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IL-1 Enhances T Cell-Dependent Antibody Production Through Induction of CD40 Ligand and OX40 on T Cells[1]

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IL-1 is a proinflammatory cytokine that plays pleiotropic roles in host defense mechanisms. We investigated the role of IL-1 in the humoral immune response using gene-targeted mice. Ab production against SRBC was significantly reduced in IL-1α/β-deficient (IL-1$^{-/-}$) mice and enhanced in IL-1R antagonist$^{-/-}$ mice. The intrinsic functions of T, B, and APCs were normal in IL-1$^{-/-}$ mice. However, we showed that IL-1$^{-/-}$ APCs did not fully activate DO11.10 T cells, while IL-1R antagonist $^{-/-}$ APCs enhanced the reaction, indicating that IL-1 promotes T cell priming through T-APC interaction. The function of IL-1 was CD28-CD80/CD86 independent. We found that CD40 ligand and OX40 expression on T cells was affected by the mutation, and the reduced Ag-specific B cell response in IL-1$^{-/-}$ mice was recovered by the treatment with agonistic anti-CD40 mAb both in vitro and in vivo. These observations indicate that IL-1 enhances T cell-dependent Ab production by augmenting CD40 ligand and OX40 expression on T cells. ***The Journal of Immunology*, 2001, 167: 90–97.**

Although IL-1 was first discovered as a major mediator of inflammation, it has gradually become evident that this cytokine has numerous functions related to host defense mechanisms, regulating not only the immune system, but also the areas of the neuronal and endocrine systems that interface with the immune system (1, 2). IL-1 is produced by various types of cells, including macrophages, dendritic cells (DC),[4] B cells, and T cells (3). It consists of two molecular species, IL-1α and IL-1β, which exert similar, although not completely overlapping, biological activities through IL-1R type I (IL-1RI; CD121a) (4). Although an IL-1R type II (IL-1RII; CD121b) has also been found, this receptor is not considered to be involved in the signal transduction, but is believed to play more of a regulatory role as a "decoy" (4). In addition, another member of the IL-1 gene family, the IL-1R antagonist (IL-1ra), binds to IL-1Rs without exerting agonistic activity (4). This molecule together with IL-1RII and the secretory forms of IL-1RI and IL-1RII are considered to be negative regulators of IL-1 signals, providing a complex regulation of IL-1 activity.

In the immune system, IL-1 is known to activate lymphocytes, monocytes, macrophages, and NK cells (3, 4). When mice were immunized with protein Ags together with IL-1, serum Ab production was enhanced, suggesting that IL-1 has an adjuvant effect (5, 6). Recently, we found that IL-1ra$^{-/-}$ mice developed chronic inflammatory arthropathy spontaneously, and production of autoantibodies against Igs, type II collagen, and dsDNA increased in these mice (7). These observations suggest an important role of IL-1 in the humoral immune responses. On the other hand, it was shown that humoral immune responses were normal in IL-1RI$^{-/-}$ mice (8, 9). Thus, the role of IL-1 in the humoral immune response is still controversial.

In this report we studied the roles of IL-1 in the humoral immune response using IL-1$^{-/-}$ and IL-1ra$^{-/-}$ mice that we had previously generated (10). Ab production to SRBC was reduced in IL-1$^{-/-}$ mice, while it was enhanced in IL-1ra$^{-/-}$ mice. We found that IL-1 was involved in T cell priming because IL-1$^{-/-}$ APCs could not fully activate Ag-specific T cells. In addition, this response was independent of CD28-CD80/CD86 cosignaling. Furthermore, we showed that IL-1 produced by APCs enhances the expression of CD40 ligand (CD40L; CD154) and OX40 (CD134) on T cells, which play an important role in CD4$^+$ T cell priming as well as Ag-specific B cells (11–15). Since the defect in Ab production in IL-1$^{-/-}$ mice was rescued by the administration of agonistic anti-CD40 mAb, suggesting that IL-1 promotes humoral immune response by inducing these cosignaling molecules on T cells.

Materials and Methods

Mice

IL-1$^{-/-}$ and IL-1ra$^{-/-}$ mice were generated by homologous recombination as described previously and backcrossed to BALB/cA mice for seven or eight generations (10). DO11.10 transgenic (Tg) mice (BALB/c background) were provided by Dr. D. Y. Loh. All the mice were housed under specific pathogen-free conditions in an environmentally controlled clean room at the Center for Experimental Medicine, Institute of Medical Science, University of Tokyo (Tokyo, Japan). The experiments were conducted according to the institutional ethical guidelines for animal experiments and the safety guideline for gene manipulation experiments. Sex- and age-matched 8- to 12 wk-old adult mice were used for the experiments.

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[4] Abbreviations used in this paper: DC, dendritic cells; IL-1RI and IL-1RII, IL-1R types I and II; IL-1ra, IL-1R antagonist; CD40L, CD40 ligand; OX40L, OX40 ligand; TD Ag, T-dependent Ag; TI Ag, T-independent Ag; SAC, splenic adherent cells; TNP, trinitrophenyl; KLH, keyhole limpet hemocyanin; Tg, transgenic; NP-CGG, nitrophenyl-conjugated chicken γ globulin.

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Immunization of mice

Mice were immunized with 1×10^8 SRBC in PBS i.p. Secondary responses were examined after immunization with SRBC. For in vivo reconstitution analysis, agonistic rat anti-mouse CD40 mAb (200 μg; LB429) (16) or rat IgG (200 μg) was injected i.p. 1 day both after the primary and secondary immunizations with SRBC. Goat anti-mouse IgM $F(ab')_2$ (200 μg; ICN Biomedical, Aurora, OH) or goat IgM $F(ab')_2$ (200 μg) was administrated i.p. 1 day after the primary immunization with SRBC, then rabbit anti-mouse IgG $F(ab')_2$ (200 μg; Rockland, Gilbertsville, PA) or rabbit IgG $F(ab')_2$ (200 μg) was injected i.p. 1 day after the secondary immunization. Blood samples were collected from the tail vein before the immunization. At 15 days after the primary immunization, mice were given the secondary immunization, and blood samples were collected 2 wk later.

Measurement of Ab titers

Ig levels in sera or culture supernatants were measured by ELISA as described previously (17). Soluble SRBC Ag (2 μg/ml), prepared as described previously (18), was coated on Falcon 3912 Micro Test III Flexible Assay Plates (Becton Dickinson, Franklin Lakes, NJ). To measure OVA-specific Ab levels in culture supernatants, 96-well plates for ELISA were coated with OVA peptide (10 μg/ml), and 50 μl of the test sample was added to each well. After incubation for 1 h the well was washed with Tris-buffered saline and 0.05% Tween 20 three times, followed by addition of 50 μl of alkaline phosphatase-conjugated goat anti-mouse IgM, IgG, IgG1, IgG2a, IgG2b, IgG3 (Zymed, San Francisco, CA), or alkaline phosphatase-conjugated rat anti-mouse IgE (Southern Biotechnology Associates, Birmingham, AL). Alkaline phosphatase activity was measured using Substrate Phosphatase SIGMA104 (Sigma, St. Louis, MO) as the substrate, and the OD_{415} is shown.

Preparation of cells from lymphoid tissues

Cells were prepared from the spleen or lymph nodes (axillary, inguinal, and brachial) by grinding the tissues with the plunger of a 1-ml disposable syringe and were then suspended in RPMI 1640 (Life Technologies, Gaithersburg, MD) medium containing 50 μM 2-mercaptoethanol (Life Technologies), 50 μg/ml streptomycin (Meiji, Tokyo, Japan), 50 U/ml penicillin (Meiji), and 10% FCS (JRH Bioscience, Lenexa, KS). Spleen cells were treated with a hemolysis buffer (17 mM Tris-HCl and 140 mM NH_4Cl, pH 7.2) to remove RBC. Adherent cells and nonadherent cells were separated after incubation for 1 h on a 10-cm dish. For APCs in the primary T cell response assay, B220$^+$ and Thy1.2$^+$ cells were removed from splenic adherent cells (SACs) using a MACS column (Miltenyi Biotec, Bergisch Gladbach, Germany). To prepare splenic and lymph node T cells, nonadherent cells were passed through a nylon wool column. CD4$^+$ T cells were purified by treating the T cell preparation with anti-mouse CD8, anti-mouse B220, and anti-mouse Mac-1 magnetic beads (Miltenyi Biotec) and then passing them through a MACS column. B cells were prepared by treating splenic nonadherent cells with anti-Thy1.2 Ab (Serotec, Oxford, U.K.) and rabbit complement (Cedarlane Laboratories, Ontario, Canada). The purity of CD4$^+$ cells and B220$^+$ cells was monitored by FACScan and was usually approximately 90%.

T cell proliferative response

In the OVA-specific T cell proliferative response assay, splenic and lymph node CD4$^+$ T cells (5×10^4 cells/well) from DO11.10 Tg mice were cocultured with irradiated APCs (5×10^3 cells/well) for 3 days in the absence as well the presence of $OVA_{323–339}$ peptide (0.1 μM; gift from Dr. T. Saito) in a final volume of 200 μl RPMI 1640/10% FCS. The effects of recombinant mouse IL-1α (125 pg/ml) and IL-1β (125 pg/ml; PeproTech, London, U.K.), or CTLA-4 Ig (30 μg/ml; gifted by Dr. R. Abe) were examined by incubating the culture with those Abs for 72 h, followed by incorporation of [^{3}H]thymidine (0.25 μCi/ml; Amersham, Little Chalfont, U.K.) for 6 h. Then, cells were harvested with a Micro 96 cell harvester (Skatron, Lier, Norway), and [^{3}H]thymidine radioactivity in the acid-insoluble fraction was measured with Micro Beta (Pharmacia Biotech, Piscataway, NJ).

B cell proliferative response

Splenic B cells (1×10^5 cells/well) and mitomycin C (Sigma)-treated DO11.10 T cells (1×10^5 cells/well) were cocultured for 3 days in the presence or the absence of OVA peptide (0.2 μM), and cells were labeled with [^{3}H]thymidine for 6 h. To examine the effects of agonistic anti-mouse CD40 mAb (HM40–3; 1 μg/ml; PharMingen, San Diego, CA), cells were cultured for 3 days with this Ab, and proliferation and OVA-specific Ab levels were measured. Isotype IgG was used as a control.

Measurement of cytokine levels

IL-2 levels in the culture supernatant were determined by Titer Zyme enzyme immunoassay kit (PerSeptive Diagnostics, Cambridge, MA). As a standard recombinant cytokine, mouse IL-2 (Genzyme, Cambridge, MA) was used. TMB One-Step Substrate System was purchased from Dako (Carpinteria, CA).

Flow cytometric analysis

In the OVA-specific T cell and B cell proliferative response, cells were harvested at the point when expression of each molecule reached peak levels. Staining of I-A^d (72 h after stimulation), CD80 (72 h), CD86 (72 h), and CD40 (72 h) on SACs; CD40L (12 h), OX40 (72 h), and IL-2Rα (60 h) on CD4$^+$ T cells; and OX40 ligand (OX40L; 72 h) on B cells was performed according to the standard protocol. Detection of CD40L on KJI-26$^+$CD4$^+$T cells was conducted as described previously (19). Briefly, the biotin-labeled anti-mouse CD40L mAb was added to OVA-specific T cell proliferation culture, and at 12 h after stimulation cells were harvested and stained with PE-anti-mouse CD4 mAb, anti-mouse DO11.10 (KJI-26), and CyChrome-streptavidin (PharMingen). After washing, cells were stained with second Ab, FITC-anti-mouse Ig (PharMingen). To examine the effects of rIL-1 on CD40L and OX40 expression on CD4$^+$ T cells, CD4$^+$ T cells (1×10^6 cells/well) were cultured with rIL-1 only, with plate-coated anti-CD3 (145-2C11; 0.1 μg/ml) in the presence or the absence of rIL-1, or with plate-coated anti-CD3 (0.1 μg/ml). To detect IL-1RI expression on CD4$^+$ T cells, CD4$^+$ T cells (1×10^6 cells/well) were cultured with plate-coated anti-CD3 (0.1 μg/ml). Cells were incubated for 12 h for analysis of CD40L and IL-1RI expression and for 72 h for analysis of OX40 expression.

Anti-mouse CD16/CD32 (2.4G2), FITC- or PE-anti-mouse CD4 (GK1.5), PE-anti-mouse B220 (RA3-6B2), PE-anti-mouse CD25 (IL-2Rα; 3C7), biotinylated anti-mouse I-A^d (AMS-32.1), biotinylated anti-mouse CD121a (IL-1RI; 12A6), and FITC-streptavidin were purchased from PharMingen, and FITC-anti-mouse CD80 (16-10A1) was obtained from BioSource (Camarillo, CA). PE-anti-mouse CD40 (3.23) and PE-anti-mouse OX40 (OX86) were purchased from Immunotech (Marseilles, France). Anti-mouse CD86 (GL-1) mAb was provided by Dr. H. Nariuchi (Institute of Medical Science, University of Tokyo, Tokyo, Japan). Biotinylated anti-mouse OX40L, MGP34, was provided by Dr. K. Sugamura (Department of Microbiology and Immunology, Tohoku University School of Medicine, Sendai, Japan) and RM134L was purchased from PharMingen.

Statistics

Student's *t* test was used for statistical evaluation of the results.

Results

Ab production to SRBC in IL-1$^{-/-}$ and IL-1ra$^{-/-}$ mice

Although adjuvant effects of IL-1 on Ab production are well known, it is not clear if IL-1 deficiency causes any defect in the humoral immune response, because Ab production was normal in IL-1RI$^{-/-}$ mice immunized trinitrophenyl (TNP)-keyhole limpet hemocyanin (KLH) together with alum or CFA. Thus, we examined whether IL-1 is involved in Ab production using IL-1$^{-/-}$ and IL-1ra$^{-/-}$ mice of the BALB/c background. After immunization with SRBC i.p., SRBC-specific serum Ab levels were measured by ELISA. SRBC-specific Ab levels of IgM, IgG, and IgE classes in IL-1$^{-/-}$ mice were significantly lower than those in wild-type mice after secondary immunization (Fig. 1*A*). In contrast, SRBC-specific IgG and IgE levels in IL-1ra$^{-/-}$ mice were increased compared with those in wild-type mice, although IgM levels were comparable in both mice (Fig. 1*B*). The suppression in IL-1$^{-/-}$ mice and the augmentation in IL-1ra$^{-/-}$ mice were observed in all IgG subclasses, showing no polarization to either Th1- or Th2-type response.

The physiological levels of serum Igs (IgM, IgG, and IgE) without immunization were similarly low in these IL-1$^{-/-}$ mice (data not shown). These results indicate that IL-1 plays an important role in T cell-dependent Ab production under physiological conditions.

We did not detect any difference in the number and composition of the immune cells from the thymus, spleen, lymph nodes, and peritoneal cavity between IL-1$^{-/-}$ and wild-type mice when we

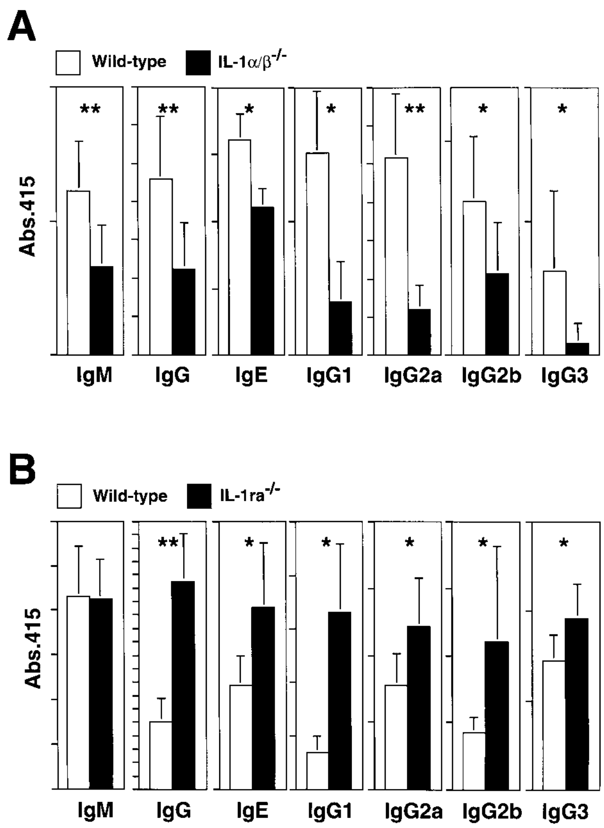

FIGURE 1. Efficiency of Ab production against SRBC in IL-$1^{-/-}$ and IL-$1ra^{-/-}$ mice. Mice were immunized with SRBC, and sera were collected 2 wk after the secondary immunization. After appropriate dilution of the serum (IgM and IgG, 1/100; IgE, 1/2; IgG1, 1/100; IgG2a, IgG2b, and IgG3, 1/10), SRBC-specific Ab levels in the sera were measured by ELISA. *A*, Wild-type mice, $n = 10$; IL-$1^{-/-}$ mice, $n = 10$. *B*, IL-$1ra^{-/-}$ mice, $n = 7$. The average and SD are shown. A graduation of the ordinate (OD_{415} (Abs.415)) is 0.1. $*$, $p < 0.05$; $**$, $p < 0.005$.

examined various cell surface markers (CD4 and CD8 on thymocytes; CD4, CD8, CD3ϵ, B220, CD62L, and CD44 on lymph node cells; CD4, CD8, CD3ϵ, B220, IgM, CD11b, CD11c, CD80, CD86, I-A^d, CD54, CD40, CD16/CD32, CD21/CD35, CD62L, and CD44 on splenocytes; B220, IgM, CD11b, F4/80, CD16/CD32, CD21/CD35, and CD5 on peritoneal cells; data not shown). This indicates that IL-1 does not affect the development and maturation of T cells, B cells, and APCs. Intrinsic B cell functions, such as proliferative response to LPS or anti-IgM mAb and Ab production against T-independent Ag TNP-LPS, were normal in IL-$1^{-/-}$ mice (data not shown). Intrinsic T cell functions, such as proliferative response and cytokine production to plate-coated anti-CD3 mAb or plate-coated anti-CD3 mAb plus soluble anti-CD28 mAb, were also normal in IL-$1^{-/-}$ mice (data not shown). Moreover, the phagocytic activity of macrophage and DCs of IL-$1^{-/-}$ mice was comparable with that of wild-type mice using FITC-latex beads, FITC-dextran, and Lucifer Yellow. The Ag-processing ability of these cells was also normal (data not shown). These results indicate that intrinsic B cell, T cell, and APC function was not affected by the deficiency of IL-1.

Role of IL-1 in T cell-APC interaction

Next we examined the roles of IL-1 in T cell-APC interaction. To assess the role of IL-1 in T cell priming upon interaction with APCs, the Ag-specific primary T cell proliferative response was assayed using T cells from DO11.10 Tg mice, who express TCR specific for the $OVA_{323-339}$ peptide, and SACs from IL-$1^{-/-}$ mice. The proliferative response of DO11.10 T cells was reduced in IL-$1^{-/-}$ SACs (Fig. 2*A*). On the other hand, using IL-$1ra^{-/-}$ SACs, the response was slightly increased (Fig. 2*A*; wild-type, 100%; IL-$1^{-/-}$, 41 $\pm$ 10% ($p < 0.01$); IL-$1ra^{-/-}$, 135 $\pm$ 12% ($p < 0.05$); average $\pm$ SD from three independent experiments). When recombinant mouse IL-1α and IL-1β were added to this culture, the response of IL-$1^{-/-}$ SACs was recovered, indicating that the defect is not developmental (Fig. 2*B*). Similar IL-1-dependent activation of T cells was observed when B cells were used as APCs (data not shown). These results suggest that IL-1 from SACs play an important role in T cell priming.

Effects of IL-1 deficiency on the expression of cell surface molecules on lymphocytes

Because the above-mentioned results have suggested that T cell activation through T cell-APC interaction is impaired in IL-$1^{-/-}$ mice, we examined the molecules involved in cell-cell interaction on APCs and T cells. The expression levels of I-A^d on SACs from IL-$1^{-/-}$ and IL-$1ra^{-/-}$ mice were comparable with those from wild-type mice (Fig. 3*A*). The expression levels of CD80 and CD86 on I-A^{d+} APCs were also similar among these mice (Fig. 3*A*). Moreover, despite the inhibitory effect of CTLA-4 Ig, which inhibits CD28-CD80/CD86 cosignaling, T cell responses were still reduced with IL-$1^{-/-}$ APCs and enhanced with IL-$1ra^{-/-}$ APCs (Fig. 3*B*; wild-type, 100%; IL-$1^{-/-}$, 29 $\pm$ 10% ($p < 0.001$); IL-$1ra^{-/-}$, 199 $\pm$ 29% ($p < 0.05$); average $\pm$ SD from three independent experiments). In these cultures IL-2 levels were well correlated to the proliferative response, consistent with the impairment of T cell activation (Fig. 3*B*). These results indicate that IL-1 acts on T cell priming independently of CD28-CD80/CD86 costimulatory signals.

We next investigated the expression of CD40-CD40L and OX40-OX40L, which are also suggested to be involved in T cell priming. The expression levels of CD40 on I-A^{d+} APCs were

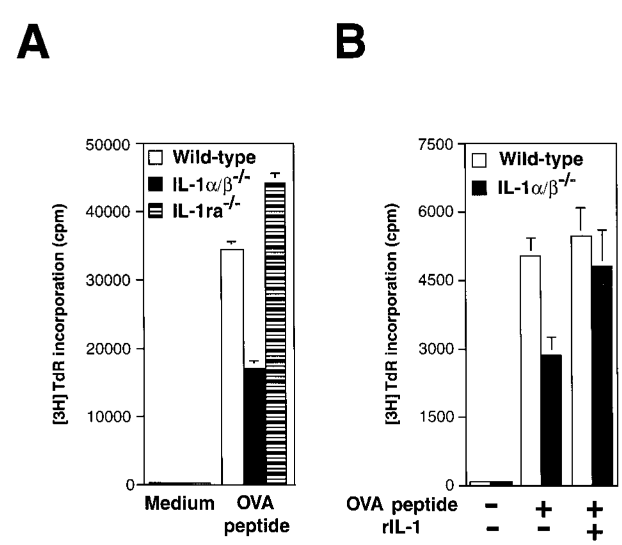

FIGURE 2. Effects of IL-1 on primary T cell proliferative response. Proliferative responses against the $OVA_{323-339}$ peptide were assessed by measuring the incorporation of [^{3}H]thymidine after 3-day culture. *A*, Effects of IL-1 on T cell priming were evaluated using DO11.10 T cells and SACs from wild-type, IL-$1^{-/-}$, and IL-$1ra^{-/-}$ mice. *B*, Effects of exogenous IL-1 (rIL-1α and rIL-1β) on T cell priming were examined using DO11.10 T cells and SACs from wild-type and IL-$1^{-/-}$ mice. The average $\pm$ SD of triplicate experiments are shown. These results were reproducible three independent experiments.

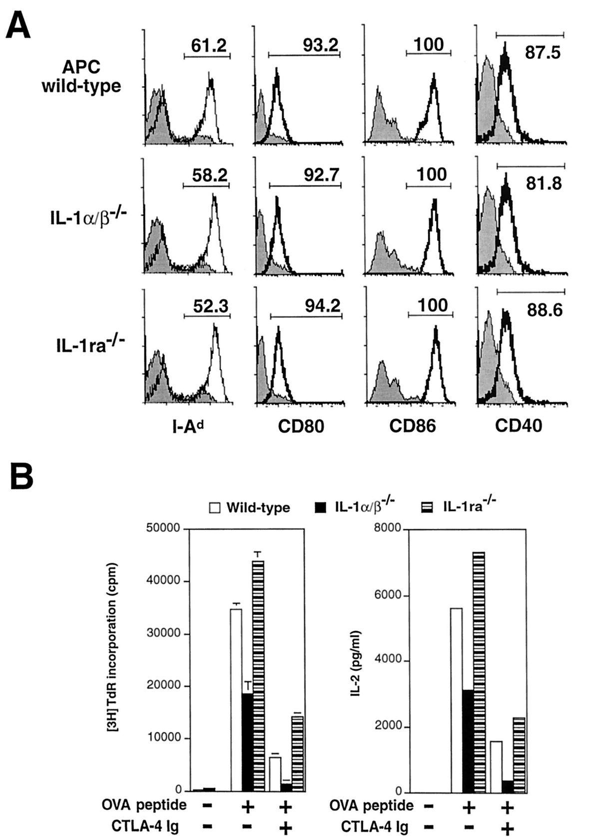

FIGURE 3. Costimulatory activity of IL-1 independent on CD28-CD80/CD86 pathway. *A*, Expression levels of I-A^d on B220-negative SACs from wild-type, IL-1$^{-/-}$, and IL-1ra$^{-/-}$ mice and of CD80, CD86, and CD40 on I-A^{d+} SACs from wild-type, IL-1$^{-/-}$, and IL-1ra$^{-/-}$ mice were analyzed by flow cytometry under the conditions of primary T cell proliferation assay described in Fig. 2*A*. The shaded area shows isotype-matched control Ig staining. *B*, Effects of CTLA-4 Ig on primary T cell response were assessed using DO11.10 T cells and SACs from wild-type, IL-1$^{-/-}$, and IL-1ra$^{-/-}$ mice. The average ± SD of triplicate experiments are shown. IL-2 levels in pooled triplicate supernatant from the proliferative response assay were examined by ELISA. These results were reproducible in three independent experiments.

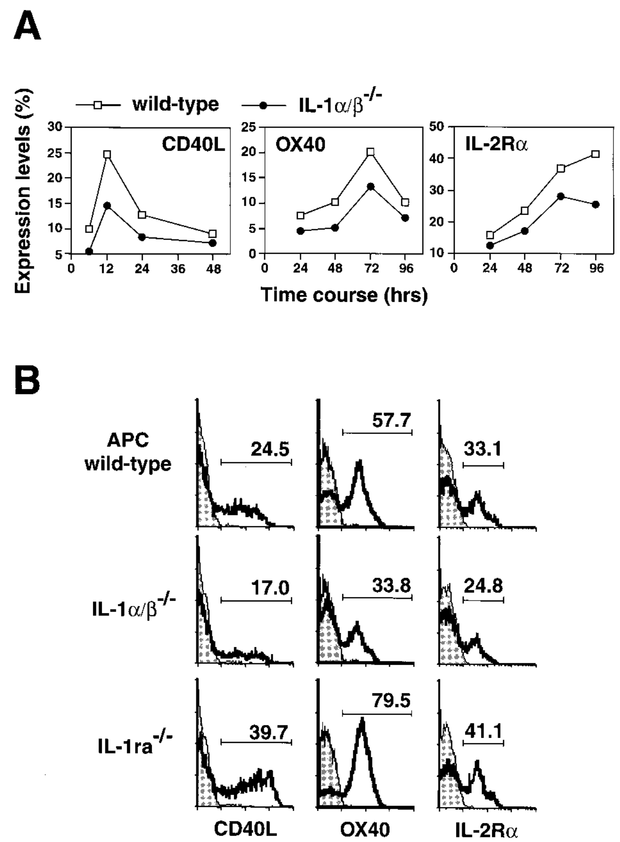

FIGURE 4. Effects of IL-1 deficiency on the expression of cell surface molecules on T cells. The expression levels of surface molecules on CD4$^+$ DO11.10 T cells after stimulation with OVA$_{323-339}$ peptide in the presence of wild-type, IL-1$^{-/-}$, or IL-1ra$^{-/-}$ APCs were analyzed by flow cytometry. *A*, The time kinetics of CD40L, OX40, and IL-2Rα induction on CD4$^+$ DO11.10 T cells. □, Wild-type APCs; ●, IL-1$^{-/-}$ APCs. The results were confirmed in another experiment. *B*, Expression levels of CD40L, OX40, and IL-2Rα on CD4$^+$ DO11.10 T cells at the peak time points. The expression of CD40L, OX40, and IL-2Rα was analyzed by flow cytometry after 12, 60, and 72 h, respectively. The shaded area shows isotype-matched control Ig staining. One of three representative results is shown.

normal both in IL-1$^{-/-}$ and IL-1ra$^{-/-}$ mice (Fig. 3*A*). On the other hand, the expression levels of CD40L and OX40 on CD4$^+$ DO11.10 T cells stimulated with IL-1$^{-/-}$ APCs were low compared with wild-type APCs (Fig. 4; CD40L, wild-type, 100%; IL-1$^{-/-}$, 68 ± 2% ($p < 0.01$); OX40, 56 ± 16% ($p < 0.01$); average ± SD from three independent experiments). In contrast, the expression levels of these molecules on T cells were enhanced when IL-1ra$^{-/-}$ APCs were used (Fig. 4*B*; CD40L, wild-type, 100%; IL-1ra$^{-/-}$, 154 ± 10% ($p < 0.01$); OX40, 133 ± 7% ($p < 0.01$); average ± SD from three independent experiments). In support for the involvement of IL-1 in the CD40L induction, we found that this reduced CD40L expression could be rescued by the addition of recombinant mouse IL-1α and IL-1β in the culture (Fig. 5*A*). In addition, the expression level of IL-2Rα (CD25), an activation marker of T cells, on CD4$^+$ DO11.10 T cells upon incubation with IL-1$^{-/-}$ APCs was reduced, and that of IL-1ra$^{-/-}$ APCs was enhanced compared with levels after incubation with wild-type APCs (Fig. 4*B*; wild-type, 100%; IL-1$^{-/-}$, 71 ± 4% ($p < 0.01$); IL-1ra$^{-/-}$, 123 ± 10% ($p < 0.005$); average ± SD from three independent experiments). The expression levels of IL-1RI on CD4$^+$ DO11.10 T cells cultured with IL-1$^{-/-}$ and IL-1ra$^{-/-}$ APCs did not differ from that observed with wild-type APCs (data not shown). These observations suggest that IL-1 produced by APCs plays a crucial role in T cell priming by enhancing the expression of CD40L and OX40.

We next examined whether IL-1 directly induces CD40L and OX40 expression on CD4$^+$ T cells. We found that rIL-1 did not induce CD40L and OX40 expression on naive CD4$^+$ T cells. However, activation of T cells with plate-coated anti-CD3 mAb (0.1 μg/ml) made these cells responsive to rIL-1 in a dose-dependent manner (Fig. 5*B*). This effect of rIL-1 was not observed when high concentrations of anti-CD3 mAb (1 and 10 μg/ml) were used (data not shown). High doses of rIL-1 (10 and 100 ng/ml) were, instead, inhibitory on the induction of CD40L and OX40 expression (data not shown). We found that IL-1RI was not expressed on naive CD4$^+$ T cells, and it was induced by the treatment with anti-CD3 mAb (Fig. 5*C*). Thus, these results clearly show that IL-1 directly induces CD40L and OX40 expression on CD4$^+$ T cells, although induction of IL-1RI through TCR signaling is necessary in advance.

Dependency on CD40-CD40L signaling in IL-1-deficient mice

We, then, examined whether activation of CD40 can recover the Ag-specific B cell response in IL-1-deficient mice. When splenic B

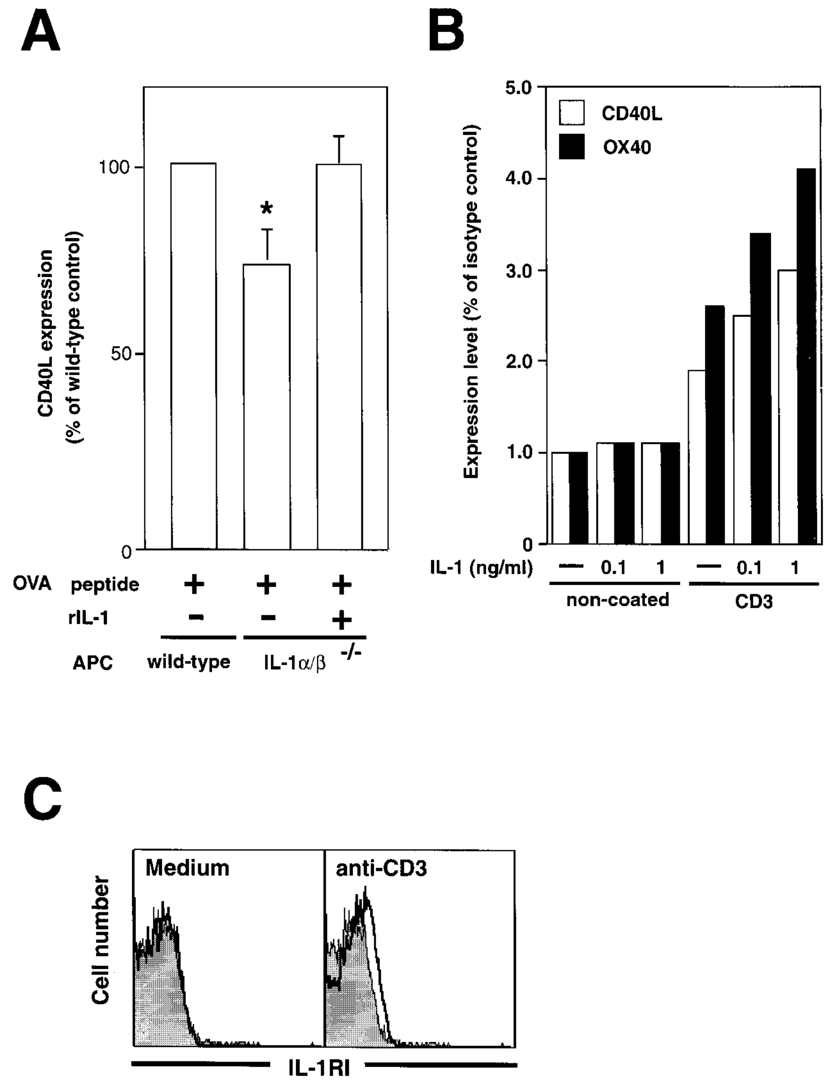

FIGURE 5. Induction of CD40L and OX40 expression on CD4$^+$ T cells with rIL-1. *A*, The defect of CD40L expression on CD4$^+$ DO11.10 T cells cultured with IL-1$^{-/-}$ APC is rescued by rIL-1α/β treatment. A relative value of CD40L-positive T cells in IL-1$^{-/-}$ APC culture against those in wild-type APC culture is shown. The average $\pm$ SD of four independent experiments are shown. $*$, $p < 0.01$. *B*, Naive T cells are not responsive to rIL-1 treatment. Purified splenic CD4$^+$ T cells were treated with rIL-1 with or without stimulation with plate-coated anti-CD3 mAb (0.1 μg/ml). Expression of CD40L and OX40 on CD4$^+$ T cells was analyzed by flow cytometry at 24 and 72 h after the treatment, respectively. Expression levels of CD40L and OX40-positive T cells relative to the isotype-matched control Ig staining are shown. *C*, IL-1RI is induced on T cells by the treatment with plate-coated anti-CD3 mAb. Purified splenic CD4$^+$ T cells were stimulated with plate-coated anti-CD3 mAb (0.1 μg/ml). After 24 h, expression of IL-1RI on CD4$^+$ T cells was examined by flow cytometry. The shaded area shows isotype-matched control Ig staining.

cells from wild-type and IL-1$^{-/-}$ mice were cultured with mitomycin C-treated T cells from DO11.10 Tg mice in the presence of the OVA peptide, the proliferative response of B cells from IL-1$^{-/-}$ mice was reduced compared with those from wild-type mice (Fig. 6*A*, wild-type, 100%; IL-1$^{-/-}$, 48 $\pm$ 12% ($p < 0.05$); average $\pm$ SD from three independent experiments). The OVA-specific Ig levels in the culture supernatant of IL-1$^{-/-}$ B cells were also reduced to 40% (Fig. 6*A*). Under this culture condition, the CD40L-expressing T cell population was less in the culture with IL-1$^{-/-}$ B cells than in the culture with wild-type B cells (Fig. 6*B*). Moreover, OX40L expression of IL-1$^{-/-}$ B cells was reduced compared with that of wild-type B cells, indicating that IL-1$^{-/-}$ B cells were activated only weakly (wild-type, 100%; IL-1$^{-/-}$, 69% $\pm$ 3% ($p < 0.01$); average $\pm$ SD from three independent experiments; Fig. 6*C*). Since CD40 activation is necessary for the induction of OX40L on B cells (20), this result is consistent with the observation that CD40L expression was reduced on T cells activated with IL-1-deficient APCs. Then, we tried to recover the immune response of IL-1$^{-/-}$ B cells by treating cells with agonistic anti-CD40 mAb. As shown in Fig. 6*D*, the reduced proliferative response and Ab production of the mutant B cells were recovered to normal levels when agonistic anti-CD40 mAb was added to the culture.

The enhancing effect of anti-CD40 mAb was also observed in vivo; SRBC-specific Ab production was recovered to the wild-type levels, when anti-CD40 mAb was administered to IL-1$^{-/-}$ mice during SRBC immunization (Fig. 7*A*). On the other hand, anti-IgM F(ab$'$)$_2$ plus anti-IgG F(ab$'$)$_2$ administration was not effective to recover SRBC-specific IgG production in IL-1$^{-/-}$ mice to the wild-type levels, although SRBC-specific IgG levels were increased in both IL-1$^{-/-}$ and wild-type mice (Fig. 7*B*). Thus, it was shown that IL-1 can be substituted by CD40 activation. These results suggest that IL-1 produced by APCs plays an important role in T cell priming and Ab production by enhancing the expression of CD40L and OX40 on T cells.

Discussion

In this report we analyzed the mechanisms of humoral immune response activation by IL-1 using IL-1$^{-/-}$ and IL-1ra$^{-/-}$ mice and showed that IL-1 plays an important role in enhancing T cell-APC interaction through inducing CD40L and OX40 on T cells. The intrinsic functions of B cells, T cells, and APCs from IL-1$^{-/-}$ mice were normal. However, the proliferative response as well as IL-2 production of CD4$^+$ DO11.10 T cells against OVA peptide was reduced when IL-1$^{-/-}$ SACs were used as APCs, suggesting that T cell-APC interaction is impaired in IL-1$^{-/-}$ mice. We found that the expression levels of CD40L and OX40 were reduced in the coculture of CD4$^+$ DO11.10 T cells with IL-1$^{-/-}$ APCs. Furthermore, we showed that rIL-1 added exogenously to the culture can induce the expression of CD40L on CD4$^+$ T cells, indicating that

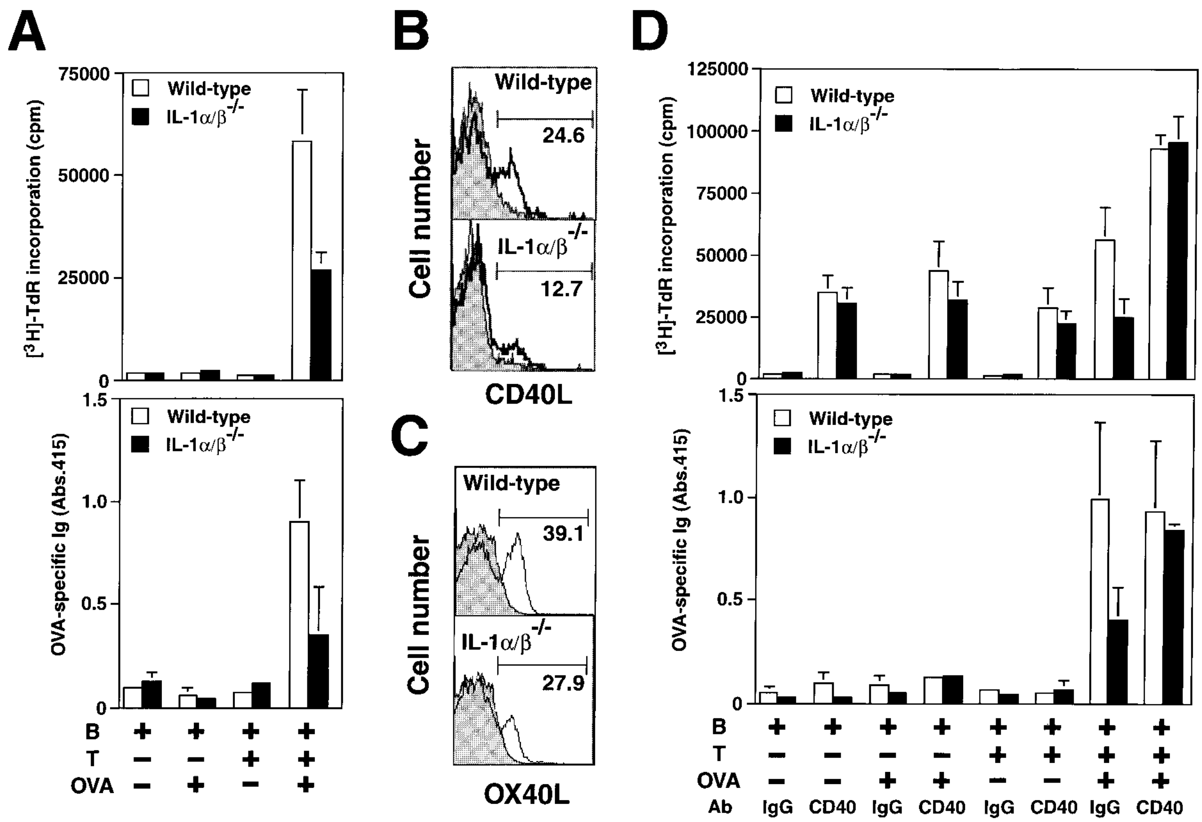

FIGURE 6. Rescue of the immune response of IL-1$^{-/-}$ B cells by agonistic anti-CD40 mAb treatment. *A*, The OVA-specific immune response is impaired in IL-1$^{-/-}$ B cells and is rescued by agonistic anti-CD40 mAb treatment. Splenic B cells from wild-type and IL-1$^{-/-}$ mice were cultured with mitomycin C-treated CD4^{+} DO11.10 T cells in the presence or the absence of OVA peptide. The effects of agonistic anti-CD40 mAb or control isotype IgG on B cell proliferation (*upper panel*) and OVA-specific Ab production (*lower panel*) were measured. The average of triplicate determinations and SD are shown. The result was confirmed in another experiment. *B*, CD40L expression levels in T cells cultured with IL-1$^{-/-}$ B cells. CD4^{+} DO11.10 T cells were cultured as described in *A*, then CD40L expression was analyzed by flow cytometry after 24 h. The shaded area represents isotype-matched control Ig staining. One of three representative results is shown. *C*, OX40L expression levels in IL-1$^{-/-}$ B cells. B cells were cultured as described in *A*, and after 72 h OX40L expression was analyzed by flow cytometry. The shaded area shows isotype-matched control Ig staining. Representative data from three independent experiments are shown.

IL-1 is an inducer of this cosignaling molecules. Since agonistic anti-CD40 mAb could rescue the deficiency observed in IL-1$^{-/-}$ mice both in vivo and in vitro, we conclude that IL-1 enhances T cell priming through induction of cosignaling molecules that are important in both T cell-APC and T cell-B cell interactions.

The importance of CD40-CD40L interaction in T cell priming and B cell activation has been amply documented (11–13, 21). It has also been shown that neither CD40$^{-/-}$ mice nor CD40L$^{-/-}$ mice are able to react with T-dependent (TD) Ags to produce IgG Ab efficiently (11, 22, 23). CD40L$^{-/-}$ mice also showed a profound reduction in the primary IgM Ab responses to SRBC (23), although the IgM anti-KLH response was not completely absent in CD40L$^{-/-}$ mice.

The OX40-OX40L signaling system has been suggested to play an important role in the humoral immune response. OX40 ligation with OX40L activates naive T cells to produce Th2 cytokines and differentiate into Th2 cells (24, 25), and promotes Ab production against TD Ags (26). Murata et al. (20) reported that Ab production against KLH was impaired in OX40L$^{-/-}$ mice. However, other investigators reported that serum Ag-specific Ig levels were similar to those in wild-type mice when OX40$^{-/-}$ or OX40L$^{-/-}$ mice were immunized with various TD Ags, including vesicular stomatitis virus, lymphocytic choriomeningitis virus, Theiler's murine encephalomyelitis virus, *Leishmania major*, *Nippostrongylus brasiliensis*, nitrophenyl-conjugated chicken γ globulin (NP-CGG), TNP-KLH, and TNP-OVA, indicating that the OX40-OX40L system is not required under certain conditions of immunization (27–29). These observations indicate that the CD40L-CD40 and OX40-OX40L cosignaling systems play an important, but not absolute, role in T cell-priming and Ab production. Thus, it is suggested that the inefficiency of TD Ab production and T cell priming in IL-1$^{-/-}$ mice is caused by the reduced expression of CD40L and OX40 on T cells upon interaction with IL-1$^{-/-}$ APCs. In support of this idea, we showed that the defects in T cell-APC interaction could be rescued by the addition of agonistic anti-CD40 Ab both in vivo and in vitro.

The CD28-CD80/CD86 cosignaling system is known to be important for T cell proliferation and cytokine secretion in humoral immune responses (30). Both primary and secondary T cell responses and Th2 type cytokine secretion are impaired in CD80/CD86$^{-/-}$ mice (31). However, the expression levels of CD80 and CD86 on APCs were normal in IL-1$^{-/-}$ mice. Moreover, we showed that CTLA-4 Ig suppressed CD4^{+} DO11.10 T cell proliferation independently of the IL-1 deficiency. These results strongly suggest that IL-1 has a previously unknown T cell activation mechanism that differs from the CD28-CD80/CD86 system.

IL-1 function in the humoral immune response has been recently examined using IL-1RI$^{-/-}$ mice (8, 9). These reports showed that specific serum Ab levels were normal in IL-1RI$^{-/-}$ mice when these mice were immunized with TNP-KLH/alum or TNP-KLH/

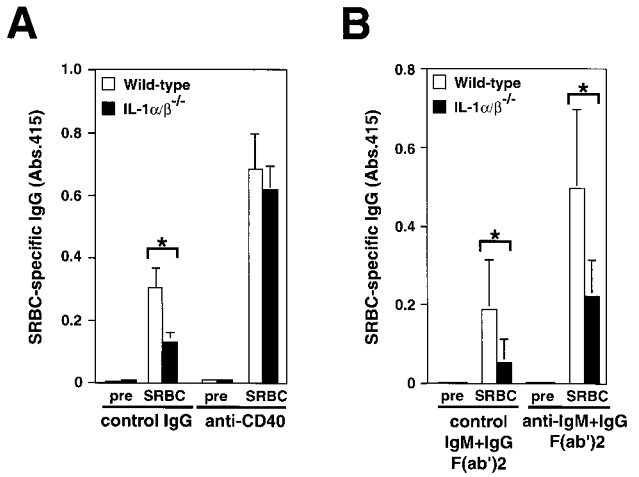

FIGURE 7. Recovery of SRBC-specific Ab production in IL-$1^{-/-}$ mice by agonistic anti-CD40 mAb treatment. Rescue of Ab production against SRBC in IL-$1^{-/-}$ mice by injection of anti-CD40 mAb (*A*) or anti-IgM $F(ab')_2$ plus anti-IgG $F(ab')_2$ (*B*). Mice were immunized with SRBC, then injected with anti-CD40 mAb or isotype IgG 24 h after the primary and secondary immunizations. Sera were collected before immunization (pre) and 1 wk (SRBC) after the secondary immunization. SRBC-specific IgG levels in the sera were measured by ELISA. The average and SD are shown. *A*, Wild-type, $n = 6$; IL-$1^{-/-}$, $n = 7$. *B*, Wild-type, $n = 8$; IL-$1^{-/-}$, $n = 8$. $*$, $p < 0.05$.

CFA either i.p. or s.c. Furthermore, they showed that KLH-specific secondary T cell proliferative responses were normal in these mice. These results apparently contradict our findings. This discrepancy is probably not due to a difference in the mouse strains used in the experiments, because we obtained the same results using IL-$1^{-/-}$ mice on the C57BL/6 background as those obtained using the BALB/c background mice. Another possibility could be that there were differences in the immunization method.

Many studies indicate that Ag dosage, adjuvant, and the route of immunization (e.g., i.p., i.v., or s.c.) affect the efficiency of the immune response and the Ig subclasses of the Abs. Different immune responses depending on the immunization program employed among investigators have been reported using CD80/CD$86^{-/-}$ mice (32), IL-$6^{-/-}$ mice (33–36), TNF-$\alpha^{-/-}$ mice (17), and OX$40^{-/-}$ and OX40$L^{-/-}$ mice as described previously (20, 27–29). Regarding this, it has been reported that the s.c. route of immunization may cause local inflammation (37, 38). Adjuvants also cause inflammation at the site of injection, which could potentially induce the production of various inflammatory cytokines, including IL-1. Since the functions of these inflammatory cytokines overlap partially, it is conceivable that the adjuvant effect of IL-1 could be substituted by some other cytokines, such as TNF-α or IL-6. In a recent study it was reported that using alum as an adjuvant could induce Th2 responses independently from IL-4- and IL-13-mediated signals (39). It is conceivable that the effects of IL-1 deficiency might not be observed when mice were immunized with adjuvant or soluble Ags, as in the reports by Glaccum et al. (8) and Satoskar et al. (9).

Another possibility is that the discrepancy is caused by a difference between protein Ags and particle Ags. With regard to this, it was reported that SRBC-specific IgG production was impaired in LT$\alpha^{-/-}$ mice, which show deficiencies in LNs formation, splenic microarchitecture, germinal center formation, and follicular dendritic cell network. Whereas IgG production against high dose NP-OVA was observed normally in this mutant mouse, suggesting that Ab production against SRBC depends on the follicular dendritic cell clustering, while that against NP-OVA does not (40–42).

This discrepancy is not caused by the difference in the mutant mice we used. We examined SRBC-specific Ab production in IL-1$RI^{-/-}$ mice without using any adjuvant and found that the immune response was reduced in both IL-1$RI^{-/-}$ and IL-$1^{-/-}$ mice of the C57BL/6J background and accelerated in IL-1$ra^{-/-}$ mice of the same background (data not shown).

In conclusion, these observations indicate that IL-1 is a potent activator of the humoral immune response, and that IL-1ra has important regulatory functions in the immune system. Immune-modulating activity via IL-1 is clearly of benefit for host defenses, as IL-1 produced upon infection with bacteria or viruses would enhance the immune response against these pathogens. Any deficiency of the IL-1/IL-1ra system, then, will probably cause serious problems in immunologic response. Our recent finding that IL-1$ra^{-/-}$ mice develop autoimmune arthritis supports this idea (7). This suggests that the balance between IL-1 and IL-1ra is of great importance in maintaining the homeostasis of the immune system. Involvement of IL-1/IL-1ra in various autoimmune diseases, such as rheumatoid arthritis, ulcerative colitis, systemic lupus erythematosus, psoriasis, lichen sclerosis, alopecia areata, and Sjogren's syndrome has also been suggested (1, 43). Further elucidation of the control mechanisms of the IL-1/IL-1ra system should provide us with important cues in the quest to develop therapeutics for these diseases.

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