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Induction of IgG2a Class Switching in B Cells by IL-27

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IL-27 is a novel IL-12 family member that plays a role in the early regulation of Th1 initiation. However, its role in B cells remains unexplored. We here show a role for IL-27 in the induction of T-bet expression and regulation of Ig class switching in B cells. Expression of WSX-1, one subunit of IL-27R, was detected at the mRNA level in primary mouse spleen B cells, and stimulation of these B cells by IL-27 rapidly activated STAT1. IL-27 then induced T-bet expression and IgG2a, but not IgG1, class switching in B cells activated with anti-CD40 or LPS. In contrast, IL-27 inhibited IgG1 class switching induced by IL-4 in activated B cells. Similar induction of STAT1 activation, T-bet expression and IgG2a class switching was observed in IFN-γ-deficient B cells, but not in STAT1-deficient ones. The induction of IgG2a class switching was abolished in T-bet-deficient B cells activated with LPS. These results suggest that primary spleen B cells express functional IL-27R and that the stimulation of these B cells by IL-27 induces T-bet expression and IgG2a, but not IgG1, class switching in a STAT1-dependent but IFN-γ-independent manner. The IL-27-induced IgG2a class switching is highly dependent on T-bet in response to T-independent stimuli such as LPS. Thus, IL-27 may be a novel attractive candidate as a therapeutic agent against diseases such as allergic disorders by not only regulating Th1 differentiation but also directly acting on B cells and inducing IgG2a class switching. The Journal of Immunology, 2004, 173: 2479–2485.

Materials and Methods

Reagents

Mouse rIL-4 and rCD28 (37.51), rIL-12, anti-FLAG (M2) and anti-actin, anti-CD3 (145-2C11) and anti-IL-2 (S4B6), anti-T-bet (4B10) and anti-STAT1, and anti-phosphotyrosine (pY)-STAT1 were purchased from BD Biosciences. 

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Biosciences (Franklin Lakes, NJ), R&D Systems (Minneapolis, MN), Sigma-Aldrich (St. Louis, MO), American Type Culture Collection (Manassas, VA), Santa Cruz Biotechnology (Santa Cruz, CA), and Cell Signaling Technology (Beverly, MA), respectively. Anti-CD40 (1C10) and LPS were purchased from R&D Systems and Sigma-Aldrich, respectively. Mouse rIFN-γ was kindly provided by Shionogi (Osaka, Japan).

**Cell culture and mice**

B cells and naive CD4⁺ T cells were cultured in RPMI 1640 medium supplemented with 10% FBS and 50 μM 2-ME. HEK293-F cells were purchased from Invitrogen Life Technologies (Carlsbad, CA) and cultured in the serum-free medium (FreeStyle 293 Expression Medium, Invitrogen Life Technologies). BALB/c and C57BL/6 mice were purchased from Japan SLC (Hamamatsu, Japan). IFN-γ-deficient mice (19) of C57BL/6 background were established and maintained at our animal facility. STAT1⁻/⁻ and STAT1⁻/⁺ mice (20) of a mixed background of 129/Sv and C57BL/6 were kindly provided by Dr. R. D. Schreiber (Washington University, St. Louis, MO). T-bet-deficient mice (17) of BALB/c background were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were maintained under specific pathogen-free conditions and used in accordance with our Institutional Guidelines.

**Preparation of purified rIL-27 protein**

rIL-27 was prepared as a soluble tagged fusion protein by flexibly linking EB13 to p28, which was reported to show a similar activity to the naive heterodimeric complex (1). Mouse IL-27 EB13 and p28 cDNAs were isolated by RT-PCR using total RNA prepared from Con A-activated spleen cells. For preparation of single-chain (sc) IL-27 expression vector, fragments encoding the mature part of EB13, followed by the (Gly4 Ser)₃ linker (Sigma-Aldrich vector), which has preprotryospin signal peptide and 3xFLAG-epitope-tag sequences at N-terminal. HEK293-F cells were then transiently transfected with the expression vector, fragments encoding the mature part of EB13, followed by the (Gly4 Ser)₃ linker (21), and then by the mature coding sequence of p28 were generated by using standard PCR methods and cloned into p3xFLAG-CMV-9 (Sigma-Aldrich vector), which has preprotryospin signal peptide and 3xFLAG-epitope-tag sequences at N-terminal. HEK293-F cells were then transiently transfected with the expression vector by using 293fectin (Invitrogen Life Technologies) according to the manufacturer’s instructions. After 3 days, culture supernatant was harvested and 3xFLAG-tagged rscIL-27 was purified by affinity chromatography using anti-FLAG (M2) affinity gel (Sigma-Aldrich). Protein concentration of purified 3xFLAG-tagged rscIL-27 was determined by titration in Western blotting with anti-FLAG (M2) using 3xFLAG-tagged rscIL-12 prepared similarly as described above as a standard. The concentration of 3xFLAG-tagged rscIL-12 was determined using rIL-12 as a standard in ELISA as described previously (22).

**Preparation of purified B and T cells**

Purified primary B cells (B220⁺ cells >99%) were isolated from spleen by negative selection with a B cell isolation kit containing biotin-conjugated mAbs to CD34, CD4, and Ter-119 (Miltenyi Biotec, Bergisch Gladbach, Germany). Primary T cells were purified by passing spleen cells depleted of erythrocytes through nylon wool. The flow-through fraction was incubated with biotin-conjugated anti-CD8a, anti-B220, anti-Mac-1, anti-Ter-119, and anti-DX5, followed by incubation with anti-biotin magnetic beads (Miltenyi Biotec), and passed through a magnetic cell-sorting column (Miltenyi Biotec). The negative fraction containing purified CD4⁺ T cells (CD4⁺ cells >95%) was collected and then incubated with anti-CD62 ligand magnetic beads (Miltenyi Biotec). The positive fraction was collected and used as purified naive CD4⁺ T cells (CD62L⁺ cells >99%).

**RT-PCR**

Total RNA was extracted by using a guanidine thiocyanate procedure, cDNA was prepared using oligo(dT) primer and SuperScript RT (Invitrogen Life Technologies), and RT-PCR was performed using TaqDNA polymerase as described (22). Cycle conditions were 94°C for 40 s, 60°C for 20 s, and 72°C for 40 s. The following primers were used: WSX-1 sense primer, 5'-ACCCAAATGGAAGCCACAC-3'; WSX-1 antisense primer, 5'-CAAGACAAGGCTTTGGTCTC-3'; T-bet sense primer, 5'-GTGGACGGATAACACAGATG-3'; T-bet antisense primer, 5'-GCGCCCCGCTTCCTTTACAC-3'. Primers used for IgG1 and IgG2a germline trans-...
RT-PCR analyses for T-bet mRNA expression. The T-bet mRNA expression was enhanced by IL-27 in a dose-dependent manner as by IFN-γ (200 ng/ml; Fig. 2A). Similar induction of T-bet expression at protein level as well as at mRNA level was observed in spleen B cells activated by anti-CD40 or LPS (Fig. 2, A and B). Although protein recovery from spleen B cells stimulated by IL-27 without activation by anti-CD40 or LPS was much less than that with the activation, increased T-bet expression at protein level was also observed without the activation (data not shown). Because T-bet is important for IgG2a class switching in B cells (13, 14), we next examined whether IL-27 induces IgG2a class switching in activated B cells. Induction of IgG2a germline transcripts by IL-27 was detected by RT-PCR in spleen B cells activated with anti-CD40 or LPS for 72 h as that by IFN-γ (Fig. 2A). Next, activated spleen B cells were incubated in the presence and absence of various amounts of IL-27 for 6 days, and the culture supernatant was analyzed for IgG2a production in ELISA. Correlating with the induction of IgG2a germline transcripts, IgG2a production was markedly enhanced by the incubation with increasing amounts of IL-27 in B cells activated with LPS with as with IFN-γ (Fig. 2C). These results suggest that IL-27 induces T-bet expression and IgG2a class switching in primary spleen B cells.

**Inhibition of IL-4-induced IgG1 class switching by IL-27 in primary spleen B cells**

IFN-γ is well known to inhibit IgG1 class switching as well (12) and T-bet was recently suggested to play a role in the inhibition of IgG1 class switching (13, 18). Therefore, we next examined the effect of IL-27 on IgG1 class switching. Primary spleen B cells were stimulated with various amounts of IL-27 (0.1, 1, and 10 ng/ml) and anti-CD40 or LPS in the presence or absence of IL-4 for 72 h and 6 days and subjected to RT-PCR, Western blotting, and ELISA, respectively, for analyses of T-bet expression and IgG1 class switching. IL-27 did not induce IgG1 class switching (Fig. 3, A and C) and the induction of T-bet expression by IL-27 was greatly diminished in the presence of IL-4 at both mRNA and protein levels (Fig. 3, A and B). In contrast, IL-27 partially but constantly inhibited IgG1 class switching induced by IL-4 in a dose-dependent manner (Fig. 3, A and C). These results suggest

![FIGURE 2. Induction of T-bet expression and IgG2a class switching by IL-27 in primary spleen B cells. A, Expression of T-bet and germline IgG2a mRNA detected by RT-PCR. Primary spleen B cells (5 x 10⁶ cells/ml) obtained from wild-type BALB/c mice were stimulated by IL-27 (0.1, 1, and 10 ng/ml) in the presence and absence of anti-CD40 (1 μg/ml) or LPS (10 μg/ml) for 72 h, and total RNA was prepared and subjected to RT-PCR analysis for T-bet, germline IgG2a and HPRT mRNA expression. B, T-bet expression at protein level detected by Western blotting. The spleen B cells (2 x 10⁶ cells/ml) were stimulated by IL-27 (0.1, 1, and 10 ng/ml) in the presence of anti-CD40 (1 μg/ml) or LPS (10 μg/ml) for 72 h, and total cell lysate was prepared and subjected to Western blotting using anti-T-bet and anti-actin. C, IgG2a production measured by ELISA. The spleen B cells (5 x 10⁶ cells/ml) were stimulated by IL-27 (0.1, 1, and 10 ng/ml) and anti-CD40 (1 μg/ml) or LPS (10 μg/ml) in the presence or absence of IL-4 (10 ng/ml) for 72 h, and the culture supernatant was analyzed for IgG2a production in ELISA in triplicate. Data are shown as the mean ± SD. IFN-γ (200 ng/ml) was used as a positive control in all these experiments. Similar results were obtained in four to five independent experiments.](http://www.jimmunol.org/)

![FIGURE 3. Inhibition of IL-4-induced IgG1 class switching by IL-27 in primary spleen B cells. A, Expression of T-bet and germline IgG1 mRNA detected by RT-PCR. Primary spleen B cells (5 x 10⁶ cells/ml) obtained from wild-type BALB/c mice were stimulated by IL-27 (0.1, 1, and 10 ng/ml) and anti-CD40 (1 μg/ml) or LPS (10 μg/ml) in the presence or absence of IL-4 (10 ng/ml) for 72 h, and total RNA was prepared and subjected to RT-PCR analysis for T-bet, germline IgG1 and HPRT mRNA expression. B, T-bet expression at protein level detected by Western blotting. The spleen B cells (2 x 10⁶ cells/ml) were stimulated by IL-27 (0.1, 1, and 10 ng/ml) and anti-CD40 (1 μg/ml) or LPS (10 μg/ml) in the presence or absence of IL-4 (10 ng/ml) for 72 h, and total cell lysate was prepared and subjected to Western blotting using anti-T-bet and anti-actin. C, IgG1 production measured by ELISA. The spleen B cells (5 x 10⁶ cells/ml) were stimulated by IL-27 (0.1, 1, and 10 ng/ml) and anti-CD40 (1 μg/ml) or LPS (10 μg/ml) in the presence or absence of IL-4 (10 ng/ml) for 72 h, and the culture supernatant was analyzed for IgG1 production in ELISA in triplicate. Data are shown as the mean ± SD. IFN-γ (200 ng/ml) was used as a positive control in all these experiments. Similar results were obtained in three independent experiments.](http://www.jimmunol.org/)
These results suggest that STAT1 activation, T-bet expression, and production (Fig. 4) similar to those in wild-type spleen B cells. T-bet expression and IgG2a class switching induced by IL-27 in primary spleen B cells are independent of IFN-γ

IFN-γ also induces STAT1 activation, T-bet expression, and IgG2a class switching in B cells (Figs. 1 and 2). IL-27 induces IFN-γ production in activated naive CD4⁺ T cells in collaboration with IL-12 (1, 4). Therefore, to exclude the possibility of potential involvement of IFN-γ in the effects of IL-27 as described above, we next used IFN-γ-deficient mice. First of all, we confirmed the inability of IFN-γ-deficient mice to produce IFN-γ using CD4⁺ T cells activated by anti-CD3 and IL-12 (Fig. 4A). Then, we compared the STAT1 activation, T-bet expression at mRNA and protein levels, and IgG2a production between IL-12 and wild-type mice. Even in IFN-γ-deficient spleen B cells, IL-27 induced tyrosine phosphorylation of STAT1 (Fig. 4B), T-bet expression at both mRNA and protein levels (Fig. 4, C and D), and IgG2a production (Fig. 4E) similar to those in wild-type spleen B cells. These results suggest that STAT1 activation, T-bet expression, and IgG2a class switching induced by IL-27 are independent of IFN-γ.

T-bet expression and IgG2a class switching induced by IL-27 in primary spleen B cells are dependent on STAT1

We have very recently found that STAT1 is indispensable for IL-27-mediated T-bet and IL-12Rβ2 expression but not for proliferation in naive CD4⁺ T cells.⁴ Therefore, we next investigated the role of STAT1 in IL-27-mediated T-bet expression and IgG2a class switching using primary spleen B cells obtained from STAT1⁻/⁻ mice and STAT1⁺/⁺ mice. We first confirmed the absence of STAT1 protein and tyrosine-phosphorylated STAT1 even after stimulation with IL-27 for 20 min in STAT1⁻/⁻ spleen B cells but the presence of them in STAT1⁺/⁺ spleen B cells (Fig. 5A). Then, these spleen B cells were activated with anti-CD40 or LPS in the presence of IL-27 for 72 h, harvested and subjected to RT-PCR and Western blotting to detect T-bet expression at mRNA and protein levels. Although IL-27 induced T-bet expression at both mRNA and protein levels in STAT1⁺/⁺ spleen B cells, IL-27 barely induced it in STAT1⁻/⁻ spleen B cells (Fig. 5, B and C). Next, the spleen B cells were activated with LPS in the presence of IL-27 for 6 days, and the culture supernatant was analyzed for IgG2a production in ELISA. Consistent with the T-bet expression, IgG2a production was greatly reduced in STAT1⁻/⁻ spleen B cells as compared with that in STAT1⁺/⁺ spleen B cells (Fig. 5D). These results suggest that STAT1 is important for T-bet expression and IgG2a class switching induced by IL-27 in spleen B cells.

IgG2a class switching induced by IL-27 in primary spleen B cells activated with LPS is dependent on T-bet

Because T-bet was recently demonstrated to be important for T-independent IgG2a class switching (14), we finally investigated the role of T-bet in IL-27-mediated IgG2a class switching using T-bet-deficient mice and wild-type BALB/c mice. T-bet-deficient spleen B cells activated with anti-CD40 or LPS were first confirmed not to express T-bet at all even after stimulation with IL-27 or IFN-γ for 72 h (Fig. 6A). However, unexpectedly, IL-27 still induced tyrosine phosphorylation of STAT1 in these B cells similar to that in wild-type B cells (Fig. 6B). Then, IL-27-induced IgG2a switching was examined. The induction of IgG2a germline transcripts by IL-27 appeared to be reduced but was still observed in T-bet-deficient spleen B cells activated by anti-CD40 (Fig. 6C). In contrast, the induction of germline IgG2a transcripts and IgG2a production by IL-27 were almost completely abolished in T-bet-deficient spleen B cells activated by LPS (Fig. 6, C and D). These results suggest that T-bet is required for IL-27-induced IgG2a class switching.

* S. Kamiya, T. Owaki, N. Morishima, F. Fukai, J. Mizuguchi, and T. Yoshimoto. An indispensable role for STAT1 in IL-27-induced T-bet expression but not proliferation of naive CD4⁺ T cells. Submitted for publication.
switching in B cells in response to T-independent stimuli such as LPS, but does not appear to be essential for it in T-dependent stimuli such as anti-CD40.

Discussion

In the present study, we have elucidated a role for IL-27 in B cells. Primary mouse spleen B cells were found to express functional IL-27R (Fig. 1), and IL-27 induced T-bet expression and IgG2a, but not IgG1, class switching in these B cells activated with anti-CD40 or LPS (Figs. 2 and 3). In contrast, IL-27 inhibited IgG1 class switching induced by IL-4 in activated B cells (Fig. 3). Similar induction of tyrosine phosphorylation of STAT1, T-bet expression and IgG2a class switching by IL-27 was observed in IFN-γ-deficient spleen B cells (Fig. 4), but not in STAT1-deficient ones (Fig. 5). The induction of IgG2a class switching was abolished in T-bet-deficient spleen B cells activated with LPS (Fig. 6). These results suggest that IL-27 play a role in the induction of T-bet expression and IgG2a, but not IgG1, class switching in B cells. Regarding the role of T-bet in the regulation of IgG2a class switching induced by IFN-γ, T-bet was reported to be necessary for IgG2a class switching in response to T-independent signaling via LPS, but not necessary for it in response to T-dependent signaling through CD40 (13, 14). Although IL-27 appears to have the similar
property to IFN-γ, further studies are necessary to clarify the regulation of IgG2a class switching by IL-27 in response to T-dependent signaling through CD40. The IgG2a Ig subclass plays a critical role in the pathogenesis of humoral autoimmunity and protection against pathogens. The IgG2a is often pathogenic in autoantibody-mediated diseases like lupus, particularly in relationship to IFN-γ production (26–28). Antiviral Abs elicited by infection with a variety of common viruses are largely restricted to the IgG2a isotype (29). However, molecular mechanisms underlying the regulation of IgG class switching to IgG2a have not been fully elucidated. IFN-γ was previously demonstrated to selectively stimulate IgG2a class switching in B cells (12). A number of reports have confirmed the critical role of this cytokine in both in vitro and in vivo IgG2a responses, including after viral and parasitic infections (30–34). However, several reports have also demonstrated a partial IFN-γ independence of specific or total IgG2a responses in the course of infections, suggesting that there may be different pathways leading to the secretion of IgG2a isotype (31, 35–38). Thus, it has been speculated that other cytokines may also regulate the IgG2a class switching in some circumstances. IL-12 is produced after viral infection and increases IgG2a secretion but in an IFN-γ-dependent manner (39–41). IFN-α is largely produced after viral infection from plasmacytoid DC (42) and has been demonstrated to induce Ig class switching to IgG2a in an IFN-γ-independent fashion (13, 37, 43). However, in the infection with a certain virus such as influenza A virus, mice lacking both IFN-αR and IFN-γR were demonstrated to still produce comparable levels of virus-specific IgG2a Abs to wild-type mice (44), suggesting that cytokines other than IFN-γ and IFN-α may possibly regulate IgG2a class switching in a certain infection. Because IL-27 is produced by macrophages and DC (1) and able to induce IgG2a class switching in activated B cells as shown in the present study, IL-27 may regulate the IgG2a class switching in the infected mice lacking both IFN-αR and IFN-γR. However, the role of endogenous IL-27 in the regulation of IgG2a class switching under physiological conditions remains to be clarified.

It has been very recently reported that IL-27 activates JAK1, STAT1, -3, and -5 in naive CD4+ T cells (4–7). We have also found that IL-27 activates JAK1, -2, tyrosine kinase 2, STAT1, -2, -3, and -5 in naive CD4+ T cells and that STAT1 plays an indispensable role in IL-27-induced T-bet and IL-12Rβ2 expression but not proliferation of naive CD4+ T cells (manuscript submitted). As JAK/STAT signaling molecules, IFN-αβR use JAK1, tyrosine kinase 2, STAT1, -2, and -3, and IFN-γ uses JAK1, -2, and STAT1 (45–51), notably, whose patterns are similar to those activated by IL-27. If two cytokines activate the same JAK/STAT signaling molecules, it is reasonable to expect similar biological actions between them. Indeed, IL-27 has been very recently shown to induce T-bet and IL-12Rβ2 expression in naive CD4+ T cells (4, 5) as IFN-γ does (52, 53). In addition, the present study revealed the roles of IL-27 in B cells including induction of STAT1 activation, T-bet expression and IgG2a class switching presumably similar to IFN-γ, further supporting the notion that IL-27 may have functional similarities to IFN-γ. If IL-27 could mimic IFN-αβR, then the pattern of their receptors will be important. Although IFN-αβR/γRs are ubiquitously expressed on various tissues and cell types, the expression of one subunit of IL-27R, type I cytokine receptor, was reported to be detected in all cell types examined including CD4+ and CD8+ T cells, B cells, NK cells, and macrophages with highest levels in CD4+ T cells and NK cells (2), but the other IL-27R subunit(s) remains to be identified. Taken together, the present study suggests that IL-27 plays a role in the induction of IgG2 class switching in B cells, as well as in the early regulation of Th1 initiation in naive CD4+ T cells through mainly the induction of T-bet expression. Thus, IL-27 may be a novel attractive candidate as a therapeutic agent against diseases such as allergic disorders by not only regulating Th1 differentiation but also directly acting on B cells and inducing IgG2a class switching.

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References


