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Cutting Edge: Inducible Costimulator Protein Regulates Both Th1 and Th2 Responses to Cutaneous Leishmaniasis

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The CD28 family member inducible costimulator protein (ICOS) has an important role in T cell differentiation and Ig class switching. To investigate the role of ICOS in vivo, ICOS−/− mice were infected s.c. with Leishmania mexicana. While wild-type mice developed large, cutaneous lesions, the growth of lesions and tissue histopathology was significantly delayed in ICOS−/− mice. ICOS−/− mice exhibited marked decreases in both Th1 and Th2 cytokine production and profound defects in L. mexicana-specific Ig isotype class switching to IgG1 and IgG2a and reduced total IgE levels. Our findings indicate that ICOS is a key regulator of both Th1 and Th2 responses and has a role in controlling cutaneous L. mexicana infection. The Journal of Immunology, 2002, 168: 991–995.

Two signals are required for optimal activation of T cells. The first signal is provided by the engagement of the TCR with the MHC-peptide complex on APCs, and the second signal is delivered by costimulatory molecules. Although the B7:CD28/CTLA-4 costimulatory pathway is pivotal in T cell activation, other costimulatory pathways may be critical in Th cell differentiation. The homolog of CD28, inducible costimulator protein (ICOS), provides important costimulatory signals to T cells (1–3). ICOS enhances T cell cytokine production, up-regulation of CD40 ligand, and help for Ig production by B cells (1, 4).

There are several features of ICOS that distinguish it from CD28. While CD28 is constitutively expressed on most T cells, ICOS is induced upon TCR engagement. Expression of ICOS is enhanced by CD28 costimulation (4). In contrast to CD28 and CTLA-4, ICOS does not bind B7-1 or B7-2 (1). The ligand for ICOS is B7h (GL50, B7RP-1, LICOS, B7H2), which is expressed on APCs as well as other cell types (5–7).

The importance of the ICOS:B7h pathway in vivo is only beginning to be appreciated (2, 3, 8). The inducible expression of ICOS suggests that ICOS regulates recently activated and effector T cell responses. Functional studies of ICOS have suggested that ICOS is important for Th2 immune responses, preferentially inducing IL-4 and IL-10 production (3, 4, 9–11). The role of ICOS in Th2 responses has been demonstrated in studies of allergic airway disease. The most profound effects on Th2 cytokine production were observed when ICOS was blocked during airway challenge in primed mice (11). ICOS−/− mice were susceptible to induction of inflammatory lung disease induced by airway challenge in primed mice, but they produced less IL-4 and IL-13 (3). Lung inflammation and airway reactivity were reduced by ICOS blockade following adoptive transfer of polarized Th2 cells to naive mice (9). These studies demonstrate a critical role for ICOS in Th2 immune responses.

However, recent studies suggest that ICOS may also regulate Th1 immune responses (12–14). Leishmania mexicana infection in mice provides a sensitive probe of Th differentiation during the immune response to infection. During L. mexicana infection, progression of lesions is determined by a balance of Th1 and Th2 responses (15). IFN-γ production is required for protective host immunity after L. mexicana infection (16–18). In L. mexicana-infected IL-4−/− mice, skewing toward Th1 is observed and correlates with healing of the lesions (16). Similarly, in the absence of STAT6, a component for IL-4 signaling, mice produce high levels of IFN-γ and do not develop cutaneous lesions after L. mexicana infection (15). IFN-γ is a potent stimulus of macrophage NO pathways which produce leishmanicidal activity (16, 19–21). IL-4 decreases IFN-γ production; thus, IL-4 production exacerbates disease by inhibiting a protective Th1 immune response.

Our studies demonstrate that L. mexicana-infected ICOS−/− mice exhibit pronounced defects in Ig class switching and T cell cytokine production. Development of cutaneous lesions and inflammatory infiltrates is impaired in ICOS−/− mice. Our findings demonstrate that ICOS is a critical regulator of both Th1 and Th2 immune responses in vivo following infection with L. mexicana.

Materials and Methods

Mice

Wild-type (WT) and ICOS−/− 129S4/SvJae mice were maintained in accordance with the institutional guidelines of Brigham and Women’s Hospital and Harvard Medical School (Boston, MA). Female 6- to 8-wk-old mice were used for our studies.

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3 Abbreviations used in this paper: ICOS, inducible costimulator protein; WT, wild type.

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**Infection**

*L. mexicana* parasites (MYNC/BZ62/M379) were maintained in the rumps of BALB/c mice. Amastigotes were isolated as previously described (22). Mice were infected with 5 × 10^6 amastigotes of *L. mexicana* by s.c. injection into the rump. Lesion diameter was measured at 1-wk intervals for up to 12 wk. Lesions were examined histologically by H&E staining.

**Serum ELISA**

Mice were bled weekly for 12 wk after inoculation with *L. mexicana*. Sera were tested for total IgE and *L. mexicana*-specific IgM, IgG1, and IgG2a by ELISA (2). The limit of detection for IgE was 40 ng/ml.

**In vitro cell preparations**

Inguinal lymph nodes were collected at the indicated time following infection with *L. mexicana*. To measure proliferative responses and cytokine production, 5 × 10^5 lymph node cells were cultured in 96-well plates with 0–40 μg/ml *L. mexicana* freeze-thaw Ag from stationary phase promastigotes. Data reflect the mean of triplicates from each Ag concentration. Culture supernatants were collected on days 1–8 following infection, and cytokine levels were determined by ELISA (23).

**Statistical significance**

Student’s paired *t* test was used to determine the statistical significance of the values obtained.

**Results**

The development of cutaneous lesions and tissue histopathology is delayed in ICOS^-/-^ mice as compared with WT mice following *L. mexicana* infection

Following *L. mexicana* infection, lesions developed in WT mice as early as 3 wk; however, lesions were not detected in ICOS^-/-^ mice until wk 4 (Fig. 1, A and B). At wk 6, WT mice had lesions that were 7.5 ± 0.33 mm (Fig. 1A), but lesions in the ICOS^-/-^ mice were markedly reduced (2.7 ± 0.55 mm). However, by wk 12, lesion size in WT and ICOS^-/-^ mice was similar (Fig. 1B). Thus, lesion development is delayed in the absence of ICOS.

In WT mice, lesions had marked tissue damage with abundant eosinophils, neutrophils, and parasitized macrophages at 6 wk postinfection (Fig. 1C). In contrast, the inflammatory response was modest and fewer parasitized macrophages were observed in ICOS^-/-^ mice at wk 6 (Fig. 1D). At 12 wk postinfection, massive inflammatory lesions and extensive tissue destruction of s.c. tissue and muscle were seen in WT mice, whereas ICOS^-/-^ mice had much less tissue damage (data not shown). However, the nature of the inflammatory infiltrates was similar in WT and ICOS^-/-^ mice. Thus, the development of inflammatory lesions following *L. mexicana* infection is delayed and less severe in ICOS^-/-^ mice.

**Ab class switching is markedly impaired in *L. mexicana*-infected ICOS^-/-^ mice**

Recent studies have shown that ICOS plays a critical role in Ab responses (2, 3, 8). Levels of *L. mexicana*-specific IgM, IgG1, and IgG2a were analyzed following infection with *L. mexicana* (Fig. 2A). High titers of *L. mexicana*-specific IgG2a and IgG1 were detected in WT mice at 4–12 wk (Fig. 2, B–E). In contrast, *L. mexicana*-specific IgG2a and IgG1 were at the limit of detection in ICOS^-/-^ mice at 6 wk following infection (Fig. 2, B and C). At 12 wk, low levels of *L. mexicana*-specific IgG1 were detected in ICOS^-/-^ mice (Fig. 2D). *L. mexicana*-specific IgG2a was not detectable in ICOS^-/-^ mice at 12 wk postinfection (Fig. 2E). At 6 and 12 wk, total IgE was elevated in WT mice, but IgE was not detected in ICOS^-/-^ mice at 6 wk and was markedly reduced at 12 wk (Fig. 3). Before infection, IgE in WT and ICOS^-/-^ was below the limit of detection (data not shown). These results demonstrate that ICOS is important for Ig class switching during infection with *L. mexicana*.

**Proliferative responses and cytokine production are impaired in *L. mexicana*-infected ICOS^-/-^ mice**

Because Ig class switching depends on T cell responses, T cell responses were analyzed following infection with *L. mexicana*. T cell proliferation was reduced at a lower Ag concentration (4 μg/ml) in ICOS^-/-^ mice as compared with WT mice at 6 wk (Fig. 4A). At
12 wk, WT and ICOS−/− mice exhibited comparable proliferative responses (Fig. 4B). At 6 wk postinfection, levels of IL-4 were slightly lower at day 3 in ICOS−/− mice as compared with WT mice, while IFN-γ was reduced in ICOS−/− mice to 50% of WT levels (Fig. 4C). At 12 wk, IL-4 and IFN-γ production remained low in ICOS−/− mice but was markedly elevated in WT mice (Fig. 4, C and D). Higher levels of IL-4 and IFN-γ mRNA were detected by RNase protection assay in WT mice as compared with ICOS−/− mice from lymph nodes collected 6 and 12 wk postinfection (data not shown). Thus, ICOS promotes both Th1 and Th2 responses in vivo.

Discussion

The outcome of L. mexicana infection in ICOS−/− mice reflects the complex interplay between Th1 and Th2 cytokines. WT mice develop nonhealing cutaneous lesions. L. mexicana-infected ICOS−/− mice exhibit impaired IL-4 and IFN-γ production. The delay in disease progression reflects the low levels of IL-4 produced in the ICOS−/− mice. However, IFN-γ production is also low and, in the absence of a vigorous Th1 response, cutaneous lesions on the ICOS−/− mice are not cured. Thus, the balance of Th1 and Th2 cytokines is key in determining the outcome of disease.

Our findings demonstrate that ICOS can regulate both Th1 and Th2 responses during infection to L. mexicana. Ab class switching also was impaired, consistent with a role for ICOS in both Th1 and Th2 responses, as profound defects in both Th1- and Th2-associated class switching to IgG1, IgE, and IgG2a were observed. Levels of IgM are comparable in WT and ICOS−/− mice, suggesting that B cell responsiveness is intact in the absence of ICOS. Our results are consistent with the defect in Ig class switching observed following immunization with a model protein Ag and are remarkable because parasites present a more complex challenge to the host (2). Although the defects in Ig isotype class switching persist.

FIGURE 2. L. mexicana-specific Ig isotype class switching is defective in ICOS−/− mice at 0, 2, 4, 6, and 12 wk postinfection with L. mexicana. A, Sera were analyzed for L. mexicana-specific IgM at 2 wk postinfection. Data represent relative IgM levels for WT (■) and ICOS−/− (○) mice at indicated serum dilutions. Values are shown for four individual mice per group and are representative of three experiments. *, Statistically similar values between groups (p > 0.05). B and C, Sera (1/50 dilution) were analyzed at 0, 2, 4, and 6 wk for L. mexicana-specific IgG2a and IgG1. Data represent the relative levels of specific IgG2a and IgG1. Values are shown for four individual mice per group for WT (■) and ICOS−/− (○) mice. *, Statistically significant differences between groups (p < 0.05). D and E, Sera were analyzed at 12 wk for L. mexicana-specific IgG2a and IgG1. Data represent the relative levels of specific IgG2a and IgG1. Values are shown for four individual mice per group for WT (■) and ICOS−/− (○) mice at the indicated serum dilutions. *, Statistically significant differences between groups (p < 0.05). Data are representative of three experiments.

FIGURE 3. Defects in total IgE are observed in ICOS−/− mice at 6 and 12 wk following infection with L. mexicana. Values are shown as the mean ± SD for four to five mice per group for WT (filled bars) and ICOS−/− (open bars) mice at the indicated time. *, Statistically significant differences between groups (p < 0.05). Data are representative of three experiments.
to 12 wk in the ICOS−/− mice following *L. mexicana* infection, the humoral immune response is not critical for clearing of the parasitic infection. Circulating Ab promotes the internalization of *L. mexicana* complex amastigotes via the FcR, thus prolonging infection (24). The primary host defense mechanism for clearing *L. mexicana* appears to be IFN-γ production.

The cross-talk between ICOS and CD28 signals is only beginning to be understood. CD28−/− mice mount effective T cell responses against several parasites (25–28). The presence of highly conserved microbial structures on infectious pathogens stimulates the innate immune response via pattern recognition receptors which enhance the function of APCs and stimulate T cell responses (29). The B7h:ICOS pathway may provide distinct co-stimulatory signals which lead to competent T cell responses in the absence of CD28. Our results are consistent with a study showing that administration of ICOS-Ig following *Nippostrongylus brasiliensis* infection decreased production of both Th1 and Th2 cytokines during in vitro restimulation (30). Blockade of ICOS in the CD28−/− mice led to further reductions in cytokine production following *N. brasiliensis* infection, suggesting that the B7:CD28 and B7h:ICOS pathways have synergistic effects during effector T cell immune responses to infectious pathogens.

A role for ICOS in Th1 responses was not observed in models examining primary and recall responses (3). However, recent studies show a role for ICOS in Th1 responses in models of cardiac allograft survival and experimental autoimmune encephalomyelitis. Cardiac allograft rejection was delayed after ICOS blockade and was associated with reduced IFN-γ and IL-10 within allografts (12). ICOS blockade during priming of autoimmune encephalomyelitis exacerbates disease (increased IFN-γ), but ICOS blockade during the effector phase abrogates disease (decreased IFN-γ) (13). Thus, ICOS may have distinct roles during priming and effector responses that can regulate Th1- and Th2-mediated pathologies. Our studies demonstrate that ICOS can contribute to IFN-γ production during a chronic infection. Thus, ICOS is a key regulator of both Th1 and Th2 responses.

**References**


