Activation of Dendritic Cells through the Interleukin 1 Receptor 1 Is Critical for the Induction of Autoimmune Myocarditis

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Abstract

Dilated cardiomyopathy, resulting from myocarditis, is the most common cause of heart failure in young patients. We here show that interleukin (IL)-1 receptor type 1–deficient (IL-1R1$^{-/-}$) mice are protected from development of autoimmune myocarditis after immunization with α-myosin-peptide(614–629). CD4$^+$ T cells from immunized IL-1R1$^{-/-}$ mice proliferated poorly and failed to transfer disease after injection into naive severe combined immunodeficiency (SCID) mice. In vitro stimulation experiments suggested that the function of IL-1R1$^{-/-}$CD4$^+$ T cells was not intrinsically defect, but their activation by dendritic cells was impaired in IL-1R1$^{-/-}$ mice. Accordingly, production of tumor necrosis factor (TNF)-α, IL-1, IL-6, and IL-12p70 was reduced in dendritic cells lacking the IL-1 receptor type 1. In fact, injection of immature, antigen-loaded IL-1R1$^{+/+}$ but not IL-1R1$^{-/-}$ dendritic cells into IL-1R1$^{-/-}$ mice fully restored disease susceptibility by rendering IL-1R1$^{-/-}$ CD4$^+$ T cells pathogenic. Thus, IL-1R1 triggering is required for efficient activation of dendritic cells, which is in turn a prerequisite for induction of autoreactive CD4$^+$ T cells and autoimmunity.

Key words: dendritic cells • interleukin 1 • interleukin 1 receptor type 1 • autoimmunity • myocarditis

Introduction

Idiopathic cardiomyopathy is the most common cause of heart failure in young patients. A growing body of evidence suggests that idiopathic cardiomyopathy may result from chronic myocarditis in predisposed individuals (1–3). Experimental autoimmune myocarditis (EAM) is a model for CD4$^+$ T cell–mediated inflammatory heart disease and can be induced in mice by immunization with α-myosin-heavy chain–derived peptides (4–6) together with CFA or CpG oligonucleotides. Proinflam-

matory cytokines like IL-12, IFN-γ, and TNF-α are critically involved in the pathogenesis of autoimmune myocarditis. Whereas TNF-α (7) and IL-12 (8, 9) are essential for the development of disease, IFN-γ protects by a mechanism that probably involves nitric oxide (10).

IL-1 is a multifunctional player during host defense and disease. It stimulates the acute phase response, the secretion of matrix metalloproteinases, chemokines, and other proinflammatory cytokines, acts as endogenous pyrogen, and activates lymphocytes (11, 12). In the context of autoimmunity, IL-1 promotes collagen-induced arthritis in mice (13–15) and plays an important role in the pathogenesis of rheumatoid arthritis in humans (16). In fact, IL-1 antagonists are already used to treat patients with rheumatoid arthritis. IL-1 is produced by a variety of cell types including macrophages, B cells, T cells, and

dendritic cells (DCs).* Two forms of biologically active IL-1 exist, IL-1α and IL-1β, which exert similar activities through the IL-1R type 1 (IL-1R1; CD121a). The IL-1R type 2 (IL-1R2; CD121b) is not considered to be involved in the signal transduction, but acts as a "decoy" receptor that can be shed from the cell surface and prevents IL-1 binding to the IL-1R type 1 (17). In addition, an endogenous IL-1R antagonist (IL-1ra) has been identified that binds to IL-1 receptors and blocks IL-1 binding and signaling (12).

The development of Coxsackie virus B3 (CVB3)-induced myocarditis is associated with the infiltration of the heart with inflammatory cells that secrete IL-1, and treatment with recombinant IL-1 enhances CVB3 myocarditis in partially resistant mice (18, 19). Furthermore, expression of IL-1R antagonist in the mouse heart by plasmid DNA decreases myocardial inflammation in CVB3 myocarditis (20). However, the role of IL-1 and the IL-1R1 during inflammatory heart disease has not been established on the genetic level. Our data provide the first in vivo evidence that IL-1R1 triggering on DCs is critical for expansion of autoreactive CD4$^+$ T cells and subsequent induction of autoimmune heart disease.

Materials and Methods

Mice and Immunization Procedures. IL-1R1$^{-/-}$ mutant mice (provided by M. Labow, Hoffmann-La Roche, Nutley, NJ; reference 21) were backcrossed into BALB/c background for more than eight generations in a specific pathogen free facility at the Basel Institute for Immunology. Wild-type BALB/c mice were purchased from Biological Research Laboratories Ltd. and SCID (BALB/c) mice from Taconics. Female mice in the age of 8–10 wk were immunized at days 0 and 7 with 50 μg of a murine specific α-myosin-heavy chain–derived peptide (myhc-α 614–629 [Ac-SLKLMATLFSTYASAD-OH]) in a 1:1 emulsion with CFA (1 mg/ml; H37Ra; Difco) (5) All experiments were in accordance with Swiss federal legislation and Canadian law and had been approved by the local authorities.

Histopathology and Immunohistochemistry. 21 d after the immunization, hearts were removed and processed for hematoxylin-eosin staining. Myocarditis was scored on a semiquantitative scale using grades from 0 to 4 (0: no inflammatory infiltrates; 1: small foci of inflammatory cells between myocytes; 2: larger foci of >100 inflammatory cells; 3: >10% of a cross section involved; 4: >30% of a cross section involved). For immunohistochemistry, OTC embedded frozen hearts were fixed in acetone and then processed for antibody staining according to standard protocols. The following antibodies were used: anti-MHC II (biotinylated; Serotec; MCA46B), anti-CD45R (RA3–6B2), anti-CD3 (KT3–1.1), anti-CD4 (YTS 191), anti-CD8 (YTS 169), anti-VCAM-1 mAb (0.5 mg/ml, clone 429; BD Biosciences), anti-ICAM-1 (20 mg/ml, clone 3E2; BD Biosciences); anti-Mac-1 (rat IgG, biotinylated), anti-CD11c (2.5 mg/ml, clone HL3; BD Biosciences), anti-Gr-1/Ly-6G (0.2 mg/ml, clone RB6–8C5; BD Biosciences).

Proliferation Assays. CD4$^+$ T cells were purified from draining lymph nodes of immunized mice using magnetic beads (CD4$^+$ T cell isolation kit; Miltenyi Biotech) and cultured for 72 h together with irradiated (2,000 rad) syngenic splenocytes, with or without 10 μg/ml of myhcα 614–634 in serum-free AIM-V/myhc(614–629) (GIBCO BRL) medium. Proliferation was assessed by measuring [^{3}H]methyl-thymidine incorporation. IFN-γ, IL-2, IL-10, and IL-4 levels in supernatants were measured using commercially available ELISA kits (Quantikine; R&D Systems) after 40 h of culture in the presence of myhc-α. For in vitro stimulation assays of primary CD4$^+$ T cells, naive CD62L-positive CD4$^+$ T cells were isolated from lymph nodes by depletion/positive selection with magnetic beads (CD4$^+$ isolation kit, CD62L microbeads, MACS; Miltenyi Biotech). 10^5 CD4$^+$ CD62L$^+$ cells were then stimulated by 5 μg/ml soluble anti-CD3ε, 5 μg/ml anti-CD3ε and 1 μg/ml anti-CD28, 50 ng/ml PMA and 500 ng/ml Ionomycin, or with 1 μg/ml Concanavalin A together with 0.25 × 10^5 irradiated (1,500 rad) purified nonstimulated DCs. Proliferative responses were assessed after 24 or 48 h in serum free AIM-V (GIBCO BRL) medium at 37°C/5% CO$_2$ by measuring [^{3}H]methyl-thymidine incorporation.

Generation of DCs, Cytokine Measurements, and FACS® Analysis. DCs were generated as described (22). CD11c positive cells were further enriched by positive selection using magnetic beads (MACS; Miltenyi Biotech). Generally, FACS® analysis for CD11c expression and microscopic assessment of the typical cell morphology revealed over 80% of DCs. For cytokine measurements, DCs were plated at 10^6/ml in 24 well plates and incubated for 24 h with various stimuli including 5 μg/ml anti-CD40, 1 μg/ml LPS, 5 μg/ml anti-CD40, and 1 μg/ml LPS, 500 U/ml TNF-α, or 10 ng/ml of IL-1β. TNF-α, IL-1β, IL-12p70, and IL-6 were measured using Quantikine ELISA kits (R&D Systems). For FACS® analysis, DC preparations were preincubated for 30 min at 4° with Fc-block (BD Biosciences) and 1% rat serum in BD Biosciences staining buffer before incubation with the appropriate fluorochrome labeled antibodies from BD Biosciences.

Adoptive Transfer of In Vitro–restimulated CD4$^+$ T Cells and DC Treatment Protocol. Spleens from donor mice were removed 21 d after the first immunization. CD4$^+$ T cells were enriched using magnetic beads (MACS; Miltenyi Biotech) and cultured for 48 h on antigen pulsed and irradiated (1,500 rad) syngenic DCs. 5 × 10^6 CD4$^+$ T cells per mouse (>98% CD4$^+$ cells) were intraperitoneally injected in to SCID (IL-1R1$^{+/+}$) mice. Recipients were killed 10 d after transfer, and myocarditis severity was assessed.

For in vivo reconstitution with antigen-pulsed DCs, we generated immature DCs by adding 20 ng/ml of IL-10 to the culture medium. 1 d prior to harvesting, cells were pulsed overnight with the myhc-α peptide at 10 μg/ml. After enrichment of CD11c-positive

Table I. *Myocarditis Prevalence and Severity in IL-1R1$^{+/+}$ and IL-1R1$^{-/-}$ Mice*

Mice	Treatment	Prevalence Day 21	Severity grade (at day 21) Median range
IL-1R1$^{+/+}$	None	9/9^a	2 (1–4)
IL-1R1$^{-/-}$	None	0/11^a	0

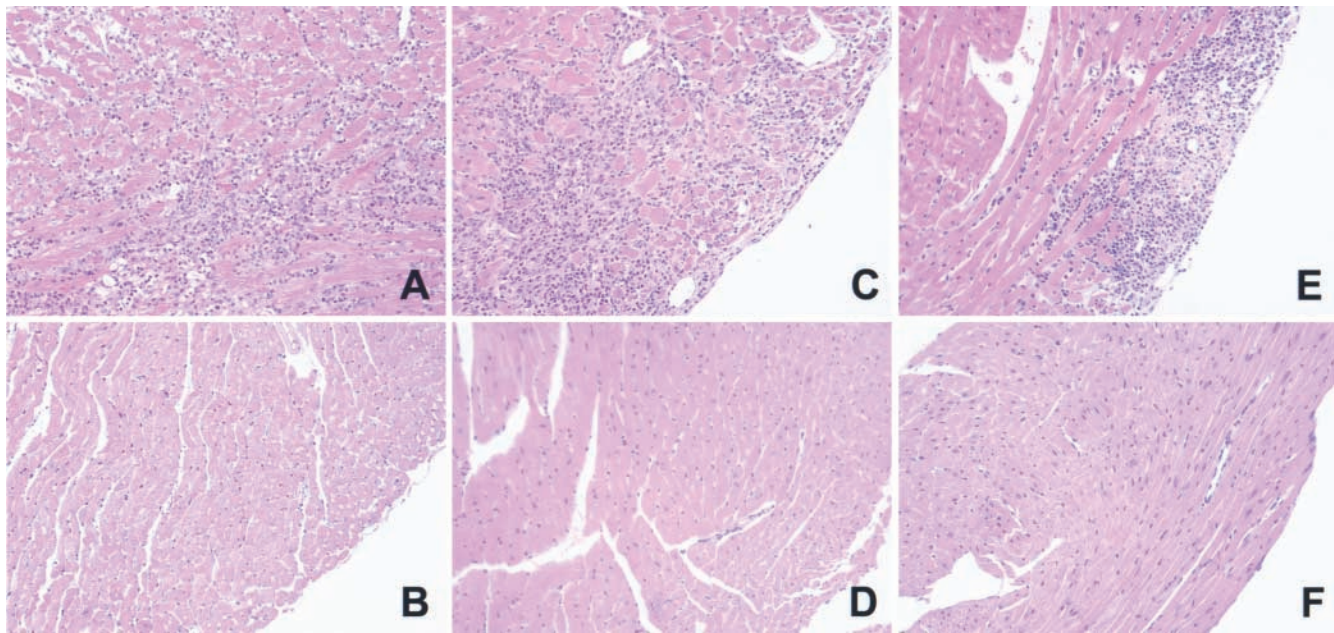^{a}P < 0.0001 for IL-1RI$^{+/+}$ vs. IL-1RI$^{-/-}$; Fisher's exact test.

Figure 1. IL-1R1$^{-/-}$ mice are protected from autoimmune myocarditis and rendered susceptible by transfer of IL-1R1$^{+/+}$ DCs. (A and B) Mice were immunized with myhc-α and hearts were evaluated at day 21. Inflammatory infiltrates are present in hearts of IL-1R1$^{+/+}$ (A) but not IL-1R1$^{-/-}$ mice (B). (C and D) IL-1R1$^{-/-}$ mice were reconstituted with immature myhc-α–pulsed bone marrow DCs derived from naive IL-1R1$^{+/+}$ mice (wt-DC→ko-mice) (C) or IL-1R1$^{-/-}$ mice (ko-DC→ ko-mice) (D) before immunization with myhc-α. Myocarditis is seen in wt-DC → ko-mice (C) but not in ko-DC → ko-mice (D). (E and F) CD4$^+$ T cells purified at day 21 after immunization of mice described in C and D were restimulated in vitro with myhc-α for 48 h before transfer into naive IL-1R1$^{+/+}$ SCID (BALB/c) recipients. At day 10 after adoptive transfer, unimmunized SCID mice develop myocarditis by transfer of IL-1R1$^{-/-}$ CD4$^+$ T cells isolated from wt-DC → ko-mice (E) but not from ko-DC → ko-mice (F). 480× original magnification.

cells using magnetic beads, 2×10^5 CD11c$^+$MHC class II^{low+} DCs per mouse were intraperitoneally injected 6 h before immunization with myhc-α and CFA on days 0 and 7.

Results

IL-1R1$^{-/-}$ Mice Are Protected from Autoimmune Myocarditis. To investigate the role of IL-1R1–mediated effects on the pathogenesis of murine autoimmune myocarditis, homozygous female IL-1R1$^{-/-}$ mice and IL-1R1$^{+/+}$ controls on a BALB/c background were immunized with a peptide derived from mouse α-myosin (myhc-α). Disease prevalence and severity were assessed by histology and immunohistochemistry 21 d after the first immunization. IL-1R1$^{+/+}$ mice developed severe cardiac inflammation. Inflammatory infiltrates mainly consisted of mononuclear cells including macrophages, CD4$^+$ T cells, and CD8$^+$ T cells, as well as some granulocytes and eosinophils (Fig. 1). In contrast, inflammatory infiltrates were absent in immunized IL-1R1$^{-/-}$ mice (Table I). This effect was not dose dependent, because disease prevalence and severity did not increase in IL-1R1$^{+/+}$ and IL-1R1$^{-/-}$ mice with higher antigen doses (data not depicted).

Reduced Activation of Autoreactive CD4$^+$ T Cells. Autoimmune myocarditis is a CD4$^+$ T cell mediated disease (6). To test the function of myosin-specific CD4$^+$ T cells in immunized IL-1R1$^{-/-}$ mice, we purified CD4$^+$ T cells from draining lymph nodes and restimulated them with the myhc-α peptide on irradiated IL-1R1$^{+/+}$ antigen-present-

ing cells in vitro. Compared with controls, self-specific CD4$^+$ T cells from IL-1R1$^{-/-}$ mice proliferated poorly (Fig. 2 A), and produced little IL-2 and IFN-γ, whereas IL-4 and IL-10 production appeared to be intact (Fig. 2 B).

To further investigate the function of autoimmune CD4$^+$ T cells in vivo, we performed adoptive transfer experiments. CD4$^+$ T cells from immunized IL-1R1$^{-/-}$ mice and IL-1R1$^{+/+}$ controls were isolated at day 21, were expanded in vitro in the presence of specific antigen, and were injected into SCID mice. As shown previously (4),

Table II. *Myocarditis Prevalence after Adoptive Transfer of In Vitro Antigen-restimulated CD4$^+$ T Cells in SCID (A^d) Mutant Mice*

CD4$^+$ donor mice	Immunization of donor mice	Prevalence in SCID recipients — Day 10 after transfer
IL-1R1$^{+/+}$	myhc-α and CFA	6/6 group A
IL-1R1$^{-/-}$	myhc-α and CFA	0/5 group B
IL-1R1$^{-/-}$	myhc-α/CFA/IL-1RI$^{+/+}$ DC	5/6 group C
IL-1R1$^{-/-}$	myhc-α/CFA/IL-1RI$^{-/-}$ DC	0/6 group D

P = 0.022 group A vs. group B, P = 0.0152 group C vs. group D, P = 0.0152 group B vs. group C; Fisher's exact test.
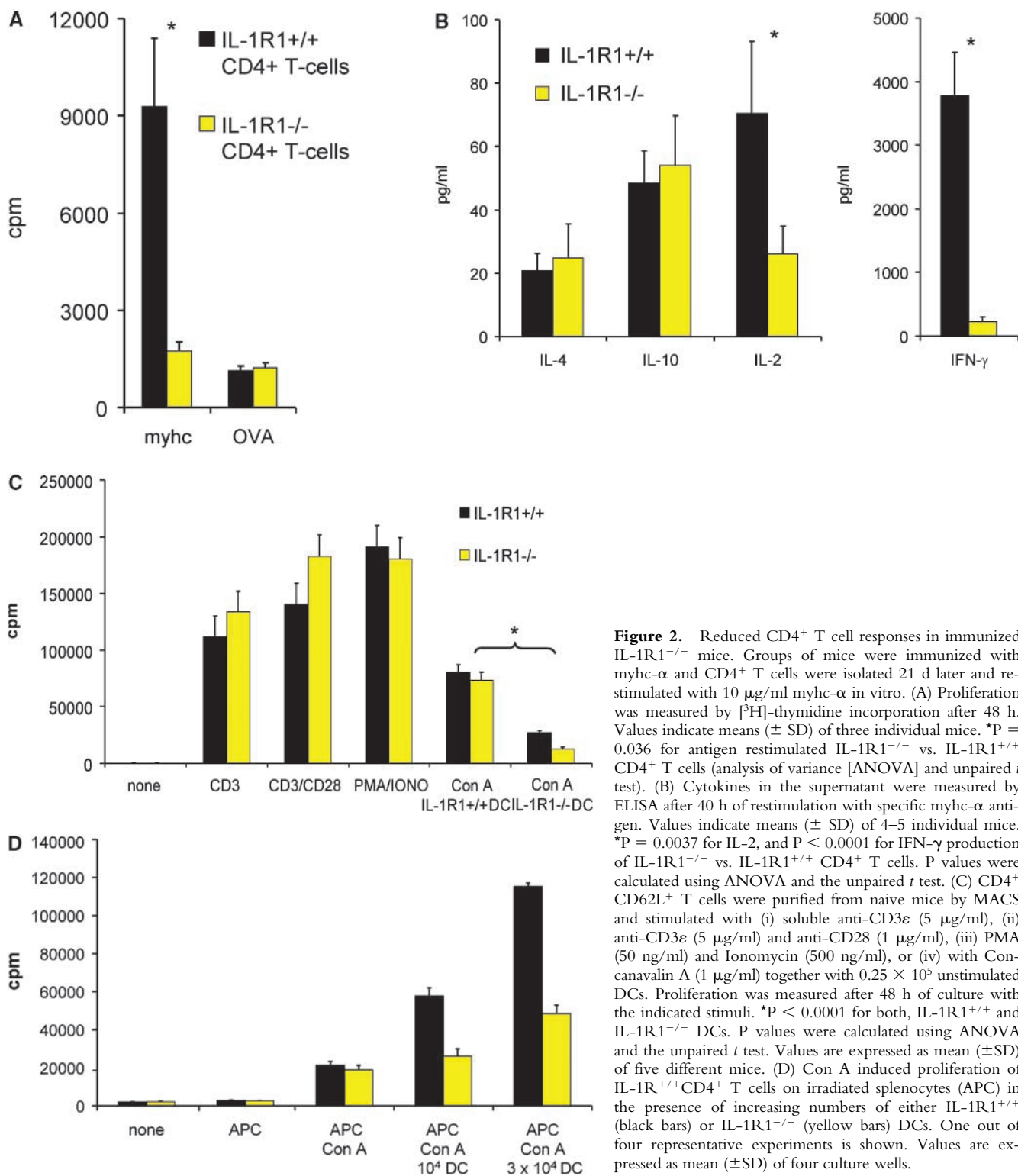
Figure 2. Reduced CD4$^+$ T cell responses in immunized IL-1R1$^{-/-}$ mice. Groups of mice were immunized with myhc-α and CD4$^+$ T cells were isolated 21 d later and re-stimulated with 10 μg/ml myhc-α in vitro. (A) Proliferation was measured by [^{3}H]-thymidine incorporation after 48 h. Values indicate means ($\pm$ SD) of three individual mice. *P = 0.036 for antigen restimulated IL-1R1$^{-/-}$ vs. IL-1R1$^{+/+}$ CD4$^+$ T cells (analysis of variance [ANOVA] and unpaired t test). (B) Cytokines in the supernatant were measured by ELISA after 40 h of restimulation with specific myhc-α antigen. Values indicate means ($\pm$ SD) of 4–5 individual mice. *P = 0.0037 for IL-2, and P < 0.0001 for IFN-γ production of IL-1R1$^{-/-}$ vs. IL-1R1$^{+/+}$ CD4$^+$ T cells. P values were calculated using ANOVA and the unpaired t test. (C) CD4$^+$ CD62L$^+$ T cells were purified from naive mice by MACS and stimulated with (i) soluble anti-CD3ε (5 μg/ml), (ii) anti-CD3ε (5 μg/ml) and anti-CD28 (1 μg/ml), (iii) PMA (50 ng/ml) and Ionomycin (500 ng/ml), or (iv) with Concanavalin A (1 μg/ml) together with 0.25 $\times$ 10^5 unstimulated DCs. Proliferation was measured after 48 h of culture with the indicated stimuli. *P < 0.0001 for both, IL-1R1$^{+/+}$ and IL-1R1$^{-/-}$ DCs. P values were calculated using ANOVA and the unpaired t test. Values are expressed as mean ($\pm$SD) of five different mice. (D) Con A induced proliferation of IL-1R$^{+/+}$CD4$^+$ T cells on irradiated splenocytes (APC) in the presence of increasing numbers of either IL-1R1$^{+/+}$ (black bars) or IL-1R1$^{-/-}$ (yellow bars) DCs. One out of four representative experiments is shown. Values are expressed as mean ($\pm$SD) of four culture wells.

transfer of autoreactive CD4$^+$ T cells from IL-1R1$^{+/+}$ BALB/c mice resulted in myocarditis in SCID recipients (Table II). In contrast, IL-1R1$^{-/-}$CD4$^+$ T cells did not induce any disease after adoptive transfer into SCID mice (Table II). These data clearly confirm that autoimmune myocarditis can be induced by autoreactive CD4$^+$ T cells

and that the IL-1R1 is essential for their optimal activation in vivo. Given the impaired in vivo CD4$^+$ T cell response in IL-1R1$^{-/-}$ mice, we asked whether the activation of CD4$^+$ T cells intrinsically requires IL-1R1 signaling. To this end, we isolated naive CD62L$^+$CD4$^+$ T cells from healthy donor mice and triggered them for 24 or 48 h with
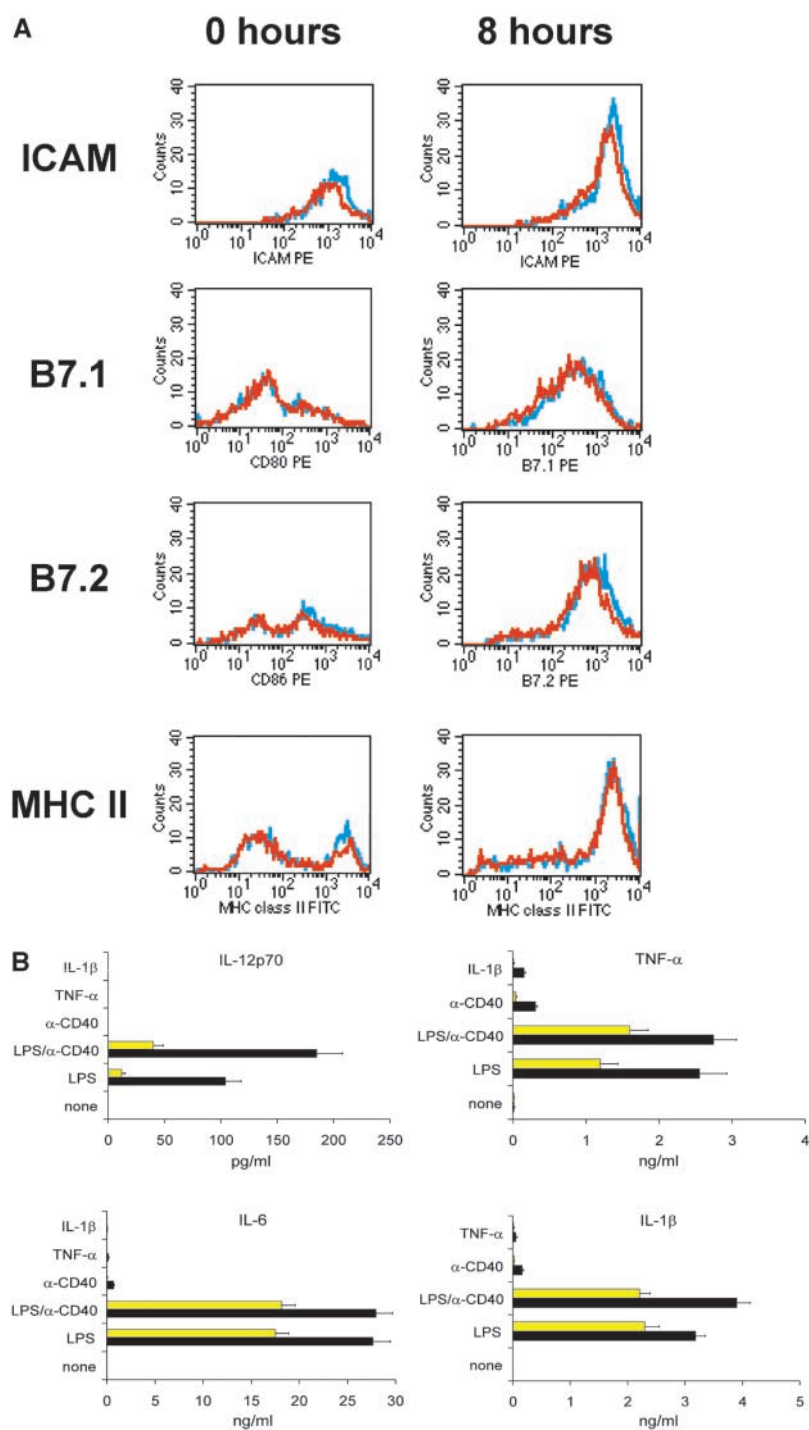
Figure 3. Up-regulation of costimulatory molecules and production of proinflammatory cytokines in IL-1R1$^{+/+}$ and IL-1R1$^{-/-}$ DCs. (A) FACS® profiles representing only minimal differences in the expression of costimulatory molecules on IL-1R1$^{+/+}$ (blue) and IL-1R1$^{-/-}$ (red) DCs before and after stimulation with LPS/anti-CD40 for 8 h. Histogramms were gated on CD11c$^+$MHC class II$^+$ live cells (ICAM, B7.1, B7.2) or CD11c$^+$ live cells. (B) Mature BM-DCs were stimulated for 24 h as indicated. IL-12p70, TNF-α, IL-6, and IL-1β were measured by ELISA. Data for IL-1R1$^{+/+}$ DCs (black bars) and IL-1R1$^{-/-}$ DCs (yellow bars) are expressed as mean ($\pm$SD) from quadruplicate culture wells. Differences between IL-1R1$^{+/+}$ and IL-1R1$^{-/-}$ DCs were highly significant for all cytokines following LPS, or LPS/anti-CD40 stimulation. IL-1R1$^{-/-}$ DCs also produced significantly reduced levels of TNF-α, IL-1β, and IL-6 after stimulation with anti-CD40 alone, significantly reduced levels of IL-6 and IL-1β after TNF-α stimulation, and significantly reduced TNF-α levels after IL-1β stimulation. (All P values 0.0001 or <0.0001, following ANOVA and unpaired t test). The data are representative for several independent experiments with similar results.

various stimuli. As shown in Fig. 2 C, naive IL-1R1$^{+/+}$ and IL-1R1$^{-/-}$CD4$^+$ T cells showed comparable proliferation upon stimulation with anti-CD3ε, anti-CD3ε/anti-CD28, PMA/Ionomycin, or Concanavalin A presented by wild-type DCs. Interestingly, both IL-1R1$^{+/+}$ and IL-1R1$^{-/-}$CD4$^+$ T cells showed impaired proliferation upon Con A stimulation in the presence of DCs derived from IL-1R1$^{-/-}$ mice. However, IL-1R1$^{-/-}$ DCs do not suppress CD4$^+$ T cell proliferation upon Con A in the presence of irradiated splenocytes. This is illustrated in Fig. 2

D, where increasing numbers of IL-1R1$^{-/-}$ DCs enhance IL-1R1$^{+/+}$CD4$^+$ T cell proliferation, albeit to a much lesser extent than IL-1R1$^{+/+}$ DCs. Taken together, these data suggest that reduced CD4$^+$ T cell activation in IL-1R1$^{-/-}$ mice immunized with self-peptide results from a defect at the level of DCs rather than from an intrinsic defect of CD4$^+$ T cells in the absence of IL-1R1.

Impaired Production of Proinflammatory Cytokines by IL-1R$^{-/-}$ DCs after In Vitro Stimulation. To test whether the absence of the IL-1R1 on DCs affected their immune stim-
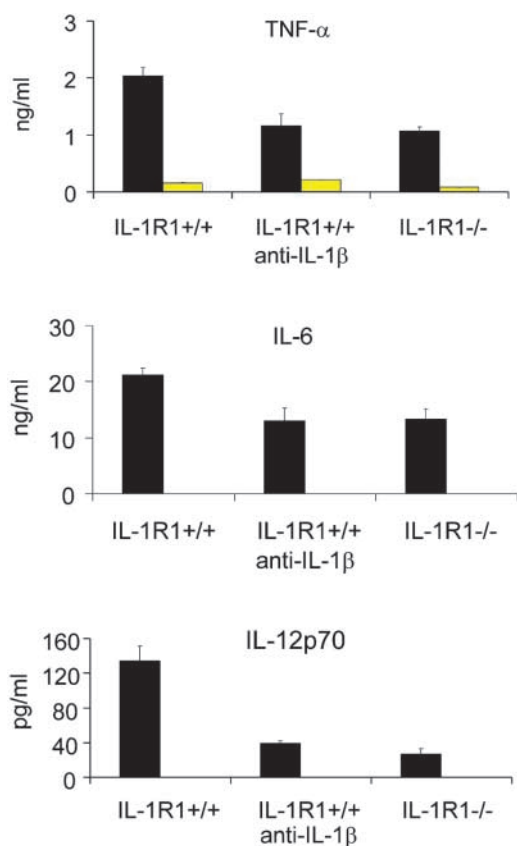
Figure 4. Neutralization of IL-1β reduces production of proinflammatory cytokines by BM-DCs. Mature bone marrow–derived IL-1R1$^{+/+}$ and IL-1R1$^{-/-}$ DCs were cultured for 24 h in the presence or absence of an anti–IL-1β antibody. Cytokines were detected by ELISA. Data for CD40/LPS stimulated DCs (black bars) and nonstimulated DCs (yellow bars) are expressed as mean (±SD) from triplicate culture wells.

Table III. *Myocarditis Prevalence and Severity in Immunized IL-1R1$^{+/+}$ and IL-1R1$^{-/-}$ Mice after Supplementation with Immature, myhc-α–loaded DCs*

Mice	Dendritic cells	Prevalence Day 21	Severity grade (at day 21) Median range
IL-1R1$^{-/-}$	IL-1R1$^{-/-}$	0/5[a]	0
IL-1R1$^{-/-}$	IL-1R1$^{+/+}$	5/6[a]	3 (0–4)
IL-1R1$^{+/+}$	IL-1R1$^{+/+}$	6/6[b]	4 (3–4)[c]
IL-1R1$^{+/+}$	IL-1R1$^{-/-}$	4/5[b]	2 (0–3)[c]

[a]P = 0.0152.
[b]P = 0.4545, Fisher's exact test.
[c]P = 0.081, Mann-Whitney U test.

anti–IL-1β blocking antibody to DC cultures during stimulation inhibited production of proinflammatory cytokines by IL-1R1$^{+/+}$ DCs similar than the IL-1R1 genetic defect (Fig. 4). Taken together our results suggest that triggering of the IL-1R1 is required for optimal activation of DCs to secrete proinflammatory cytokines including IL-12, which promote CD4^{+} activation.

Injection of IL-1R1$^{+/+}$ Antigen Pulsed DCs Restores Myocarditis Susceptibility of IL-1R1$^{-/-}$ Mice. We next asked whether susceptibility to myocarditis could be restored in IL-1R1$^{-/-}$ mice by reconstitution with IL-1R1$^{+/+}$ DCs. We grew immature BM-DCs in the presence of IL-10 to prevent activation and possibly tolerance induction (23) and pulsed them with myhc-α before injection in IL-1R1$^{-/-}$ mice. Subsequently, mice were immunized with myhc-α/CFA. Intriguingly, disease susceptibility was completely restored in IL-1R1$^{-/-}$ mice receiving IL-1R1$^{+/+}$ DCs but not in those receiving IL-1R1$^{-/-}$ DCs (Fig. 1, and Table III), and histology of the hearts of DC reconstituted IL-1R1$^{-/-}$ mice was not different from diseased IL-1R1$^{+/+}$ controls (Fig. 1). Importantly, disease severity in IL-1R1$^{+/+}$ mice was not significantly reduced after injection of immature IL-1R1$^{-/-}$ DCs excluding a relevant tolerogenic role for IL-1R1$^{-/-}$ immature DCs in our experimental setting. Efficient in vivo priming of autoreactive T cells in IL-1R1$^{-/-}$ mice reconstituted with immature IL-1R1$^{+/+}$ DCs was finally confirmed by the fact that CD4^{+} T cells isolated from these mice transferred disease upon injection into SCID (IL-1R1$^{+/+}$) recipients (Fig. 1, and Table II). In conclusion, our data provide strong evidence that triggering of IL-1R1 on DCs is critical for induction of a pathogenic autoreactive CD4^{+} T cell response and development of autoimmune heart disease.

Discussion

Our data demonstrate an essential role of the IL-1 pathway in the development of autoimmune inflammatory

ulatory capacity, we measured the expression of costimulatory molecules and the production of proinflammatory cytokines in response to various stimuli. Surface expression of MHC class II, CD40, ICAM-1, CD80, and CD86 differed only minimally on bone marrow–derived DCs (BM-DCs) from IL-1R1$^{-/-}$ and IL-1R1$^{+/+}$ mice before and after (8 and 24 h) stimulation with either LPS, anti-CD40 mAb, or LPS/anti-CD40 mAb (Fig. 3 A). Furthermore, we did not observe any differences in the proportions of living and apoptotic CD11c^{+} BM-DCs in cell cultures after 24 h (not shown). Production of inflammatory cytokines including IL-6, TNF-α, IL-1β, and IL-12p70 by wild-type BM-DCs were considerably induced only after stimulation of Toll-like receptor (TLR)4 with LPS but not by stimulation with either anti-CD40, IL-1β, or TNF-α. Interestingly, stimulation of both TLR4 and CD40 further augmented production of IL-12p70 but not IL-6 and TNF-α. As illustrated in Fig. 3 B, production of IL-6, TNF-α, IL-1β and, in particular, IL-12p70 was largely impaired in IL-1R1$^{-/-}$ DCs upon stimulation with LPS or LPS/anti-CD40. Preliminary data from our laboratory indicate that stimulation of TLR9 with CpG also resulted in impaired IL-12p70 production by IL-1R1$^{-/-}$ DCs (not shown). Coadministration of an

heart disease. Mice lacking the IL-1R1 were protected from myocarditis and showed impaired priming of heart myosin specific CD4$^+$ T cells, which resulted from defective activation of DCs. To our knowledge, this is the first publication showing that inefficient DC activation prevents induction of autoimmune disease.

Members of the IL-1R/Toll-like receptor (TLR) superfamily including IL-1R1 and IL-18R commonly induce the signaling cascade MyD88 → IRAK1/IRAK2 → TRAF6 leading to the activation of the transcription factor nuclear factor (NF)-κB, p38 mitogen-activated protein kinase (MAPK), and Jun-N terminal kinase. The NF-κB pathway appears to play an important role in the development of autoimmune diseases and inactivation ameliorates EAE (24) and collagen-induced arthritis (25, 26), which has been implicated with the inhibition of Th1 responses. NF-κB may promote the production of IFN-γ by T cells directly by interaction with a functional NF-κB site in the IFN-γ promoter or by activation of p38 MAPK through regulation of GADD45β in response to IL-18 (27). In our studies, we also observed reduced IFN-γ production by antigen-specific CD4$^+$ T cells of immunized IL-1R1$^{-/-}$ mice. However, this does not provide a direct explanation for the protection from heart inflammation, because IFN-γ$^{-/-}$ mice develop exacerbated myocarditis (8–10). On the other hand, IL-1 and NF-κB activation has also been associated with the development of Th2 responses (28–30), but we found that IL-4 and IL-10 production was unaffected after restimulation of CD4$^+$ T cells from immunized IL-1R1$^{-/-}$ mice excluding immune deviation and the induction of IL-10$^+$ regulatory T cells. Furthermore, Th2 cells do not play a crucial role in autoimmune myocarditis, although they can modulate the disease (8). Regardless whether IL-1 and NF-κB regulate IFN-γ or IL-4 production in CD4$^+$ T cells, our data clearly show that pathogenic CD4$^+$ T cells in the IL-1R1$^{-/-}$ mice were fully activated and induced disease when they encountered IL-1R1$^{+/+}$ DCs presenting the antigen. A normal Th1 and Th2 subset polarization was also observed when naive IL-1R1$^{-/-}$CD4$^+$ specific for OVA$_{323–339}$ were stimulated with cognate antigen in the presence of wild-type DCs (unpublished data).

Transfer of immature BM-DCs from wild-type mice completely restored disease in IL-1R1$^{-/-}$ mice suggesting that DCs lacking the IL-1R1 are incapable to prime CD4$^+$ T cells and induce autoimmunity. It is conceivable that IL-1 acts on DCs by stimulating their capacity to up-regulate CD40L and OX40 on CD4$^+$ T cells (31). DCs might require IL-1R1 triggering for proper development, migration, antigen processing/presentation, or/and for optimal activation. In preliminary experiments, some of these possibilities were addressed. We purified CD11c$^+$ DCs from lymph nodes and spleen of naive mice and analyzed them by flow cytometry. The frequency of CD8$^+$ and CD8$^-$ DCs and the expression levels of MHC class II, CD86, and CD40 were comparable in IL-1R1$^{+/+}$ and IL-1R1$^{-/-}$ mice indicating that DC development was not grossly affected (not shown). Our experiments using BM-DCs sug-

gest that IL-1R1 triggering is required for production of a panel of pro-inflammatory cytokines including IL-12p70, IL-1, IL-6 and TNF-α, which are all known to be targets of NF-κB (32, 33). Reduced production of these cytokines was also observed when BM-DCs from wild-type mice were stimulated in the presence of neutralizing anti-IL-1β mAb (Fig. 4). This finding is in keeping with results obtained with human DC subsets (34).

Interestingly, IL-1R1$^{-/-}$ DCs seem not to have a relevant defect to mature upon stimulation, as the up-regulation of CD80, CD86, and ICAM-1, the latter also activated by NF-κB, was almost comparable to IL-1R1$^{+/+}$ DCs.

Nevertheless, we cannot exclude that other DC functions such as their migratory capacity is reduced in the absence of IL-1R1 (35, 36). Regardless, the impaired production of TNF-α, IL-6, and IL-12 itself may explain disease resistance of IL-1R1$^{-/-}$ mice, because each of them is indispensable for the induction of autoimmune myocarditis (7, 8, 37).

The phenotype of in vitro–activated IL-1R1$^{-/-}$ DCs appears somewhat reminiscent to the TNF-α–induced semimature DCs described by Menges et al. (23), which showed impaired IL-12p70 production but normal up-regulation of costimulatory molecules upon stimulation. These semimature DCs were tolerogenic in a model of CD4$^+$ T cell–mediated autoimmune disease. Despite the fact that injection of immature IL-1R1$^{-/-}$ DCs did not prevent disease in immunized IL-1R1$^{+/+}$ mice, we cannot exclude a potential tolerogenic role for IL-1R$^{-/-}$ DCs because our experimental setting is different from the published tolerance induction protocol (23).

N. Rose and coworkers had shown that IL-1 treatment renders otherwise resistant mouse strains susceptible to viral myocarditis and suggested an important role for IL-1 and TNF-α in up-regulation of adhesion molecules on endothelial cells (18). ICAM-1 expression has been shown to be critical for the recruitment of inflammatory cells in Coxsackie B3–induced myocarditis (38). Our data do not rule out a role for IL-1R1 signaling in endothelia activation or target organ homing of antigen-specific T cells. The fact that reconstitution with IL-1R1$^{-/-}$ DCs alone is sufficient in restoring myocarditis susceptibility and normal expression of endothelia activation markers in reconstituted IL-1R1$^{-/-}$ mice, suggest that IL-1R1–mediated mechanisms are either not decisive in mediating the access of autoreactive CD4$^+$ T cells to the heart or may be compensated by redundant pathways.

Recently, it has been shown that structural proteins of several microorganisms potentially affecting the human heart show homology to the pathogenic α-myosin peptide in BALB/c mice and to the human α-myosin (39). Our findings contribute to the understanding of how DC activation may be initially modulated by microbial products in the absence of T cells and in the presence of innate signals. Indeed, the initial activation of DCs phagocyting debris and microbes containing potential self-peptide homologues might determine whether a pathogenic autoreactive response evolves or not. In this context, IL-1R1 sig-

naling may play an important role bridging innate and adaptive immunity.

Taken together, our data show that IL-1R1 signaling induces autoimmunity by critically enhancing the capacity of antigen-presenting DCs to prime autoreactive T cells. Given the availability of clinically effective drugs targeting IL-1, our findings open new therapeutic perspectives in the treatment of inflammatory heart disease. Meanwhile, our data are a major step forward in understanding the mechanisms underlying autoimmunity and cardiac inflammation.

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