Exacerbation of Granuloma Formation in IL-1 Receptor Antagonist-Deficient Mice with Impaired Dendritic Cell Maturation Associated with Th2 Cytokine Production

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Dendritic cells (DCs) are specialized APCs that play an essential role in the activation of T lymphocytes (1, 2). Their function is regulated by their state of maturation. Originating from bone marrow, DCs migrate to the periphery as immature cells (2, 3). However, the molecular mechanisms of DC homing to various organs and accumulation of DCs in inflamed tissues are still poorly understood (4). Proinflammatory cytokines such as TNF-α and IL-1 have been identified as regulatory factors of DC migration in inflamed tissues (2, 5, 6). IL-1β, a product of skin Langerhans cells (LC), contributes to the initiation and regulation of LC migration in response to skin sensitization (7). Ag-capturing DCs in afferent lymphatics express IL-1 type I receptor (IL-1RI) (8, 9). These reports suggest that IL-1/IL-1RI interaction is important for DC mobilization from inflamed tissue to draining lymph nodes (LNs). In addition, the intracellular domain of IL-1RI is similar to TLRs, a pattern recognition receptor for pathogen-associated molecular patterns, which regulate immunoreactions during pathogen-induced inflammation (10). Because cross-talk between TLRs and IL-1RI has been found (11), the role of the IL-1R system in innate immunity at sites of inflammation is clearly of interest.

Both IL-1α and β are produced by various types of cells; they exhibit pleiotropic biological effects on a variety of cells, and have been implicated as major mediators of tissue destruction in many inflammatory diseases, including septic shock and rheumatoid arthritis (12, 13). The damaging effects of IL-1 in inflammatory diseases have prompted many investigators to search for naturally occurring inhibitors of IL-1. Several lines of evidence indicate that IL-1ra is produced in various types of diseases, such as rheumatoid arthritis and infectious diseases, and that it is an important element of host defense in these conditions (16).

Previously, we observed that a large amount of IL-1ra was produced mainly by hepatocytes during the course of LPS-induced acute hepatitis in Propionibacterium acnes-primed mice, and the administration of an anti-IL-1ra mAb significantly aggravated the liver injury, suggesting a protective role of IL-1ra (19). We also reported that P. acnes-induced granulomas regulate the outcome of liver injury (20, 21). During P. acnes-induced inflammation,
F4/80^-CD11c^+ DC precursors appeared in the circulation, migrated into the perisinusoidal space, and matured within newly formed granulomas. Recruited DCs later migrated to the portal area to interact with T cells in what we termed “portal tract-associated lymphoid tissue (PALT)” (22). We established that DC-movement between the two anatomical compartments (sinusoidal granulomas and PALT) was important to successfully eliminate *P. acnes* (22). However, molecular mechanism of intrahepatic DC movement has not been fully understood yet.

Mature DCs preferentially migrate to the T cell area of regional lymphoid tissues, where they induce activation and proliferation of Th and CTLs (1). Immature and mature DCs commonly express CD11c and TLR2 (22–24). Mature DCs show increased mRNA expression of CCR7, IL-12p40, but reduced mRNA expression of TLR3 and CCR1 in comparison with immature DCs (22–26). Maturation of DCs is also regulated by DNA-reactivation protein 12 (DAP12)-triggering receptors expressed by myeloid cells (TREM), involving TREM-2 signal transduction, via induction of CCR7 expression in DCs (27). Recently, it was reported that activation of DCs is altered in IL-1R1 gene-deficient mice during *α*-myosin-peptide-induced autoimmune myocarditis (28). However, the physiological role of IL-1 in DC maturation and migration during pathogen-induced inflammation has not yet been clarified. We generated mice deficient in the IL-1Ra gene and here examined the pathological roles of IL-1Ra in this murine liver injury model, particularly in relation to the maturation of DCs.

**Materials and Methods**

**Generation of IL-1Ra-deficient mice**

An 11-kb genomic fragment of the IL-1Ra gene was subcloned into pBlue-script SK II plasmid vector. This region spans from 6 kb 5’ from the first exon of the secreted form of the IL-1Ra (sIL-1Ra) gene to the HinClI site in the fourth exon (29). A 2.0-kb fragment containing the second, third, and a part of the fourth exons, which encodes the functional domain of IL-1Ra, was deleted and replaced with a 1.1-kb XhoI-BamHI fragment of the neo-mycin phosphotransferase gene derived from pMC1Neo-poly (A) (Fig. 1A). The MC1-HSV thymidine kinase was inserted into the unique SalI site in the 5’ end of the targeting vector (31). Ten million E14-1 embryonic stem (ES) cells were electroporated with 32 μg of linearized targeting vector. Antibiotic selection procedures with G418 (400 μg/ml) and tetracycline (1 μg/ml) were begun 24 and 48 h after the transfection, respectively. G418- and tetracycline-resistant colonies were picked up 10–12 days later, scored for homologous recombination by PCR and subsequently confirmed by genomic Southern blot hybridization (Fig. 1B). Chimeric mice were generated by microinjection of the targeted ES cells into C57BL/6 blastocysts. Chimeric offspring were bred with C57BL/6 mice to produce heterozygous mice. Heterozygous mutant mice (F2 interbred from 129/Ola genomic Southern blot hybridization (Fig. 1C)). Chimeric mice were gen-

**Histological analysis**

Liver specimens were fixed in 10% neutral buffered formalin and embedded with paraffin for HE staining or Tissue-Tek OCT compound (Miles), snap-frozen in liquid nitrogen, and stored at −80°C for immunohistochemical analyses (35). Paraffin-embedded samples were stained with HE solution, and the numbers and the size of granulomas in the liver were determined on 10 randomly chosen microscopic fields at ×100 magnification. The area of each granuloma was determined with the help of NIH Image Analysis software (version 1.61) as previously described (21). For immunohistochemical analyses, the tissue was cut with a cryostat into 7-μm sections. The sections were incubated with a rat mAb to the mouse macrophage/Kupffer cell surface protein F4/80 (CL3-1; BMA Biomedicals), a hamster mAb to the mouse DC surface protein CD11c (HL; 3D Biosciences) (22), a rat mAb to the mouse CD4 (RM4-5; BD Biosciences), a rat mAb to the mouse B cells surface protein B220 (RA3-6B2; BD Biosciences) or negative control rat IgG at a concentration of 10 μg/ml. Samples were incubated with HRP-conjugated goat anti-rat IgG (BioSource International) or alkaline phosphatase-conjugated donkey anti-hamster IgG

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**FIGURE 1.** Targeted disruption of the IL-1ra gene by homologous recombination. A, Scheme showing the strategy for targeted disruption of the IL-1ra gene. The first line shows the structure of the genomic mouse secreted form of the IL-1-ra gene. Exons of the sIL-1Ra locus, including four exons (numbered black boxes) and three introns of sIL-1Ra. The second line depicts the linearized targeting construct, with neomycin phosphotransferase (neo) and herpes simplex thymidine kinase (HSV-TK) genes. The third line represents the targeted IL-1Ra locus. Arrowheads, Primers used for detection of the mutated allele by PCR. B, Probes for Southern blot analysis are indicated as 3’ probes. Arrows, Expected size of DNA fragments obtained by restriction enzyme digestion. Exons. Amino acid noncoding exon. Restriction enzyme sites are indicated in the maps. B, BamHI; XhoI; HindIII; XbaI; F, Southern blot analysis of DNA from transgenic mouse tail bred by heterozygous intercross. Hybridization was conducted using a 3’ probe. All bands detected corresponded to either the wild-type (+/-) or mutant allele (+/-), as expected from the genome structure. DNA from mouse tail was digested with BamHI. C, RT-PCR analysis of total liver RNA obtained at 7 days after *P. acnes* administration from individual mice sacrificed 2 h after i.v. injection of 0.1 μg of LPS.
Enrichment of DCs from liver nonparenchymal cells (NPCs)

NPCs were prepared as described by Yoneyama et al. (22), with some modifications. Liver was perfused in situ via the central vein with liver perfusion medium (137 mM NaCl, 5.4 mM KCl, 0.6 mM NaH2PO4, 0.8 mM Na2HPO4, 10 mM HEPES, 3.8 mM CaCl2, 4.2 mM NaHCO3, and 5 mM glucose, pH 7.35) for 10 min, followed by collagenase solution (1 mg/ml in liver perfusion medium; Wako Pure Chemical) for 5 min at 37°C. The liver was excised and cell suspensions (5 ml) were layered onto 5-ml columns of 16.8% Nycodenz R (Jackson ImmunoResearch Laboratories). After centrifugation (2500 rpm for 20 min at room temperature), the interface was used as NPCs. CD11c+ cells were sorted from NPCs by using Epics Elite ESP cell sorter (Beckman Coulter).

Expression analysis of DC maturation marker genes by semiquantitative RT-PCR

Total RNA was isolated from liver specimens or liver-derived CD11c+ cells using ISOGEN reagents (Wako Pure Chemical) according to the manufacturer’s instructions. cDNA was synthesized from 1 μg of total RNA using oligo(dT)15 as an mRNA-specific primer and M-MLV reverse transcriptase. The expression of CCR1, CCR7, IFN-γ, IL-4, IL-12p40, and G3PDH was determined by real-time quantitative PCR using the ABI 7700 sequence detection system (Applied Biosystems) (35). The reaction mixture contained a cDNA sample prepared according to the manufacturer’s protocol to yield final concentrations of 200 μM dATP, dCTP, dGTP, and 400 μM dUTP, 4 mM MgCl2, 1.25 U of AmpliTaq DNA polymerase, 0.5 U of Amp-Erase uracil-N-glycosylase, and 200 μM of each primer for amplification. The reaction mixtures also contained the following target hybridization probes (100 nM each) labeled with a reporter fluorescent dye, 6-carboxyfluorescein, at the 5′ end. The sets of primers and probes are shown in Table II (35). The thermal cycling conditions included 50°C for 2 min and 95°C for 15 s, and 55°C for 1.5 min for denaturing anneal-extension, respectively.

Statistical analysis

Results are expressed as the mean ± SD. Statistical significance was determined by two-way ANOVA and Scheffe’s multiple comparison methods. A value of p < 0.05 was accepted as statistically significant.
Results

Enhanced intrahepatic granuloma formation and impaired PALT formation in IL-1ra-deficient mice primed with P. acnes

To clarify the pathophysiological role of IL-1ra in granuloma formation, P. acnes was administered to wild-type mice and IL-1ra-deficient mice. Mice infected with P. acnes develop not only granulomas in the hepatic sinusoid but also PALT in the portal area (22). Histological examination demonstrated that mononuclear cells infiltrated into the hepatic lobes and small granulomas were formed in the livers of wild-type mice at 3 days after P. acnes administration (Fig. 2A). Enhanced granuloma formation was observed in the livers of IL-1ra-deficient mice compared with wild-type mice at 3 and 7 days after P. acnes administration (Figs. 2, C and D). The area of infiltrated cells was ~2-fold increased in the livers of IL-1ra-deficient mice compared with wild-type mice after P. acnes administration (Fig. 3). In contrast, PALT formation was impaired in IL-1ra-deficient mice in comparison with wild-type mice, as evidenced by reduced B cell aggregations (Fig. 4). These results suggest that endogenously produced IL-1ra controls compartmentalization of two disease foci by alleviating sinusoidal granulomas and by facilitating PALT formation.

Increased DC infiltration in P. acnes-primed IL-1ra-deficient mice

In P. acnes-induced granuloma formation, DCs pivotally regulate granuloma as well as PALT formation (20–22). To characterize the infiltrated cells after priming with P. acnes, we performed immunohistochemical analyses. Infiltrated mononuclear cells within sinusoidal granulomas were predominantly positive for F4/80, CD11c, and CD4 in both IL-1ra-deficient mice and wild-type mice (Figs. 5 and 6). The numbers of CD11c+ DCs were increased in IL-1ra-deficient mice, as compared with wild-type mice. Moreover, CD4+ cells are also increased in IL-1ra-deficient mice in comparison with wild-type mice (Figs. 5 and 6). These results suggest that infiltrated DCs were increased in the granuloma sites of P. acnes-primed IL-1ra-deficient mice.

Altered differentiation of DCs in P. acnes-primed, IL-1ra-deficient mice

Mature DCs express specific markers such as CCR7 and IL-12p40 (23, 25), while immature DCs strongly express CCR1 in comparison with mature DCs (23, 25). To clarify the differentiation levels of DCs in granuloma, we examined mRNA expression of these mature and immature DCs in wild-type mouse- and IL-1ra-deficient mouse-derived CD11c+ liver cells markers by real-time quantitative PCR (Fig. 7). IL-1ra-deficient mouse-derived CD11c+ liver cells showed increased mRNA expression of CCR1, but decreased mRNA expression of CCR7 and IL-12p40 in comparison with wild-type CD11c+ cells (Fig. 7A). Immature and mature DCs express different types of TLRs, and recognize bacterial Ags to mediate inflammatory immune responses (10, 24). Hence, we also examined the expression of TLR family mRNAs in wild-type mice and IL-1ra-deficient mouse-derived CD11c+ cells (Fig. 7B). IL-1ra-deficient mouse-derived CD11c+ cells showed increased expression of TLR3 and decreased expression of TLR5 in

**FIGURE 4.** Immunohistochemical analysis of PALT at 7 days after P. acnes administration in wild-type (A) and IL-1ra-deficient (B) mice. Immunohistochemistry was performed using mAb B220 against B lymphocytes as described in Materials and Methods (×400 magnification). These patterns are representative of the results obtained from five independent experiments.
comparison with wild-type CD11c<sup>+</sup> cells. However, both wild-type and IL-1ra-deficient mouse-derived CD11c<sup>+</sup> cells expressed a marker of immature and mature DCs, TLR2 (Fig. 7B). TREM-2 express immature DCs and TREM-2/DAP12 promotes up-regulation of CCR7 (27). It was reported that DAP12-deficient mice showed a dramatic accumulation of dendritic cells in mucocutaneous epithelia associated with decreased hapten-specific contact sensitivity (36). Despite a similar mRNA expression level of DAP12 between wild-type mouse-derived and IL-1ra-deficient mouse-derived CD11c<sup>+</sup> cells, the IL-1ra-deficient mouse-derived CD11c<sup>+</sup> cells showed increased expression of TREM-2 in comparison with wild-type CD11c<sup>+</sup> cells (Fig. 7C). These results suggest that the maturation stage of infiltrated DCs may be different in the absence of endogenous IL-1ra.

**Altered cytokine expression in the liver of IL-1ra-deficient mice after *P. acnes* administration**

Previous studies demonstrated that Th1 cytokines were mainly involved in the granuloma formation in the liver after *P. acnes* administration and that subsequent LPS injection rapidly shifted the response from Th1 to Th2 cytokines in the liver (21, 35, 37). Cytokine balance at the whole liver level is therefore important to determine the disease outcome (21, 35, 37). To characterize cytokine expression profiles in the livers of *P. acnes*-primed IL-1ra-deficient mice, we determined mRNA expression of Th1 (IFN-γ) and Th2 cytokines (IL-4) by using real-time quantitative RT-PCR (Fig. 8). In wild-type mice, IFN-γ gene expression was increased at 7 days after *P. acnes* priming. Marginal induction of IL-4 mRNA expression was observed in wild-type mice at 3 and 7 days after *P. acnes* priming (Fig. 8). In contrast, IL-1ra-deficient mice exhibited a marked increase in intrahepatic expression of these cytokines at 3 days after *P. acnes* administration (Fig. 8). These results suggest that in the absence of endogenous IL-1ra, *P. acnes* administration induced Th2 as well as Th1 cytokine production, and eventually caused massive granuloma formation.

**Discussion**

Granuloma formation is a protective response for the host to eliminate exogenous agents. However, uncontrolled granulomatous immune responses often lead to tissue damage including irreversible necrosis and fibrosis. Successful immune responses should be achieved by creating an appropriate anatomical and cytokine environment during the granulomatous reaction. In a granulomatous liver disease induced by *P. acnes*, the balances between *P. acnes* induced two distinct compartments, PALT and sinusoidal granulomas, and also between Th1 and Th2 cytokines greatly contribute to the disease outcome (22). In the present study, we show that both anatomical and cytokine balances associated with granulomatous inflammation are significantly altered in the absence of endogenous IL-1ra, demonstrating the involvement of IL-1ra in forming a disease-associated, effective local environment. Because DCs play pivotal roles in forming disease-associated anatomical compartments and in generating granuloma-forming Th cells (21, 35, 37), we consider that altered granulomatous responses shown in IL-1ra-deficient mice are attributed to impaired DC responses.

*P. acnes*-primed DC precursors migrate into hepatic sinusoids from the circulation, interact with Kupffer cells, and then activate to increase expression of CCR7 to migrate into PALT (22). Mature DCs move from PALT to hepatic LNs, and induce T lymphocyte proliferation (22). We previously reported that granuloma-forming memory T cells are generated by migrated mature DCs in the hepatic LNs (38). *P. acnes*-primed LN T cells initially produced both IFN-γ and IL-4 by day 3, but preferentially produced IFN-γ thereafter. IFN-γ-producing Th1 cells then leave the LNs and migrate into the liver to complete granuloma formation (38). In the present study, at day 3 after *P. acnes* administration, CD11c<sup>+</sup> DCs increased in the liver of IL-1ra-deficient mice in comparison with...
wild-type mice (Fig. 5). CD4⁺ cells on day 3 increased in IL-1ra−/− deficient mice (Figs. 5, C and F), suggesting that liver-homing IFN-γ⁺ IL-4⁺ T cells are already established in the hepatic LNs by highly activated DCs at this time or recruited from spleen nonspecifically. However, Th2 cytokine mRNA excessively increased in the liver of IL-1ra−/− deficient mice in comparison with wild-type mice (Fig. 8), also suggesting an aberrantly high expression of IL-4 in IL-1ra−/− deficient liver at an early phase. Such IL-4 may further affect the activation status of liver-infiltrating DCs. Although Th1 cytokines are mainly involved in liver pathology observed after priming with P. acnes, subsequent LPS injection rapidly induced cytokine expression from Th1 to Th2 in the liver, causing exacerbation (21, 35). In this respect, an early and robust intrahepatic Th2 response possibly induced by overactivated DCs may lead to dysregulated granuloma formation.

PALT formation is important for host defense, because the balance between protective PALT and injurious sinusoidal granuloma would determine the disease outcome (22). P. acnes-primed DC precursors migrate to the portal area to form PALT from sinusoidal areas after maturation (22). Therefore, the molecules directing DC positioning within the inflamed tissue space are also important, and our results show that IL-1ra is involved in this process. Previously we reported that IL-1ra is mainly produced by liver parenchymal cells around the central vein in mice after P. acnes administration (19). Anatomically, liver parenchymal cells form hepatic cord, with their poles facing toward Disse’s space, and thus they make a “pathway” for migration of DCs from sinusoidal areas to PALT. Interestingly, immature DC accumulation in P. acnes-treated liver of IL-1ra deficient mice is similar to phenotype of DAP12-deficient mice (36). IL-1ra produced by liver parenchymal cells may guide DC migration during granulomatous reactions (Fig. 9).

At day 7 after P. acnes administration, CD11c⁺ DCs were still increased in liver of IL-1ra−/− deficient mice in comparison with wild-type mice (Fig. 6). Moreover, maturation of DCs was altered in the liver of IL-1ra−/− deficient mice in comparison with wild-type mice (Fig. 7). IL-1ra-deficient mice showed massive granuloma formation in hepatic sinusoids, but PALT formation was decreased compared with wild-type mice (Fig. 4). These results indicate that the IL-1/IL-1ra system is not essential for DC entry from the circulation into liver tissue, but is required for DC migration from the sinusoid area to the portal area. After P. acnes administration, excessive activation of DCs in the early phase and altered maturation of DCs induce massive granuloma formation in IL-1ra−/− deficient mice. From these observations, we speculate that endogenous IL-1ra production regulates maturation of DCs to induce optimal immune responses against pathogens. IL-1α and IL-1β stimulate LC migration and activation (39–41). Moreover, caspase-1 is required for LC migration and optimal contact sensitization in mice (42). Eriksson et al. (28) also reported that an IL-1RI signal is required for activation of DCs, which is in turn a prerequisite for induction of autoreactive CD4⁺ T cells. Our results suggest that the IL-1/IL-1ra system is essential for the regulation of maturation and migration of DCs during inflammation.
It was reported that an IL-1RI signal is required for activation of DCs in α-myosin-induced autoimmune disease (28). However, lack of the IL-1α gene in mice induced migration of immature DCs in the liver after P. acnes administration. In P. acnes-primed, IL-1α-deficient mice, serum IL-1 biological activity increased to 3 times that of wild-type mice after LPS injection (data not shown). IL-1R-associated kinase-M has recently been identified as a TLR family-specific negative signal regulator in monocytes/macrophages (43). As IL-1 and TLR use a common signal transduction pathway, our data indicate that maturation of DCs was regulated by the IL-1 signal-induced negative feedback system. After P. acnes treatment at day 7, the expression of CCR7 in liver DCs decreased in IL-1α-deficient mice in comparison with wild-type mice (Fig. 7A). Recently it was reported that TLR signaling induces the expression of CCR7 (44, 45). Our result indicates that the expression of CCR7 may also regulate by the IL-1 signal pathway after P. acnes treatment.

In conclusion, we found that granuloma formation was enhanced in the liver after P. acnes treatment, and granuloma contained immature DCs in IL-1α-deficient mice in comparison with wild-type mice. PALT formation was altered in the liver of IL-1α-deficient mice after P. acnes treatment. These results suggest that the IL-1α/IL-1 system regulates maturation and migration of DCs during pathogen-related inflammation in mouse liver.

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Disclosures
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References
18. Dripps, D. J., B. J. Brandhuber, R. C. Thompson, and S. P. Eisenberg. 1991. Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. J. Biol. Chem. 266:10331.


