

Inducible costimulator-positive T cells are required for allergen-induced local B-cell infiltration and antigen-specific IgE production in lung tissue

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Background: Airway inflammation plays a critical role in the pathogenesis of asthma. In susceptible individuals, airway allergen exposure results in the recruitment of inflammatory cells into lung tissue, leading to a local inflammatory response. Central to the induction and regulation of this process are T lymphocytes. **Objective:** Blocking of the newly discovered costimulatory T-cell molecule inducible costimulator (ICOS) with monoclonal antibodies was shown to ameliorate allergic airway inflammation in models of murine asthma. Although these observations indirectly support an association between ICOS and the development of allergic inflammation, the role of the ICOS⁺ T cell in the pathogenesis of allergic airway disease remains unclear. **Methods:** We used an adoptive transfer model to analyze further the role of antigen-specific ICOS⁺ T cells during the effector phase of allergic airway inflammation. *In vitro* stimulated CD4⁺ T cells from mice transgenic for an ovalbumin-specific T-cell receptor (DO11.10) were sorted into ICOS-enriched and ICOS-depleted T-cell fractions and transferred into BALB/c recipient mice. **Results:** Transfer of the ICOS-enriched T-cell population followed by allergen airway challenges induced pronounced infiltration of recipient T and B cells and local production of allergen-specific IgE by intrapulmonary plasma cells. In contrast, transfer of the ICOS-depleted T-cell fraction resulted in the recruitment of significantly lower numbers of B cells and no local IgE production. **Conclusion:** These data indicate that expression of ICOS defines a subset of T effector cells that are required for B-cell infiltration and local IgE production in lung tissues on allergen airway exposure. (J Allergy Clin Immunol 2004;114:775-82.)

Key words: Asthma, allergy, IgE, ICOS, T cell, B cell

Abbreviations used

APAAP: Alkaline phosphatase antialkaline phosphatase
BALT: Bronchus-associated lymphoid tissue
FITC: Fluorescein isothiocyanate
ICOS: Inducible costimulator
TCR: T-cell receptor

The respiratory tract holds an extensive network of lymphoid tissue, referred to as bronchus-associated lymphoid tissue (BALT), which can respond to various inhaled allergens. Like other secondary lymphoid tissues, such as lymph nodes and Peyer patches, BALT contains T cells, B cells, dendritic cells, macrophages, and high endothelial venules.¹ Through the interaction between these cells and components, BALT functions as the inductive primary site for presentation of airborne antigens to the immune system.

Central to the induction and regulation of the allergen-induced inflammation are T lymphocytes. T cells in lung tissue of individuals with atopic asthma are predominantly of a T_H2 phenotype, characterized by enhanced secretion of IL-4, IL-5, and IL-13.² These T_H2 cytokines promote the recruitment, activation, and survival of leukocytes, especially eosinophils, in the airway tissues, and the production of IgE, the major mediator of allergen-induced immediate-type hypersensitivity reactions.³ Concordantly, allergen-specific IgE levels are increased in serum and in bronchoalveolar lavage fluids from patients with asthma and correlate with disease severity.⁴

Allergen-induced T-cell effector functions are triggered by the T-cell receptor (TCR), mediating antigen specificity, and a combination of various costimulatory signals that modulate the direction of the immune response.⁵ CD28 costimulation is critical for the activation of naive T cells, whereas memory T cells are far less dependent on the CD28/B7 costimulatory pathway,^{6,7} suggesting that either antigenic stimulation via the TCR alone is sufficient or that other costimulatory molecules are able to compensate. The newly discovered inducible costimulatory (ICOS) molecule is structurally and functionally related to CD28.⁸⁻¹⁰ In contrast with the constitutively expressed CD28, ICOS is upregulated after T-cell activation and shows sustained expression on T cells of the

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effector/memory phenotype.¹¹⁻¹³ Blockade of ICOS in murine models of active allergen sensitization and airway challenges was shown to ameliorate allergic airway inflammation and development of airway hyperreactivity.¹⁴⁻¹⁶ Although these observations suggest an association between ICOS expression and the development of allergic airway inflammation, the mode of action of the ICOS⁺ T cell in the intrapulmonary compartment has not been revealed. To delineate further the function of ICOS⁺ effector T cells in the primary sites of airway inflammation, we used a murine model of adoptive transfer of allergen-specific CD4⁺ T cells from DO11.10 mice carrying a TCR specific for ovalbumin into BALB/c recipient mice followed by repeated allergen airway challenges. By using this protocol, in which the focus is the effector and not the sensitization phase, we show that antigen-specific ICOS⁺ T cells promote pulmonary B-cell infiltration and local, allergen-specific IgE production in lung tissues. This defines ICOS as a promising novel target for local inhibition of allergen-induced IgE-mediated airway diseases.

METHODS

Mice

DO11.10 mice transgenic for an ovalbumin-specific TCR¹⁷ and normal BALB/c mice were bred under SPF conditions. All animal experiments were performed in accordance with the national research ethical institution.

In vitro stimulation of DO11.10 T cells

Spleen cells from DO11.10 mice were magnetically depleted of CD8⁺ cells (Miltenyi Biotec, Bergisch Gladbach, Germany). Because naive DO11.10 T cells were unable to induce allergic airway inflammation on subsequent ovalbumin airway challenges in this protocol (reviewed in Lloyd et al¹⁸), the CD8-depleted DO11.10 mononuclear cells were prestimulated *in vitro* with the peptide ovalbumin₃₂₃₋₃₃₉ (0.2 μ M) at 2×10^6 cells/mL in complete RPMI medium for 6 days. Dead cells were removed by Ficoll-gradient centrifugation (Ficoll Histopaque-1083; Sigma-Aldrich, St Louis, Mo).

Magnetic cell sorting

For some experiments, the *in vitro* stimulated DO11.10 T cells were separated into ICOS-depleted and ICOS-enriched fractions by magnetic cell sorting. Cells were incubated with digoxigenized anti-ICOS mAb (MIC-280)¹¹ followed by anti-digoxigenin Fab conjugated to magnetic beads (Miltenyi Biotec). To prevent unspecific binding of the anti-ICOS mAb, the cells were preincubated with blocking anti-Fc γ RII/III mAb (200 μ g/mL; 2.4G2, American Type Culture Collection, Manassas, Va) and purified rat IgG (500 μ g/mL; Nordic, Tilburg, The Netherlands). Cell fractions were separated with LS magnetic cell-sorting columns (MACS; Miltenyi Biotec). Cell fractions were purified to $\geq 90\%$.

Flow cytometry

Single cell suspensions were stained with digoxigenized anti-murine ICOS mAb (MIC-280), fluorescein isothiocyanate (FITC)-labeled anti-CD4 mAb (GK1.5; American Type Culture Collection), biotinylated mAb against the activation marker CD69 (H1.2F3; BD Biosciences, Franklin Lakes, NJ), and ovalbumin-TCR specific mAb (KJ1-26).¹⁹ Cy5-conjugated anti-digoxigenin Fab (Roche

Biochemicals, Mannheim, Germany) and streptavidin-phycoerythrin (BD Biosciences) were used as secondary reagents. Samples ($\sim 200,000$ cells) were analyzed on a FACSCalibur (BD Biosciences).

Flow-cytometric analysis of intracellular cytokines

Cells were stimulated with phorbol 12-myristate 13-acetate (10 ng/mL) and ionomycin (1 μ g/mL). Brefeldin A (5 μ g/mL; all reagents from Sigma-Aldrich) was added after 2 hours. After 5 hours, the cells were fixed with 2% formaldehyde, permeabilized with 0.5% saponin (Sigma-Aldrich), stained with phycoerythrin-conjugated anti-IL-13 (38213.11; R&D Systems), and analyzed by flow cytometry.

Cytokine ELISA

Supernatants from cell cultures stimulated with phorbol 12-myristate 13-acetate and ionomycin for 20 hours were analyzed for different cytokines by sandwich ELISA. For the quantification of IL-4, IL-5, and IL-10, we used 11B11, TRFK5, and SXC-1 as coating mAb and biotinylated BVD6-24G2, TRFK4, and JES5-2A5 as detection mAb, respectively.

Adoptive transfer model

Unseparated, ICOS-depleted, and ICOS-enriched fractions containing identical numbers of CD4⁺ KJ1-26⁺ T cells (1.5×10^6 cells/mouse) were injected intravenously into the tail vein of recipient BALB/c mice (5 animals per group). To facilitate homing of the transferred T cells to the lungs, the recipient mice had been exposed for 20 minutes to an aerosol of 1% ovalbumin (Sigma-Aldrich) in PBS or, for negative control, to PBS without ovalbumin 24 hours before cell transfer, as previously described.²⁰ Challenges were repeated 24 hours and 48 hours after cell transfer, and the mice were killed 48 hours after the last challenge. The lungs were infused with 800 μ L 50% Tissue-Tek (Miles Diagnostics, Elkhart, Ind) in PBS by intratracheal injection. The right lower lobe was embedded in Tissue-Tek, snap-frozen in liquid nitrogen, and stored at -80°C .

Immunohistochemistry

Serial cryostat sections of the right lower lobes of the lungs were cut in vertical direction to the central bronchus, air-dried, and fixed in pure acetone. Sections were stained with mAb against CD3 (KT3),²¹ CD45R/B220 (RA3-6B2),²² ICOS (12A7),²³ ovalbumin-TCR (KJ1-26), IgG1 (A85-3; BD Biosciences), and IgE (23G3; Southern Biotechnology Associates, Birmingham, Ala) by using the alkaline phosphatase antialkaline phosphatase (APAAP) technique.²⁴ Immunofluorescence double staining was performed with mAb against the plasma cell marker CD138 (Syndecan-1; 281-2; BD Biosciences), detected with antirat-Ig-Cy3 (Jackson Laboratories, Bar Harbor, Me), and, after extensive blocking with polyclonal rat serum, with FITC-labeled anti-IgE mAb (23G3). Double staining caused by cross-reactivity was ruled out by internal controls. Furthermore, FITC-labeled anti-IgE mAb (23G3) was used in combination with an ovalbumin-digoxigenin conjugate detected with anti-digoxigenin horseradish peroxidase and Tyramide-AlexaFluor546 (Molecular Probes, Eugene, Ore). Conjugation of ovalbumin to digoxigenin was performed as described.²⁵ Immunofluorescence staining of eosinophils was performed with a polyclonal rabbit antimouse antibody against major basic protein (kindly provided by Drs G. Gleich and J. Lee, Mayo Clinic, Scottsdale, Ariz, and Rochester, Minn),²⁶ followed by antirabbit-Ig-FITC, as previously described.²⁷ For standardization of the histologic assessment, cellular infiltrates localized between bronchi with a diameter of 80 to 100 μ m and an adjacent blood vessel were

analyzed. Area of infiltrates and number of positive cells per infiltrate were determined with Image Tool (University of Texas Health Sciences Center in San Antonio). From each of 5 animals per group, 3 separate lung sections were analyzed in a blind fashion for infiltrate size and cellular composition.

Statistical analysis

Differences between groups were determined by analysis of means by using the unpaired Student *t* test (2-tailed).

RESULTS

Transfer of allergen-specific T cells before allergen airway challenges induces airway infiltration with B and T cells

Transfer of preactivated allergen-specific T cells into BALB/c recipients followed by repeated allergen airway challenges induced a substantial airway inflammatory response in the recipients similar to that generated by conventional active sensitization and challenge protocols.^{28,29} Immunohistochemical analysis of cryosections from inflamed lungs of allergen-challenged recipient mice revealed the rapid formation of combined B-cell and T-cell infiltrates that were preferentially located between middle-sized bronchi and adjacent blood vessels (Fig 1, A and B). However, we did not observe the formation of classical germinal centers, defined by the detection of peanut agglutinin–positive germinal center B cells,³⁰ as it was described previously for the chronic model of allergic airway inflammation.³¹

Transfer of allergen-specific T cells before allergen airway challenges induces local allergen-specific IgE production

To characterize further the properties of the infiltrating B cells in the lung tissues, we discriminated local Ig production by means of immunostaining. IgG1-positive and, in smaller numbers, IgE-positive cells were detected in the infiltrates (Fig 2, A–C). Double immunostaining with anti-IgE mAb (Fig 2, E) and anti-CD138 mAb, a marker specific for plasma cells (Fig 2, F), showed that all IgE-positive cells stained double-positive for the plasma cell marker (Fig 2, G), ruling out mast cells or monocytes that had bound IgE on their cell surfaces as a source of positive IgE staining. To test for antigen specificity of the IgE production, ovalbumin was labeled with digoxigenin and detected by anti-digoxigenin AlexaFluor546 (Fig 2, I). The overlay confirmed that the IgE-producing plasma cells were indeed specific for ovalbumin (Fig 2, K).

ICOS⁺ T cells are collocated with IgE-producing plasma cells

Expression of ICOS by infiltrating T cells was detected by immunostaining. Serial sections of the lung tissues showed that after transfer of allergen-specific T cells and subsequent airway allergen challenges, the majority of T cells within the lung infiltrates of recipient mice ex-

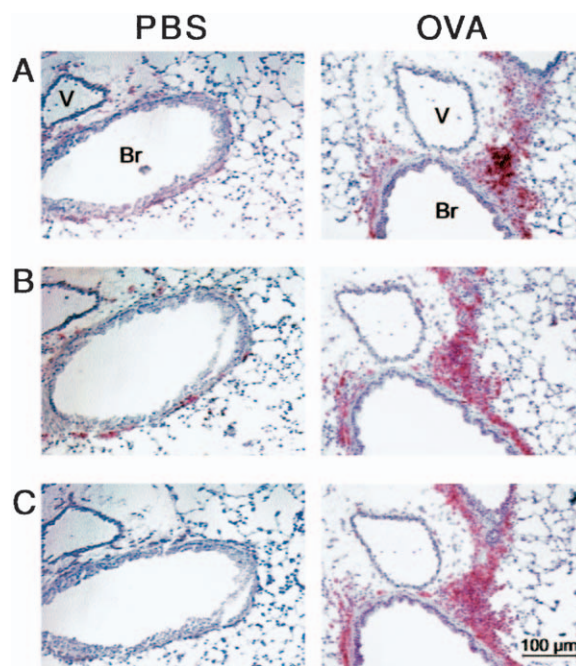


FIG 1. B-cell and T-cell infiltration in lung tissues after adoptive transfer of allergen-specific T cells and subsequent allergen airway challenge. Representative serial sections between a bronchus (Br) and a blood vessel (V) are shown for the negative control group (PBS) and the allergen-challenged group (OVA). Immunostaining (APAAP) for (A) B cells, (B) T cells, and (C) ICOS⁺ T cells is shown.

pressed ICOS (Fig 1, C). Further analysis revealed that these ICOS-expressing T cells were collocated with IgG1-producing and IgE-producing cells in the perivascular regions of the infiltrated airways of transferred and challenged recipient mice (Fig 2, D).

ICOS⁺ T cells are required for the development of local airway inflammation and IgE production

Because ICOS was highly expressed in the lung infiltrates, we wanted to delineate the role of ICOS⁺ T cells for the formation of airway inflammation and local IgE production. To address this question, CD4⁺ T cells from ovalbumin-TCR transgenic mice (DO11.10) were preactivated *in vitro* with ovalbumin_{323–339} for 6 days and sorted into unseparated, ICOS-depleted, and ICOS-enriched cell fractions. For all 3 subsets, the frequency of transgenic T cells was determined by fluorescence-activated cell sorting analysis with the ovalbumin-TCR specific mAb KJ1-26 (data not shown) to ensure that all 3 groups of recipient mice received equal numbers of ovalbumin-TCR specific T cells. Further, the different fractions were in a similar state of activation as revealed by expression of the activation marker CD69 (Fig 3, A), warranting that equal numbers of activated allergen-specific T cells were transferred into BALB/c recipients. After 2 subsequent allergen airway challenges, recipient mice were killed and analyzed.

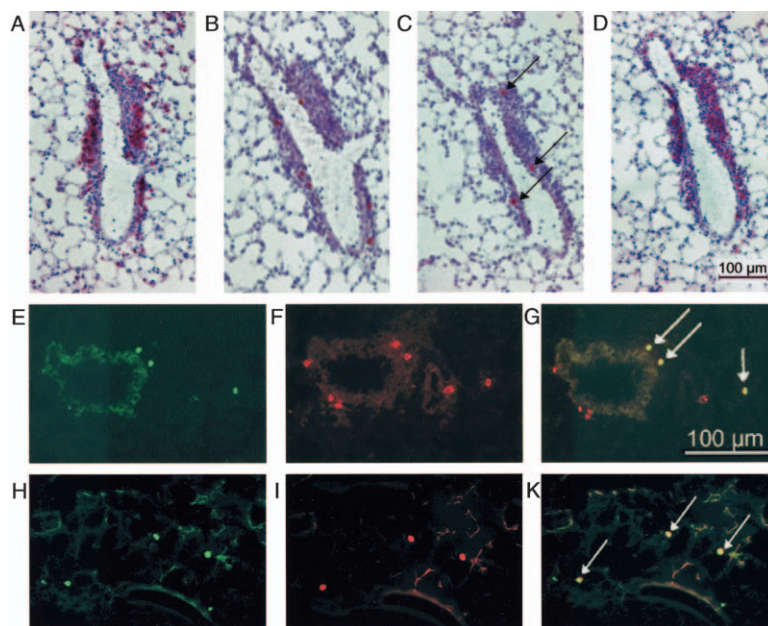


FIG 2. Local IgG1 and allergen-specific IgE production in lung tissue. Cellular infiltrates were stained for (A) B cells, (B) IgG1, (C) IgE, and (D) ICOS. IgE-positive cells are indicated by arrows. The middle row shows double staining for (E) IgE (green) and (F) the plasma cell marker CD138 (red). In the overlay (G), double-positive cells (yellow) are indicated by arrows. The bottom row shows double staining for (H) IgE (green) and (I) ovalbumin-digoxigenin conjugate (red). In the overlay (K), double-positive cells (yellow) are indicated by arrows. The illustrations are representative of 15 replicate samples each.

Transfer of the ICOS-enriched T-cell fraction before repeated airway allergen challenges led to a significant increase in the size and number of cellular airway infiltrates as compared with transfer of unseparated or ICOS-depleted CD4⁺ DO11.10 T cells ($P < .0001$; Fig 3, B). Analysis of the cellular composition of the infiltrates by immunostaining showed that the transferred ovalbumin-TCR transgenic KJ1-26 positive T cells migrated into the lung infiltrates in all of the 3 ovalbumin-challenged groups (Fig 3, C). However, remarkable differences in regard to infiltration with recipient B and T cells were observed. Although transfer of ICOS-enriched DO11.10 cells significantly enhanced the recruitment of recipient B cells compared with transfer of unseparated DO11.10 T cells, hardly any B cells infiltrated into the lungs of mice after transfer of the ICOS-depleted cell fraction (Fig 3, C). In contrast, the infiltrates in the lung tissues of these latter mice consisted mostly of recipient T cells in significantly higher numbers compared with transfer of unseparated cells (Fig 3, C).

Finally, the involvement of ICOS-expressing T cells for local IgE production was analyzed. After transfer of ICOS-enriched DO11.10 T cells, the numbers of IgE-positive cells in the perivascular and peribronchial lung infiltrates were doubled in comparison with transfer of unseparated DO11.10 T cells. In contrast, no IgE-positive cells were detected in allergen-challenged recipients of the ICOS-depleted cell fraction (Fig 4, A-D). Likewise, infiltration of eosinophils was enhanced in lungs of mice after transfer of ICOS-enriched but diminished after

transfer of ICOS-depleted T cells compared with transfer of unseparated T cells (Fig 4, E).

ICOS-expressing T cells are characterized by a specific cytokine profile

We analyzed the cytokine profile of the T-cell fractions before adoptive transfer. The ICOS-enriched T cells had an 4-fold to 5-fold increased potential to produce the T_H2 cytokines IL-4, IL-5, and IL-13, and a more than 8-fold increased potential to secrete IL-10, compared with the unsorted T-cell fraction. Accordingly, in the ICOS-depleted T-cell fraction, the potential to produce T_H2 cytokines was clearly reduced (Fig 5).

DISCUSSION

Primary foci and local IgE production in the adoptive transfer model

Although elevated IgE serum levels and airway inflammation are cardinal features of allergen-induced bronchial asthma, the precise mechanisms regulating the induction and maintenance of local immune responses after allergen airway exposure remain unclear. Until now, development of local immune responses including expansion of germinal centers and local production of antigen-specific IgA, IgG, and IgE has been observed only in a murine model of active sensitization and chronic allergen airway challenge.³¹ Here we show for the first time the local production of allergen-specific IgE in lung

tissues in an adoptive transfer model, focusing on the effector phase of allergen-induced airway inflammation. In contrast with the model of repeated airway allergen challenges of actively sensitized animals,³¹ we did not detect any germinal center B cells as defined by positive peanut agglutinin staining. Although allergen-specific IgE-positive plasma cells were present in the lung tissue as early as 4 days after transfer, allergen-specific IgE was not detectable in the serum of the recipient mice after transfer and airway challenges (data not shown), suggesting that at this time point of the protocol, IgE may be produced only locally. The kinetics of antibody production in the adoptive transfer system are markedly accelerated compared with conventional protocols by using active sensitization and airway challenge. This may be a result of direct interaction of the activated antigen-specific T cells with B cells in the lung tissue. In secondary lymphoid tissues, it has been shown that antigen-specifically activated T cells direct the trapping of antigen-specific B cells at the border between the T-cell and B-cell zones of lymph nodes, thus establishing so-called *primary foci*.³² In primary foci, T-cell–B-cell interaction during the primary immune response induces isotype switching and production of antibodies with low affinity by plasma cells occurring even before the formation of germinal centers.³³ Our current results, however, indicate that primary foci can be established in the lung tissues directly. Accordingly, in the spleen, the appearance of switched IgG2a-producing plasma blasts in primary foci has been shown to occur as early as 2 days after transfer of DO11.10 T cells and to peak on day 4.³⁴ Likewise, we demonstrate here the development of primary foci in lung tissues and the induction of ovalbumin-specific IgE production on day 4 after transfer.

Role of ICOS⁺ T cells in the formation of primary foci

CD28 costimulation is considered an important factor to equip T cells with the set of chemokine receptors that are required for homing to sites of allergic lung inflammation.³⁵ However, because CD28-deficient mice are still able to develop allergic airway inflammation, CD28 cannot be solely responsible for the development of the allergic phenotype.³⁵ The costimulatory T-cell molecule ICOS, a member of the same family of costimulatory molecules as CD28, has been suggested not only to perpetuate some of the functions of CD28 during an already established immune response but also to induce additional T-effector cell functions.¹⁶ In our model, ICOS was highly expressed in lung infiltrates of transferred and challenged recipients. ICOS⁺ T cells were located within B-cell infiltrates where antibody-producing B cells were detected. Actually, the number of ICOS⁺ T cells in the infiltrates was higher than the numbers of transferred ovalbumin-TCR transgenic cells, suggesting that not only the transferred but also the recruited recipient T cells in the infiltrates turned ICOS⁺. To delineate the role of ICOS expression on antigen-specific T cells during the effector phase of allergic inflammation, we transferred preacti-

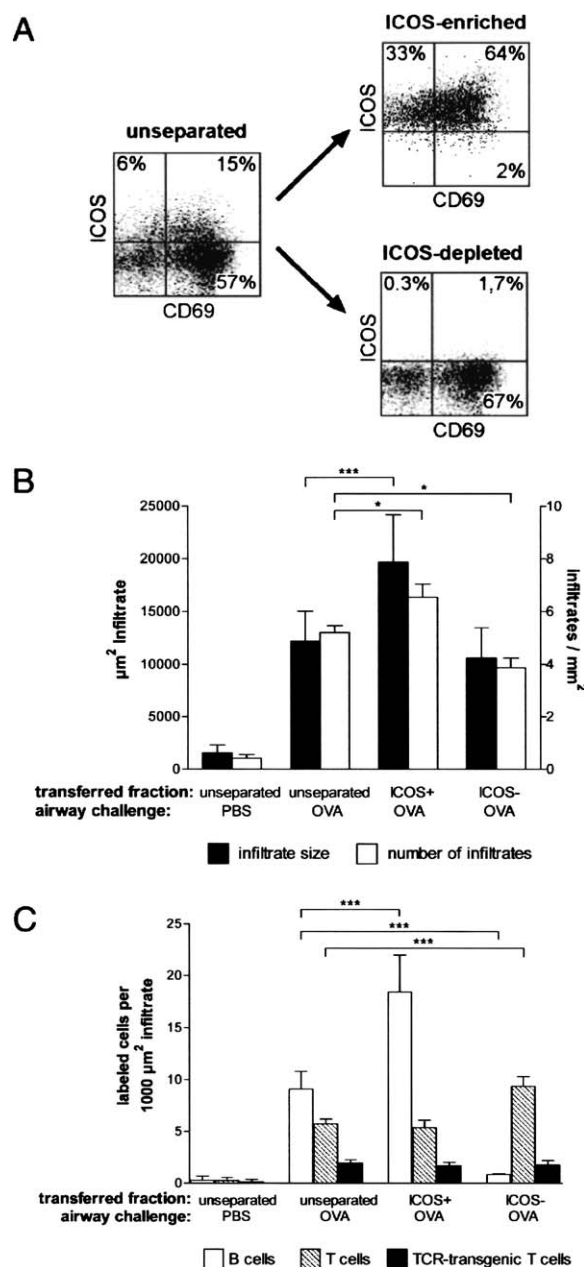


FIG 3. Size and cellular composition of infiltrates after transfer of different T-cell fractions. Preactivated, CD8-depleted DO11.10 spleen cells were sorted into ICOS-enriched (ICOS⁺) and ICOS-depleted (ICOS⁻) fractions. **A**, The activation status of unseparated and sorted T-cell fractions was determined by CD69 expression. **B**, The size (filled bars) and the number of the infiltrates (open bars) were determined with Image Tool. **C**, Cellular composition of infiltrates was analyzed by counting positively labeled cells per 1000 μm^2 of infiltrate (* P < .05; *** P < .0001).

vated, unseparated, ICOS-enriched and ICOS-depleted DO11.10 T-cell fractions of similar activation status. In all 3 recipient groups, the transferred ovalbumin-TCR positive T cells migrated into BALT, showing that the ICOS-depleted T cells were not impaired in their migration characteristics. However, a significant difference was

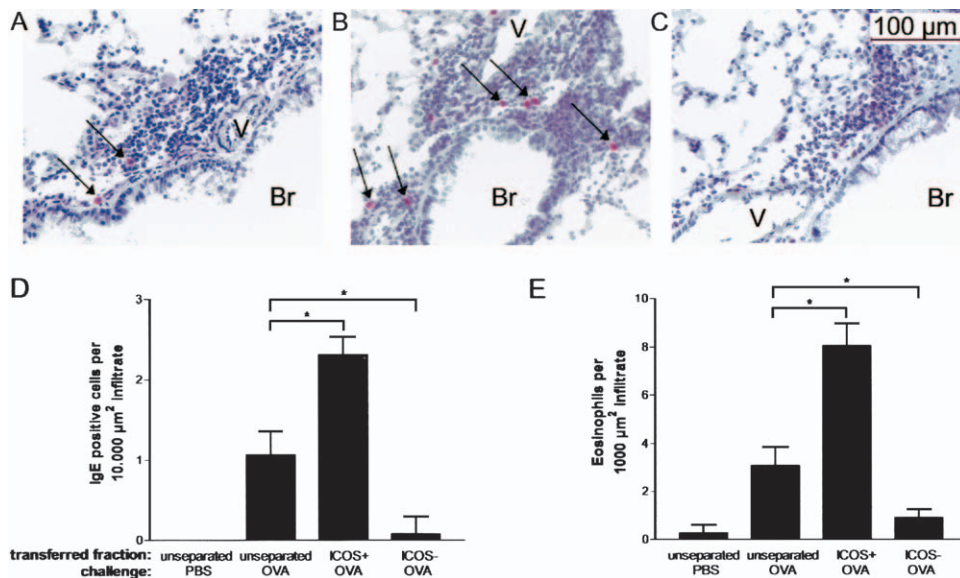


FIG 4. Local IgE production and eosinophilic infiltration is promoted by antigen-specific ICOS⁺ T cells. After transfer of (A) unseparated, (B) ICOS-enriched, and (C) ICOS-depleted DO11.10 T-cell fractions and subsequent allergen airway challenge, IgE-positive cells (indicated by arrows) were detected by APAAP staining and quantified by counting positively labeled cells (D). Eosinophils were detected by major basic protein staining (E) (**P* < .0001).

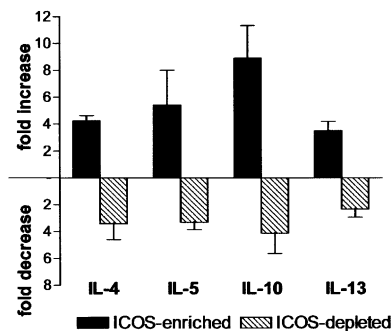


FIG 5. Cytokine profile of T-cell fractions before transfer. The cytokines IL-4, IL-5, and IL-10 were measured in the supernatants of the restimulated T-cell fractions by ELISA. IL-13 was determined by intracellular staining and flow-cytometric analysis. Data from 3 independent experiments are shown and expressed as fold increase or decrease compared with the unsorted T-cell fraction (mean $\pm$ SD).

observed in the size of infiltrates and their composition: transfer of the ICOS-enriched fraction led to massive recruitment of recipient B cells into BALT, along with some recipient T cells at relative numbers comparable with transfer of unsorted T cells. This significant increase in numbers of B cells may be the consequence of either enhanced attraction and trapping of B cells into BALT or improved proliferation of B cells because of local conditions created by antigen-specific ICOS⁺ T cells. Along with this increased B-cell infiltration, significantly higher numbers of IgE⁺ plasma cells and an enhanced eosinophil airway inflammation were observed after transfer of ICOS-enriched T cells. In contrast, in the

group that received ICOS-depleted DO11.10 T cells, infiltrates consisted almost exclusively of recipient T cells, and only few B cells, no IgE-positive cells, and hardly any eosinophils were detected. These results support the data of previous studies, in which systemic administration of ICOS-Ig fusion protein or anti-ICOS mAb in murine models of allergic airway inflammation led to a decrease of eosinophilic airway infiltration, reduced levels of IL-5 in bronchoalveolar lavage fluid, and lower serum IgE.¹⁴⁻¹⁶ Our current results extend these findings and indicate that ICOS⁺ T cells are pivotal for the recruitment of B cells to BALT and the regulation of T-cell-induced local IgE production and eosinophilic airway inflammation.

T-cell subset defined by ICOS expression

The particular capability of ICOS⁺ T cells to prepare the microenvironment for the development of primary foci and to pursue IgE production by B cells in response to antigen may be partially attributed to their specific cytokine pattern. Sorting T cells for ICOS expression enriched those cells with high potential to produce the Th2-type cytokines IL-4, IL-5, IL-10, and IL-13. In accordance with this finding, it was previously shown that ICOS expression defines a T-effector cell subpopulation in lymph nodes that produced high amounts of IL-4, IL-5, and IL-13, and, in particular, was characterized by high and stable IL-10 production.¹¹ Therefore, the enhanced eosinophilic inflammation caused by transfer of ICOS⁺ T cells may be attributed to the increased production of IL-5, the main factor for the differentiation, maturation, and recruitment of eosinophils.^{29,36} Of particular interest is the enhancement of IL-13 production in

the ICOS-enriched T-cell fraction. IL-13 is a potent inducer of airway eosinophilia and hyperresponsiveness, mucus production, and fibrotic remodeling,³⁷⁻³⁹ cardinal features of allergic airway inflammation. Further, ICOS⁺ T cells may promote the differentiation of B cells into IgE-producing plasma cells through the enhanced production of IL-4 and IL-10.⁴⁰ In support of a link between IL-10-producing T cells and IgE production, a rapid increase in IL-10-positive T cells in BAL fluids on allergen airway challenge was observed in subjects with asthma⁴¹ with kinetics similar to the increase in IgE levels.⁴²

Besides the regulation of B-cell responses through T-cell cytokines, direct interaction of ICOS⁺ T cells with B cells may also play a role for IgE synthesis. A role for this kind of direct interaction is supported by the finding that isotype switching to IgG4 and IgE in purified surface IgM⁺ B cells was enhanced in the presence of membranes of activated T cells, suggesting that an inducible cell surface molecule was involved that was shown not to be CD40-ligand.⁴³ Furthermore, the inability of ICOS-deficient mice to mount IgE responses demonstrates the major role of ICOS in the induction of IgE production.⁴⁴

Although the particular function of the ICOS/ICOS ligand interaction for T-B cooperation remains to be elucidated, our results clearly show that ICOS-expressing T cells are required for the induction of B-cell infiltration, local allergen-specific IgE production, and eosinophil airway inflammation. In conclusion, ICOS is a cell-surface marker that defines a subpopulation of effector T cells critically involved in pivotal aspects of allergen-induced airway inflammation and may therefore serve as a novel diagnostic and therapeutic target in the future.

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