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Blockade of Lymphotoxin Pathway Exacerbates Autoimmune Arthritis by Enhancing the Th1 Response

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Objective. **To study the role of the lymphotoxin (LT) signaling pathway in the development and pathogenesis of collagen-induced arthritis (CIA), and to understand the mechanisms by which blockade of the LT pathway influences the arthritogenic response to type II collagen (CII).**

Methods. **LTα-deficient and wild-type C57BL/6 mice were immunized with CII. Male DBA/1 mice were immunized with CII and treated with LTβ receptor immunoglobulin fusion protein (LTβR-Ig) or control Ig. Mice were monitored for the development and severity of arthritis. The effects of LT blockade on immune responses were evaluated by cytokine production and antigen-specific proliferation in vitro, the delayed-type hypersensitivity (DTH) response, and serum levels of CII-specific antibodies.**

Results. **CIA that developed in LTα-deficient mice was more severe and prolonged than that which developed in wild-type mice. Blocking LT signaling with LTβR-Ig significantly exacerbated the disease. Exacerbation of CIA was associated with an enhanced Th1-type response, including increased type 1 cytokine production, an enhanced DTH response, and elevated production of CII-specific IgG2a antibodies.**

Conclusion. **Blockade of the LT signaling pathway exacerbates the development and progression of CIA, probably by skewing the Th1/Th2 balance that determines the outcome of autoimmune responses.**

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Lymphotoxin (LT) is an immunoregulatory cytokine that belongs to the immediate major histocompatibility complex–linked tumor necrosis factor (TNF) family. In its secreted form, LT is a homotrimeric molecule (LTα3) that binds to TNF receptor I (TNFRI) and TNFRII (1). LTα is also present on the cell surface in association with LTβ, forming LTα1β2 and LTα2β1, which bind to the LTβ receptor (LTβR); the LTα1β2 form is the predominant ligand (2). LT is a critical mediator of T cell–dependent autoimmune diseases (3–6) such as multiple sclerosis, experimental autoimmune encephalomyelitis, experimental autoimmune myasthenia gravis, and insulin-dependent diabetes mellitus. All of these findings are consistent with the current notion that LT is a proinflammatory cytokine with activities equivalent to those of TNFα in chronic and autoimmune pathologic conditions. Therefore, the LT signaling pathway has been implicated as a target for immunotherapy in autoimmune diseases.

Rheumatoid arthritis (RA) is the most common chronic inflammatory autoimmune disease and is associated with destruction of cartilage and underlying bones in the joints. The collagen-induced arthritis (CIA) model shares important characteristics with human RA and provides a valuable tool for studying autoimmunity to a defined autoantigen present in healthy individuals and for understanding how this autoimmunity may be converted into disease pathology. It is generally considered that both RA and CIA are predominantly Th1 diseases, and there is considerable evidence supporting a critical role for Th1 cytokines in the pathogenesis of both diseases (7–10).

We studied the role of LT signaling in the development of autoimmune arthritis in LTα-deficient mice and mice treated with LTβR immunoglobulin fusion protein (LTβR-Ig) during the induction of autoimmune arthritis. Contrary to the notion that LT is a proinflammatory cytokine and the expectation that its

removal will result in a decreased arthritogenic response, blockade of LT signaling leads to more severe and prolonged autoimmune arthritis. The underlying mechanism responsible for this exacerbated arthritogenic response in the absence of LT signaling is enhanced Th1 cytokine production. Thus, the current study identifies a novel role for LT signaling in regulating inflammatory responses by skewing Th1/Th2 differentiation and balance.

MATERIALS AND METHODS

Mice. LTα-deficient mice (The Jackson Laboratory, Bar Harbor, ME) were backcrossed to C57BL/6 mice for more than 10 generations. Male DBA/1 mice, 8–12 weeks old, were purchased from The Jackson Laboratory. All animals were maintained under specific pathogen–free conditions, housed in autoclaved microisolators, provided with sterile bedding, food, and water, and maintained on a 12-hour day/night cycle. Animal experimentation was performed in accordance with protocols approved by the Animal Research Committee of Baylor College of Medicine.

Induction and evaluation of CIA. To induce CIA in wild-type or LTα-deficient mice derived from the C57BL/6 background, animals were immunized as previously described (11). Briefly, mice were injected intradermally at the base of the tail with 100 μg (in 100 μl) chicken type II collagen (CII; Sigma, St. Louis, MO) dissolved in 0.01*M* acetic acid and emulsified in an equal volume of Freund's complete adjuvant (CFA), prepared by grinding 100 mg of heat-killed *Mycobacterium tuberculosis* (strain H37Ra; Difco, Detroit, MI) in 20 ml of Freund's incomplete adjuvant (Sigma). Three weeks after the primary immunization, mice were given the same injection. Mice were observed for the onset of arthritis, and an arthritis index was derived by grading the severity (degree of swelling and periarticular erythema) in each paw on a 0–3-point scale, as previously described (12). The scores for all 4 paws were added, yielding the arthritis index.

To induce CIA in DBA/1 mice, animals were injected subcutaneously at the base of the tail with 200 μg (in 200 μl) bovine CII (Sigma) dissolved in 0.05*M* acetic acid and emulsified in an equal volume of CFA (Sigma). Three weeks after the primary immunization, mice were given an intraperitoneal booster injection of 100 μg aqueous CII mixed with 100 μg monophosphoryl lipid A–trehalose dimycolate adjuvant (Sigma). Mice were observed and scored as described above. The generation and production of recombinant LTβR-Ig were described previously (13). Human Ig was obtained from Sigma. In LT blocking experiments, 100 μg of LTβR-Ig or control human Ig in phosphate buffered saline (PBS) was given intravenously on days 0, 2, and 5 after the secondary CII immunization.

Detection of anti-CII antibodies. CII-specific antibodies in mouse sera were determined by enzyme-linked immunosorbent assay (ELISA), as previously described (14). Briefly, microplates were coated with chicken or bovine CII overnight and then blocked with 10% fetal calf serum. Samples were added, incubated for 1 hour at 37°C, and washed. Horseradish peroxidase–conjugated goat anti-mouse IgG1, IgG2a, and IgM (Southern Biotechnology, Birmingham, AL) were used as secondary detection reagents. Antibody titers were determined as the end points when optical density values were $\geq$2.5-fold of normal control sera on each plate.

Delayed-type hypersensitivity (DTH) response. Mice were immunized with 100 μg of chicken gamma globulin (CGG) in CFA, subcutaneously at the base of the tail. One week later, 5 μg of CGG in 20 μl of PBS was injected subcutaneously in the footpad (the contralateral footpad received 20 μl of PBS). The DTH response was estimated 18–36 hours later by measuring footpad swelling with a dial caliper (0.1-mm increments; Bel-Art Products, Pequannock, NJ). Antigen-induced swelling was expressed as the increase in footpad thickness (in 0.1-mm units) minus the thickness of the control contralateral paw.

In vitro recall lymphocyte proliferation. Mice were immunized with CGG as described above. One week later, draining (inguinal) lymph nodes were obtained, and a single-cell suspension was prepared. Cells (5×10^4 per well) were cultured in 96-well flat-bottomed plates with various concentrations of CGG for 4 days. Cells were pulsed with ^{3}H-thymidine (1 μCi/well) for the last 18 hours, and incorporation of ^{3}H-thymidine was measured with a 1205 Betaplate Liquid Scintillation Counter (Wallac, Gaithersburg, MD).

Cytokine assays. To determine cytokine production in cultures, 3 days after in vitro stimulation with recall antigens or concanavalin A, supernatants were assayed by ELISA for interferon-γ (IFNγ), TNFα, interleukin-4 (IL-4), and IL-10, using paired antibodies according to the manufacturer's instructions (PharMingen, San Diego, CA). For intracellular cytokine staining, cultured cells were first stimulated with 50 ng/ml of phorbol myristate acetate (Sigma) and 500 ng/ml of ionomycin (Sigma) in the presence of 10 μg/ml brefeldin A (Sigma) for 4 hours. Cells were washed and stained with monoclonal antibodies to surface markers. Cells were then washed and fixed with 4% paraformaldehyde at room temperature for 10 minutes. After fixing, cells were washed and treated with 0.5% saponin (Sigma) for 10 minutes. Next, cells were stained with the appropriate monoclonal antibodies to the cytokines. All antibodies were purchased from PharMingen. Samples were collected on a FACScan machine (Becton Dickinson, Mountain View, CA) and analyzed using FlowJo software (Tree Star, San Carlos, CA).

Statistical analysis. Nonnormally distributed data were presented as medians and compared using the Mann-Whitney U test (groups) or the Kruskal-Wallis test followed by Dunn's test for multiple comparisons. Otherwise, as appropriate, data are shown as the mean $\pm$ SEM.

RESULTS

More severe and prolonged arthritogenic response in LTα-deficient mice. To study directly the role of LT in the development of autoimmune arthritis, we investigated the induction and clinical outcome of CIA in LTα-deficient and wild-type C57BL/6 mice, using a modified immunization protocol that differs slightly from that used to elicit CIA in DBA/1 mice (11).

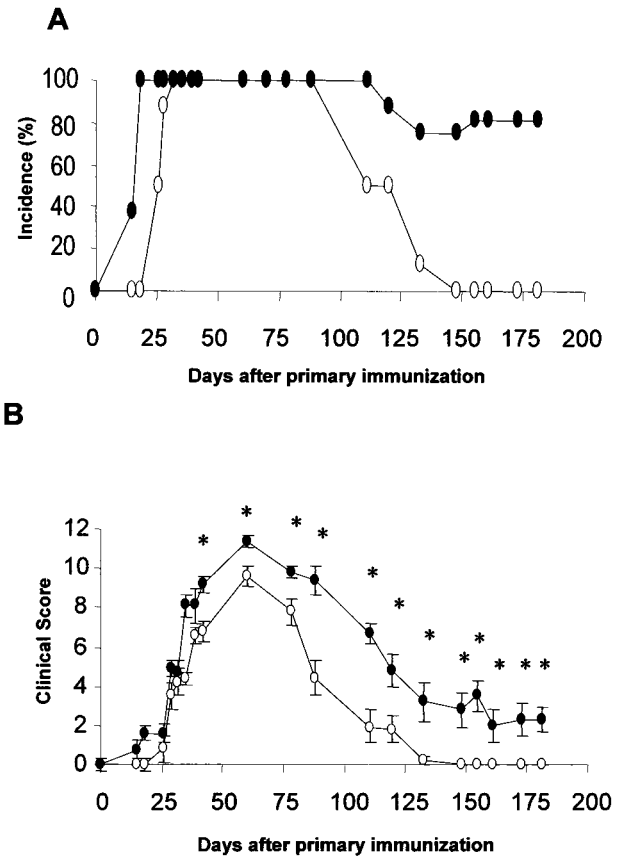

Figure 1. Exacerbation of collagen-induced arthritis in lymphotoxin α (LTα)–deficient mice. LT$\alpha^{-/-}$ and wild-type C57BL/6 mice received primary and booster immunizations with type II collagen. Clinical scores and the incidence of arthritis were determined after the primary immunization. **A,** Incidence of arthritis among LT$\alpha^{-/-}$ (solid ovals; n = 9) and wild-type C57BL/6 (open ovals; n = 8) mice. **B,** The paws of LT$\alpha^{-/-}$ (solid circles) and wild-type C57BL/6 (open circles) mice were evaluated for the severity of inflammation, and a combined clinical score was determined. Data shown are representative of 2 similar independent experiments. Values are the mean ± SEM. $*$ = $P < 0.05$.

LTα-deficient and wild-type C57BL/6 mice were immunized with chicken CII and inspected for the development of arthritis during a 6-month period. Surprisingly, the absence of LTα did not suppress the development of arthritis. Rather, LTα deficiency led to an accelerated onset of disease (Figure 1A). Because LTα-deficient mice lack lymph nodes, it is quite remarkable that local immunization could elicit a stronger response than that observed in wild-type animals. Even before the secondary immunization, a significant number of mutant mice showed clinical signs of arthritis. Fifteen days after the primary immunization, minor arthritis had already developed in 37% of the LTα-deficient mice, whereas no clinical signs of arthritis were observed in wild-type controls. Five days after secondary immunization (day 26), all of the mutant mice developed arthritis, compared with a 50% incidence in wild-type mice. In wild-type mice, the plateau lasted ~2 months. After day 88 postimmunization, arthritis declined steadily in wild-type mice. After day 141, no signs of arthritis were observed in the control group (Figure 1A). In marked contrast, in LTα-deficient mice, arthritis not only peaked faster but also lasted much longer. When the experiments were terminated on day 181, most (87%) of the LTα mutants still had various degrees of arthritis (Figure 1A).

In addition, the arthritis that developed in LTα-deficient mice was more severe than that in wild-type mice (Figure 1B). During the induction phase (days 15–32), the mean ± SEM clinical arthritis scores in LTα-deficient and wild-type mice were 3.3 ± 0.9 and 1.9 ± 0.8, respectively. During the peak of disease (days 35–78), the mean ± SEM clinical score observed in mutant mice was 9.6 ± 0.6, compared with 7.6 ± 0.9 in wild-type controls. In the declining phase of disease (days 88–148), arthritis was much more persistent in mutant mice, in which the mean ± SEM clinical score was 5.3 ± 1.2. In contrast, during this phase arthritis scores in wild-type animals decreased steadily (mean ± SEM 1.6 ± 0.8).

Exacerbation of autoimmune arthritis by blockade of LT signaling with soluble LT receptor. Because the lymphoid tissues and immune responses are disturbed in LTα-deficient mice (15–19), the elevated arthritogenic response could be attributable to changes other than the absence of LT signaling. To exclude this possibility and confirm that the exacerbation of disease is a direct consequence of defective LT function, we investigated the effect of LT signaling blockade in a conventional CIA model. DBA/1 mice were injected with LTβR-Ig (13) to interrupt in vivo LT signaling following secondary challenge with CII. The disease incidence in mice receiving LTβR-Ig was significantly higher than that in mice receiving control human Ig. In 34% of mice that received human Ig, arthritis had not developed when the experiment was terminated 68 days after immunization, whereas arthritis of various degrees developed in all of the mice that received LTβR-Ig (Figure 2A). In addition, the severity of arthritis in mice that received 3 injections of LTβR-Ig was significantly higher than that observed in mice that received injections of human Ig (Figure 2B). Thus, it is the direct effect of LT blockade, not the indirect effects of a

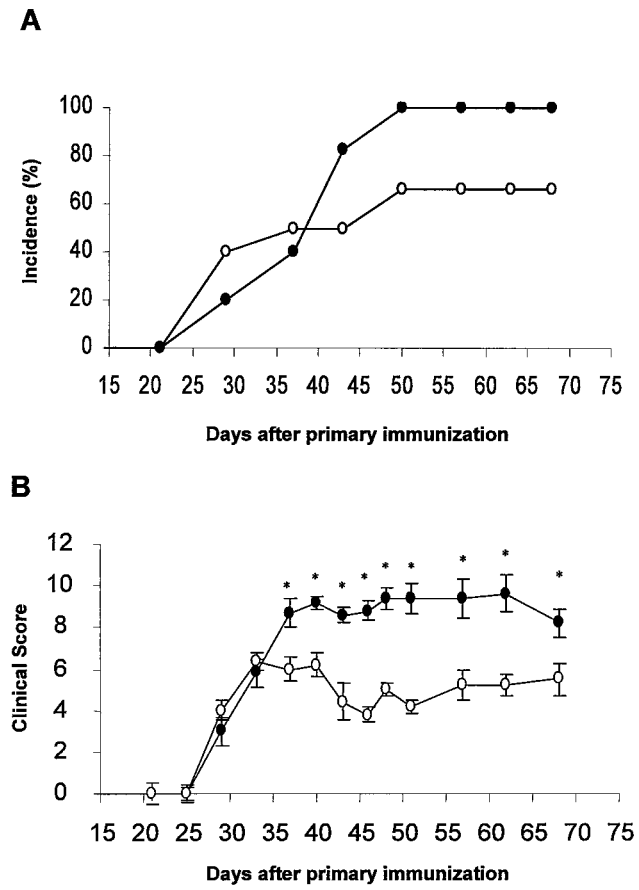

Figure 2. Exacerbation of collagen-induced arthritis by lymphotoxin β receptor immunoglobulin fusion protein (LTβR-Ig) treatment. Male DBA/1 mice were immunized, boosted with type II collagen, and treated with either LTβR-Ig (solid circles; n = 6) or control human Ig (open circles; n = 6). Clinical scores and the incidence of arthritis were determined. **A,** Incidence of arthritis among mice treated with LTβR-Ig or control human Ig. **B,** Mice that received LTβR-Ig or control human Ig were evaluated for the severity of inflammation, and a combined clinical score was recorded. Data shown are representative of 2 similar independent experiments. Values are the mean ± SEM. $* = P < 0.05$.

disturbed immune system, that leads to an enhanced arthritogenic response.

Enhanced Th1 response caused by blockade of LT signaling. Because Th1-type or proinflammatory cytokines such as IFNγ and TNFα play a dominant role in the pathogenesis of RA and CIA (7–10), we investigated whether the enhanced inflammatory response in the absence of LT signaling was associated with an elevated type 1 T cell response.

In vitro cytokine production in response to CII restimulation by draining lymph node cells from DBA/1 mice that had received injections of LTβR-Ig or control human Ig was determined. When stimulated in vitro with bovine CII, lymph node cell cultures from mice treated with LTβR-Ig produced significantly higher levels of IFNγ and TNFα than did lymph node cell cultures from control mice treated with human Ig (Figures 3A and B). In contrast, IL-4 levels in both LTβR-Ig–treated and control Ig–treated groups were very low or undetectable (Figure 3C), consistent with the notion that CIA is a pathologic condition dominated by a type 1 T cell response.

Elevated Th1 cytokine production was also observed in cell cultures from LTα-deficient mice. Because

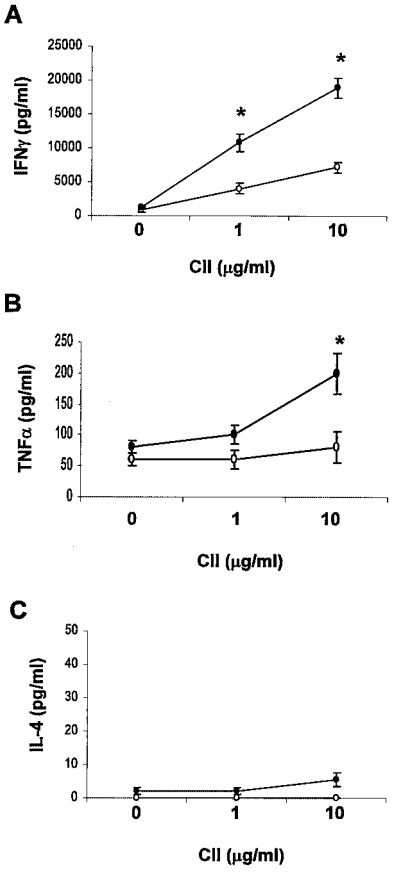

Figure 3. Promotion of type 1 cytokine production by lymphotoxin β receptor immunoglobulin fusion protein (LTβR-Ig) treatment in collagen-induced arthritis. Draining lymph node cells obtained from human Ig–treated (open circles) or LTβR-Ig–treated (solid circles) male DBA/1 mice were cultured with graded concentrations of type II collagen (CII) for 96 hours, and concentrations of interferon-γ (IFNγ) (**A**), tumor necrosis factor α (TNFα) (**B**), and interleukin-4 (IL-4) (**C**) were measured by enzyme-linked immunosorbent assay. Data shown are representative of 2 independent experiments with 6–8 mice in each group. Values are the mean ± SEM. $* = P < 0.05$.

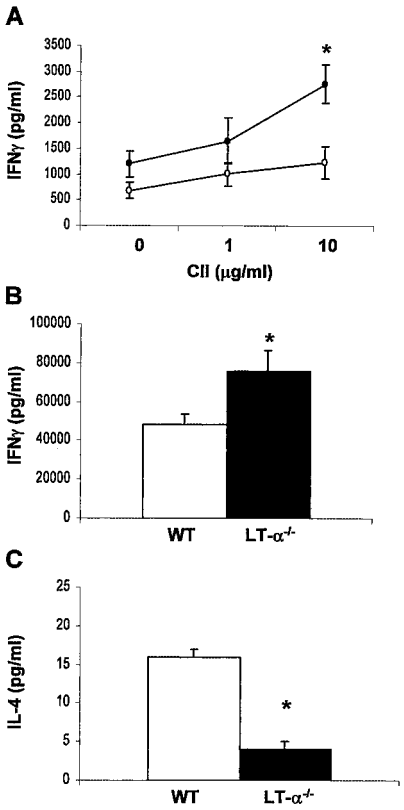

Figure 4. Biased type 1 cytokine production in vitro by lymphocytes from lymphotoxin α (LTα)–deficient mice. **A,** Splenic cells from wild-type (WT) C57BL/6 controls (open circles) or LT$\alpha^{-/-}$ mice (solid circles) were cultured with graded concentrations of type II collagen (CII) for 96 hours, and the mean ± SEM concentrations of interferon-γ (IFNγ) were measured. **B** and **C,** Levels of IFNγ and interleukin-4 (IL-4) in splenic cell cultures with 5 μg/ml of concanavalin A were determined by enzyme-linked immunosorbent assay. Values are the mean and SEM. Data shown are representative of 2 independent experiments with 6–8 mice in each group. $* = P < 0.05$.

LTα-deficient mice lack lymph nodes (15–18), we determined the cytokine profiles of spleen cells obtained from CII-immunized LTα-deficient and wild-type mice. As shown in Figure 4A, although the overall IFNγ response was much lower compared with that in lymph node cultures due to lower frequencies of CII-specific T cells in the spleens, cell cultures from LTα-deficient mice produced a significantly higher amount of IFNγ than did cell cultures from wild-type mice. IL-4 was undetectable in the same cultures (results not shown). We also compared the general potential for cytokine production between cells from wild-type and LTα-deficient mice by culturing splenic cells with the mitogen, concanavalin A. Consistently, splenic cells from LTα-deficient mice produced higher levels of IFNγ (Figure 4B) and showed decreased IL-4 production (Figure 4C) compared with splenic cells from wild-type animals.

Because humoral responses induced under Th1 or Th2 conditions are characterized by predominant production of IgG2a/IgG2b/IgG3 or IgG1/IgE antibodies, respectively (19), we determined whether blockade of LT affected the levels of specific Ig isotypes in animals immunized with CII. The serum levels of IgG1 and IgG2a CII-specific antibodies were evaluated, and the ratios of IgG2a:IgG1 were assessed as an index for Th1/Th2 dominance. In DBA/1 mice immunized with CII, the serum levels of IgG1 anti-CII antibodies were comparable between mice treated with LTβR-Ig and control mice treated with human Ig (Figure 5A). Con-

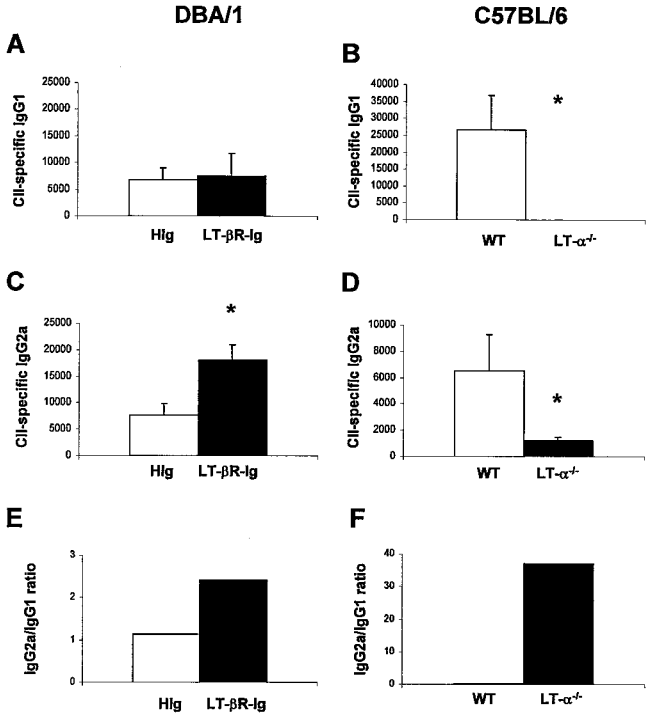

Figure 5. Enhanced IgG2a antibody production in the absence of lymphotoxin (LT) signaling. Serum samples from DBA/1 (**A**, **C**, and **E**) or C57BL/6 (**B**, **D**, and **F**) mice were collected 8 weeks after primary immunization. DBA/1 mice treated with control human Ig (HIg) or lymphotoxin β receptor immunoglobulin fusion protein (LTβR-Ig) and C57BL/6 wild-type (WT) or LT$\alpha^{-/-}$ mice were immunized as described in Materials and Methods. Type II collagen (CII)–specific serum antibodies were measured by enzyme-linked immunosorbent assay. CII-specific IgG1 (**A** and **B**) and IgG2a (**C** and **D**) antibody titers are expressed as the mean and SEM, and the levels of IgG2a in relation to levels of IgG1 are expressed as ratios (**E** and **F**). Data are representative of 2 independent experiments with 6–8 mice in each group. $* = P < 0.05$.

sistent with enhanced Th1 cytokine production, there was a significant increase in IgG2a production in mice receiving LTβR-Ig compared with that in mice receiving control human Ig (Figure 5C). Thus, the IgG2a:IgG1 ratio in LTβR-Ig–treated mice was significantly higher than that in control mice (Figure 5E).

Levels of anti-CII IgG1 in LTα mutant mice were extremely low or undetectable (Figure 5B). This diminished IgG1 antibody response is most likely attributable to the deficient humoral response, especially the diminished germinal center reaction in LTα-deficient mice (20). However, even in the near absence of IgG1 CII-specific antibodies, CII-specific antibodies of the IgG2a isotype were readily detectable in LTα mutant mice (Figure 5D), resulting in a very high IgG2a:IgG1 ratio (Figure 5F). In contrast, IgG1 production dominated in wild-type C57BL/6 mice immunized with CII. Thus, these data further demonstrate that blockade of LT function, either by disrupting the LT–LTβR interaction or ablating LT expression, promotes the development of a Th1 response.

LT signal blockade resulting in enhanced Th1 response to other conventional antigens. To confirm our findings in the CIA model and to further study immune modulation by the LT pathway, we investigated the effects of LT blockade on immune responses to other antigens. DTH is a form of cell-mediated immunity induced by Th1 cells (21). We studied the DTH response induced in mice injected with either LTβR-Ig or control human Ig after immunization with CGG. As shown in Figure 6A, when mice treated with LTβR-Ig were challenged with CGG, the severity of footpad swelling was significantly greater than that elicited in control animals. We also measured in vitro antigen-specific proliferative responses in lymph node cell cultures from mice that were primed with CGG and treated with LTβR-Ig or human Ig. The proliferative response of lymph node cells obtained from mice receiving LTβR-Ig was significantly increased compared with that of lymph nodes from control animals (Figure 6B). When IFNγ production was determined by intracellular staining, the numbers of IFNγ-producing cells in both CD4+ and CD8+ populations were increased in LTβR-Ig–treated animals (Figure 6C). Consistently, the levels of IFNγ in culture supernatants from LTβR-Ig–treated mice were significantly higher than those in cell cultures from control mice (Figure 6D). These findings confirm that blockade of LT signaling enhances type 1 responses, and that this effect is not restricted to CII.

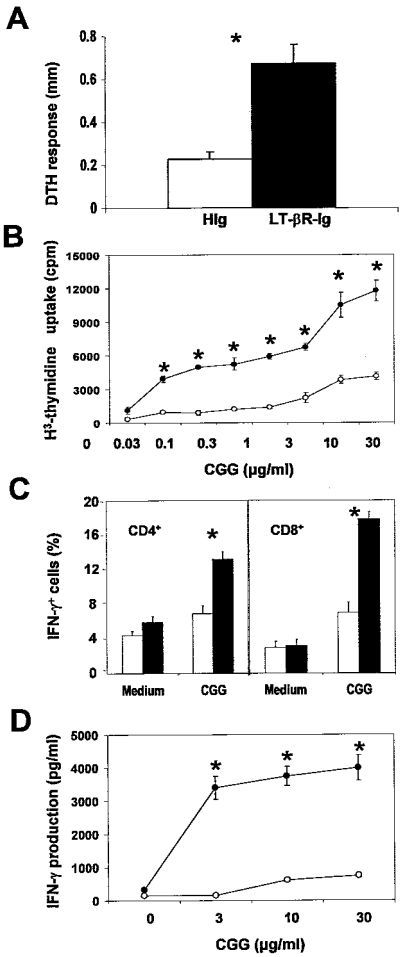

Figure 6. Enhanced Th1 response to chicken gamma globulin (CGG) in the absence of lymphotoxin (LT) signaling. C57BL/6 mice were immunized with CGG and treated with lymphotoxin β receptor immunoglobulin fusion protein (LTβR-Ig; solid bars and circles) or control human Ig (HIg; open bars and circles) on days 0, 2, and 5 after immunization. **A,** Increased delayed-type hypersensitivity (DTH) response 24 hours after challenge in mice treated with LTβR-Ig. Antigen-induced swelling was expressed as the increase in footpad thickness minus the thickness of the control contralateral paw. Data shown are representative of 2 independent experiments. **B,** Increased antigen-specific cellular proliferation in vitro. Draining lymph node cells were prepared and stimulated in culture with various concentrations of CGG. Cells were harvested 96 hours later, in the presence of ^{3}H-thymidine for the last 18 hours. **C,** Three days after in vitro restimulation with 10 μg/ml of CGG, lymph node cells were analyzed for intracellular staining. Values are the percentages of interferon-γ (IFNγ)–positive cells gated on CD4+ or CD8+ cells, respectively. **D,** IFNγ production in day 3 culture supernatants was determined by enzyme-linked immunosorbent assay. Values are the mean ± SEM. * = $P < 0.05$.

DISCUSSION

The present study has demonstrated a novel role of LT function in the initiation and development of

autoimmune arthritis through its influence on Th1/Th2 differentiation and balance. Due to its critical role in the development of certain autoimmune diseases (3–6), LT has been considered a proinflammatory cytokine. Thus, it was unexpected that the LT mutant mice would develop a more severe and longer-lasting arthritogenic response than did wild-type animals. Most investigations on the role of LT in autoimmunity suggest that the main pathogenic role of LT lies in its ability to participate in the formation of ectopic lymphoid tissue and germinal cells in targeted inflamed organs/tissues (22,23). LT function presumably mediates this effect, partly by the stimulated secretion of various chemokines such as B lymphocyte chemoattractant (CXCL13), secondary lymphoid chemokine (CCL21), and Epstein-Barr virus–induced molecule 1 ligand chemokine (CCL19), which are critical for the organization of lymphoid tissue (24). However, results of our current study suggest that LT plays an important role in regulating inflammatory responses through T cell activation and differentiation. It appears that normal LT function includes the maintenance of a proper balance between the Th1 and Th2 response, because blocking the LT–LTβR interaction pushes the response to the Th1 differentiation pathway. This notion is supported further by the results from blocking experiments with LTβR-Ig.

It is known that in addition to LT, LIGHT, one of the costimulatory molecules on T cells, is another ligand for LTβR (25–27). Thus, the effect of LTβR-Ig treatment on T cell responses could also result from signaling through LIGHT, thereby enhancing the Th1 response. However, the Th1 bias observed in LTα-deficient mice suggests that it is more likely the blockade of LT function, not the signaling through LIGHT, that causes an elevated Th1 response and more severe arthritic inflammation.

These results are in apparent contrast to a recent observation that treatment of DBA/1 mice with LTβR-Ig suppressed the development and pathogenesis of CIA (28). The discrepancy between the 2 studies may result from the differences in the treatment time and the LTβR-Ig dosage. In the previous study, LTβR-Ig was given 2 weeks before the primary immunization and was continued throughout the experiment (28). Thus, unlike blocking LT signaling after priming in the current study, the approach used in the earlier study would block initiation of the immune response and continue blocking thereafter, leading to reduction of inflammatory responses in the joints. Indeed, when LTβR-Ig was given before priming, the follicular dendritic cell network was ablated, and serum antibody titers were reduced (28). In the present study, LTβR-Ig was given 3 weeks after the primary immunization, and only 3 injections were administered (on days 0, 2, and 5). Therefore, blockade of LT signaling occurred at a time point when an immune response was well developed. Consequently, the interruption in LT signaling might result in dysregulation and an enhanced Th1 response, leading to elevated inflammatory responses in the joints.

It is interesting that more severe arthritis develops in LTα-deficient mice, while antibody responses are generally decreased. This may be attributable to the predominant Th1-driven inflammatory response in the mutant mice. Earlier studies also demonstrated that Th1-mediated airway inflammation is increased in LTα-deficient mice (29). Paradoxically, the increased airway inflammation in LTα-deficient mice is accompanied by diminished levels of IgE and can be alleviated by IgE reconstitution (29). Thus, these earlier studies demonstrated that LT is critical for IgE production, and a physiologic role of IgE in the airway is to minimize Th1-dominant inflammation. It is possible that LT-dependent production of Ig, including IgE, may play an essential role in maintaining the balance of Th1 and Th2 responses to prevent aberrant inflammation.

Overall, our data demonstrate that LT signaling may inhibit Th1 development, and that blockade of LT function may result in an elevated Th1-type response. Currently, it is not known whether membrane-bound LT can deliver a direct signal that affects T cell activation and differentiation. Much work needs to be done on elucidating the signaling motifs of membrane-bound LT and possible intracellular signal transduction pathways. Thus, the LT signaling pathway may be another "double-edged sword" for the immune system that can be turned from a proinflammatory cytokine required in building an optimal environment for autoreactivity to a regulator in Th1/Th2 differentiation and balance.

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