

Essential role of dendritic cell CD80/CD86 costimulation in the induction, but not reactivation, of T_H2 effector responses in a mouse model of asthma

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Background: Airway dendritic cells (DCs) are crucial for the generation of T_H2 cells from naive T cells during sensitization and for reactivation of primed T_H2 cells on allergen challenge in mouse models of asthma. It is unknown whether CD80/CD86 costimulation is necessary during both phases of the response because primed T cells rely less on costimulatory molecules compared with naive T cells.

Objective: We sought to study the contribution of CD80/CD86 costimulatory molecules on DCs during sensitization or challenge in a mouse model of asthma.

Methods: Naive BALB/c mice received an intratracheal injection of ovalbumin (OVA)–pulsed DCs obtained from the bone marrow of wild-type (WT) or CD80/CD86^{−/−} mice and were subsequently challenged with OVA aerosol to address the role of costimulation during sensitization. OVA-sensitized mice received OVA-pulsed WT or CD80/CD86^{−/−} DCs without OVA aerosol to address the role of costimulation during challenge.

Results: WT DCs induced the proliferation and effector T_H2 differentiation of naive OVA-specific T cells, whereas CD80/CD86^{−/−} DCs induced only proliferation. Not surprisingly, WT DCs but not CD80/CD86^{−/−} DCs induced sensitization to OVA in naive mice. In contrast, in OVA-sensitized mice intratracheal injection of CD80/CD86^{−/−} OVA-pulsed DCs led to eosinophilic airway inflammation, goblet cell hyperplasia, and effector T_H2 cytokine production that was not different from that seen after injection with WT OVA-DCs, even when the inducible costimulator ICOS was blocked or cytotoxic T lymphocyte–associated antigen 4 immunoglobulin was given.

Conclusion: CD80/CD86 costimulation on DCs is only necessary during priming of naive T cells into T_H2 cells but not during restimulation of previously primed T_H2 cells in the challenge phase. (*J Allergy Clin Immunol* 2004;114:166-73.)

Key words: Dendritic cells, antigen presentation, antigen processing, allergy, mouse asthma model costimulation

Asthma is a T_H2-mediated disease in which airway dendritic cells (DCs) play an important role.^{1,2} A prominent role was shown for airway DCs during the sensitization to inhaled allergen but also during the challenge phase, in which previously primed T_H2 cells are reactivated in the lung to become effector T_H2 cells.³⁻⁶

The ways by which DCs activate naive or primed T cells are incompletely understood. DCs interact with T cells through multiple cell-surface interactions, some of which only serve to approximate both cell types and others that lead to T-cell activation. The latter molecules are called costimulatory molecules, the most studied of which is CD80/CD86, interacting with CD28 and cytotoxic T lymphocyte–associated antigen 4 (CTLA).^{7,8} It is generally accepted that this pathway is absolutely crucial for naive T-cell activation and differentiation. However, it is still controversial whether CD28 signaling is absolutely essential for generating primary immune responses *in vivo*.⁹⁻¹⁴ The precise role of CD80/CD86 costimulation in the reactivation of primed T cells in a secondary immune response is even more complex. Primed T cells are less dependent on CD80/CD86 costimulation.^{15,16} In some disease models, however, blocking CD80/CD86 costimulation with treatment with CTLA4-Ig fusion proteins led to reduced memory T-cell reactivation.¹⁷ Recent reports have also shown that mucosal DCs interacting with primed T cells in the lung upregulate CD86 in mice with eosinophilic airway inflammation⁵ in contrast to the low expression of CD86 on airway DCs found in the steady state.¹⁸ These results suggest that CD80/CD86 costimulation on DCs might be involved in local reactivation of memory T cells in the lung, leading to eosinophilic airway inflammation. In this article we have studied the requirements of CD80/CD86 costimulation for the induction of primary and secondary immune responses to allergen in the lung. To specifically address the role of these molecules on DCs only, we used a model of asthma in which DCs were the only cells lacking expression of CD80/CD86, avoiding interference with CD80/CD86-CD28 interaction between other cells. In addition, we

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Supported by a grant of the Netherlands Asthma Foundation (NAF3.2.99.37). B.L. is a recipient of an NWO VIDI grant.

Received for publication February 20, 2004; revised March 22, 2004; accepted for publication March 29, 2004.

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0091-6749/\$30.00

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doi:10.1016/j.jaci.2004.03.044

Abbreviations used

APC: Antigen-presenting cell
BALF: Bronchoalveolar lavage fluid
CFSE: Carboxyfluorescein diacetate succinimidyl ester
CTLA4: Cytotoxic T lymphocyte-associated antigen 4
DC: Dendritic cell
LN: Lymph node
OVA: Ovalbumin-pulsed dendritic cell
TCR: T-cell receptor
WT: Wild type

compartmentalized the immunization by administering DCs in the airways in contrast to other models that use systemic sensitization routes. Our results suggest that CD80/CD86 costimulation provided by DCs in the lung is mainly required for generating effector T-cell responses during sensitization but is redundant during reactivation of primed T cells in the challenge phase. These findings have important implications for the design of immunotherapeutic strategies in asthma that aim to interfere with costimulation.

METHODS

Animals

All experiments were performed with 8- to 10-week-old female BALB/c, DO11.10 BALB/c, and CD80/CD86^{-/-} BALB/c and C57Bl/6 mice (kindly provided by Dr M. Oosterwegel, Utrecht University, The Netherlands, and B. Salomon, Pitié Salpêtrière, Paris, France).¹⁹ Mice were housed under specific pathogen-free conditions. All of the experimental procedures were approved by an ethical committee of animal experiments.

Generation and antigen pulsing of bone marrow-derived DCs

DCs were grown from bone marrow cells, as described earlier.⁴ On day 9 of culture, cells were pulsed overnight with 100 µg/mL LPS-low ovalbumin (OVA; Worthington Biochemical Corporation, Lakewood, NJ; LPS contamination of <2.9 EU/mL). At day 10, cells were washed, and any free OVA was removed. CD11c⁺/MHCII⁺ cells grown out of wild-type (WT) donors expressed 97% CD80 and 87% CD86 compared with only 1.4% and 0.4%, respectively, in CD80/CD86^{-/-} mice, representing background immunofluorescence.

Generation of eosinophilic airway inflammation with DCs

OVA-pulsed DCs or control unpulsed DCs, 1×10^6 , were injected into the airways of anesthetized naive mice at day 0 to induce sensitization to OVA, as described in earlier experiments.^{4,20} DCs were obtained from WT or CD80/CD86^{-/-} BALB/c mice to address the role of costimulation during sensitization. Ten days later, mice were challenged with a 30-minute OVA aerosol (grade III, 1% wt/vol in PBS; Sigma Chemical Co, St Louis, Mo) on 3 consecutive days.³

To address the role of costimulation during challenge, sensitized mice received an intratracheal injection of 1×10^6 OVA-pulsed DCs on day 11 after sensitization in the absence of any OVA aerosol exposure and were killed 2 days later (DC challenge protocol). DC-sensitized mice were challenged with (1) WT DCs, (2) CD80/CD86^{-/-} DCs, or (3) CD80/CD86^{-/-} DCs in combination with an

intravenous injection of 250 µg of CTLA4-Ig (BD Biosciences Pharmingen, San Diego, Calif) or 250 µg of mouse IgG2a as a control or (4) CD80/CD86^{-/-} DCs in combination with a daily (days 11 and 12) intraperitoneal injection of 100 µg of rat anti-murine ICOS (12A8)²¹ or 100 µg of rat IgG as a control.

Antigen presentation assay to OVA T-cell receptor transgenic T cells *in vivo*

Cell suspensions of pooled peripheral lymph nodes (LNs) from naive OVA T-cell receptor (TCR) Tg DO11.10 mice were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, Ore), as previously described.²² CFSE⁺ T cells, 10×10^6 , were transferred intravenously into naive mice. Twenty-four hours later, either 1×10^6 OVA-pulsed WT or CD80/CD86^{-/-} DCs were transferred intratracheally. Seventy-two hours after DC transfer, lung-draining LNs were analyzed for the proliferation of CFSE-labeled OVA-specific TCR Tg T cells. As a control, non-lung-draining LNs (brachial) were used. Transferred OVA-specific TCR transgenic CD4⁺ T cells were recognized as CD4⁺CFSE⁺ cells expressing the clonotypic TCR recognized by the mAb KJ1.26+ (Caltag, Burlingame, Calif). Propidium iodide (Sigma) was used to exclude dead cells. To prevent nonspecific binding to Fc receptors, 2.4.G2 blocking reagent was used (ATCC, Manassas, Va). Division plots were obtained with a FACScalibur flow cytometer (Becton and Dickinson Immunocytometry Systems, San Jose, Calif) and analyzed with FlowJo software (Treestar, Costa Mesa, Calif).

Collection and analysis of cells and tissues

Bronchoalveolar lavage fluid. Twenty-four hours after the last aerosol challenge or 48 hours after DC challenge in sensitized mice, bronchoalveolar lavage was performed, as previously described.⁴ The number of eosinophils in bronchoalveolar lavage fluid (BALF) cells was determined by means of FACS analysis²³ after staining with the mAbs FITC-MHCII (2G9), phycoerythrin-CCR3 (R&D systems, Abingdon, United Kingdom), cychrome-CD3 (145-2C11) and cychrome-B220 (RA3-6B2), and antigen-presenting cell (APC)—CD11c (HL3), all in combination with 2.4.G2 blocking reagent. This method was used to determine the percentage of eosinophils without other contaminating BALF cells.

LN restimulation. For LN restimulation, cells were dispersed through a 40-µm cell sieve (Becton Dickinson), plated in 96-well round-bottom plates at a density of 2×10^5 cells per well, and restimulated for 4 days with 10 µg/mL OVA. After 4 days, levels of IL-4, IL-5, IL-10, IFN-γ (OptEIA, BD Biosciences, Pharmingen), and IL-13 (R&D systems, Abingdon, United Kingdom) were measured in supernatants by means of ELISA.

Airway histology. Lungs were infused with OCT (1:1 vol/vol in PBS) and were snap-frozen in liquid nitrogen. Three-micrometer cryosections were stained with hematoxylin and eosin or with periodic acid-Schiff (Sigma) to detect goblet cells.

Statistical analysis

Comparison of means between different groups was performed with the Mann-Whitney *U* test for unpaired data (SPSS 10.0 for Windows). All experiments were performed with 6 to 8 mice per group. Differences were considered significant at a *P* value of less than .05. A score for the degree of inflammation was assigned to each section to compare histology sections. The score was based on perivascular and peribronchial infiltrates, goblet cell hyperplasia, and inflammatory cells in parenchyma in this order of importance. The scores per group were compared with a Kruskal-Wallis test and, in case of significance, pairwise with the Mann-Whitney *U* test. Differences were considered significant at a *P* value of less than .05.

RESULTS

Role of CD80 and CD86 costimulation by DCs for inducing proliferation and T_H2 differentiation of naive T cells

Mice were sensitized by means of an intratracheal injection of either OVA-pulsed WT DCs or OVA-pulsed CD80/CD86^{-/-} DCs. Mice received 10×10^6 CFSE-labeled OVA TCR transgenic T cells intravenously 1 day before DC injection to visualize naive T-cell proliferation. Flow cytometry was used to track cell division of the transferred CFSE⁺/OVA TCR Tg/CD4⁺ T cells in draining LNs of the lung. CD80/CD86^{-/-} DCs induced up to 6 divisions in OVA-specific naive T cells, and WT DCs induced up to 8 divisions. CD80/CD86^{-/-} DCs recruited significantly less cells into cell division ($P = .002$). In CD80/CD86^{-/-} DC-sensitized mice, proliferating OVA-specific T cells divided on average 2.13 times compared with 2.81 times in OVA-pulsed WT DC-sensitized mice (Fig 1, A). No division was seen in nondraining LNs (brachial) or in mice sensitized with unpulsed DCs.

We investigated whether sensitization with DCs lacking CD80/CD86 impaired the differentiation of divided cells into effector cells. We determined cytokine production by T cells in the lung-draining LNs after restimulation with OVA *ex vivo*. Despite displaying only a small reduced proliferation, T cells of mice sensitized with CD80/CD86^{-/-} DCs produced dramatically lower levels of IL-4, IL-13, and IFN- γ compared with the mice that received OVA-pulsed WT DCs ($P = .002$; Fig 1, B). Also, less IL-10 was produced after sensitization with CD80/CD86^{-/-} DCs compared with that seen in mice sensitized with OVA-pulsed WT DCs ($P = .002$).

Role of CD80/CD86 costimulation by DCs during sensitization on development of eosinophilic airway inflammation in response to inhaled OVA

The lower production of T_H2 cytokines by OVA-specific T cells after injection of CD80/CD86-deficient DCs suggested that naive T cells did not differentiate into effector cells. This was investigated by challenging OVA-pulsed DCs or OVA-pulsed CD80/CD86^{-/-} DC-immunized mice with OVA aerosols. In these experiments no OVA-TCR Tg T cells were administered. Mice were sensitized by means of an intratracheal injection of OVA-pulsed CD80/CD86^{-/-} or OVA-pulsed WT DCs. Unpulsed DCs were used as a negative control. From days 10 through 12, mice were challenged with OVA aerosols. Analysis of BALF cells revealed that OVA aerosol challenge in mice sensitized with CD80/CD86^{-/-} DCs led to a significantly lower number of total cells ($P = .02$; Fig 2, A) and eosinophils ($P = .002$; Fig 2, B) compared with numbers found in mice sensitized with WT DCs. Although airway inflammation was severely reduced in mice immunized with CD80/CD86^{-/-} OVA-pulsed DCs, it was still higher compared with the baseline level seen in mice immunized with unpulsed DCs (Fig 2, A and B).

We next determined the type and magnitude of cytokine production of T cells in the lung-draining LNs in response to the inhaled allergen. In agreement with the BALF data, T_H2 cytokine production of IL-4, IL-5, IL-13, and IL-10 ($P = .002$) was significantly less when mice were immunized with CD80/CD86^{-/-} DCs compared with OVA-pulsed WT DCs (Fig 2, C). Mice that were sensitized with unpulsed WT DCs had lower IL-13 levels than mice sensitized with CD80/CD86^{-/-} DCs ($P \leq .026$). Sensitization with CD80/CD86^{-/-} DCs induced similar IFN- γ production compared with sensitization with OVA-pulsed WT DCs. Histologic analysis of lungs of mice sensitized with OVA-pulsed WT DCs revealed intense inflammatory perivascular and peribronchial infiltrates and goblet cell hyperplasia (Fig 2, D) after OVA challenge. Lungs of mice sensitized with CD80/CD86^{-/-} DCs revealed less inflammatory perivascular and peribronchial infiltrates (Fig 2, E) compared with those seen in mice sensitized with OVA-pulsed WT DCs. As expected, eosinophilic airway inflammation and epithelial changes were almost completely absent in mice immunized with unpulsed WT DCs (Fig 2, F).

Role of CD80/CD86 costimulation by the antigen-presenting DCs during antigen challenge in sensitized mice

To determine the role of CD80/CD86 costimulation provided by DCs during the effector phase, we used a newly developed model in which sensitized mice are challenged intratracheally with injected DCs instead of the more commonly used challenge with 3 OVA aerosols. This protocol induces a secondary immune response in mice, resulting in all the hallmarks of asthma, including eosinophilic airway inflammation, T_H2 cytokine production, high IgE serum level, and an enhanced airway hyperresponsiveness to methacholine. This response was T cell dependent because applying this protocol to mice with severe combined immunodeficiency syndrome did not induce eosinophilia (manuscript submitted).

OVA-sensitized mice were challenged with an intratracheal injection of 1×10^6 WT OVA-pulsed DCs or with CD80/CD86^{-/-} OVA-pulsed DCs. As shown in Fig 3, A, the total number of BALF cells and BALF eosinophil counts were similar after challenge with either OVA-pulsed WT DCs or CD80/CD86^{-/-} DCs. Also, cytokine production of IL-4, IL-5, IL-13, and IL-10 by lymphocytes of the lung-draining LNs after restimulation with antigen was not significantly different between both groups (Fig 3, B), except for a higher IFN- γ production ($P = .009$) by CD80/CD86^{-/-} DC-immunized mice. Analysis of airway histology revealed similar levels of peribronchial and perivascular inflammatory infiltrates and goblet cell hyperplasia in mice challenged with CD80/CD86^{-/-} DCs or WT DCs (Fig 3, C and D).

To determine whether bystander cells were able to provide supplementary CD80/CD86 costimulation during antigen presentation induced by CD80/CD86^{-/-} DCs, we blocked CD80 and CD86 molecules by administering

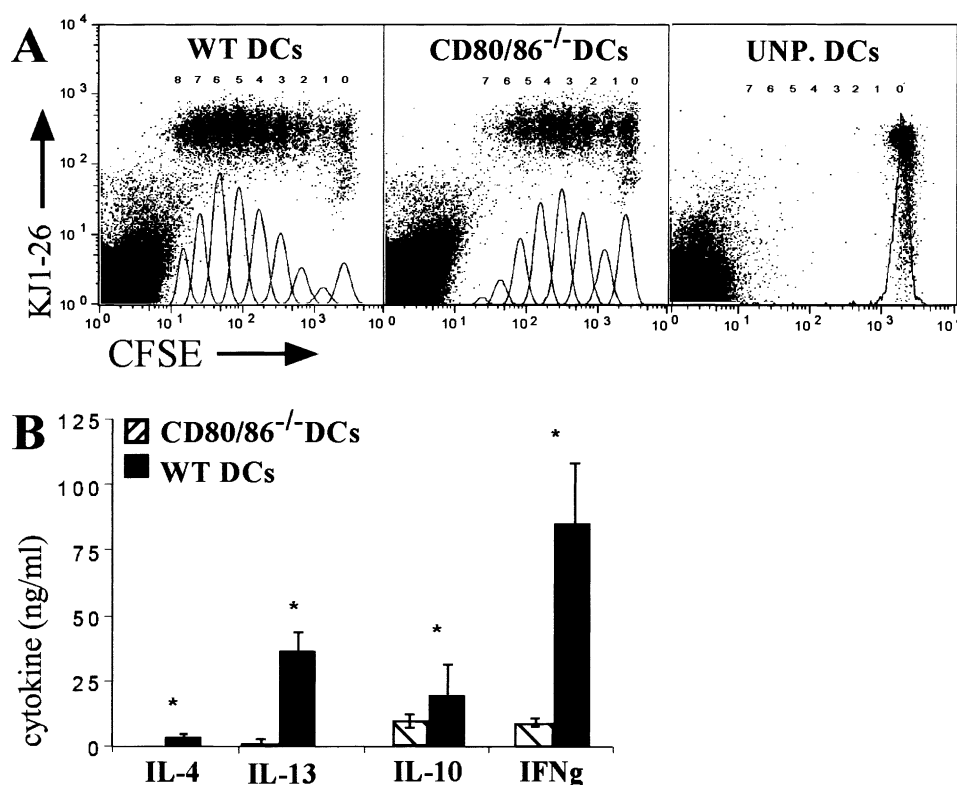


FIG 1. Proliferation and T_H2 differentiation of naive T cells induced by means of sensitization with CD80/CD86^{-/-} DCs compared with WT DCs. The number of induced divisions in adoptively transferred CFSE⁺/OVA TCR Tg/CD4⁺ T cells in mice were sensitized with either OVA-pulsed WT DCs (A; left panel) or OVA-pulsed CD80/CD86^{-/-} DCs (A; middle panel) or unpulsed DCs (A; right panel) is shown. UNP, Unpulsed. B, T_H2 cytokine production by lung-draining LN cells (*P < .05 in comparison with CD80/86^{-/-} DCs).

CTLA4-Ig to sensitized mice 24 hours before we injected OVA-pulsed CD80/CD86^{-/-} DCs. The number of recovered eosinophils from BALF after CTLA4-Ig treatment was not significantly different from that of control mice treated with mouse IgG2a (3.8×10^4 vs 5.8×10^4 , $P = .7$).

Because we found that CD80/CD86 absence did not affect the potential of DCs to induce effector responses in primed mice, we questioned whether costimulation through B7-Rp1 interacting with ICOS would be responsible for residual costimulatory capacity in the absence of CD80/CD86. These experiments were performed in C57Bl/6 mice. Mice were treated with anti-ICOS or control rat IgG blocking antibody at the time of injection with CD80/CD86^{-/-} DCs and 24 hours later. One day after the last anti-ICOS injection, analysis of BALF cells revealed a lower, although not statistically significant, number of BALF eosinophils recovered from mice challenged with CD80/CD86^{-/-} DCs in combination with ICOS blockade compared with that seen in mice challenged with CD80/CD86^{-/-} DCs only or with WT DCs (Fig 3, E).

DISCUSSION

We previously showed the involvement of DCs during sensitization and challenge in a murine model for

asthma.^{3,4,20,22} Because activation of naive T cells and primed T cells depends differentially on provision of costimulatory molecules, it was of interest to study whether CD80/CD86 costimulation provided by DCs was necessary for induction of T_H2 effector responses in the lung and whether there would be a difference in the need for costimulation during the sensitization phase (ie, when naive T cells become primed) compared with the challenge phase (when primed T cells become effector T cells).

For activation of naive T cells *in vitro*, strong costimulatory signals are required. However, it has been less clear whether the CD80/CD86–CD28 interaction is really essential for inducing primary immune responses *in vivo*. Several reports in CD28^{-/-} mice show dependence on CD28 for generation of effective immune responses,^{9–11} whereas others show that these mice mount an equally effective immune response compared with their WT littermates in models of allograft rejection,¹² graft-versus-host disease,¹³ and generation of antiviral immunity.¹⁴ These differences in outcome might be due to differences in the types of APCs that induce the T-cell response or differences in the degree of maturity that is being induced in the APCs. In our study CD80/CD86 costimulation provided by DCs was required for an efficient sensitization to OVA, illustrating that DC maturation was

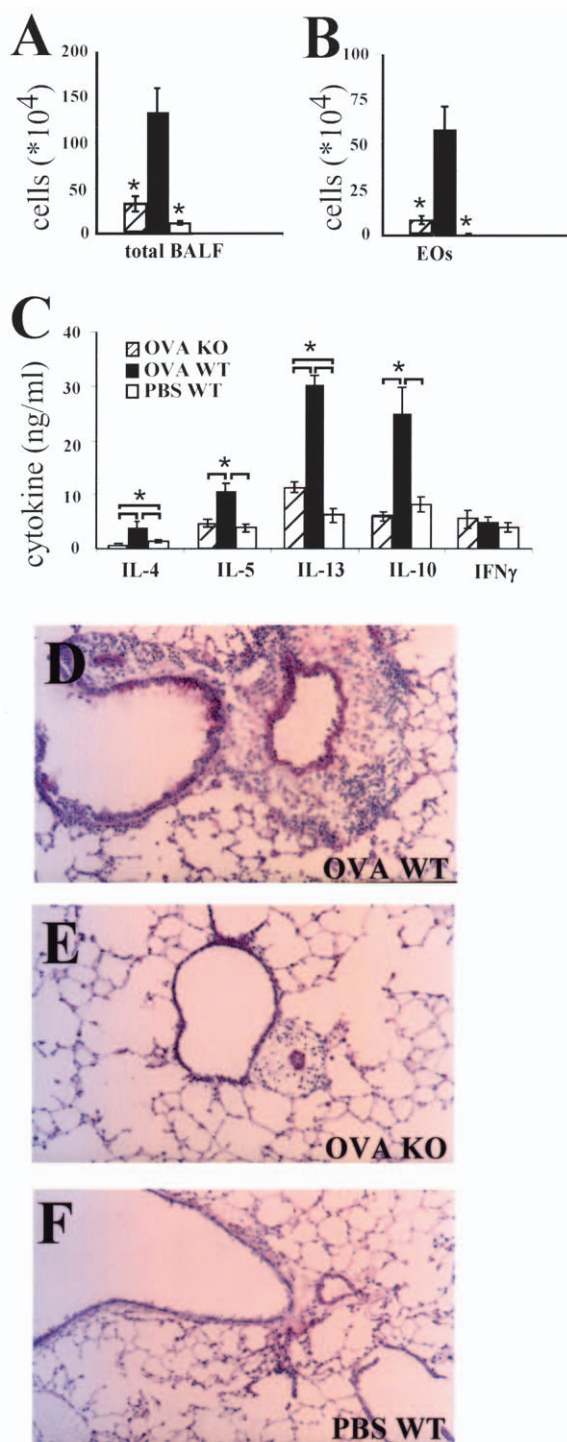
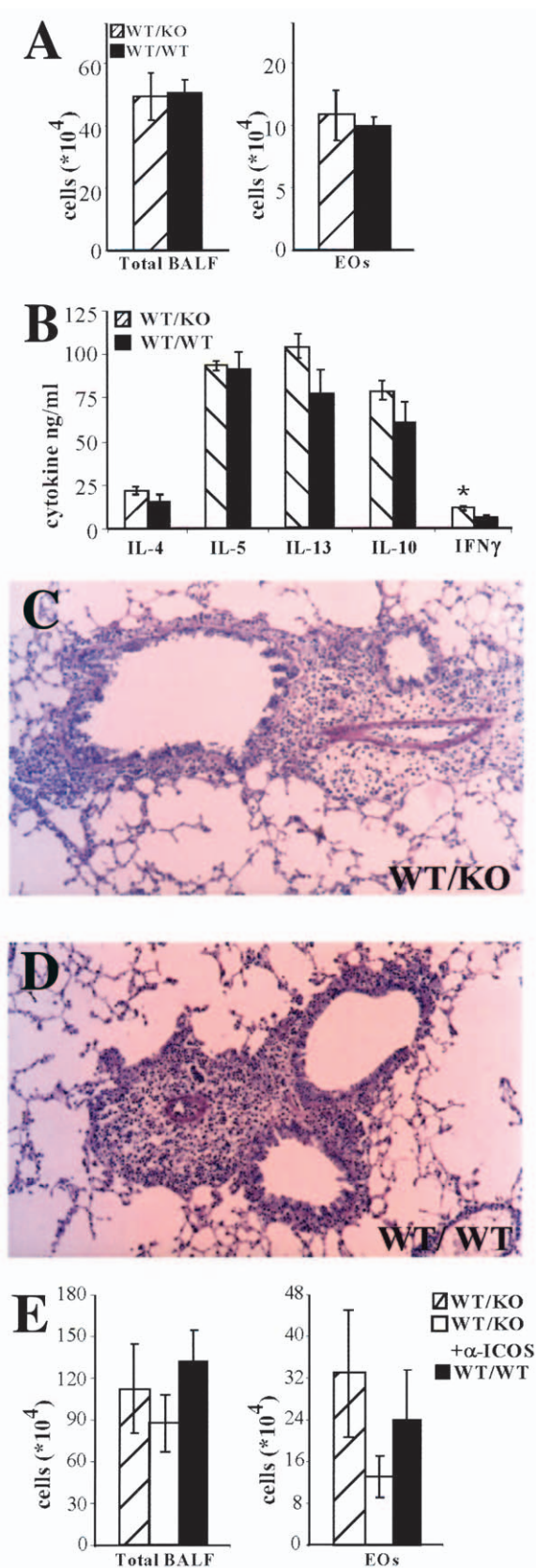


FIG 2. No induction of eosinophilic airway inflammation in response to inhaled allergen in CD80/CD86^{-/-} DC-sensitized mice. Mice were sensitized with OVA-pulsed WT DCs (OVA WT), OVA-pulsed CD80/CD86^{-/-} DCs (OVA KO), or unpulsed WT DCs (PBS WT) and were challenged with OVA aerosols. Total cell numbers (**A**) and numbers of eosinophils (Eos; **B**) in BALF were determined (**P* < .05 in comparison with OVA-pulsed WT DCs). **C**, T_H2 cytokine production by draining LN cells of the lung (**P* < .05). Sections of lungs from OVA aerosol-challenged mice sensitized with OVA-pulsed WT DCs (**D**), OVA-pulsed CD80/CD86^{-/-} DCs (**E**), and PBS-pulsed WT DCs (**F**). Lungs were stained with periodic acid-Schiff.

necessary for induction of a fully activated T-cell response. Although CD80/CD86^{-/-} DCs were able to induce strong proliferation in naive OVA-specific T cells, these T cells produced virtually no IL-4, IL-13, IL-10, and IFN-γ, which is indicative of the fact that no potent effectors were induced. Although T-cell division is often measured as a read out of T-cell activation, a lot of attention has recently been paid to the uncoupling of naive T-cell proliferation from differentiation to effector T cells.^{11,24-29} A new concept has emerged from the observation that cells receiving a suboptimal antigen, costimulatory stimulus, or both proliferated as strongly as fully activated T cells but died from neglect. The term launched by Lanzavecchia's group is "unfit T cells," representing the idea that T cells stimulated *in vitro* under suboptimal conditions show some signs of activation, such as division, but do not develop into proinflammatory effector cells and are unable to survive when the amount of antigen becomes limiting.^{30,31} Many studies have demonstrated that adoptively transferred TCR transgenic T cells can proliferate *in vivo* in response to harmless free peptide or to peripheral self antigen but without differentiation to effector function and even leading to deletional tolerance.^{22,28,32-34} In these experiments with harmless (self) antigens, DC maturation is not induced because of a failure of activation of innate immune receptors on DCs.^{34,35} Following this concept, CD80/CD86^{-/-} DCs presenting antigen in our studies might resemble immature DCs that do not engage T-cell contact of sufficient strength, leading to generation of unfit T cells, which proliferate in response to allergen but do not differentiate into effector T cells. In support of the unfit T-cell concept, rechallenge with antigen of CD80/CD86^{-/-} DC-immunized mice did not induce airway inflammation in contrast to mice sensitized with WT DCs. The partially activated T cells that were induced by CD80/CD86^{-/-} DCs *in vivo* could not be rescued into effector function by a second injection of WT DCs, illustrating that they were either deleted or rendered anergic (unpublished observations). Further T cell-tracking experiments can elucidate the fate of these unfit T cells *in vivo*.

The above data imply that DC maturation in the form of CD80/CD86 expression is important for differentiation of naive T cells into primed T_H2 cells in the lung in the process of sensitization. Whether DC maturation and CD80/CD86 expression are also important for activation of already primed T cells in the lung is currently unknown. *In vitro*, primed T cells need less costimulatory molecules compared with naive T cells for full activation^{15,16} and therefore could be activated by less mature DCs or even less professional APCs, such as B cells, macrophages, and epithelial cells, expressing MHC. In support, studies with CD80/CD86^{-/-} splenic APCs showed that memory T cells required less costimulation than naive cells for cytokine production and proliferation in response to antigen encounter.^{15,16} Recent articles have shown that CD86 expression is rapidly triggered on airway mucosal DCs after OVA inhalation in sensitized animals⁵ and on BALF DCs during ongoing eosinophilic airway



inflammation.¹⁸ When isolated from the lung, airway mucosal DCs upregulate CD86 after coculture with effector T cells and not after coculture with naive T cells.⁵ It was therefore proposed that T_H memory cells mediated this activation, suggesting a role for CD80/CD86 costimulation during secondary immune response. In our studies we addressed directly through adoptive transfer of DCs whether CD80/CD86 costimulation was absolutely necessary during the reactivation of primed T cells in the lung at times of allergen challenge and found that CD80/CD86 expression is redundant. The observation that CD86 is upregulated on DCs during allergen challenge *in vivo*, however, still suggests some functional role of CD86. Some studies in mouse models of allergy showed that blocking B7-CD28 ligation with CTLA4-Ig could partially or totally inhibit secondary immune responses.^{21,36-40} Other studies showed, however, that inhibition was not possible with CTLA4-Ig during challenge, particularly when mice were very strongly sensitized by means of intraperitoneal injection of OVA in alum or when primed T_H2 cells were adoptively transferred.^{41,42} An explanation for our findings that CD80/CD86 is of less importance when adoptively transferred DCs restimulate primed T cells in the lung would be that other endogenous cells provide CD80/CD86 costimulation during antigen presentation by transferred CD80/CD86^{-/-} DCs or that antigen would be transferred from injected to endogenous DCs. To exclude this possibility, we treated mice with CTLA4-Ig before challenge with CD80/CD86^{-/-} DCs. Mice treated with CTLA4-Ig had a similar number of recovered BALF eosinophils compared with untreated mice, making this mechanism unlikely.

It is therefore likely that other costimulatory molecules beside CD80/CD86 are involved in activating T cells in secondary immune responses. Several molecules on the surface of the DC, like B7RP-1 (also known as ICOSL), OX-40L, 4-1BBL, CD40, and intercellular adhesion molecule 1, have been reported to have costimulatory capacity and can be responsible for the induced reaction in the absence of CD80/CD86⁴³, a likely candidate in this context is B7RP-1, which is induced on mature DCs in lung-draining LNs.⁴⁴ Blockade of ICOS-ICOSL by using an ICOS-Ig fusion protein was able to completely suppress asthmatic features induced by adoptive transfer

FIG 3. Induction of eosinophilic airway inflammation in response to challenge with CD80/CD86^{-/-} DCs in WT DC-sensitized mice. Mice were sensitized with OVA-pulsed WT DCs and challenged with either OVA-pulsed CD80/CD86^{-/-} DCs (WT/KO) or OVA-pulsed WT DCs (WT/WT). **A**, Total cell numbers and number of eosinophils in BALF were determined. **B**, T_H2 cytokine production by lung-draining LN cells (**P* < .05). Sections of lungs from WT-DC-sensitized mice challenged with OVA-pulsed CD80/CD86^{-/-} DCs (**C**) or OVA-pulsed WT DCs (**D**). Lungs were stained with hematoxylin and eosin. **E**, C57Bl/6 mice were sensitized with OVA-pulsed WT DCs and challenged with either an intratracheal injection of OVA-pulsed CD80/CD86^{-/-} DCs (WT/KO), CD80/CD86^{-/-} DCs in combination with α -ICOS (WT/KO + α -ICOS), or WT DCs (WT/WT). Total cell numbers and numbers of eosinophils in BALF were determined.

of OVA-specific T_H2 cells to the airways of mice.⁴⁵ When we blocked ICOS-ICOSL interaction with an anti-ICOS antibody during CD80/CD86^{-/-} DC challenge in sensitized mice, this did not significantly reduce airway eosinophilia, although there was a trend toward reduced airway eosinophilia. One explanation for the poor efficacy of ICOS in this model in contrast to previous models would be the recent observation that DCs downregulate the expression of B7RP-1 during eosinophilic airway inflammation.¹⁸ Another costimulatory pathway able to compensate for the lack of CD80/CD86/B7RP-1 costimulation on DCs would be OX-40L. OX-40 (CD134), a member of the TNF receptor family, is a major regulator of antiapoptotic proteins, such as Bcl-xL and Bcl-2, and strongly promotes the survival of antigen-activated primary CD4 T cells. In addition, OX-40 is preferentially expressed by memory T_H2 cells. Blocking OX-40–OX-40L interaction impaired all features of asthma induced by means of adoptive transfer of OVA-specific T_H2 cells.⁴² Follow-up experiments involving *in vivo* blockade of OX-40–OX-40L will demonstrate whether this molecule is responsible for the residual stimulation of primed T_H2 cells in the combined absence of CD80/CD86 and ICOSL.

In conclusion, we have shown the dependence on DC-derived CD80/CD86 costimulation during primary immune responses *in vivo* in the lung. In the absence of CD80/CD86 costimulation, OVA-specific T cells were driven into proliferation, but this did not lead to differentiation into effector T cells, illustrating the essential role of CD28 in T_H2 differentiation. Secondary immune responses induced by DCs were not dependent on costimulation provided by B7 family members. These results have implications for inhibition of costimulatory molecules as a target for primary prevention strategies in asthma.⁴⁶ It will have to be elucidated which costimulatory molecules on DCs or other APCs are absolutely required for a secondary immune response before a strategy of costimulatory blockade can be envisaged as a therapy for established asthma.

We thank S. Baelemans and K. Leman for technical assistance with staining histology slides, K. Thielemans for kindly providing rmGM-CSF, and M. Oosterwegel, F. Hofhuis, and B. Salomon for provision of CD80/CD86^{-/-} mice.

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