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*J Immunol* 2008; 180:30-33; doi: 10.4049/jimmunol.180.1.30
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Cutting Edge: A Key Pathogenic Role of IL-27 in T Cell-Mediated Hepatitis

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The signals driving T cell activation in T cell-mediated fulminant hepatitis are not fully understood. In this study, we identify the cytokine IL-27p28/EBI3 as a major pathogenic factor in the ConA model of T cell-mediated hepatitis. We found an up-regulation of hepatic EBI3 and p28 expression and augmented levels of IL-27 in wild-type mice after ConA administration, suggesting a potential pathogenic role of this cytokine in ConA hepatitis. Consistently, IL-27 EBI3-deficient mice were almost completely protected from ConA-induced liver damage. Such protection was associated with reduced levels of IFN-γ and its signaling proteins pSTAT-1 and T-bet. Finally, in vivo blockade of IL-27 function using a soluble IL-27 receptor fusion protein led to reduced pSTAT1 levels and suppression of liver injury. Taken together, these data demonstrate a key pathogenic role of IL-27 in T cell-mediated liver injury. Furthermore, in vivo blockade of IL-27 emerges as a novel potential therapy for T cell-mediated hepatitis. The Journal of Immunology, 2008, 180: 30–33.

Interleukin-27 is a member of the IL-6/IL-12-family that consists of EBV-induced gene 3 (EBI3)2 and p28 (1–3). It is the only known ligand for the gp130/WSX-1 receptor (1). Macrophages and dendritic cells are the main producers of IL-27, and the IL-27 signaling cascade includes activation of Jak1, STAT1, STAT3, STAT4, and STAT5 in T cells, STAT1 and STAT3 in monocytes, and STAT3 in mast cells (2–4).

IL-27 may exert both pro- and anti-inflammatory functions (5, 6). It can augment T cell proliferation and enhance synthesis of IFN-γ through the induction of T-bet and the activation of STAT4 (1). IL-27 can activate the transcription of T-bet independently from STAT-1 (4), and such induction of T-bet appears to be mainly responsible for IL-27-mediated IFN-γ production (5, 7).

In addition to its proinflammatory effects, there is increasing evidence that IL-27 may suppress immune responses (8, 9). In fact, infection of WSX-1-deficient mice with Trypanosoma cruzi resulted in an immune-mediated liver injury most likely mediated by augmented production of IFN-γ and TNF-α by hepatic T cells and NKT cells (10).

Materials and Methods

Reagents and mice

ConA was purchased from Sigma-Aldrich. Anti-STAT1, anti-phospho-STAT-1, anti-STAT3, anti-phospho-STAT-3, and anti-phospho-STAT-4 Abs were obtained from Cell Signaling.

EBI3-knockout mice (9) and corresponding wild-type animals on a 129/C57BL/6 background were bred in the Animal Care Facility in Mainz, Germany. The strain has been formally denoted B6;129X1-EBI3tm Birk.

Isolation of primary hepatocytes

Hepatocytes from EBI3-deficient and control mice were isolated from mouse livers after perfusion with 0.05% collagenase (Roche) as described previously (11). After 1 day of culture, total RNA was isolated as described below.

Isolation of nonparenchymal liver cells, CD11c+ cells, CD11b+CD11c− cells, sinusoidal endothelial cells (SEGs), and hepatic CD90+ cells

Livers were perfused through the portal vein with 0.05% collagenase A (Roche), mechanically disrupted, and incubated with 0.05% collagenase IV. The fraction of nonparenchymal cells was recovered by centrifugation steps (2 × 400 rpm and 1 × 1500 rpm). Isolation of CD11c+ cells, CD11b+CD11c− cells, CD90+ cells, and CD45− (liver SEGs) was done by magnetic cell separation (Miltenyi Biotech) according to the manufacturer’s instructions. To select liver SEGs, negatively selected CD11b+CD11c− cells were incubated with CD45 Microbeads and CD45− cells were seeded in collagen-coated wells.

Quantitative real-time PCR for EBI3, p28, WSX-1, and gp130

Total cellular RNA was isolated from cells and organs with RNeasy columns (Qiagen), including DNase I digestion. Quantitative real-time PCR analysis for EBI3, p28, WSX-1, gp130, and hypoxanthine phosphoribosyltransferase was performed by using specific Quantitect primer/probe assays (Qiagen).

RT-PCR of hepatic EBI3, IFN-γ, and soluble WSX (WSX) mRNA

C57BL/6 mice were killed 4 h after i.v. injection of ConA and total RNA from the liver was isolated by using QIAamp tissue kit (Qiagen). Specific primers

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Received for publication January 24, 2007. Accepted for publication October 30, 2007.

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2 Abbreviations used in this paper: EBI3, EBV-induced gene 3; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SEG, sinusoidal endothelial cell; WSX, soluble WSX.

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were as follows: EB3, 5'-GAATCTAAGCCGGACCT-3' and 5'-GAATCATCAGCAGGACCT-3'; IFN-γ, 5'-ACACTGACATCTTG GCCTTGG-3' and 5'-GGGATGAGCTCATTTAATGCT-3'.

For detection of liver sWSX mRNA, wild-type mice were hydrodynamically injected with pcDNA-sWSX and control pcDNA vectors as described below. After 4 h, total liver RNA was extracted and RT-PCR was performed using following primers: WSX, 5'-CGTGAAGAGCTCAAACCCAGA-3' and 5'-GGGAAAGGAGAAGCTGAGG-3'.

ConA-induced liver injury

ConA-induced hepatitis was performed as previously described (12–14). Transaminase levels were measured in the clinical chemistry laboratory at the University of Mainz. Liver specimens of the large anterior lobe were cut to 5-μm-thin slices and then stained with H&E.

Western blot analysis

Western blotting was performed using anti-STAT-1 Ab, anti-phospho-STAT-3, anti-phospho-STAT-1, or anti-T-bet Ab at 1/1000 dilution at room temperature. HRP-labeled anti-rabbit or anti-goat IgGs (DakoCytomation) at 1/1000 dilution was used as a secondary Ab. An enhanced luminescence kit (Amersham Biotechnology) was used.

Hydrodynamic injection of plasmid DNA

Ten micrograms of pcDNA 3.1-WSX and 10 mg of pcDNA3.1 were diluted in 1.6 ml of PBS and injected into the tails of wild-type mice. The injection took no longer than 10 s, and the apnea period in mice ranged from 4 to 6 s.

Statistics

Data were compared by Student’s t test for independent events. P < 0.05 was considered statistically significant.

Results and Discussion

Expression of EB3 and p28 in hepatic cells and serum levels of IL-27 in ConA-induced hepatitis

To determine the role of IL-27 in T cell-mediated hepatitis, we analyzed the hepatic expression of the EB3 subunit of IL-27 upon ConA administration in wild-type mice. Expression levels of EB3 and p28 were increased in APCs such as CD11b+CD11c- cells and CD11c+ cells and to a much lesser extent in CD90+ T cells from the liver 6 h after injection of ConA, whereas hepatocytes and SECs did not reveal an augmented p28/EB3 expression (Fig. 1A). Moreover, we found a significant increase of serum IL-27 levels 4 and 8 h after the administration of ConA as compared with control mice (Fig. 1B), consistent with a potential regulatory role of this cytokine in T cell-mediated liver injury.

IL-27/EB3-deficient mice are protected from ConA-induced liver injury

To address the functional role of IL-27 in the development of ConA-induced liver injury, we took advantage of IL-27/EB3-deficient mice (9). Injection of 25 mg/kg ConA in IL-27/EB3-deficient mice resulted in significantly lower serum liver values (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) as compared with those in wild-type mice. Injection of 25 mg/kg ConA revealed no signs of inflammation or necrosis (Fig. 2B).

Decreased expression of IFN-γ and IFN-γ signaling proteins in IL-27/EB3-deficient mice upon injection of ConA

The IFN-γ signaling pathway plays a key pathogenic role in ConA-induced liver injury because IFN-γ, STAT-1, and T-bet-knockout mice are protected from ConA-mediated hepatitis (12, 13). In contrast, activation of the transcription factor STAT-3 by IL-6 results in protection against ConA-mediated hepatitis (14). Interestingly, IL-27 can activate both STAT-1 and STAT-3 in naïve CD4+ T cells and NK cells (15, 16). Accordingly, we determined the expression of IFN-γ and STAT signaling proteins upon ConA administration in wild-type and IL-27/EB3-deficient mice. We found a decreased hepatic IFN-γ expression (Fig. 3A) and reduced STAT-1 activation (Figs. 3, B and C) in ConA-treated IL-27/EB3 deficient mice as compared with wild-type mice. Furthermore, the STAT-1-dependent T-box transcription factor T-bet was markedly suppressed in the former mice as compared with the latter mice, suggesting that IL-27 induces STAT-1 and T-bet activation in T cell-mediated hepatitis. Finally, we determined the activation levels of STAT-3 and STAT-4. However, analysis of liver lysates from EB3-deficient mice revealed no differences in STAT-3 expression and phospho-STAT-3/phospho-STAT-4 levels between wild-type and knockout mice (Fig. 3B). To address the question of which cell type the changes of IFN-γ-related proteins occur, we isolated CD11b+CD11c- cells, CD11c+ cells, CD90+ lymphocytes, and primary hepatocytes from the livers of ConA-treated EB3-deficient mice and control mice. Decreased phospho-STAT-1 expression was observed in CD90+ cells, CD11b+CD11c- cells, CD11c+ cells, and hepatocytes, whereas no reduction in phospho-STAT-1 expression was detected in SECs (Fig. 3C). Taken together, these
data suggest a critical role of IL-27 EBI3 in controlling IFN-γ production and signaling in T cell-mediated hepatitis via STAT-1 and T-bet.

**Hydrodynamic injection of IL-27 receptor fusion protein DNA ameliorates ConA-mediated liver injury in wild-type mice**

To further analyze the functional role of IL-27/EBI3 in the pathogenesis of T cell-mediated liver pathology, we aimed at blocking IL-27 function in vivo. Accordingly, we performed i.v. hydrodynamic injection of a cDNA encoding for a blocking, soluble IL-27 receptor fusion that has been previously shown to block IL-27 function in vivo and in vitro (17). Upon the administration of ConA, the levels of AST and ALT were significantly lower in mice given the plasmid expressing the soluble receptor fusion protein as compared with mice treated with the control vector (Fig. 4A), indicating that a blockade of IL-27 signal transduction suppresses T cell-mediated hepatitis in vivo. Histological analysis of liver sections revealed fewer pathological changes in sWSX expression vector-treated mice compared with control vector-treated mice (Fig. 4B). Because the i.v. injection of DNA for the soluble IL-27 receptor fusion protein resulted in the intrahepatic expression of sWSX mRNA (Fig. 4C), our data are consistent with a model in which sWSX is expressed upon hydrodynamic injection followed by the suppression of ConA-mediated hepatitis in vivo. Consistently, Western blot analysis revealed lower levels of phospho-STAT-1 and T-bet expression. In contrast, no changes in phospho-STAT-4 levels were noted, suggesting that STAT-4 can be activated by other cytokines in the absence of IL-27 (Fig. 4D).

Finally, we analyzed the expression of WSX and gp130 on different liver cell types using real-time PCR. We detected no significant differences in cellular WSX and gp130 expression between EBI3-deficient and wild-type mice (Fig. 4E), suggesting that both EBI3-deficient and wild-type cells in the liver are susceptible to IL-27-dependent activation.

**A key regulatory role for IL-27 in T cell-mediated hepatitis**

The signals driving T cell activation in the ConA-mediated model of hepatitis are not fully understood. In this study, we have shown a key role of IL-27 in controlling T cell-mediated liver injury in this model. IL-27 is rapidly produced by dendritic cells and macrophages in vivo (1), and its serum levels have shown a key role of IL-27 in controlling T cell-mediated immunity in T cell-mediated hepatitis.

Mice deficient in IL-27/EBI3 were markedly protected against ConA-induced liver injury, whereas WSX-1-knockout mice showed augmented ConA hepatitis (18), suggesting marked differences in the phenotype between these mice. Indeed, previous studies have identified differences between IL-27/EBI3- and WSX-1-deficient mice. For instance, Yoshida and colleagues demonstrated by using WSX-1-deficient mice that WSX-1 is required for the normal production of IFN-γ by primary naive CD4+ T cells (5). In contrast, EBI3-deficient T cells showed diminished IL-4 and augmented IFN-γ synthesis (9). Although the reason for these differences is not entirely clear, our data unequivocally identify IL-27 as a central pathogenic factor in the ConA model of T cell-mediated liver injury.

**FIGURE 2.** EBI3-deficient mice are protected from severe liver injury upon challenge with ConA. **A**, Eight hours after injection of 25 mg/kg (n = 16) or 10 mg/kg (n = 12) ConA, blood was taken from EBI3-deficient mice and wild-type mice. Levels of liver enzymes (AST and ALT) from the indicated groups of mice are shown as mean values ± SD from three independent experiments (*, p < 0.001). **B**, H&E-stained liver sections of ConA-treated EBI3-deficient mice and wild-type (wt) mice. Representative sections from each group are shown. Administration of PBS in mice served as control. Injection of 10 mg/kg ConA in EBI3-deficient mice did not result in any histopathological changes.

**FIGURE 3.** The IFN-γ signaling pathway is down-regulated in ConA-treated EBI3-deficient mice. **A**, Expression of hepatic IFN-γ mRNA was analyzed by RT-PCR 4 h after injection of ConA (25 mg/kg ConA). β-Actin expression served as control. **B**, Levels of STAT-1, T-bet, and STAT-3 proteins and phospho-STAT proteins in liver lysates from EBI3-deficient and wild-type mice after injection of ConA were analyzed by Western blotting. Levels of phospho-STAT-1 and T-bet were lower in EBI3-deficient mice compared with wild-type controls, while levels of STAT-3, pSTAT-3 and pSTAT-4 were virtually unchanged. **C**, Analysis of phospho-STAT-1 expression was performed in different liver cell types 6 h after administration of ConA (25 mg/kg) using immunoprecipitation. Expression levels were lower in CD11c+ cells, CD11b+ cells, hepatocytes, and CD90+ cells isolated from EBI3-deficient mice when compared with wild-type controls.
Furthermore, because the blockade of IL-27 using an IL-27 receptor fusion protein suppressed ConA-mediated hepatitis in vivo, the targeting of IL-27 emerges as a potential novel approach for therapy of T cell-mediated hepatitis.

**Acknowledgments**
We thank Sonja Klein for excellent technical assistance.

**Disclosures**
The authors have no financial conflict of interest.

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