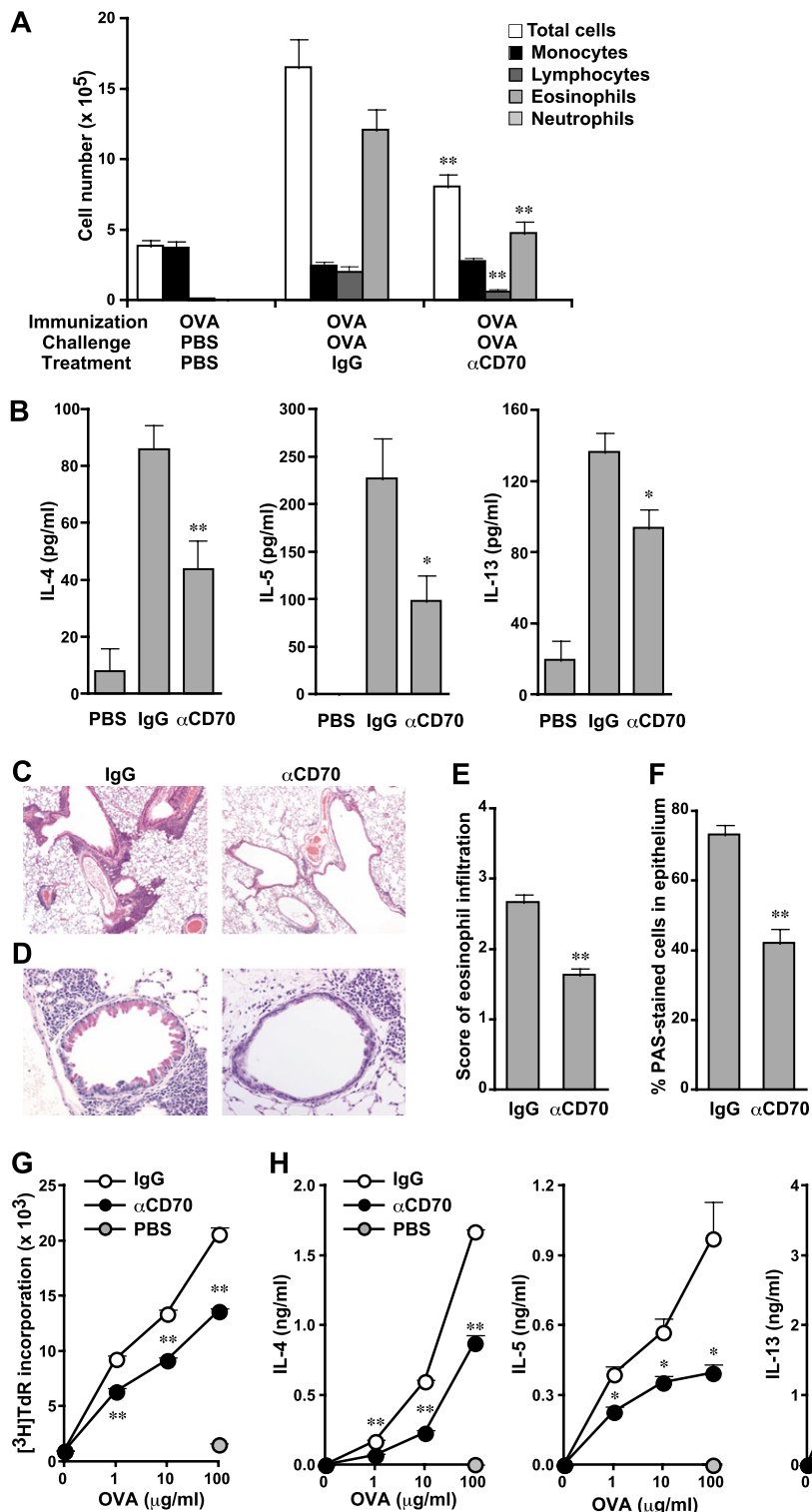


**Figure 2.** Effect of anti-CD70 mAb treatment during the induction phase of allergic lung inflammation. BALB/c mice were immunized and challenged, as described in Figure 1. Some groups of mice were intraperitoneally injected with anti-CD70 mAb (αCD70), control rat IgG (IgG), or PBS from Day 0 to Day 18. (A) BAL fluid was collected from individual mice, and the cellular composition of airway infiltrates is shown. (B) Cytokine production (IL-4, IL-5, and IL-13) in BAL fluid was determined by ELISA. Lung tissue was stained with (C) hematoxylin and eosin (×40) and (D) PAS (×200). Representative sections are shown. (E) The infiltration of eosinophils is defined as the average of the scores. (F) Airway mucus levels are expressed as the percentage of PAS-positive cells in total epithelial cells. Bronchial lymph node cells were isolated and cultured with the indicated concentrations of OVA. (G) For estimating proliferation, 0.5 μCi [<sup>3</sup>H]TdR was added during the last 6 hours of a 48-hour culture. (H) Cytokine production in the culture supernatants at 72 hours was determined by ELISA. Results are expressed as means ± SEM. Similar results were obtained in three independent experiments (*n* = 7–8 mice per group). \**P* < 0.05 and \*\**P* < 0.01, compared with control IgG.

CD4 T cells were intravenously infused into naive BALB/c mice. These mice were exposed daily for 6 days to OVA/PBS, and treated with anti-CD70 mAb or control IgG on Days 1, 3, and 5. Lung inflammation was assessed on Day 7. Again, anti-CD70 mAb treatment exerted no significant effect on the accumulation of eosinophils in the lung (Figure 3D), or on the OVA-specific proliferative response (Figure 3E) and production of Th2 cytokines (IL-4, IL-5, and IL-13) (Figure 3F) in LN cells. Taken together, these results indicate a critical contribution of the CD70–CD27 interaction to the induction phase of allergic lung inflammation,

but it does not play a major role in the effector phase, including the migration and activation of pathogenic Th2 cells in the lung.

#### Antigen-Specific CD4 T Cells Are Separated into CD27<sup>+</sup> and CD27<sup>−</sup> Populations upon Immunization

The amelioration of allergic lung inflammation by anti-CD70 mAb may result from the modulation of OVA-specific CD4 T-cell responses. To address this possibility, we examined the expression of CD27 on OVA-specific CD4 T cells using DO11.10/Rag-2<sup>−/−</sup>

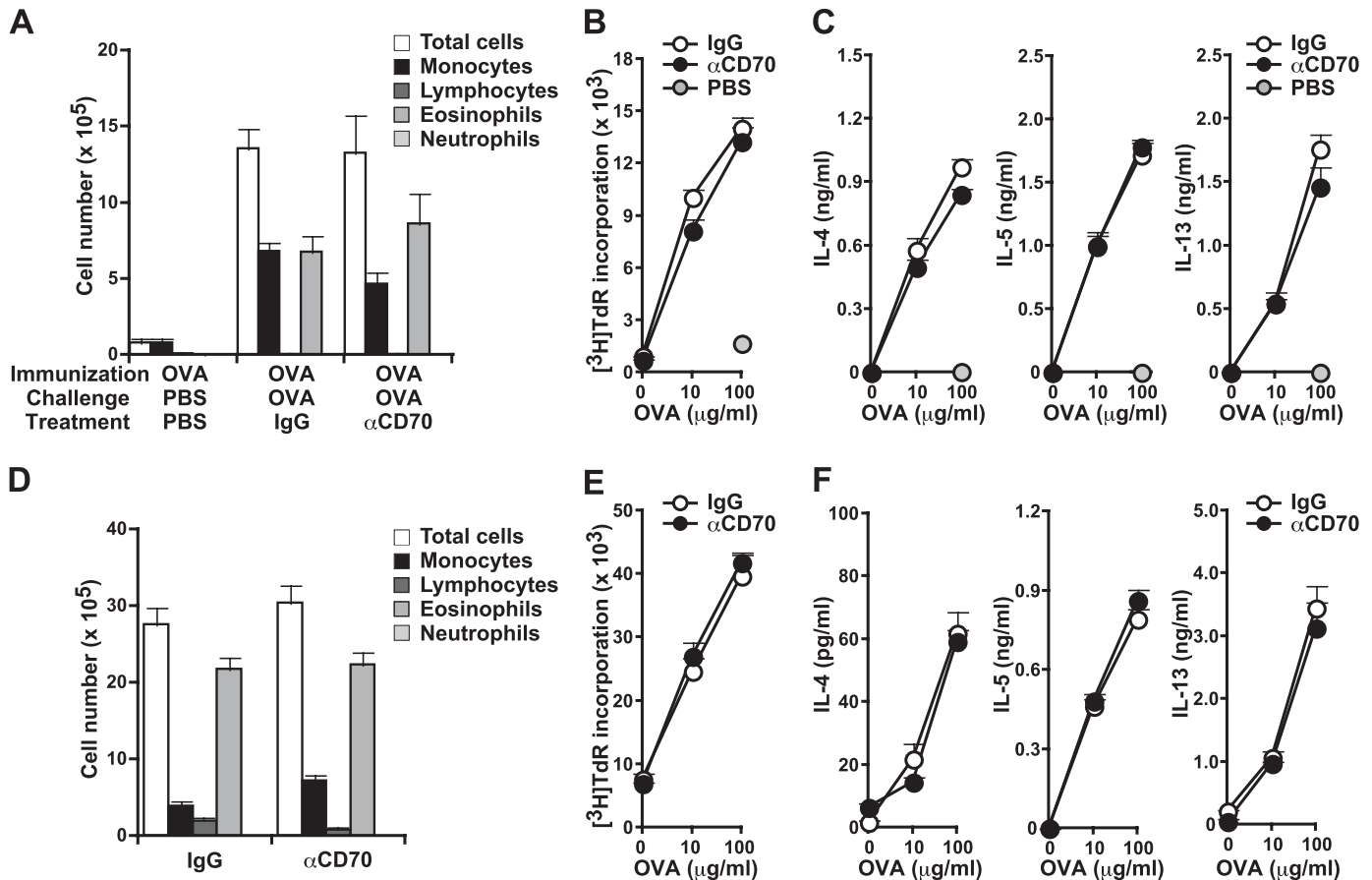


**Figure 3.** Effect of anti-CD70 mAb treatment during the effector phase or during adoptively transferred lung inflammation. (A–C) Effector phase of allergic lung inflammation. BALB/c mice were immunized and challenged, as described in Figure 1. Some groups of mice were intraperitoneally injected with anti-CD70 mAb ( $\alpha$ CD70), control rat IgG (IgG), or PBS from Day 22 to Day 28. (D–F) Adoptively transferred lung inflammation. BALB/c mice were adoptively transferred with OVA-immunized CD4 T cells, challenged with OVA on Day 1 to Day 6, and treated with anti-CD70 mAb ( $\alpha$ CD70) or control rat IgG (IgG) on Days 1, 3, and 5. (A and D) BAL fluid was collected from individual mice, and the cellular composition of airway infiltrates is shown. Bronchial lymph node cells were isolated and cultured with the indicated concentrations of OVA. (B and E) For estimating proliferation, 0.5  $\mu$ Ci [<sup>3</sup>H]TdR was added during the last 6 hours of a 48-hour culture. (C and F) Cytokine production in culture supernatants at 72 hours was determined by ELISA. Results are expressed as means  $\pm$  SEM. Similar results were obtained in two independent experiments ( $n = 7$ –8 mice per group).

mice, which express an I-A<sup>d</sup>-restricted TCR specific for an OVA<sub>323–339</sub> peptide, but not endogenous TCR. We confirmed that the administration of anti-CD70 mAb to OVA-immunized DO11.10/Rag-2<sup>-/-</sup> mice significantly suppressed the allergic lung inflammatory responses (Figure E4). DO11.10/Rag-2<sup>-/-</sup> mice were subcutaneously immunized with OVA/alum, and LN cells were harvested 7 days after immunization. As shown in Figure 4A, naive DO11.10 TCR<sup>+</sup> T cells in the LN of DO11.10/Rag-2<sup>-/-</sup> mice before immunization constitutively expressed CD27, but not inducible costimulator (ICOS), which is an activation marker (24, 25). A substantial level of ICOS expression was found in DO11.10 TCR<sup>+</sup> T cells in the LN of OVA-immunized DO11.10/Rag-2<sup>-/-</sup> mice. Notably, DO11.10 TCR<sup>+</sup> T cells were clearly separated into two populations by CD27 expression, namely CD27<sup>+</sup> and CD27<sup>-</sup>, after OVA immunization (Figure 4A). Therefore, we further examined cytokine production by isolated CD27<sup>+</sup> and CD27<sup>-</sup> DO11.10 TCR<sup>+</sup> T cells (Figure 4A) after stimulation with anti-CD3 and anti-CD28 mAbs *in vitro*. As shown in Figure 4B, the CD27<sup>+</sup> cells produced a high level of IFN- $\gamma$  and low levels of IL-4 and IL-13. In contrast, CD27<sup>-</sup> cells produced high levels of IL-4, IL-5, and IL-13, and a low level of IFN- $\gamma$ . A similar pattern was observed in the spleens

of OVA-immunized DO11.10/Rag-2<sup>-/-</sup> mice (data not shown). These results indicate that the CD27<sup>-</sup> DO11.10 TCR<sup>+</sup> population represents Th2 cells in OVA-immunized DO11.10/Rag-2<sup>-/-</sup> mice.

#### Anti-CD70 mAb Treatment Inhibits the Development of CD27<sup>-</sup> Th2 Cells *In Vivo*

To determine the effects of anti-CD70 mAb on the development of CD27<sup>-</sup> Th2 cells *in vivo*, DO11.10/Rag-2<sup>-/-</sup> mice were subcutaneously immunized with OVA/alum and treated with anti-CD70 mAb or control IgG on Days 0 and 3, and LN cells were collected on Day 7. As depicted in Figure 5A and compiled in Figure 5B, the control IgG-treated mice developed substantial percentages of CD27<sup>-</sup> cells within their total DO11.10 TCR<sup>+</sup> T cells (21.4%  $\pm$  1.3%) upon OVA/alum immunization. The anti-CD70 mAb treatment significantly reduced the CD27<sup>-</sup> population (9.2%  $\pm$  0.8%). Similar results were obtained with spleen cells when DO11.10/Rag-2<sup>-/-</sup> mice were intraperitoneally immunized with OVA/alum and treated with anti-CD70 mAb (data not shown). Taken together, these results suggest that the CD70–CD27 interaction plays a critical role in the development of effector Th2 cells in this system.

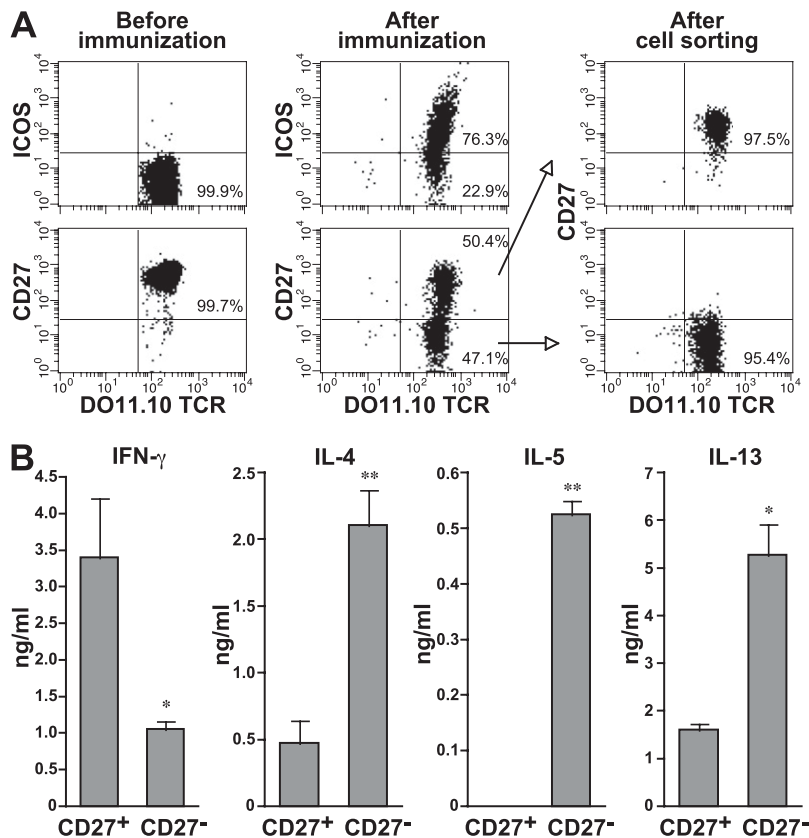


**Figure 4.** Separation of DO11.10 TCR<sup>+</sup> T cells into CD27<sup>+</sup> and CD27<sup>-</sup> populations after OVA immunization. (A) Expression of CD27 and inducible costimulator (ICOS) on DO11.10 TCR<sup>+</sup> T cells. Lymph node cells were isolated from DO11.10/Rag-2<sup>-/-</sup> mice on Day 0 before immunization and on Day 7 after immunization, and stained with anti-ICOS, anti-CD27, and anti-DO11.10 TCR mAbs. The dot plots are representative of five mice with similar results. CD27<sup>+</sup> and CD27<sup>-</sup> DO11.10 TCR<sup>+</sup> T cells were isolated from the lymph nodes on Day 7 after immunization by FACS sorting. (B) Isolated CD27<sup>+</sup> and CD27<sup>-</sup> cells were stimulated with immobilized anti-CD3 and anti-CD28 mAbs for 48 hours. Concentrations of the indicated cytokines in culture supernatants were determined by ELISA. Data are expressed as means  $\pm$  SEM. Similar results were obtained in three independent experiments. \* $P$  < 0.05 and \*\* $P$  < 0.01, compared with CD27<sup>+</sup>.

## DISCUSSION

The anti-CD70 mAb-treated mice showed a substantial reduction of allergic lung inflammation. Moreover, the administration of anti-CD70 mAb during the induction phase effectively ameliorated allergic responses, although it was not effective during the effector phase. The *in vitro* restimulation of draining LN cells showed that anti-CD70 mAb treatment significantly reduced the OVA-specific proliferative response and production of Th2 cytokines. These results suggest that the CD70–CD27 interaction plays a major role in the development of pathogenic Th2 cells during the induction phase, but not in the recruitment or activation of Th2 cells in the lung during the effector phase. This finding is inconsistent with a recent report that CD27 was dispensable for the development of asthma in CD27-deficient C57BL/6 mice (23). At present, the reason for this discrepancy is not clear, but it may be attributable to differences in murine strains, as shown in Figures E1–E3. To support this notion, we previously indicated that the administration of mAb against murine OX40L, which is another TNF family member, differentially regulated the allergic airway inflammation in BALB/c and C57BL/6 mice (26, 27). In our further study (28), chemokine C-X-C motif receptor 5 (CXCR5<sup>+</sup>) follicular B helper CD4<sup>+</sup> T (T<sub>FH</sub>) cells in the LN of C57BL/6 mice uniquely expressed OX40, whereas those from BALB/c mice did not. Consistent with the expression of OX40, the development of both CXCR5<sup>+</sup> CD4<sup>+</sup> T cells and germinal center (GC) B cells was partly inhibited by anti-OX40L mAb treatment in the LN of C57BL/6, but not BALB/c, mice. These results indicate that the expression of OX40 in T<sub>FH</sub> cells and the contribution of the OX40/OX40L interaction to the development of T<sub>FH</sub> cells and GC B cells are variable among murine strains.

Previous studies showed that the CD70–CD27 signal was important for IFN- $\gamma$  production by CD4 T cells, but not for the induction of Th2 cytokines (29, 30). On the other hand,

a recent study showed that human CD70, expressed in myeloid dendritic cells, promoted not only the Th1 but also the Th2 differentiation of naive CD4 T cells (31). In the present study, we show that anti-CD70 mAb treatment significantly reduced

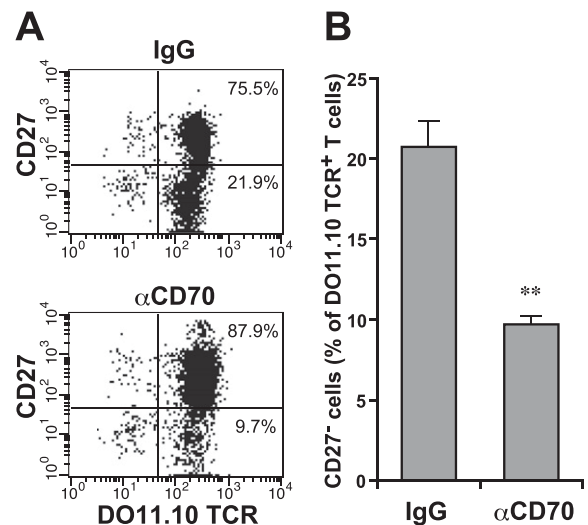


**Figure 5.** Anti-CD70 mAb inhibits the induction of CD27<sup>-</sup> Th2 cells in the lymph node. DO11.10/Rag-2<sup>-/-</sup> mice were immunized with OVA on Day 0, and received anti-CD70 mAb ( $\alpha$ CD70) or control rat IgG (IgG) on Days 0 and 3. Lymph node cells were collected on Day 7. (A) The induction of CD27<sup>-</sup> Th2 cells was determined by staining with anti-CD27 and anti-DO11.10 TCR mAbs. The dot plots are representative of six mice in each group. (B) The data are compiled and expressed as mean percentages  $\pm$  SEMs of CD27<sup>-</sup> Th2 cells within DO11.10 TCR<sup>+</sup> T cells. Similar results were obtained in three independent experiments. \*\* $P$  < 0.01, compared with control IgG.

the CD27<sup>-</sup> DO11.10 TCR<sup>+</sup> cell population. The loss of CD27 expression was reported to coincide with the differentiation into effector T cells (13). Therefore, these results suggest that the CD27<sup>-</sup> population represents effector Th2 cells. In accordance with these findings, Wambre and colleagues (32) recently demonstrated that pathogenic responses (chemoattractant receptor-homologous molecule expressed on Th2 cells expression and Th2 cytokine production) were specifically associated with CD27<sup>-</sup> allergen-specific CD4<sup>+</sup> T cells, which dominated in alder pollen allergic subjects but were absent in nonallergic subjects. In contrast, CD27<sup>+</sup> allergen-specific CD4<sup>+</sup> T cells were present at low frequencies in both allergic and nonallergic subjects, and reflected classic features of the protective immune response, with a high expression of IL-10 and IFN- $\gamma$ . We should noted that it remains unclear whether CD27 signaling directly induces the Th2 cells. Our previous study indicated that OX40 plays a critical role in Th2 development in experimental leishmaniasis (15). Therefore, some other signals may be required for the development of Th2 cells in addition to the CD27 signal. On the other hand, anti-CD70 was reported to inhibit the priming of CD4 and CD8 T cells localized in the gut mucosa in response to infection with *Listeria monocytogenes* (33). The priming of these T cells depends on a unique population of APCs in the lamina propria that expresses high levels of CD70. In the spleen, CD70 plays an important role in the DEC-205<sup>+</sup> dendritic cell (DC) subset (30). DEC-205 belongs to the mannose receptor family, and behaves as an antigen uptake/processing receptor for DCs (34). When antigen was targeted specifically to the DEC-205<sup>+</sup> DC subset by anti-DEC-205 mAb, the priming and IFN- $\gamma$  production of cognate CD4 T cells were independent of IL-12, and fully depended on CD70 expression in this DC subset. In contrast, when the same antigen was targeted to another DC subset expressing a distinct uptake receptor, DCIR2, recognized by the 33D1 mAb (35), Th1 differentiation did not depend on CD70, but required IL-12 (30). Therefore, the CD70-CD27 interaction may be sufficient to induce CD4 T-cell differentiation to effector T cells in particular circumstances. The critical APC expressing CD70 for the development of Th2 in response to OVA/alum remains to be identified in future studies.

CD27-deficient mice infected with influenza virus have shown a reduced number of CD4 and CD8 effector cells that infiltrate the lung (36). CD27 was not required for entry into the cell cycle upon T-cell activation, or for differentiation into IFN- $\gamma$ -producing or cytolytic effector T cells. Instead, CD27 promoted the survival of activated T cells throughout successive rounds of division, and contributed to the accumulation of effector T cells in the lung (37). In the present study, the anti-CD70 mAb treatment during the induction phase of allergic lung inflammation significantly reduced the accumulation of lymphocytes in BAL fluid. Thus, the amelioration of lung inflammation by anti-CD70 mAb treatment may result from the suppression of not only the differentiation of pathogenic Th2 cells, but also the accumulation of newly activated CD4 T cells. Further studies are needed to address these possibilities.

In conclusion, a potent inhibition of the inflammatory response in a murine model of allergic lung inflammation was achieved with the administration of anti-CD70 mAb. The CD70-CD27 interaction appears to play a critical role in the development of pathogenic Th2 cells, and thus represents a novel target for intervention during allergic lung inflammation in humans.

**Author disclosures** are available with the text of this article at [www.atsjournals.org](http://www.atsjournals.org).

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