

B7RP-1 Is Not Required for the Generation of Th2 Responses in a Model of Allergic Airway Inflammation but Is Essential for the Induction of Inhalation Tolerance¹

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The recently described ICOS-B7RP-1 costimulatory pathway has been implicated in the generation of effector Th2 responses and, hence, has become an attractive therapeutic target for allergic diseases. In the present study, we used B7RP-1-deficient mice to investigate the role of B7RP-1 in the generation and maintenance of Th2 responses in a model of mucosal allergic airway inflammation. We found that exposure of B7RP-1 knockout mice to aerosolized OVA in the context of GM-CSF leads to airway eosinophilic inflammation. This response was long lasting because rechallenge of mice with the same Ag recapitulated airway eosinophilia. Moreover, significant expression of T1/ST2 on T cells and production of Th2-affiliated cytokines (IL-5, IL-4, and IL-13) and Igs (IgE and IgG1) conclusively demonstrate the generation of a Th2 response in the absence of B7RP-1. In addition, expression of two major Th2-associated costimulatory molecules—CD28 and ICOS—indicates T cell activation in the absence of B7RP-1 signaling. Finally, B7RP-1 knockout mice are resistant to the induction of inhalation tolerance as indicated by the sustained eosinophilia in the lung and IL-5 production. In summary, our results demonstrate that in a model of mucosal allergic sensitization, the ICOS-B7RP-1 pathway is redundant for the generation of Th2 responses but essential for the induction of inhalation tolerance. *The Journal of Immunology*, 2005, 174: 3000–3005.

Optimal T cell activation requires TCR engagement with peptide-MHC on APC and a second Ag-independent signal referred to as a costimulatory signal (1, 2). The importance of costimulatory pathways in both the generation and control of immune responses is undisputed. In particular, various costimulatory pathways such as CD28-B7, OX40-OX40L, and CD40-CD40L pathways appear to play important roles in different phases of the generation of Th2 responses (2, 3). Recently, new costimulatory pathways have been discovered, including ICOS-ICOSL (4, 5). ICOS interaction with its ligand ICOSL (B7RP-1, LICOS, B7h, B7H2, and GL50) has been implicated in the development of Th2 effector activity because disruption of this interaction results in the attenuation of Th2 responses both in vitro and in vivo (6, 7). The most persuasive evidence has been obtained in mice with a genetically disrupted ICOS gene. Indeed, ICOS-deficient mice are characterized by impaired germinal formation, have a profound defect in isotype class switching in T cell-dependent B cell responses, and are defective in IL-4 and IL-13 production (8, 9). Furthermore, blockade of ICOS in a conventional model of allergic sensitization involving i.p. sensitization before airway

challenge attenuated eosinophilic accumulation in the lungs (10). Although it has been argued that ICOS is involved primarily in Th2-mediated effector functions rather than in Th2 differentiation per se (11, 12), others have proposed that B7RP-1 is essential for both Th2 differentiation and effector function (13, 14). Moreover, ICOS has also been implicated in inhalation tolerance, suggesting that this costimulatory pathway may be involved in regulating lung homeostasis (15).

The objective of this study was to investigate the role of B7RP-1 in a model of mucosal allergic sensitization. To this end, we subjected mice deficient in B7RP-1 to a protocol that involves repeated aerosolization of Ag (OVA) in the context of a GM-CSF-rich airway environment achieved by the intranasal delivery of a replication-deficient adenoviral (Ad)³ vector carrying the GM-CSF transgene (16). Expression of GM-CSF allows for allergic mucosal sensitization under conditions that otherwise lead to the induction of inhalation tolerance (17). The ensuing inflammatory response is characterized by airway and lung eosinophilia and expression of Th2 cytokines and OVA-specific IgE, all hallmarks of allergic asthma. That GM-CSF is expressed in allergic disease, including asthma and allergic rhinitis, attests to its relevance in the experimental protocol (18). Our data demonstrate that the absence of B7RP-1 does not impede the generation of activated T cells expressing CD28, ICOS, and the Th2-effector cells' marker T1/ST2. In addition, B7RP-1-deficient mice are able to produce Th2-affiliated cytokines and Igs. Moreover, the development of Th2-type inflammatory responses in the airway either in the primary or secondary phase is completely intact in B7RP-1-deficient mice. Interestingly, B7RP-1 knockout (KO) mice generated an enhanced Th2 immune response, as compared with littermates, which could

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³ Abbreviations used in this paper: Ad, adenoviral; KO, knockout; i.n., intranasally; BAL, bronchoalveolar lavage; WT, wild type; DC, dendritic cell.

suggest the impairment in the maintenance of lung homeostasis. Indeed, B7RP-1 KO mice were unable to establish inhalation tolerance and instead developed eosinophilia and produced IL-5. In summary, our data indicates the redundancy of B7RP-1 in the establishment of Th2 responses in a mucosal model of allergic inflammation but the essential role of this molecule interaction with its ligand for the generation of inhalation tolerance.

Materials and Methods

Animals

B7RP-1-deficient mice and control littermates were generated as described before (13) and obtained from Dr. T. Mak (University of Toronto, Toronto, Ontario, Canada). Mice were housed in a specific pathogen-free environment following a 12-h light-dark cycle. All experiments performed were approved by the Animal Research Ethics Board of McMaster University.

Model of respiratory mucosal allergic sensitization

As previously described, a replication-deficient human type 5 Ad construct encoding murine GM-CSF cDNA in the E1 region of the viral genome (Ad/GM-CSF) was delivered intranasally (i.n.) 24 h before the first OVA exposure. Ad/GM-CSF was administered i.n. at a dose of 3×10^7 PFU in 30 μ l of PBS vehicle (two 15- μ l administrations, 5 min apart) into anesthetized animals. Over a period of 10 consecutive days (days 0–9), mice were placed in a Plexiglas chamber (10 $\times$ 15 $\times$ 25 cm) and exposed for 20 min daily to aerosolized OVA (1% w/v in 0.9% saline). OVA aerosol was generated by a Bennet nebulizer at a flow rate of 10 L/min. Inhalation tolerance was induced by exposing animals to OVA only. For in vivo rechallenge with OVA, mice were re-exposed to a 1% OVA aerosol for 20 min daily for 3 consecutive days, following complete resolution of initial airways inflammation.

Tolerance induction

Mice were placed in a Plexiglas chamber, as described before, and exposed for 20 min daily over a period of 10 consecutive days to aerosolized OVA (1% w/v in 0.9% saline) (17, 19). Control mice were exposed to saline only. Two days after tolerance induction, mice were subjected to respiratory mucosal allergic sensitization.

Collection and measurement of specimens

At various time points, mice were killed and bronchoalveolar lavage (BAL) was performed according to a standard protocol (16). Briefly, the lungs were dissected and the trachea was cannulated with a polyethylene tube (BD Biosciences). The lungs were lavaged twice with PBS (0.25 ml followed by 0.2 ml); $\sim$ 0.3 ml of the instilled fluid was recovered consistently. Total cell counts were determined using a hemocytometer. Cell pellets were resuspended in PBS, and smears were prepared by cytocentrifugation (Thermo Shandon) at 300 rpm for 2 min. Hema 3 (Biochemical Sciences) was used to stain all smears. Differential counts of BAL cells were determined from at least 500 leukocytes using standard hemocytological criteria to classify the cells as neutrophils, eosinophils, lymphocytes, or macrophages/monocytes. Additionally, blood was collected by retroorbital bleeding. Serum was obtained by centrifugation after incubating whole blood for 30 min at 37°C. Finally, lung tissue was fixed in 10% formalin and embedded in paraffin. Three-micrometer thick sections were stained with H&E.

Splenocyte culture

Spleens were harvested into sterile tubes containing sterile HBSS (Invitrogen Life Technologies). Tissue was triturated between the ends of sterile,

frosted slides, and the resulting cell suspension was filtered through nylon mesh (BSH Thompson). RBC were lysed with ACK lysis buffer (0.5 M NH_4Cl , 10 mM KHCO_3 , and 0.1 mM Na_2EDTA at pH 7.2–7.4). Remaining splenocytes were washed twice with HBSS and then resuspended in RPMI supplemented with 10% FBS (Invitrogen Life Technologies), 1% L-glutamine, and 1% penicillin/streptomycin. Cells were cultured in medium alone or with 40 μ g of OVA/well at 8×10^5 cells/well in a flat-bottom, 96-well plate (BD Biosciences). After 5 days of culture, supernatants were harvested for cytokine measurements.

Cytokine and Ig measurement

ELISA kits for murine IL-13, IL-4, IFN- γ , and IL-5 were purchased from R&D Systems. Each of these assays has a threshold of detection of 3–5 pg/ml. Levels of OVA-specific IgE and IgG1 were measured using a previously described Ag-capture ELISA method (16).

Flow cytometric analysis

Flow cytometric analysis was performed on lung cells isolated as previously described with slight modifications (16). Briefly, total lung mononuclear cells were obtained by collagenase digestion (collagenase type III; Invitrogen Life Technologies) followed by discontinuing gradient centrifugation in 30 and 60% Percoll (Pharmacia Biotech). The interface containing mononuclear cells was collected, washed twice with PBS, and stained with a panel of Abs. The following Abs were purchased from BD Pharmingen: anti-CD3 (PE-conjugated 145-2CII), anti-CD4 (biotin-conjugated L3T4), and anti-CD28 (FITC-conjugated 37.51). T1/ST2 (3E10) and ICOS Ab were provided by Millennium Pharmaceuticals and were FITC-labeled in-house. To minimize nonspecific binding, 10^6 cells were preincubated with FcBlock (CD16/CD32; BD Pharmingen). For each Ab combination, 10^6 cells were incubated with mAbs at 0–4°C for 30 min; the cells were then washed and treated with second stage reagents. Streptavidin-cy5 (BD Pharmingen) was used as a second step reagent for detection of biotin-labeled Abs. Titration was used to determine the optimal concentration for each Ab. Cells were fixed in 1% paraformaldehyde, counted on a FACScan, and analyses were performed using WinMDI software (The Scripps Research Institute). Twenty thousand to 30,000 events were acquired.

Data analysis

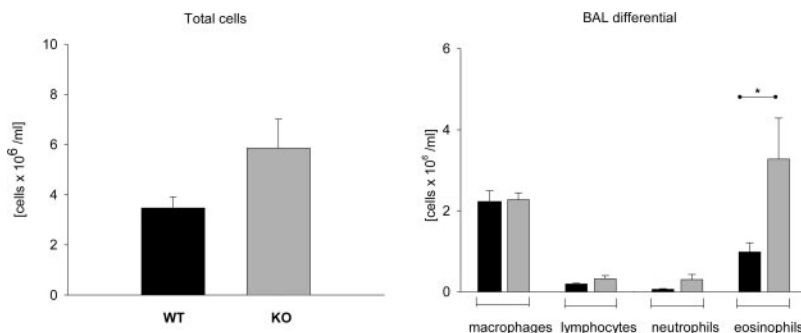
Data are expressed as mean $\pm$ SEM. Statistical interpretation was performed using ANOVA with Fisher post hoc test or Student *t* test. Differences were considered statistically significant when $p < 0.05$.

Results

Impact of the B7RP-1 deficiency on the generation of primary Th2 responses

B7RP-1 KO mice and control littermates subjected to our protocol of respiratory mucosal sensitization were sacrificed 48 h after the last OVA aerosol challenge, and the BAL content was assessed. Both strains mounted an overall inflammatory response in the lungs of a similar degree. The absolute number and percentage of eosinophils ($43 \pm 6\%$ B7RP-1KO vs $27 \pm 4\%$ wild type (WT)) in the BAL of B7RP-1 KO mice were in fact greater than that in WT mice and statistically different between groups (Fig. 1, *a* and *b*). Upon histopathological examination, the extent of the lung eosinophilic inflammatory infiltrate was, in agreement with the BAL findings, greater in B7RP-1KO than in control littermate mice

FIGURE 1. Airway eosinophilia in BAL of control littermates (WT) and B7RP-1-deficient (KO) mice exposed to aerosolized OVA in the context of GM-CSF expression. Over a period of 10 consecutive days, mice were exposed daily to OVA. Twenty-four hours before first exposure, mice were infected i.n. with an Ad construct expressing GM-CSF. Data were obtained 48 h after last exposure to OVA. Total cell number and differential cell count acquired in BAL fluid (mean $\pm$ SEM; $n = 7$ –8; statistical analysis was performed using Student's *t* test; *, $p < 0.05$).



(Fig. 2). The accumulation of inflammatory cells, primarily eosinophils and mononuclear cells, was apparent in both perivascular and peribronchial areas.

Additionally, to assess the ability of B7RP-1 KO mice to develop Th2 responses, we restimulated splenocytes collected 48 h after last OVA exposure with OVA Ag. Effector Th2-associated cytokines were measured in the supernatants of these cultures. As shown in Fig. 3, the levels of IL-4, IL-5, and IL-13 detected were in fact greater in B7RP-1 KO mice as compared with control littermates. Thus, the absence of the ICOS-B7RP-1 interaction in mice mucosally sensitized not only does not preclude Th2 differentiation but insinuates even greater Th2 polarization. A compromised IFN- γ production that would counterbalance Th2 responses (20) could potentially explain the particularly elevated production of Th2 cytokines. However, as demonstrated in Fig. 3d, the levels of IFN- γ were similar in B7RP-1 KO and control littermates, indicating that another yet unidentified mechanism is responsible for the increased production of Th2 cytokines in B7RP-1 KO.

To examine the ability of mucosally sensitized B7RP-1 KO mice to generate humoral responses, we measured serum OVA-specific IgE and IgG1 48 h after the last OVA aerosol challenge. Fig. 4 shows that IgE and IgG1 production in B7RP-1 KO mice is comparable to that observed in control littermate mice.

Impact of the B7RP-1 deficiency on the generation of memory responses

The importance of costimulatory pathways in the generation of memory T cell responses is controversial (21). Therefore, although not playing a major role in Th2 differentiation, the ICOS-ICOSL interaction might be important for the generation of Th2 effector memory. To investigate this aspect, mice sensitized to OVA were left for 35 days to allow a complete resolution of the acute inflammatory response and were then re-exposed to aerosolized OVA on 3 consecutive days. Seventy-two hours after the last exposure, mice were sacrificed and the BAL cellular response was assessed. As shown in Fig. 5a, B7RP-1 KO and control littermate mice mounted an eosinophilic airway inflammatory response that was quantitatively similar. To determine whether the infiltration of T cells reflected a preferential accumulation of Th2 cells, lung mononuclear cells were subjected to flow cytometric analysis. T1/ST2,

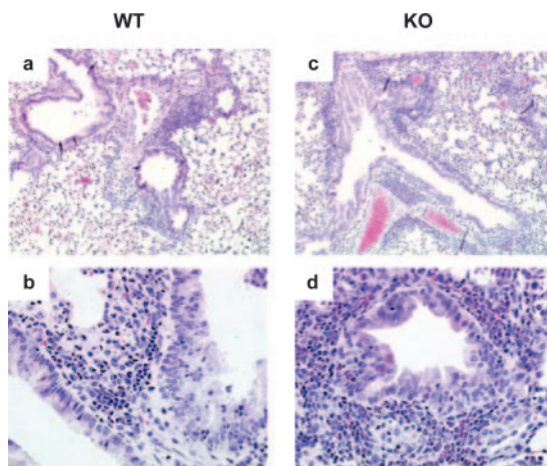


FIGURE 2. Light photomicrograph of paraffin-embedded sections of lung tissues. B7RP-1-deficient mice (KO) and control littermates (WT) were exposed to aerosol over a period of 10 days following i.n. delivery of Ad/GM-CSF. Tissues were obtained 48 h after the last OVA exposure. Sections were stained with H&E. Panels represent WT (a and b) and KO (c and d) mice; magnification of panels: a and c, $\times 50$; b and d, $\times 200$.

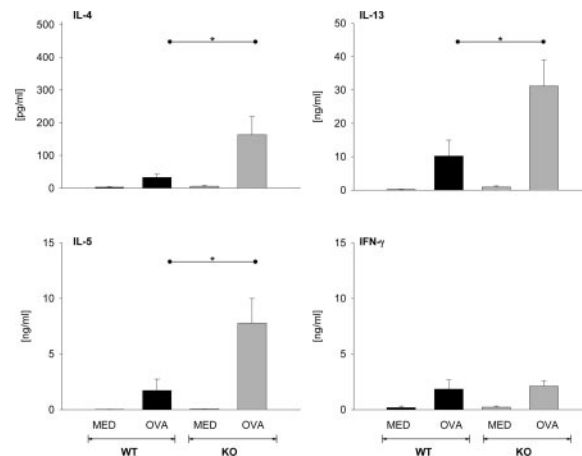


FIGURE 3. Cytokine production by control littermates (WT) and B7RP-1-deficient (KO) mice. For cytokine measurement, splenocytes were obtained from both strains 48 h after the last OVA exposure. Cells were cultured for 5 days in medium alone or stimulated with OVA (mean $\pm$ SEM; $n = 7-8$; statistical analysis was performed using one-way ANOVA with Fisher's post hoc test; *, $p < 0.05$).

a putative marker of Th2 effector cells (22), was expressed on CD3/CD4 cells in both B7RP-1 KO and control mice (Fig. 5b). In addition, the expression of T1/ST2 in B7RP-1KO was significantly higher than in WT controls.

Expression of costimulatory molecules in lung T cells

We next evaluated the expression of two costimulatory molecules, namely CD28 and ICOS, on lung T cells. To this end, lungs were subjected to enzymatic digestion followed by the isolation of mononuclear cell fraction. As shown in Table I, the expression of CD28 and ICOS was similar in both mouse strains, indicating activation of T cells, which is independent on signaling generated by B7RP-1.

Generation of inhalation tolerance

The increased responses that we observed in B7RP-1 KO mice as compared the control littermates could be explained by the decreased ability to control homeostatic conditions in the lung. Therefore, we examined the induction of inhalation tolerance that constitutes the major controlling mechanism of Th2 responses in the airways (19). Fig. 6 demonstrates that although the tolerance was induced in control mice (diminished eosinophilia and IL-5 production), B7RP-1 KO mice presented sustained eosinophilic response and production of IL-5, indicating inability to mount inhalation tolerance.

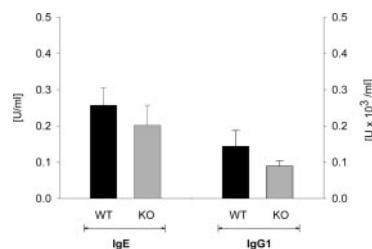


FIGURE 4. Ig production by control littermates (WT) and B7RP-1-deficient (KO) mice. Levels of OVA-specific IgE and IgG1 were measured in serum of WT and KO mice obtained 48 h after the last OVA exposure (mean $\pm$ SEM; $n = 7-8$; statistical analysis was performed using Student's t test; *, $p < 0.05$).

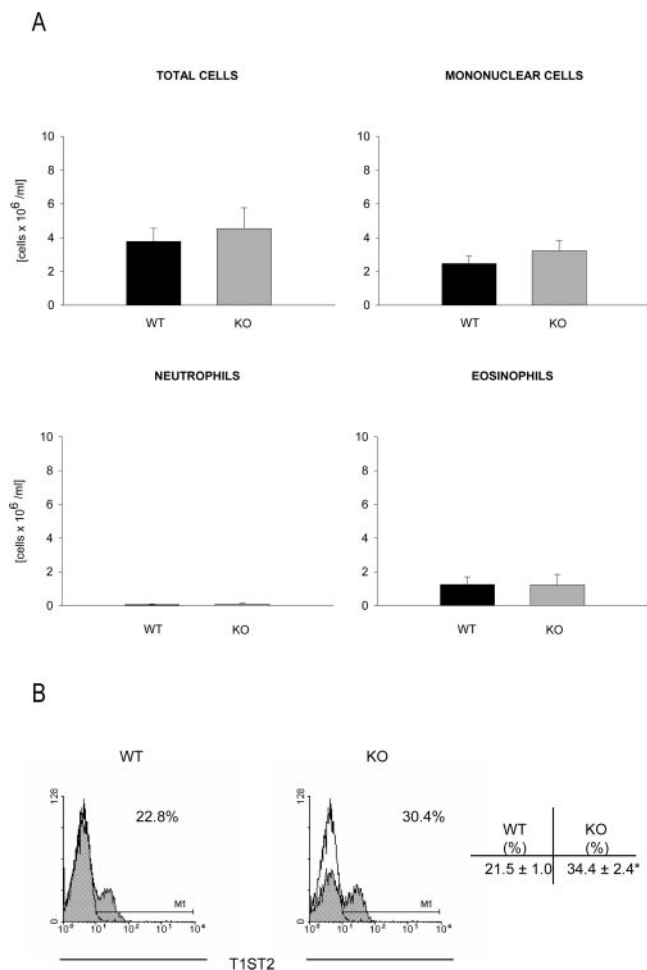


FIGURE 5. In vivo rechallenge of control littermates (WT) and B7RP-1-deficient (KO) mice exposed to OVA in the context of GM-CSF. At day 60, after complete resolution of the airway inflammation, mice were re-exposed to aerosolized OVA for 3 consecutive days. *a*, Data show total cell number and differential of cells obtained 72 h after last challenge from BAL (mean ± SEM; *n* = 4; statistical analysis was performed using Student's *t* test; *, *p* < 0.05). *b*, T1/ST2 expression on T cells obtained from lung tissues. Lungs were collected 72 h after last exposure and subjected to enzymatic digestion. Cells were stained with mAbs against CD3, CD4, and T1/ST2. Histograms were gated on CD3⁺/CD4⁺ T lymphocytes. Open histograms represent the background staining with the isotype control Ab. Histograms are representative of four independent measurements (*n* = 4/group) for individual lungs. Data in table shows mean ± SEM for WT and KO (*n* = 4; statistical analysis was performed using Student's *t* test; *, *p* < 0.05).

Discussion

It has been postulated that ICOS is important for the generation of Th2 cells because ICOS mRNA is expressed at high levels on in vitro-differentiated Th2 cells (6), ICOS ligation mediates IL-4 ex-

Table I. Flow cytometric analysis of T cells (CD3⁺CD4⁺) obtained from lungs of control littermates (WT) and B7RP-1-deficient (KO) mice^a

	WT	KO
CD28	90.5 ± 0.6	90.2 ± 2.9
ICOS	55.5 ± 1.9	68.8 ± 3.8

^a Lung mononuclear cell fraction was obtained by enzymatic digestion of whole lung, and, expression of CD28 and ICOS was examined on lung T cells. Thirty thousand events were collected. Data are representative of four independent measurements (*n* = 4 mice/group) for individual lungs (mean ± SEM).

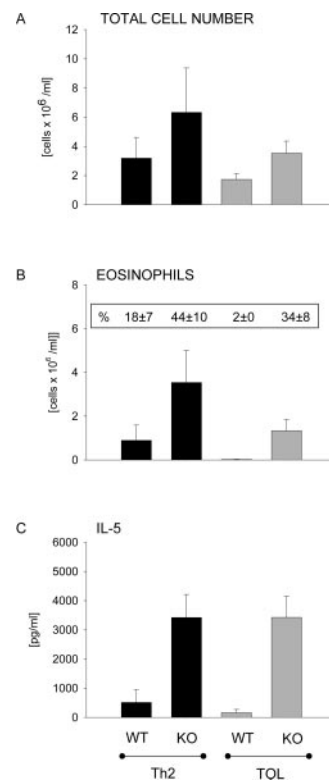


FIGURE 6. Induction of inhalation tolerance in control littermates (WT) and B7RP-1-deficient (KO) mice. Mice were exposed to saline (Th2) or OVA (tolerance (TOL)) for 10 consecutive days, and 1 wk later, they were re-exposed to OVA in the context of GM-CSF. Data were obtained 48 h after last exposure to OVA. *a*, Total cell number acquired in BAL fluid (mean ± SEM; *n* = 4). *b*, Percentage and cell number of eosinophils present in the BAL (mean ± SEM; *n* = 4). *c*, Levels of IL-5 production by splenocytes (mean ± SEM; *n* = 4).

pression (7), and IL-4 expression is deficient in ICOS-KO mice (8, 9). Thus, the prediction was that the absence of ICOS ligand, namely B7RP-1 and, therefore, the disruption of the ICOS-ICOSL costimulatory pathway, would compromise the generation of Th2-associated responses. However, our experiments with mice genetically engineered for the absence of B7RP-1 (B7RP-1KO) demonstrate that efficient Th2 sensitization and effector function occur in the absence of this costimulatory pathway.

B7RP-1-deficient mice subjected to a model of mucosal allergic sensitization responded efficiently to OVA and generated productive Th2 response at both the cellular and humoral levels. Indeed, OVA aerosolization in the context of GM-CSF led to the accumulation of eosinophils in both airways and lung parenchyma and Th2-affiliated cytokine production (IL-4, IL-5, and IL-13). Interestingly, the extent of cellular lung infiltration, the levels of cytokine production, and T1/ST2 expression on T cells were greater in B7RP-1KO than in control mice. We considered whether decreased IFN- γ production in B7RP-1 KO could explain these heightened Th2 responses; however, B7RP-1KO and control littermate mice produced similar amounts of IFN- γ in agreement with a recent report by Nurieva et al. (14). Therefore, our data indicate another unidentified mechanism that is responsible for the increased production of Th2 cytokines in B7RP-1KO mice.

The ICOS-B7RP-1 pathway appears to be important in humoral immunity as ICOS-deficient mice subjected to different immunization protocols revealed deficits in IgG1, IgG2a, and IgE levels (8, 23–25). Our data demonstrate an intact production of Ag-specific immunoglobulins (IgG1 and IgE). Although apparently at

variance with the results observed in ICOS KO mice, it must be noted that the impairment in Ab production observed in ICOS-deficient mice can be overcome by the use of a strong adjuvant such as CFA (25). Similarly, administration of polyethylene glycol-GM-CSF in B7RP-1KO led to partial rescue of IgG1 production (13). In this regard, the presence of intact humoral responses in B7RP-1 KO in our mucosal model can be explained by the nature of a protocol that involves repeated exposure to Ag (OVA) in the context of a GM-CSF-rich airway microenvironment.

That the generation of primary and memory Th2 responses is completely intact in B7RP-1 KO mice suggests that the importance of the ICOS costimulatory pathway is redundant under our experimental conditions. Our findings are at variance with those recently reported by Mak et al. (13). The discrepancy can be explained, at least in part, by the nature of the experimental models used in both studies. Whereas Mak et al. (13) used a conventional model involving two i.p. injections of OVA/alum, followed by OVA aerosol challenge, we used a model of mucosal allergic sensitization that entails delivery of OVA directly to airways through aerosolization in the context of locally expressed GM-CSF. We have elaborated recently in detail on the importance of GM-CSF to allergic airway inflammation (18). More specifically, that GM-CSF is involved in the expansion of a particular Th2-associated dendritic cell (DC) subset (myeloid DC; DC2) is documented extensively in the literature. Of direct relevance to the work presented here, Mak et al. (13) showed that delivery of polyethylene glycol-GM-CSF to B7RP-1-deficient mice led to robust expansion of DC2 and restoration of IgG1 production in 50% of mice. Unfortunately, no other components of Th2 responses were evaluated in these mice.

It is known that expression of ICOS on T cells is dependent on TCR and CD28 signals and that absence of CD28 results in diminished levels of ICOS (7). To this end, we examined the expression of CD28 and ICOS on CD3/CD4 cells isolated from the lungs in our recall protocol. As shown on Fig. 6, both molecules are expressed in B7RP-1 KO. Two potential explanations can emerge from our studies: either the CD28-B7 pathway can fully substitute for the absence of a secondary signal, namely ICOS-ICOSL, or there is a second ligand for ICOS, which is distinct from B7RP-1, that interacts with ICOS expressed on T cells. Previous studies using either CD28KO or B7.1/2 antagonists have shown that the CD28-B7 pathway is absolutely necessary for the generation of Th2 responses, supporting the former notion (26). In addition, current literature postulates that blockade of the ICOS-ICOSL pathway with ICOS-Ig does not prevent Th2 differentiation but can reduce acute airways inflammation (11, 27). In addition, evidence of eosinophilic infiltration in a model of allergic airway inflammation in ICOS KO (9) supports the notion that CD28/B7 is the primary pathway for Th2 responses, whereas ICOS-ICOSL serves as an enhancing arm.

The issue that remains unresolved is the potential role of B7RP-1 in T cell priming. Although it is clear that B7RP-1 is expressed on resting B cells and macrophages, it is still controversial whether this molecule is also expressed on murine DC. Human DC express B7RP-1, which is down-regulated upon stimulation with LPS or TNF- α (3). Similarly, airway exposure to OVA leads to the decrease of B7RP-1 expression on lung DC (28, 29). Both studies imply that activated DC might not use B7RP-1 for the purpose of activating naive T cells but rather use the B7RP-1 pathway to sustain homeostasis. Indeed, many peripheral tissues, among them endothelial cells, constitutively express low levels of B7RP-1 and presumably can interact with ICOS⁺ T cells producing regulatory IL-10 (29). The involvement of ICOS/ICOSL pathway in regulating homeostasis remains, however, unresolved and requires additional investigations. That mice trans-

genic for B7RP-1Fc develop T cell hyperplasia, plasmocytosis, and hypergammaglobulinemia suggest that the contribution of B7RP-1 involves primarily a direct interaction between T and B cells rather than with DC (24). However, the presence of Ag-specific Abs in our model supports the notion that stimulation through CD40-CD40L is sufficient in the absence of in vivo ICOS signaling to execute an efficient humoral response (25).

The increased Th2 deviation observed in the B7RP-1 KO was quite perplexing, especially in light of evidence claiming that ICOS-B7RP-1 pathway is important for the generation of Th2 responses. Therefore, we have decided to examine whether the enhancement of Th2 responses in our mucosal model might result from the interference with ICOS/B7RP-1-dependent regulatory mechanisms in the lung. To this end, we tested whether B7RP-1KO mice are able to generate inhalation tolerance in response to OVA. Under these experimental conditions, exposure of mice to OVA leads to the establishment of tolerance because the subsequent exposure to OVA in the context of GM-CSF results in diminished airway eosinophilia and negligible levels of Th2-associated cytokines (17). Our data show that B7RP-1KO mice, unlike the control animals, did develop airway eosinophilia and were able to produce IL-5. Our findings are in agreement with those by Akbari et al. (15), who postulated that intact ICOS-ICOSL pathway is necessary for the effective generation of regulatory T cells and inhalation tolerance. Therefore, the absence of B7RP-1 signaling could lead to the impairment of homeostatic regulation in the lung that results in the generation of default Th2 responses.

In summary, our data shows that we could efficiently trigger Th2-associated cellular and humoral responses in B7RP-1-deficient mice in a model of allergic airway sensitization. In sharp contrast, ICOS-B7RP-1 signaling is absolutely essential for the induction of inhalation tolerance. That the absence of this signaling pathway leads to the enhancement of Th2 responses indicates the importance of costimulatory pathways in the balancing the development of specific immune responses.

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Disclosures

The authors have no financial conflict of interest.

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