Control of functional T helper cell polarization by dendritic cells
Limao Vieira, P.

Citation for published version (APA):
ICOS-mediated Costimulation of Human T Helper Cells Promotes both Th1 and Th2 Cytokine Production but Selectively Promotes the Expansion of Th2 cells

Pedro L. Vieira, Lianne Wassink, L. Mary Smith, Samuel Nam, Gillian A. Kingsbury, Eddy A. Wierenga, Jose Carlos Gutierrez-Ramos, Anthony J. Coyle, Martien L. Kapsenberg

Ligation of the third member of the CD28 family, inducible costimulator (ICOS) has recently been demonstrated to provide a CD28 independent signal for activation of CD4+ T cells and to regulate T cell-dependent immune responses in vivo. The expression and functional importance of ICOS during APC-T cell interaction on human Th cells is not fully understood. In the present paper, we report that human ICOS is induced upon activation of Th cells at all stages of maturation, including Th1 and Th2 cells. Blocking of B7-related protein 1 (B7RP-1)-induced ICOS costimulation by an ICOS-lg fusion protein revealed that ICOS is essential for the production of the anti-inflammatory cytokine IL-10 and contributes significantly to the production of signature Th1 (IFN-γ) and Th2 (IL-4, IL-5 and IL-13) cytokines, but not IL-2. Despite its role in the production of Th1 and Th2 cytokines, ICOS does not affect the differentiation of Th cells into Th1 or Th2 cells. However, ICOS preferentially promotes Th2 responses by selectively controlling the proliferation of established effector Th2 cells. These data underline the importance of ICOS in the expression of Th1- and Th2-mediated effector functions and define a novel role for ICOS in selectively favoring the clonal expansion of Th2 cells.

Submitted for publication
INTRODUCTION

The capacity of APC to initiate effective Th cell responses relies on the presentation of specific peptides in the context of class II MHC in combination with the appropriate costimulatory signals. Naive T cells are costimulated by the ligation of CD28 through CD80 and/or CD86 expressed on professional APC. In contrast to CD28, that is expressed constitutively by almost all resting human Th cells, the second member of the family, CTLA-4, is induced upon activation and delivers a negative signal to the activated T cell, opposing CD28-mediated costimulation (1, 2). However, while CD28 plays an essential role in both clonal expansion and IL-2 production from naive CD4^+ Th cells, activation of effector Th cells is less dependent on CD28-CD80/86 costimulation (3). Recently the third member of the CD28 family, inducible costimulator (ICOS), was identified, which similar to CTLA-4 is expressed only by activated T cells (3-5). Crosslinking of the ICOS receptor, in combination with TCR activation delivers a signal for the production of effector cytokines (5-7). The ligand of ICOS, counter receptor B7-related protein 1 (B7RP-1, also known as B7h, GL-50, ICOS ligand, LICOS, and B7-H2) (Refs. 5, 8-12), is expressed by several APC types, including dendritic cells (DC) and B cells (5, 8, 10, 12-14) as well as non-professional APC including fibroblasts and CD34^+ progenitor cells (8, 14).

Although ICOS was first identified in human T cells (6), most data on the function of ICOS has been obtained in murine models in vivo. Murine ICOS was initially cloned after subtractive hybridization of Th1 and Th2 cDNA, since in the mouse ICOS is preferentially expressed by Th2 cells (7). Indeed, activation of mouse naive Th cells induces ICOS expression that is maintained during Th2 cell development, but is downregulated during Th1 cell development (7, 15). While initial studies in the mouse suggested that ICOS regulates Th2 cell function, more recent data has suggested that the situation is more complex as inhibition of ICOS also inhibits Th1-associated experimental allergic encephalomyelitis and allograft rejection (7, 16-19). Furthermore, ICOS-deficient mice exhibit impaired germinal center formation and defective Ig class switch indicating a critical role for ICOS in T cell-dependent Ab responses (20-22). Interestingly, ICOS deficiency regulated not only the IL-4-dependent production of IgE and IgG1, but also IgG2a production is dramatically impaired suggesting that ICOS regulates Th1- and Th2-mediated B cell responses. In contrast, ICOS-deficient animals or administration of anti-ICOS mAb during
ICOS regulates human T helper cell function

Immunization has been demonstrated to dramatically augment the severity of experimental autoimmune encephalomyelitis (EAE) (7, 16, 17, 19, 22, 23).

While the role of ICOS in T cell-dependent immune regulation in vivo remains controversial, the role and relative importance of B7RP-1–ICOS pathway in human Th cell activation remains unknown. Indeed, recent studies by Aicher and colleagues suggested that ICOS does not play an important role as compared to CD28 in the activation of human T cells. However, it is unknown if at other stages of maturation and activation whether ICOS costimulation provides an important signal for naive and/or memory cell activation and to what extent ICOS preferentially supports the development of Th1 vs Th2 cells.

In the present series of experiments, we show that ICOS was upregulated in all Th cells subsets tested. Although ICOS has no effect on the differentiation of either Th1 or Th2 cells, ICOS costimulation selectively supports the expansion of established effector Th2 cells. B7RP-1–mediated ICOS costimulation allows for the full expression of signature Th1 and Th2 cytokines by naive and resting memory Th cells and effector Th1 and Th2 cells. In effector Th cells, ICOS also mediates the production of other cytokines. These data underscore that the B7RP-1–ICOS costimulatory pathway is important for the full expression of the effector function of both Th cells at all stages of maturation, including Th1 and Th2 cells, but plays an essential role for Th2 responses by supporting their clonal expansion.

MATERIALS AND METHODS
Generation of ICOS-Ig
A fusion protein of human ICOS with human IgG1 (ICOS-Ig) was generated as previously described by Coyle and colleagues for the mouse ICOS-Ig (7). Briefly, a DNA sequence containing the extracellular domain of human ICOS was PCR-amplified and cloned into a vector containing the CD5 signal sequence and the human IgG1 constant region. COS cells were transiently transfected using lipofectamine (GIBCO-BRL, Gaithersburg, MD), and the recombinant protein was purified over a protein A column. The purity of ICOS-Ig was subsequently assessed by coomassie-stained SDS-PAGE and was determined to be greater than 90%. The identity of the ICOS-Ig was further confirmed by mass spectrometry by comparing the
trypsin peptides generated from the extracted gel band to a theoretical trypsin digest (peptide mass fingerprinting by MALDI-TOF analysis)

**Mice and immunizations**

C3H female, 6-8 week old mice were obtained from Taconic Farm (Germantown, NY). Mice were immunized with plasmid DNA containing ICOS-Ig. Briefly, 50 mg of gold microcarriers were coated with 100 μg of plasmid DNA according to manufacture’s instructions (Bio-Rad Laboratories, Hercules, CA). The chest and abdomen of each animal were shaved and particles were delivered to both using the Helios® Gene Gun system (Bio-Rad Laboratories). Each animal received approximately 0.5 mg of gold-coated beads containing approximately 1 μg of DNA/shot. The animals were injected every other day for a total of 5 days (10 shots). Three days prior to fusion, the animals were immunized intravenously with 25 μg of ICOS-Ig in PBS.

**Generation of anti-ICOS mAb**

B cell hybridomas were constructed by fusing the immune spleen cells with the nonsecreting murine fusion partner SP2/0 as described previously (24). Hybridoma supernatant fluids were screened for specific antibodies by flow cytometry. Briefly, L cells transfected with ICOS were incubated with hybridoma supernatant fluid. Specific antibodies were detected using a PE-conjugated goat F(ab’)2 anti-mouse IgG and IgM (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Hybridomas that were secreting ICOS specific Ab were cloned using limiting dilution.

**Generation of DC from peripheral blood monocytes and induction of their maturation**

Immature DC were generated from peripheral blood monocytes by culturing them in IMDM (Life Technologies, Paisley, U.K.) containing 1% FCS (Hyclone, Logan, UT), rGM-CSF (500 U/ml; Schering-Plough, Uden, The Netherlands) and rIL-4 (250 U/ml; Pharma Biotechnologie Hannover (PBH), Hannover, Germany) (25). At d 6 the obtained immature CD1a+CD83− DC (data not shown) were obtained and were induced to mature by a 2-d exposure to a combination of the cytokines rIL-1β (10 ng/ml, sp. act. 2x10⁸ U/mg; PBH) and rTNF-α (50 ng/ml, sp. act. 1x10⁸ U/mg; PBH)
ICOS regulates human T helper cell function (25). All subsequent tests were performed after harvesting the cells at d 8 and removal of residual GM-CSF, IL-4, IL-1β, and TNF-α, by extensive washing. These mature DC abundantly express B7RP-1, as confirmed by ICOS-Ig labeling and FACS® (data not shown).

Isolation of lymphocyte populations
CD4⁺ Th cells were purified from PBL using anti-CD4 coated Dynabeads (Dynal AS, Oslo, Norway), followed by treatment with Detach-a-beads (Dynal AS) according to the manufacturer's instructions. This method yielded highly purified CD4⁺ Th cells as assessed by flow cytometry (>98%, data not shown). Naive CD4⁺ CD45RA⁺ CD45RO⁻ Th cells were isolated from PBL, with the negative selection human CD4⁺/CD45RO⁻ column kit (R&D Systems, Minneapolis, MN). This method yielded highly purified naive Th cells as assessed by flow cytometry (>98%, data not shown).

Generation of polarized Th1 and Th2 cells
Polarized Th cell populations were generated in IMDM supplemented with 10% FCS, gentamycin (86 μg/ml; Duchefa, Haarlem, The Netherlands) and rIL-2 (10 U/ml; Cetus Corporation, Emeryville, CA). To this aim naive CD45RA⁺CD4⁺ Th cells were stimulated with immobilized anti-CD3 mAb (1 μg/ml; CLB-T3/3) and soluble anti-CD28 mAb (2 μg/ml; CLB-CD28/1, CLB) both obtained from Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB, Amsterdam, The Netherlands), and cultured for 10 d in the presence of either rIL-4 (1000 U/ml; PBH, Hannover, Germany) for Th2-polarizing conditions, or in the presence of rIL-12 (100 U/ml; a gift from Dr. M. K. Gately, Roche, Nutley, NJ) and neutralizing anti-IL-4 mAb (1 μg/ml; CLB-IL-4/6, CLB) for Th1 polarizing conditions.

Evaluation of human ICOS expression by flow cytometry
Th cells were washed, resuspended and stained on ice for 30 min 1% BSA in PBS containing 0.05% NaN₃. The cells were stained with the mouse anti-human ICOS mAb 9C8 (IgG1, 2 μg/ml) or the isotype-matched control Ab MOPC-21 (Sigma-Aldrich, St. Louis, MO) followed by FITC-conjugated goat F(ab')₂ anti-mouse IgG and IgM (Jackson ImmunoResearch Laboratories). Samples were analyzed on a
FACSCalibur®. Data were analyzed using WinMDI software (freely available at http://facs.scripps.edu/).

**Induction and measurement of proliferative response in different populations of Th cells**

Th cells (2.5x10^4 cells/200μl) were cocultured in 96-well flat-bottom culture plates (Costar, Cambridge, MA) with increasing numbers of allogeneic DC or autologous DC (10^1 to 10^4) in the additional presence of the superantigen *Staphylococcus aureus* enterotoxin B (SEB; Sigma-Aldrich) (0.1 ng/ml or 1 ng/ml). In all experiments DC were preincubated for 1 h at 37 °C with either human IgG1 (control IgG1) (Sigma-Aldrich) or ICOS-Ig prior to the addition of the Th cells and the cocultures were performed in the continuous presence of IgG1 or ICOS-Ig. Pilot experiments had demonstrated that ICOS-Ig is effective in inhibiting Th cell proliferation and cytokine production in the range of 2-25 μg/ml (data not shown). Therefore, in the experiments described hereafter we used the control IgG1 and ICOS-Ig at a final concentration of 25 μg/ml. Cell proliferation was assessed by measuring the incorporation of [³H]-TdR (Radiochemical Centre, Amersham, Little Chalfont, U.K.) by liquid scintillation spectroscopy after a pulse with 13 KBq/well during the last 16 h of a 3-d or a 7-d culture.

**Induction and measurement of cytokine production in different populations of Th cells**

Th cells (1x10^5 cells/200μl) were cocultured in 96-well flat-bottom culture plates (Costar) either with 2.5x10^4 DC/well in the presence of SEB (0.1 ng/ml or 1 ng/ml). The DC were preincubated for 1 h at 37 °C with either control IgG1 (Sigma-Aldrich) or ICOS-Ig before coculture with the Th cells still in the presence of IgG1 or ICOS-Ig. Cytokine concentrations were determined by specific solid-phase sandwich ELISA in supernatants collected after 24h (figure 3B, except for IL-10 that was determined in 72h supernatants) or after 72h (figure 3A). Pairs of specific mAbs and recombinant cytokine standards were obtained from BioSource International (Camarillo, CA) for the determination of GM-CSF, IL-2, and TNF-α, and from BD Pharmingen for the determination of IL-10. IL-13 was determined using a specific ELISA kit (CLB) according to manufacturer's instructions. IFN-γ, IL-4 and IL-5 were determined as
ICOS regulates human T helper cell function

described before (25). The limits of detection of these ELISA are as follows: GM-CSF, 40 pg/ml; IFN-γ, 100 pg/ml; IL-2, 5 U/ml; IL-4, 60 pg/ml; IL-5, 50 pg/ml; IL-10, 25 pg/ml; IL-13, 3 pg/ml; and TNF-α, 20 pg/ml.

Alternatively, the cytokine profiles were determined by intracellular staining. To this aim, at d 5 of coculture the Th cells were further expanded for 7 d in medium containing IL-2. Resting Th cells were harvested on d 12, washed and restimulated with PMA (10 ng/ml, Sigma-Aldrich) and ionomycin (1 μg/ml, Sigma-Aldrich) for 6 h, the last 5 h in the additional presence of brefeldin A (10 μg/ml, Sigma-Aldrich). Thereafter, the cells were washed in PBS, fixed for 15 min at room temperature in PBS containing 2% paraformaldehyde and stained in permeabilization buffer (PBS containing 1% BSA, 0.5% saponin, and 0.05% NaN₃) with FITC-labeled mouse anti-human IFN-γ (IgG2b) and PE-labeled mouse anti-human IL-4 (IgG1), or the respective isotype-matched controls (all from BD Pharmingen). Subsequently, the cells were washed and suspended in PBS containing 1% BSA and 0.05% NaN₃ and analyzed with a FACSCalibur®. Data were analyzed using WinMDI software.

Statistical analysis
ICOS expression and proliferation data were analyzed for the area defined by the respective curves using the following equation: \[ \text{area} = \frac{1}{2} (x_2-x_1) (y_1 + y_2) \]. The areas were then compared for statistical significance with the GraphPad InStat® software (version 3.00, GraphPad InStat, Inc., San Diego, CA) using a two-tailed paired Student's t-test. Data on cytokine production were analyzed for statistical significance using a two-tailed Student's t-test (Welch-corrected when applicable). A \( P \) value of <0.05 was considered as the level of significance.

RESULTS
Regulation of ICOS expression at the different stages of Th cell maturation
In order to obtain a more precise insight in the regulation of ICOS expression at the various stages of Th cell maturation, we compared the kinetics of ICOS expression after activation of naïve Th cells, memory Th cells and effector Th1 and Th2 cells. For this purpose Th cells were activated in vitro in an APC-free system using mAbs directed against CD3 and CD28 (26), taking into account that CD28 triggering optimizes ICOS expression (15). Flow cytometry analyses consistently showed that
ICOS is virtually absent on resting CD69^-CD25^- naive and memory Th cells (Fig. 1A, d 0). Upon activation, both populations acquired transiently substantial levels of ICOS, which peaked at d 3 to 4 and reached base line levels again by d 7 (Fig. 1A). Quiescent Th1 and Th2 cells at d 10 after the last restimulation also expressed virtually no ICOS. Upon activation, both Th1 and Th2 cells transiently acquired substantial ICOS levels. Peak levels were reached more rapidly than in resting naive and memory T cells (Fig. 1B), but also reached base line levels within 7 d. In contrast to mouse cells, ICOS was upregulated on both Th1 cells and Th2 cells, even stronger on Th1 cells compared to Th2 cells.

**Figure 1.** Kinetics of ICOS expression by human Th cells. (A). Naive Th cells and CD4^+ Th cells or (B) Th1 cells and Th2 cells were activated with anti-CD3 and anti-CD28 mAbs. Cells were harvested at the indicated time points post activation and the expression of ICOS was analyzed by flow cytometry. Results are expressed as net mean fluorescence intensity (ΔMFI) ± SD of three independent experiments. The areas defined by the ICOS expression curves were compared for statistical significance using a two-tailed paired Student's t-test. P values <0.05 are given in the respective figure.

The role of B7RP-1-mediated ICOS ligation in the costimulation of Th cell proliferation
ICOS ligand B7RP-1 is expressed by several APC types including mouse B cells and human B cells and DC (5, 8-11, 13). The relative contribution of the B7RP-1–ICOS costimulatory pathway in the DC-mediated activation of Th cells was studied at the different stages of Th cell maturation, using monocyte-derived DC with abundant expression of B7RP-1, and an inhibitory ICOS-Ig fusion protein blocking the B7RP-1–ICOS interaction. The Th cells were activated either by allogeneic DC (MLR) or by
ICOS regulates human T helper cell function

Figure 2. B7RP-1–ICOS costimulation is essential for Th2 cell proliferation. Th cells were activated with graded numbers of APC in the presence of either control IgG1 (●) or the presence of ICOS-Ig (○). (A) Naive Th cells and CD4+ memory Th cells were activated with allogeneic DC (MLR) for 7 d, or with autologous DC and SEB either 0.1 ng/ml (SEB 0.1) for 7 d, or 1 ng/ml (SEB 1) for 3 d. (B) Th1 cells and Th2 cells were activated either with allogeneic DC (MLR) for 7 d or with autologous DC in the presence of 1 ng/ml SEB (SEB) for 3 d. The proliferative response was determined after a pulse with [³H]-TdR during the last 16 h of culture. Results, expressed as mean cpm ± SD of triplicate cultures, are from one representative experiment of at least four. The areas defined by the proliferation curves were compared for statistical significance using a two-tailed paired Student’s t-test. P values <0.05 are given in the respective figures.

superantigen SEB. In a first series of experiments we evaluated the role of ICOS in the induction of proliferation of Th cells. The proliferation of naive Th cells was not affected by ICOS (Fig. 2A, upper panels). Similarly, the proliferation of resting memory CD4+ Th cells was only marginally dependent on ICOS costimulation as
indicated by a small, and statistically not significant reduction of proliferation in the presence of ICOS-Ig (Fig. 2A, lower panels) (10). In addition, ICOS-Ig did not inhibit the proliferation of the effector Th1 cells activated by DC either in the MLR or with SEB (Fig. 2B, upper panels). Surprisingly, the proliferation of effector Th2 cells was strongly inhibited by ICOS-Ig, irrespective of the proliferation induction model, and was almost completely abrogated in the MLR (Fig. 2B, lower panels). These data indicate a critical and unique role for B7RP-1–mediated ICOS costimulation in the proliferation of effector Th2 cells.

The role of B7RP-1–mediated ICOS ligation in the costimulation of Th cell cytokine production
To further investigate the role of B7RP-1–mediated ICOS costimulation in the activation of primary, memory and effector Th cell responses, we analyzed the changes in production of (anti-) inflammatory and Th1/Th2 signature cytokines upon ICOS-Ig addition to SEB-primed DC-Th cell cocultures. ICOS-Ig significantly (~ 30-50 %) inhibited the production of the signature cytokines IFN-γ, IL-4, IL-5, IL-13 and the anti-inflammatory cytokine IL-10 in primary naive Th cells and resting CD4⁺ Th cells (Fig. 3A). In sharp contrast, the production of IL-2, GM-CSF, and TNF-α was unaffected. In a similar set of experiments, ICOS-Ig significantly inhibited the production of the signature cytokines and IL-10 in both Th1 and Th2 cells without affecting IL-2 production (Fig. 3B). Remarkably, IL-10 was always most strongly inhibited and almost completely abrogated in the Th1 and Th2 cells.

Figure 3. B7RP-1–ICOS costimulation is essential for Th cell cytokine production but does not imprint a specific polarized Th phenotype. (A and C) Naïve Th cells and CD4⁺ Th cells were activated with DC and 0.1 ng/ml SEB. (B and D) Th1 cells and Th2 cells were activated with DC and 1 ng/ml SEB. In every case, APC-T cell cocultures were performed in the presence of either control IgG1 (■) or ICOS-Ig (□). Cytokine levels were determined by ELISA in (A) 72 h and (B) 24 h (72 h for IL-10) supernatants. Results are expressed as mean ± SD of triplicate cultures and are from one representative experiment of at least four. Alternatively, (C and D) Th cells were expanded from d 5-12 in medium containing IL-2 and restimulated with PMA and ionomycin in the presence of brefeldin A for the assessment of IFN-γ and IL-4 production at the single cell level. Results are mean ± SD of at least three independent experiments. Data were analyzed for statistical significance using a two-tailed Student’s t-test. aP<0.001, bP<0.001.
ICOS regulates human T helper cell function

A

<table>
<thead>
<tr>
<th>naive</th>
<th>CD4⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2 (U/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>IFN-γ (ng/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>IL-5 (pg/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>IL-13 (pg/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>GM-CSF (pg/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>TNF-α (ng/ml)</td>
<td>![Graph</td>
</tr>
</tbody>
</table>

B

<table>
<thead>
<tr>
<th>Th1</th>
<th>Th2</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2 (U/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>IFN-γ (ng/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>IL-5 (pg/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>IL-10 (ng/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>IL-13 (ng/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>GM-CSF (pg/ml)</td>
<td>![Graph</td>
</tr>
<tr>
<td>TNF-α (ng/ml)</td>
<td>![Graph</td>
</tr>
</tbody>
</table>

C

<table>
<thead>
<tr>
<th>naive</th>
<th>CD4⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of IL-4⁺-positive T cells</td>
<td>![Graph</td>
</tr>
<tr>
<td>% of IFN-γ⁺-positive T cells</td>
<td>![Graph</td>
</tr>
</tbody>
</table>

D

<table>
<thead>
<tr>
<th>Th1</th>
<th>Th2</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of IL-4⁺-positive T cells</td>
<td>![Graph</td>
</tr>
<tr>
<td>% of IFN-γ⁺-positive T cells</td>
<td>![Graph</td>
</tr>
</tbody>
</table>
Chapter 4

The role of B7RP-1–mediated ICOS costimulation in the differentiation of Th cells

Since ICOS costimulation influences the level of cytokines in naive and memory Th cells, we also investigated whether ICOS costimulation affects their development into effector Th1 and Th2 cells, i.e. the commitment of these cells to produce signature cytokines upon subsequent restimulation. To this aim, the SEB-stimulated naive or memory Th cells were further expanded in medium containing IL-2, until they became quiescent. The obtained effector cells were subsequently restimulated with PMA and ionomycin in the presence of brefeldin A and analyzed for the intracellular expression of IFN-γ and IL-4. Although the blocking of the B7RP-1–ICOS costimulatory pathway by ICOS-Ig during the priming of Th cells affected the immediate cytokine production (Fig. 3A), it did not affect their commitment to produce these cytokines upon restimulation (Fig. 3C). Th cells that originate from cocultures in the presence of ICOS-Ig are primed for the secretion of similar ratios of IFN-γ to IL-4, as the Th cells that originate from parallel cultures primed in the presence of control IgG1. Also, the commitment of the memory CD4+ Th cells and effector Th1 and Th2 cells was not affected by ICOS-Ig (Fig. 3C and 3D). As expected, in these conditions the Th2 cells produce some IFN-γ upon restimulation as a result of DC-derived IL-12 (Fig. 3D) (27). In these experiments with naive and memory Th cells the commitment of the effector cells to produce IL-2 and TNF-α was also not affected (data not shown).

In order to study whether these naive or memory Th cell-derived effector Th cells obtained in the presence or absence of ICOS-Ig still required ICOS costimulation, they were restimulated with autologous DC and SEB, again in the presence or absence of ICOS-Ig. The inhibitory effect of ICOS-Ig on cytokine production observed in the first stimulation appeared not to commit these cells to an ICOS insusceptibility, as ICOS-Ig again inhibited cytokine production during the second stimulation, regardless of whether they were generated with or without ICOS-Ig (data not shown). This finding strengthens the concept that B7RP-1–mediated ICOS costimulation regulates Th cell cytokine production instantly and transiently and does not commit these cells for restricted cytokine profiles.
ICOS regulates human T helper cell function

DISCUSSION

The present results show that the expression of ICOS is transiently induced on resting naive and resting memory Th cells and both on effector Th1 and Th2 cells. Like for many other molecules, ICOS is more rapidly induced on effector cells than on resting cells. In contrast to previous mouse data, ICOS is expressed on both Th1 and Th2 cells and even higher on the Th1 cells. There seems to be no consequence of this differential regulation of ICOS expression on Th1 and Th2 cells, as ICOS-Ig inhibited cytokine production in Th1 and Th2 cells to the same extent.

Our study was focused further on the relative contribution of the B7RP-1–ICOS costimulatory pathway to the activation of human Th cells at different stages of maturation by testing the effects of a blocking ICOS-Ig fusion protein in DC-Th cell cocultures. The low, but significant levels of ICOS shortly after stimulation of the naive Th cells seem to be functional, as blocking of the B7RP-1–mediated ICOS costimulation resulted in the decreased production of Th1/Th2 signature cytokines also by these Th cells. A functional consequence of the production of these cytokines is not apparent, as the proliferation of these naive Th cells was not affected by ICOS-Ig, probably because it did not block the production of the autocrine T cell growth factor IL-2. This finding indicates that ICOS ligation is not strictly necessary for the expansion of naive Th cells, which is in line with the notion that proliferation of naive Th cells is effectively costimulated through CD28 (3).

In agreement with the data reported by Aicher and colleagues (10), the expansion of freshly isolated CD4+ Th cells was affected by inhibition of ICOS costimulation in the allogeneic MLR, although only slightly. Surprisingly, whereas established Th1 cells were not affected, proliferation of established Th2 cells was strongly inhibited by blocking of the B7RP-1–ICOS costimulatory pathway. These data show for the first time that ICOS is selectively critical for the expansion of effector Th2 cells and therefore, may strongly determine the strength of Th2-mediated immune responses. It is tempting to speculate that this Th-specific inhibition results from inhibition of Th2 cell-specific autocrine growth factor(s).

Indeed, blocking of the B7RP-1–ICOS costimulatory pathway had immediate consequences for the production of signature and (anti-) inflammatory cytokines by the Th cell populations studied. The production of specific signature cytokines was significantly inhibited in all cell types. Similar to the results from studies with mouse
cells, the production of IL-4, IL-5 and IL-10 was inhibited, as was the production of IL-13. In contrast to these studies, we found that IFN-γ was also significantly inhibited in the human cells. ICOS-Ig also blocked GM-CSF and TNF-α production, but only in effector Th1 and Th2 cells. These results suggest that the B7RP-1–ICOS costimulatory pathway is important in both Th1 cell- and Th2 cell-mediated effector functions through the upregulation of the signature cytokines at all the stages of Th cell maturation. Conversely, our data suggest that ICOS may be critically involved in the subsequent downregulation of Th1/Th2-mediated inflammation through the upregulation of IL-10 production, which is known to peak later than the other cytokines (28). ICOS-Ig did not affect the production of IL-2 by any of the cell types tested, supporting the observations that ICOS ligation fails to costimulate for IL-2 production (15, 21, 29, 30) and explains that ICOS costimulation is not required for the expansion of naive, memory and Th1 cells, which in all cases depends on autocrine IL-2. It is noteworthy that the effect of the human B7RP-1–ICOS costimulatory pathway on the production of the other cytokines is transient. ICOS costimulation does not affect the differentiation of naive Th cells into Th1 or Th2 cells, nor does it affect the polarization profile of memory and effector Th1 and Th2 cells upon subsequent restimulation.

The data presented here contribute to the understanding of the role of ICOS in the regulation of Th1 and Th2 cell responses. It indicates that ICOS costimulation affects Th2 responses more firmly than Th1 responses. ICOS costimulation not only promotes the production of Th2 cytokines, but also selectively promotes the expansion of established Th2 cells. Although ICOS also promotes the production of Th1 cytokines, it does not promote the expansion of Th1 cells. Indeed, murine ICOS was implicated in Th1-associated experimental allergic encephalomyelitis and allograft rejection (18, 19), but the role of murine ICOS was more readily demonstrable in Th2-mediated responses. In fact, the murine model for allergic airway inflammation indicates that ICOS costimulation is critical in both Th2 effector function and expansion (7, 16, 17). Taken together our data suggest that ICOS-mediated costimulation provides a unique signal for the expansion of Th2 cells and as such ICOS is an important target for therapy of T cell-mediated inflammatory diseases such as allergic asthma that are characterized by inappropriate activation of Th2 cells.
Acknowledgments
This work was financially supported by the Fundação for Science and Technology, Lisbon, Portugal (grant PRAXIS XXI/BD/9195/96 to P.L.V.) and by the Netherlands Asthma Foundation (grant 32.00.51 to L.W.).

References