Inducible Costimulator Regulates Th2-Mediated Inflammation, but Not Th2 Differentiation, in a Model of Allergic Airway Disease

Amanda G. Tesciuba, Sumit Subudhi, Russell P. Rother, Susan J. Faas, Aric M. Frantz, David Elliot, Joel Weinstock, Louis A. Matis, Jeffrey A. Bluestone and Anne I. Sperling

doi: 10.4049/jimmunol.167.4.1996

http://www.jimmunol.org/content/167/4/1996

**References**

This article cites 30 articles, 15 of which you can access for free at:
http://www.jimmunol.org/content/167/4/1996.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Inducible Costimulator Regulates Th2-Mediated Inflammation, but Not Th2 Differentiation, in a Model of Allergic Airway Disease

Amanda G. Tesciuba,*† Sumit Subudhi, Russell P. Rother, Susan J. Faas, Aric M. Frantz, David Elliot, Joel Weinstock, Louis A. Matis, Jeffrey A. Bluestone,*‡ and Anne I. Sperling*‡

A novel member of the CD28 family, inducible costimulator (ICOS), has been identified by Kroczek and colleagues (1). ICOS, a homodimer on the surface of T cells, shares 24% amino acid identity with CD28 and 17% identity with CTLA-4. Like CTLA-4, ICOS is expressed on activated cells (1, 2). In addition, ICOS, CD28, and CTLA-4 share several structural (Ig-like folds) and functional similarities (e.g., phosphatidylinositol 3-kinase binding sequence) (1) and are genetically linked, mapping to the same chromosome (chromosome 1 in mice and chromosome 2 in humans) (2). ICOS has been shown to have potent costimulatory effects on T cell proliferation and cytokine production (1, 3). Similar to CD28, ICOS-mediated costimulation can induce human peripheral-blood CD4+ T cells to increase IL-4, IL-5, IL-10, IFN-γ, TNF-α, and GM-CSF secretion. However, unlike CD28, ICOS costimulation has not been shown to dramatically augment IL-2 production (1, 4). The initial studies on ICOS demonstrated the absence of the B7-binding MYIYYY motif that is found in CD28 and CTLA-4, suggesting that ICOS was not a receptor for B71/B72. Subsequently, it has been shown that a novel B7-like molecule termed B7-related protein-1 (B7RP-1) (also referred to as B7h, GL50, and LICOS; Refs. 2 and 5–8) binds to ICOS. B7RP-1 shares ~20% identity with B71/B72 and is expressed on both normal and sensitized APCs, whereas B71/2 molecules are up-regulated on activated cells.

The majority of naive T cells will express ICOS after activation. However, Th2 cells continue to express ICOS, whereas Th1 cells have decreased expression (9, 10). Studies have suggested that signaling through ICOS may induce Th2 cell differentiation (1, 9, 11–13), and in vivo analysis from several groups demonstrate that ICOS-mediated costimulation may also regulate Th2 effector cells (10, 14). In the present study, we have examined the role of ICOS in a Th2-dependent model of allergic airway disease (AAD) induced by sensitization with inactivated Schistosoma mansoni eggs and local antigenic challenge with S. mansoni soluble egg Ag (SEA) in the lungs. Injection of S. mansoni eggs i.p. has been shown to result in early T cell-independent IL-4 production within the periphery (15, 16). As activated precursor T cells expand, this IL-4 production leads to polarization toward a Th2 lineage. These T cells can be found in both the primary and secondary lymphoid organs and produce Th2 cytokines when restimulated with SEA in culture (17). In addition, a strong IgE response, a characteristic trait of parasitic infections, is induced by S. mansoni egg injection (17). In our model of AAD, we use the Th2-skewing effect of S. mansoni eggs by immunizing with inactivated eggs that do not induce an infection. Following challenge in the lungs with purified SEA, local cells respond by making chemokines and cytokines that in turn cause Th2 cells to enter the site of challenge (18). Cytokines, such as IL-5, lead to the eosinophilia, which is a hallmark of allergic airway inflammation. Other physiologic features of AAD include goblet cell hyperplasia, smooth muscle hyperplasia, and...
excess mucous production within the lungs (19). We have shown that both the initial sensitization with *S. mansoni* eggs and the subsequent challenge with SEA are necessary to achieve eosinophilic inflammation in the lung (20). However, IgE levels are increased following i.p. sensitization alone, suggesting that activated systemic Th2 cells provide help for B cells.

In previous studies, we demonstrated that CD28 blockade inhibits Th2-mediated inflammation by skewing the response normally made to *S. mansoni* from a Th2 phenotype toward a Th1 phenotype (20). These results suggested that CD28 signaling was important during the primary immune response. Thus, it was of interest to examine the effect of ICOS blockade on the response made during primary stimulation. To test whether ICOS/B7RP-1 interactions are required for promoting Th2 inflammatory responses, mice received an i.p. injection of ICOS-Ig, a soluble form of ICOS, during sensitization and challenge. In this system, we found decreased airway inflammation in ICOS-Ig-treated mice as compared with control mice. Interestingly, unlike CTLA4-Ig treatment, no change in T-cell priming or differentiation was observed when mice are treated with ICOS-Ig. Cytokine production from restimulation of ex vivo Th2 effector lung cells was inhibited by ICOS-Ig. These results suggest that ICOS-mediated costimulation may regulate Th2 effector cell function without affecting Th2 differentiation to a Th2-type antigenic stimulation.

**Materials and Methods**

**Mice**

Female 4- to 6-wk-old C57BL/6 mice were purchased from The Division of Cancer Treatment at the National Cancer Institute (Frederick, MD). Animals were housed in a specific pathogen-free biohazard level 2 facility maintained by the University of Chicago Animal Resources Center (Chicago, IL). The studies detailed herein conformed to the principles set forth by the Animal Welfare Act and the National Institutes of Health guidelines for the care and use of animals in biomedical research.

**Cloning of ICOS and production of rICOS-Ig**

A partial amino acid sequence containing homology with CD28 and CTLA-4 was obtained by searching the murine expressed sequence tag database with CD28 or CTLA-4 protein sequences as queries using the tblastn program found on the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/). Two gene-specific PCR primers were designed based on the expressed sequence tag nucleic acid sequence, and 5' and 3' RACE were performed on mouse spleen Marathon-Ready cDNA as described in the manufacturer's protocol (Clontech, Palo Alto, CA). PCR products were cloned into the pcR2.1-TOPO vector using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) and sequenced by the DNA Sequencing and Synthesis Facility (Iowa State University, Ames, IA). Full-length ICOS cDNA was obtained by PCR using the mouse spleen Marathon-Ready cDNA as a template and primers that flanked the start and stop codons of the molecule. The construct for ICOS-Ig was made by fusing the extracellular region of murine ICOS (1–427 bp) to the CH2 and CH3 regions of murine IgG2a Fc in the eukaryotic expression vector pCDNA3.1*. The construct was expressed in HEK 293/TSA-O-A cells. Cells were maintained in a RESCU-Primer bioreactor (Cellex Biosciences, Minneapolis, MN) and grown in 1.25% complete medium (DMEM, 1.25% Minnisotra, MN) and 25 mM HEPES, 2 mM L-glutamine, 100 U/ml pen-streptomycin, 2 mM nonessential amino acids, and 5 × 10⁻⁸ M β2-ME). ICOS-Ig was purified by passing the harvest supernatant over a protein A column (Repligen, Boston, MA) using standard techniques.

**Ag sensitization and challenge**

Inactivated *S. mansoni* eggs and Ag were prepared as previously described (21, 22). The protocol used for animal sensitization and challenge was modified from that of Padrid et al. (20) and Lukacs et al. (23). Briefly, mice were immunized i.p. on day 0 with 5 × 10⁵ inactivated *S. mansoni* eggs. Mice were challenged on day 7 via intratracheal delivery of 10 µg SEA. Previously, we have found that AAD is not induced in mice left unchallenged or challenged with PBS only (20). Where indicated, mice received an i.p. injection of either 50 µg ICOS-Ig, 100 µg ICOS-Ig, 1× sterile PBS, or 50 µg IgG2a (Southern Biotechnology Associates, Birmingham, AL) beginning the day before *S. mansoni* egg sensitization (day –1) and every other day thereafter (days 1, 3, 5, 7, and 9) until animals were sacrificed on day 11.

**Bronchoalveolar lavage (BAL)**

Mice were sacrificed on days 4–6 after challenge via i.p. injection of 30 mg/mouse ketamine-HCl. We have found that airway inflammation peaks on these days in our model and is completely resolved by 14 days after intratracheal challenge (A. M. Frantz and A. I. Sperling, unpublished observations). BAL was performed by delivering 0.8 ml cold PBS into the airway via a trachea cannula and gently aspirating the fluid. The lavage was repeated three times to recover a total volume of 2–3 ml. The cells were stained with trypan blue to determine viability, and total nucleated cell counts were obtained using a hemocytometer. Cytospin slides were prepared from the BAL and were then fixed and stained using Diff-Quick (Dade Diagnostics, Aguada, PR). Differential cell counts were determined by counting a minimum of 200 cells/slide using standard morphological criteria. The total number of eosinophils, macrophages, and lymphocytes were calculated as follows: (total number of nucleated cells within airway) × (percentage of cell type within airway). The percentage of cell types found within the BAL fluid was calculated as follows: (number of cell type/total number of cells within cytospin).

**Histology**

A portion of the lung was fixed in 4% paraformaldehyde. Lobes were sectioned sagittally, embedded in paraffin, cut into 5-µm sections, and stained with H&E for routine analysis. Scoring was based on the intensity of inflammation on a scale of 0–5, with 0 being negative for airway inflammation and 5 being the most severe. Lungs were considered inflamed if the following were detected: eosinophilic and lymphocytic infiltration around bronchioles and vessels, as well as goblet-cell hyperplasia and smooth muscle thickening. The slides were coded and scored blindly to prevent bias.

**Isolation of lung lymphocytes**

Lungs were disassociated by agitating the tissue for 1 h in 20 ml digestion buffer (hyaluronidase at 85 U/ml Sigma, St. Louis, MO), DNaasel at 50 U/ml (Boehringer Mannheim, Mannheim, Germany), and collagenase P at 1.0 mg/ml (Boehringer Manheim). The digest was passed through nylon filter, and RBCs were depleted with ammonium chloride-potassium lysing buffer. To enrich for lymphocytes, cells were centrifuged through a Percoll step gradient (Amersham Pharmacia Biotech, Uppsala, Sweden). The cells at the 50–75% interface were collected and washed in 5% complete medium.

**Lung lymphocytes and splenocyte restimulation ex vivo**

Cells were plated at 2 × 10⁶ cells/well (lung cells) or 4 × 10⁶ cells/well (splenocytes) in a 96-well U-bottom plate. T cell-depleted, irradiated splenocytes (APCs) were mixed with lung cells at a 1:1 ratio. Cells were cultured with SEA (10 µg/ml), IgG2a (20 µg/ml), or ICOS-Ig (20 µg/ml) as indicated in the text. The final volume of all wells was 200 µl. Plates were incubated for 48 h, after which time the supernatants were collected for cytokine analysis by ELISA. Percentage of inhibition of cytokine production in the presence of ICOS-Ig or in the presence of an isotype control was calculated by first subtracting the spontaneous cytokine production from the levels with SEA and then determining the percentage of inhibition with ICOS-Ig or control IgG2a. To assess proliferation, additional plates were pulsed with 1 µCi/well of [³H]thymidine at 48 h and harvested 8–12 h later.

**Cytokine analysis**

Cytokine production by lung lymphocytes and splenocytes was measured by ELISA according to the manufacturer’s protocol (BD Pharmingen, San Diego, CA). Sera were collected by cardiac puncture and used to measure total serum IgE levels by ELISA (BD Pharmingen).

**Statistical analysis**

All statistics were done using an unpaired Student two-tailed t test. Error bars represent SEM.

**Results**

**ICOS-Ig treatment decreases the severity of Th2-mediated airway inflammation in C57BL/6 mice**

To induce airway inflammation, female C57BL/6 mice were sensitized i.p. on day 0 with 5 × 10⁵ inactivated *S. mansoni* eggs, and...
on day 7, the mice were challenged with 10 μg SEA delivered by intratracheal instillation. In addition, mice were treated with nothing, PBS (data not shown), 50 μg ICOS-Ig, or with an IgG2a isotype control on days -1, 1, 3, 5, 7, and 9. Throughout our studies, we found that treatment with nothing, PBS, or an IgG2a isotype control gave similar results (Fig. 1A and data not shown); therefore, not all controls were used for each experiment. On day 11, the animals were sacrificed and analyzed for airway inflammation. Total nucleated cell counts were determined from the BAL fluid recovered from the airways. A 66–73% decrease in the severity of inflammation within the airways was found in ICOS-Ig-treated animals compared with control Ig-treated animals and untreated animals, respectively (Fig. 1A). Thus, blockade of ICOS-mediated costimulation dramatically reduced cellular infiltration of cells into the airways of inflamed lungs.

We further examined the extent of airway inflammation in the mice by determining the cellular populations within the airways. When BAL is performed on mice that have been left unsensitized and unchallenged or on mice that have been only sensitized or only challenged, macrophages are the predominant cell type found in the BAL fluid. Mice that have been both sensitized and challenged (SCH) typically have a high level of eosinophilia in the BAL, an increased number of lymphocytes, and a small percentage of macrophages. The observed eosinophilia has been shown to depend on the local production of Th2 cytokines, such as IL-4, IL-5, and IL-13 (24–27). In the present study, ICOS-Ig-treated mice were found to have significantly fewer eosinophils (75% decreased; p < 0.0001) and lymphocytes (59% decreased; p < 0.001) than untreated animals (Fig. 1B). The number of macrophages followed the trend of decreased cell number (39% decrease), although the difference was not statistically significant compared with control mice. These data are supported by the finding that local IL-5 production measured in the BAL fluid from SCH mice was lower in the ICOS-Ig-treated group vs the control group (Fig. 1C).

Although the total number of cells was decreased, the percentages of eosinophils, macrophages, and lymphocytes found within the BAL of ICOS-Ig-treated mice were similar to those found in the BAL of control-treated animals (Fig. 1D). These results are in contrast to previous observations in a similar experimental model system, where treatment with CTLA4-Ig not only blocked total inflammation, but also significantly lowered the relative percentages of eosinophils found while increasing the number of lymphocytes (20). Thus, treatment with ICOS-Ig did not selectively alter the Th2 phenotype of the inflammatory response, but rather diminished the severity of the inflammation.

The attenuated severity of inflammation in ICOS-Ig-treated animals was further supported by experiments to evaluate inflammation by histological analysis of lung sections (Fig. 2). Lung sections were scored for infiltration based on a system previously described in detail by Padrid et al. (20). Briefly, the lungs are scored on a scale of 0–5, in which a score of 0 was given when there is no inflammation, and a score of 5 was given when massive infiltration is found around both the vessels and the bronchioles. Because inflammation can vary from lobe to lobe, each lobe was scored separately, and an average inflammatory score was obtained for the entire lung tissue. Both control and ICOS-Ig-treated lungs

![Figure 1](http://www.jimmunol.org)
displayed characteristics typical of lung inflammation, including eosinophilic and lymphocytic infiltration around bronchioles and vessels as well as goblet-cell hyperplasia and smooth muscle thickening (data not shown). However, the extent of infiltration was markedly reduced in ICOS-Ig-treated lungs as compared with those from control-treated mice.

Blocking ICOS-B7RP-1 interactions does not prevent Ag-specific T cell priming or Th2 cell differentiation

One possible explanation for the observed decrease in inflammation with ICOS-Ig treatment is that blockade of ICOS-mediated costimulation during sensitization inhibits T cell priming to Ag. Alternatively, the blockade of ICOS costimulation may lead to the induction of T cell anergy. To investigate these possibilities, splenocytes from mice SCH in the presence or absence of ICOS-Ig were examined for their ability to respond to restimulation with SEA ex vivo. Total splenocytes from SCH mice were cultured either with SEA or medium alone, and proliferation was measured by [3H]thymidine uptake. No differences in proliferation were observed (Fig. 3A). These data suggest that ICOS-Ig treatment did not induce T cell anergy, nor did it block T cell priming to SEA.

Although ICOS-Ig treatment did not block the priming of T cells to Ag, it may have altered T cell differentiation into Th2 cells, as is seen when mice are treated with CTLA-4-Ig (20). To determine whether or not ICOS-Ig prevented Th2 differentiation in response to SEA, Th2-type cytokine production was examined ex vivo using T cells isolated from control and ICOS-Ig-treated animals. Splenocytes were prepared from SCH mice 4 days after the final antigenic challenge and stimulated with SEA in vitro as described above. Supernatants from the cultures were harvested at 48 h and tested for cytokine content by ELISA. No difference in IL-5, IL-4, or IL-10 production was observed between mice that were treated with ICOS-Ig and control mice (Fig. 3B). Together, these results demonstrate that blockade of ICOS-mediated costimulation throughout sensitization did not affect the ability of systemic T cells to be activated by Ag or to differentiate into Th2-type cells.

Blockade of ICOS costimulation does not affect serum IgE levels

In addition to eosinophilia, elevated levels of serum IgE are indicative of a Th2 response to S. mansoni eggs. However, whereas the number of eosinophils was decreased by ICOS-Ig treatment, there was no statistically significant difference in the levels of IgE in ICOS-Ig-treated vs control animals (Fig. 4). Nevertheless, ICOS-Ig-treated mice were found to have reduced levels of IgE (3000 vs 4700 ng/ml in control-treated mice). These data suggest that B cells are still capable of being activated during the primary response; thus, Ig class switching is not prevented in the absence of ICOS signaling.

ICOS costimulation regulates cytokine production by effector Th2 cells

The results presented thus far have demonstrated that ICOS-mediated costimulation is not required for development of Th2 immune responses following the initial sensitization to S. mansoni eggs. Rather, our data revealed that ICOS-Ig treatment severely inhibits airway inflammation, suggesting that ICOS-mediated costimulation may play a role in the function of differentiated Th2 cells in the present model of AAD. To test whether T cells that make it to the lung tissue under ICOS blockade are able to produce Th2 cytokines, T cells isolated from ICOS-Ig-treated and untreated inflamed lung tissue were restimulated in vitro with SEA. We found that ex vivo restimulation of T cells from the inflamed lungs of ICOS-Ig-treated mice resulted in the production of significant amounts of the Th2 cytokine IL-5 (Fig. 5). There was no increase in the Th1-type cytokine IFN-γ, which remained below the level of detection (data not shown). These data suggest that S. mansoni-specific Th2 cells that have migrated to the lungs in response to challenge are capable of effector functions. However, in the absence of ICOS signaling, far fewer cells successfully enter the lungs following secondary stimulation with Ag (Fig. 1B).

Taken together, the data suggest that the ICOS-B7RP-1 pathway may be important in regulating effector Th2 function, in particular cytokine production, once cells reach the site of challenge. To investigate this, mice were SCH as described earlier, but in the absence of ICOS-Ig treatment. On day 11, the SCH mice were sacrificed, and lung lymphocytes were enriched for in vitro studies. Restimulation of the lung lymphocytes with SEA for 48 h demonstrated an Ag-specific increase of the Th2 cytokines IL-4, IL-5, and IL-10, whereas IFN-γ was undetectable (Fig. 6 and data not shown). This Ag-specific response was strongly inhibited by the addition of ICOS-Ig to the culture, but not by the presence of an irrelevant IgG2a mAb. Therefore, ICOS-mediated costimulation regulates Th2 cytokine production upon restimulation with Ag.

We also observed this effect in splenocytes isolated from these mice (data not shown), suggesting that the effect of ICOS-B7RP-1 blockade is not tissue specific, but rather, that ICOS signaling is generally important for the effector function of Th2 cells.

Discussion

In the current study, the role of ICOS in the development of a Th2 response was evaluated in an animal model of AAD. ICOS-Ig administration throughout the entire sensitization and challenge period resulted in no alterations in the primary immune response to S. mansoni; proliferation and cytokine production of splenocytes from ICOS-Ig-treated or untreated mice were similar upon restimulation in vitro (Fig. 3, A and B). In contrast to recent reports (1, 9), these results suggest that ICOS-mediated costimulation does not alter the ability of precursor Th2 cells to proliferate and differentiate into Th2 effector cells. It is possible that, because S. mansoni is a potent Th2-inducing Ag, the blockade of ICOS-B7RP-1 interactions may not be strong enough to prevent the development of Th2 cells in our in vivo model. It is also possible that the ICOS-Ig used in these experiments did not completely prevent the interaction between ICOS and B7RP-1. However, ICOS-B7RP-1 blockade did lead to a reduction in the number of Th2 cells and eosinophils infiltrating the lungs following local challenge with
SEA. These data suggest that Th2 function such as cytokine production and/or Th2 migration may be more sensitive to ICOS blockade than to Th2 differentiation. The decrease in IL-5 production could, for instance, explain the decrease in eosinophilia within the inflamed lungs, whereas a decrease in responsiveness to chemokines could explain the decrease in Th2 cells that are recruited to the lung following challenge. Together, these events may offer an explanation for the decreased inflammation and lung eosinophilia observed in ICOS-Ig-treated mice.

Serum IgE levels, which are increased due to *S. mansoni* infection, were not significantly decreased in ICOS-Ig-treated mice (Fig. 4). We and others have found that high serum IgE levels can be found in mice that have been sensitized but not challenged (data not shown and Ref. 17), suggesting that much of the IgE response can be attributed to the primary response to *S. mansoni* eggs. Thus, the inability of ICOS-Ig to block IgE production is consistent with our findings that ICOS costimulation is not required for the primary Th2 response to *S. mansoni* eggs. Likewise, Coyle et al. (10) have shown that ICOS blockade does not inhibit Ig production upon primary stimulation. Similarly, Kopf et al. (14) found that there was no inhibition of IgG subclasses when mice infected with lymphocytic choriomeningitis virus were treated with ICOS-Ig during the course of the infection. Although Hutloff et al. (1) found that ICOS-B7RP-1 interactions induced up-regulation of CD40 ligand on T cells, and Coyle et al. (10) showed that ICOS blockade partially inhibits some isotypes after secondary immunization, our data support recent studies that suggest that this signaling cascade is not exclusively required for T cell help of Ig switching and production.

Recent reports have shown a defect in the ability of ICOS<sup>−/−</sup> mice to mount a Th2 response (11–13). In particular, IL-4 and IgE production seem to be defective in culture and in vivo, whereas IFN-γ is either augmented or equal in the ICOS<sup>−/−</sup> mice. The defect in Th2 differentiation could be overcome in part by the
addition of exogenous IL-4 in vitro or by the use of a strong adjuvant in vivo. In our model, we use a strong Th2-inducing Ag. In addition, it has been found that *S. mansoni* egg injection into the peritoneal cavity results in early IL-4 production that is not due to CD4+ T cells, and in fact, may be the result of an innate immune response by mast cells to the parasitic Ag (15). This early, “exogenous” IL-4 may serve to skew CD4+ T helper precursors toward a Th2 phenotype and account for the development of Th2 cells in the absence of ICOS signaling. Our data suggest that although differentiation of Th2 cells can be accomplished in the absence of ICOS signaling, the requirement for ICOS costimulation for Th2 effector function is more stringent.

Blockade of ICOS signaling resulted in decreased severity of inflammation in our model. Interestingly, although the total number of cells recovered from the airway was reduced in the ICOS-Ig-treated group, the percentage of the various cell populations recovered from the BAL did not differ from that of control mice (Fig. 1D). These results differ from experiments that examined the role of CD28-mediated costimulation in airway inflammation. In those studies, the airway inflammation was not only reduced, but the cellular composition of the inflammation was significantly altered (20); CTLA4-Ig treatment led to a decrease in eosinophil infiltration with a concomitant increase in lymphocytic infiltration. In addition, restimulated lung T cells exhibited reduced Th2 cytokine production, whereas Th1 (IFN-γ) cytokine production was enhanced (20). In contrast, treatment of mice with ICOS-Ig did not change the cytokine profile produced by the lung lymphocytes upon ex vivo restimulation when the total T cell numbers in each well were equalized between samples (Fig. 5). Thus, our data suggest that Th2 development was not affected by ICOS blockade. This finding is in direct contrast to those presented by Kopf et al. (14). In those studies, ICOS-Ig treatment was found to cause a slight decrease in the development of Th2 cells in response to the nematode *Nippostrongylus brasiliensis*. It should be noted that the two systems differ in many ways, such as the types of Ag used, as well as in the administration of ICOS-Ig (i.v. vs i.p.).

Th2 effector functions, such as cytokine production, were shown to be dependent on ICOS-mediated costimulation (Fig. 6). This suggests that Th2 cells that successfully migrate to the site of challenge upon restimulation with Ag may produce less cytokines in the absence of ICOS signaling. Decreased cytokine production...
in the lungs by the residual Th2 cells could have a profound effect on the recruitment of additional Th2 cells as well as eosinophils into the tissue. Li et al. (18) have shown that supernatant from Th2 cells, when instilled directly in to the lungs, can induce chemokine production and the recruitment of eosinophils. IL-13, for example, can induce eotaxin expression by epithelial cells, and local production of IL-5 has been demonstrated to be necessary for effective migration of eosinophils (18, 28). Thus, blockade of ICOS-B7RP-1 interactions may result in fewer cytokine-producing T cells migrating into the lung tissue and, therefore, to a decrease in eosinophilia. However, the effect of ICOS-Ig on cytokine production that was observed in vitro may not fully correspond to an in vivo situation. For instance, in vivo ICOS-Ig may be less potent due to protein degradation or to a lower “final” concentration, whereas, in vitro, these issues are less of a concern. Furthermore, we cannot rule out the possibility that the observed decrease in cellular infiltration (Fig. 1B) is due to Th2 cells that may be less receptive to chemokines. Studies have shown differences in the ability of Th1/Th2 cells to respond to chemokines. For example, CCR7, which recognizes secondary lymphoid chemokine and EB11-ligand chemokine, is specifically expressed on Th1 cells (29), whereas CCR3 and CCR4 have been implicated in the recruitment of Th2 cells to the lungs during allergic inflammation (30). Thus, it is possible that ICOS signaling is important for the up-regulation of specific Th2 chemokine receptors.

Coley et al. (10) have found that ICOS-Ig treatment of mice in an adoptive transfer model of airway inflammation effectively blocked the Th2-type eosinophilic, but not the Th1-type neutrophilic, inflammatory response. In their model, the T cells were first differentiated in vitro. The resulting Th1 and Th2 populations were independently transferred into naive BALB/c recipients that were then challenged with inhaled Ag. Their finding that ICOS signaling is important in the migration and/or function of these effector Th2 cells supports our findings. We have further demonstrated that ICOS blockade can significantly decrease inflammation without affecting Th2 differentiation. Together, these data suggest that ICOS signaling may play a more important role in effector function than in Th2 differentiation.

In contrast to the findings presented herein and the studies of Coyle and colleagues, Dong et al. (11) found that the absence of ICOS (in ICOS−/− mice) did not lead to a significant reduction in the number of eosinophils and lymphocytes within the airways of mice with OVA-induced AAD. T cells from the mediastinal lymph nodes made less IL-4 and IL-13 when restimulated, as compared with wild-type cells, whereas no defect in IL-5 production was noted. The reasons for the difference between the results with the ICOS−/− mice and the ICOS-Ig administration remain to be determined. It is possible that the effect of blocking ICOS function on normally differentiated ICOS-sufficient Th2 cells may be completely different from what is seen in the ICOS−/− mice. In these mice, the “Th2-type” response is abnormal because the T cells make no IL-4, little IL-13, and normal levels of IL-5. These cells may not be conventional Th2 cells, but an alternative type of response that is not normally predominant in the presence of ICOS signaling. Additional experiments are required to definitively resolve these apparently contradictory findings.

Our data have important implications for the treatment of patients with asthma or with other Th2-related conditions. As opposed to immune suppressants that disrupt all immune responses, ICOS blockade may only affect migration and effector function of Ag-specific Th2 cells activated during secondary stimulation in an allergic response. Thus, our findings that ICOS and B7RP-1 are involved in sustaining a Th2 response during re-stimulation may lead to the development of useful therapeutics for Th2 diseases.

Acknowledgments

We thank Drs. Philip Padrid, Dayang Wu, Rebecca Shilling, and Diwakar Balachandran for their contributions to these studies. We also thank Donna Decker, Gregory Szot, Michelle Giannoni, and Cheri Kresecker for technical assistance.

References


20. Padriz, P. A., M. Mathur, X. Li, K. Herrmann, Y. Qin, A. Cattamanchi, J. Weinstock, D. Elliott, A. I. Sperling, and J. A. Bluestone. 1998. CTLA4Ig inhibits airway eosinophilia and hyperresponsiveness by regulating the develop-


