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Inducible costimulator (ICOS)-B7 homologous protein (B7h) is a new member of the CD28-B7 family of costimulatory molecules that regulates T cell-dependent humoral immune responses. In this study, we examined the involvement of this costimulatory pathway in the development and progression of lupus in NZB/W F1 mice. Expression of ICOS on T cells was enhanced with disease progression, whereas B7h expression on B cells was down-regulated. Administration of anti-B7h mAb before the onset of renal disease significantly delayed the onset of proteinuria and prolonged survival. Blockade of B7h effectively inhibited all subclasses of IgG autoantibody production and accumulation of both Th1 and Th2 cells. Hypercellularity and deposition of IgG and C3 in glomeruli were significantly reduced. B7h blockade after the onset of proteinuria prevented the disease progression and improved the renal pathology. Our results demonstrated the involvement of the ICOS-B7h costimulatory pathway in the pathogenesis of lupus nephritis, and the blockade of this pathway may be beneficial for the treatment of human systemic lupus erythematosus.


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3 Abbreviations used in this paper: SLE, systemic lupus erythematosus; ICOS, inducible costimulator; B7h, B7 homologous protein; MFI, mean fluorescent intensity; LN, lymph node; PAS, periodic acid-Schiff.

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Syste

mic lupus erythematosus (SLE) is a prototypic systemic autoimmune disease. SLE is characterized by dysregulated activation of both T and B cells, leading to generation of autoantibodies, particularly to dsDNA, that are critically involved in tissue damage (1, 2). The characteristic deposition of IgG in the kidney and other organs implies the involvement of IgG autoantibodies in the pathogenesis of SLE. NZB × NZW (NZB/W) F1 mice develop a spontaneous autoimmune disease characterized by production of IgG antinuclear Abs, accumulation of immune complexes, and subsequent development of a fatal glomerulonephritis, which are reminiscent of human SLE (3). In both human SLE and the murine model of lupus nephritis, it has been shown that activation of autoantibody-producing B cells is dependent on T cell help through cytokines and costimulatory molecules (4). Recent reports demonstrated that the anergic state of autoreactive B cells is reversed by CD4+ T cell help and regulated by T cell-mediated suppression (5). In the cognate interactions between T and B cells, both CD28-CD80/CD86 and CD154-CD40 are crucial costimulatory pathways for initial immune responses (6). Engagement of TCR by Ags presented by B cells rapidly induces CD154 expression on T cells, and its binding to CD40 on B cells promotes germinal center formation, B cell proliferation and differentiation, and isotype switching (7). The engagement of CD40 also induces CD80 and CD86 expression on B cells, and ligation of CD28 on T cells by the binding of CD80 and CD86 induces a strong costimulatory signal that promotes T cell activation and survival (8). Blockade of CD28 and/or CD40 pathways could inhibit the onset of murine lupus nephritis (9–13), suggesting the importance of both costimulatory pathways.

In addition to these pathways, other receptor/ligand pairs that are also involved in the T-B cognate interactions and may influence the pathogenesis of systemic autoimmune diseases include CD134-CD134 ligand (14), BAFF-BAFF ligands (15), and inducible costimulator (ICOS)-ICOS ligand (16, 17). ICOS is induced on T cells after activation (18). Its ligand B7 homologous protein (B7h) (19)/B7-related protein-1 (20)/GL50 (21)/ligand of ICOS (22) (hereafter designated B7h) is constitutively expressed on B cells and inducible on monocytes and dendritic cells at low levels (23). In addition to the expression on professional APCs, B7h is also inducible on fibroblasts (19) and endothelial cells (24) by proinflammatory cytokines such as IFN-γ and TNF-α. Ligation of ICOS strongly enhances the production of IL-4, IL-5, IFN-γ, and IL-10, but not IL-2 (18, 25, 26), suggesting a distinct costimulatory effect from CD28. IL-4 production by T cells was extremely impaired in the absence of ICOS (27). ICOS knockout mice exhibit profound defects in Th cell-dependent B cell responses, germinal center formation, and Ig class switching (28, 29). This deficit could be overcome by CD40 stimulation, suggesting that ICOS promotes isotype switching by regulating the CD40-CD154 pathway (28). Although these studies have substantiated a pivotal role of the ICOS-B7h costimulatory pathway in regulating humoral immune responses, its contribution to autoantibody production has not been well characterized. To investigate the involvement of the ICOS-B7h costimulatory pathway in the development and progression of lupus nephritis, we have examined the effects of anti-ICOS or anti-B7h mAb administration in lupus-prone NZB/W F1 mice.
Materials and Methods

Mice and mAb treatment

NZB/W F₁ and C.B-17 scid (SCID) female mice were purchased from SLC (Shizuoka, Japan) and Charles River Breeding Laboratories (Kanagawa, Japan), respectively, and maintained in the animal facility at Tokyo Medical and Dental University. Mice transgenic for the OVA233-247-specific and I-A⁻restricted DO11.10 TCR-αβ on a RAG⁻²⁻ background were generously supplied by Dr. S. Koyasu (Keio University, Tokyo, Japan). All procedures were reviewed and approved by the Animal Care and Use Committee of the Tokyo Medical and Dental University. The anti-mouse B7h (HK5.3, rat IgG2a) (30) and ICOS (B10.5, rat IgG2a) (31) mAbs were generously supplied by Dr. S. Koyasu (Keio University, Tokyo, Japan). All procedures were reviewed and approved by the Animal Care and Use Committee of the Tokyo Medical and Dental University. The anti-mouse B7h (HK5.3, rat IgG2a) (30) and ICOS (B10.5, rat IgG2a) (31) mAbs were generated as described previously. HK5.3 blocked the binding of mouse ICOS-Ig to mouse B7h transfectants (30), and both B10.5 and HK5.3 could inhibit the ICOS-B7h-mediated proliferative responses by CD4⁺ T cells in vitro (Ref. 30; H. Iwai, unpublished observation). In the experiments to see the effect of mAb treatment on the development of lupus, 2-mo-old NZB/W F₁ females were randomly divided into three groups and treated with control reagents, anti-B7h mAb (200 µg/body), or anti-ICOS mAb (100 µg/body) i.p. twice per week for 19 wk as described previously (10). Optimal doses for anti-B7 and anti-ICOS mAbs were determined according to our previous studies (10, 30). As the control reagents, PBS or rat IgG (200 µg/body; Sigma-Aldrich, St. Louis, MO) was used. As we observed similar results between the mice treated with PBS or rat IgG in all experiments, the results from the mice treated with PBS and rat IgG were combined and presented as a control group. In the experiments to see therapeutic effects, a cohort of NZB/W F₁ female mice was monitored for proteinuria every week from 5 mo of age. The treatment was started after observation of two consecutive proteinuria of >100 mg/dl. Three groups of mice received control reagent, anti-B7h mAb (200 µg/body), or anti-ICOS mAb (100 µg/body) i.p. three times per week until death or up to 12 mo of age.

Assessment of nephritis

For monitoring the development of nephritis, proteinuria was measured every week as described previously (32). Protein concentrations of >100 mg/dl of urine were considered positive. For histological evaluation of renal disease, mice were sacrificed at 7 mo of age or at 3 mo after the initial treatment. Kidneys were either fixed in formalin or snap-frozen in Tissue Tek (Sakura Finetechanical, Tokyo, Japan) for cryostat sectioning. Formalin-fixed tissue was embedded in paraffin, sectioned, and stained by the periodic acid-Schiff (PAS) method. The cellularity of 12 randomly selected glomeruli in each kidney cross-section was counted, and the severity of change was scored from 0 to 3 as follows: grade 0, normal (35–40 cells/glomerulus); grade 1, mild (41–50 cells/glomerulus); grade 2, moderate (51–60 cells/glomerulus); grade 3, severe (>60 cells/glomerulus) as described (33).

Hormonal injections were given s.c. at 100 µg/kg of OVA233-247 and 1 mg/kg of OVA323-390 peptide every week as described (34). The mean cellularities (ICOS) and MFI (B7h) of each section was averaged.

ELISA of serum anti-dsDNA Ab

Serum samples were collected at 3, 5, and 7 mo of age, and the levels of dsDNA total IgG, IgG1, IgG2a, and IgG2b Abs were measured by ELISA as described previously (35). A pooled serum from 7-mo-old NZB/W F₁ mice with severe nephritis was used as a standard. The titer of the standard serum was defined as 1 U, and the levels in individual samples are expressed as unit values.

mAbs and flow cytometry

mAbs against the following Ags were used for immunofluorescence analysis: CD3 (145-2C11, hamster IgG), CD4 (RM-4.5, rat IgG2a), CD5 (53-73, rat IgG2a), CD8 (53-6.7, rat IgG2a), CD69 (H1.2F3, hamster IgG), CD25 (PC61, rat IgG1), CD45RB/B220 (RA3-6B2, rat IgG2a), B7 (HK5.3), ICOS (B10.5), IFN-γ (XMG1.2, rat IgG1), IL-4 (BV4D4.11, rat IgG2b), and IL-10 (JES5-16E3, rat IgG2b). All FITC-, PE-, or PerCP-conjugated mAbs and control Ig were obtained from BD Biosciences (San Diego, CA). Biotinylated anti-B7 and anti-ICOS mAbs were prepared in our laboratory and detected by PE-streptavidin (BD Biosciences). Multi-color staining for intracellular cytokine and cell surface Ags were prepared as described previously (36). Immunofluorescence, flow cytometry, and data analysis were performed using FACSCalibur and CellQuest software (BD Biosciences).

Adoptive transfer experiments using DO11.10 TCR-transgenic T cells

To investigate the effect of in vivo treatment with anti-ICOS mAb on Ag-specific T cell responses, we performed the adoptive transfer of OVA-specific T cells from DO11.10 TCR-transgenic mice (37, 38). SCID (8-wk-old female) mice received a mixture of splenocytes and lymph node (LN) cells containing 3 × 10⁵ of CD4⁺ KJ1-26⁺ T cells from RAG⁻²⁻/⁻ DO11.10 mice. After 24 h, 1 mg of chicken OVA (grade V; Sigma-Aldrich) in IFA (Difco, Detroit, MI) was injected i.p. One hundred micrograms of control rat IgG or anti-ICOS mAb (B10.5) was administered i.p. on days 0 and 2. Five days later, mice were sacrificed and the draining LNs were collected. Total LN cell numbers were counted, and the expansion and activation of OVA-specific T cells were examined using PE-conjugated anti-CD25 mAb and biotinylated anti-DO11.10 TCR (KJ1-26, mouse IgG2a; Caltag, Burlingame, CA) mAb, followed by allophycocyanin-streptavidin (BD Biosciences), or Annexin VFITC (BD Biosciences) by flow cytometry. Alive cells, apoptotic cells, and large activated alive cells were determined to be annexin V⁻FSC−medium-high, annexin V⁻FSC−low, and annexin V⁻FSC⁺high cells, respectively. Furthermore, isolated LN cells (5 × 10⁶ cells/ml) were cultured in the presence of 2 µg/ml OVA323-390 peptide (Bachem, Bubendorf, Switzerland), and cytokine production in the supernatants after 48-h culture was measured by ELISA. ELISA for IFN-γ, IL-4, and IL-10 was performed using ELISA kits (Ready-SET-Go; eBioscience, San Diego, CA) according to the protocols recommended by the manufacturer.

Statistical analysis

The log-rank and Mann-Whitney U tests were used. Values of p < 0.05 were considered significant.

Results

Expression of ICOS and B7h on splenocytes from NZB/W F₁ mice

We first examined the expression of ICOS and B7h on splenocytes from NZB/W F₁ mice before (at 2 mo of age) and after (at 7 mo of age) the onset of disease. A considerable percentage of T cells expressed ICOS in the splenocytes from NZB/W F₁ mice at 2 mo of age (Fig. 1). The expression of ICOS on T cells was gradually up-regulated as the age increased, and most T cells expressed ICOS at 7 mo of age in NZB/W F₁ mice. Similar results were obtained with PBLs (data not shown). Consistent with a previous observation (25), most B cells constitutively expressed B7h at all time points analyzed. Interestingly, the MFI was significantly (p < 0.03) decreased at 7 mo of age compared with 2 mo of age. These results prompted us to investigate the involvement of ICOS-B7h interactions in the development of lupus nephritis.

FIGURE 1. Expression of ICOS and B7h on splenocytes from NZB/W F₁ mice. Splenocytes from 2- and 7-mo-old NZB/W F₁ mice were stained with biotinylated anti-ICOS or anti-B7h mAb and FITC-labeled anti-CD3 or anti-CD45R mAb or with the appropriate fluorochrome-conjugated control Ig, followed by PE-streptavidin. Staining was analyzed by flow cytometry. An electronic gate was set on CD3⁺ T cells or CD45R⁻ B cells, and the expression of ICOS on T cells and B7h on B cells are represented as MFI with the control Ig staining as a dotted line. The values of mean percentages (ICOS) and MFI (B7h) ± SEM are indicated in the upper right. Data are representative of three separate experiments.
Administration of anti-B7h mAb before the onset of disease prevents the development of lupus

We examined the effects of anti-B7h or anti-ICOS mAb administration before the onset of lupus nephritis. We started the treatments from 2 mo of age when an obvious renal disease was not observed. At the cessation of treatment (6.5 mo of age), about one-half of the mice treated with control reagents or anti-ICOS mAb developed renal disease (proteinuria, >100 mg/dl), but none of the anti-B7h mAb-treated mice developed the disease (Fig. 2A). Although the mice treated with anti-B7h mAb eventually developed proteinuria after the cessation of treatment, the disease course was significantly ($p = 0.08$) different from that of the control group (Fig. 2A). The final survival rate of the anti-B7h mAb-treated mice at 10 mo of age was 80%, whereas all of the mice treated with control reagents or anti-ICOS mAb died of severe nephritis until this time point (Fig. 2B). The improvement of nephritis by the anti-B7h mAb treatment was verified by histological examination. Renal tissue sections from the control or anti-B7h mAb-treated mice at 7 mo of age were examined for the glomerular hypercellularity and the deposition of IgG and C3. The sections from the control mice showed a severe change including membranoproliferative changes and sclerosis of glomeruli (Fig. 3Aa), whereas these manifestations were not observed in the anti-B7h mAb-treated mice (Ab). Deposition of IgG (Fig. 3A, c and d) and C3 (not shown) to glomeruli was obvious in the control mice, but this was clearly inhibited in the anti-B7h mAb-treated mice. In the quantitative analyses, the anti-B7h mAb treatment showed a significant inhibition in the hypercellularity and the IgG and C3 deposition in glomeruli (Fig. 3B). These results indicated that the treatment with anti-B7h mAb before the onset of disease prevented the development of nephritis, although this preventive effect was lost upon cessation of the treatment.

Effect of anti-B7h or anti-ICOS mAb treatment on lymphocyte status

To examine the effects of anti-B7h or anti-ICOS mAb treatment on lymphocytes in circulation and secondary lymphoid organs, we examined the cell number and lymphocyte population in peripheral blood and spleen at 7 mo of age. Consistent with our previous observation that T lymphopenia was observed at this age of NZB/W F1 mice (39, 40), the control NZB/W F1 mice exhibited a reduction of T lymphocytes in peripheral blood (Tables I and II). In the anti-B7h mAb-treated mice, this T lymphopenia was significantly ameliorated. In addition, splenomegaly and a high CD4/CD8 ratio of splenic T cells were characteristic features at this age of NZB/W F1 mice, and these features were not observed in the mice treated with anti-B7h mAb (Tables I and II). The treatment with anti-ICOS mAb did not clearly affect the lymphocyte count and phenotypes as compared with the control mice (not shown).

We next examined the effects of mAb treatment on activational state of T cells. The percentages of CD69$^+$ or CD25$^+$ cells in the control mice at 5 mo of age and became prominent at 7 mo of age (Fig. 4). All of these isotypes of Ab production were significantly suppressed in the mice treated with anti-B7h mAb. These results suggested that the anti-B7h mAb treatment inhibited both Th1- and Th2-mediated autoantibody production. In contrast, the treatment with anti-ICOS mAb did not consistently inhibit the production of anti-dsDNA Ab. The evaluation of respective IgG subclasses revealed that IgG1 was rather enhanced in the mice treated with anti-ICOS mAb at 3.5, and 7 mo of age, whereas IgG2a and IgG2b were significantly inhibited. These results suggested a differential effect of the anti-ICOS mAb treatment on Th1- vs Th2-mediated immune responses.

We have monitored the serum Ab against the respective administered rat IgGs. We failed to detect a substantial serum Ab against the anti-B7h rat IgG2a in the mice treated with anti-B7h mAb, whereas we observed a slight increase of Abs against the administered control rat IgG and the anti-ICOS rat IgG2a in the control IgG- and anti-ICOS mAb-treated mice, respectively (data not shown). These results suggest an efficient inhibition of the anti-rat host responses by the treatment with anti-B7h mAb.

Inhibition of anti-dsDNA IgG1, IgG2a, and IgG2b Ab production by anti-B7h mAb treatment

Consistent with our previous observation (10), the production of anti-dsDNA IgG Abs, including IgG1, IgG2a, and IgG2b subclasses, was first detectable in the control mice at 5 mo of age and became prominent at 7 mo of age (Fig. 4). All of these isotypes of Ab production were significantly suppressed in the mice treated with anti-B7h mAb. These results suggested that the anti-B7h mAb treatment inhibited both Th1- and Th2-mediated autoantibody production. In contrast, the treatment with anti-ICOS mAb did not consistently inhibit the production of anti-dsDNA Ab. The evaluation of respective IgG subclasses revealed that IgG1 was rather enhanced in the mice treated with anti-ICOS mAb at 3.5, and 7 mo of age, whereas IgG2a and IgG2b were significantly inhibited. These results suggested a differential effect of the anti-ICOS mAb treatment on Th1- vs Th2-mediated immune responses.

The improvement of nephritis by the anti-B7h mAb treatment was verified by histological examination. Renal tissue sections from the control or anti-B7h mAb-treated mice at 7 mo of age were examined for the glomerular hypercellularity and the deposition of IgG and C3. The sections from the control mice showed a severe change including membranoproliferative changes and sclerosis of glomeruli (Fig. 3Aa), whereas these manifestations were not observed in the anti-B7h mAb-treated mice (Ab). Deposition of IgG (Fig. 3A, c and d) and C3 (not shown) to glomeruli was obvious in the control mice, but this was clearly inhibited in the anti-B7h mAb-treated mice. In the quantitative analyses, the anti-B7h mAb treatment showed a significant inhibition in the hypercellularity and the IgG and C3 deposition in glomeruli (Fig. 3B). These results indicated that the treatment with anti-B7h mAb before the onset of disease prevented the development of nephritis, although this preventive effect was lost upon cessation of the treatment.

FIGURE 2. Effect of anti-B7h or anti-ICOS mAb treatment on the development of lupus nephritis. NZB/W F1 female mice ($n = 10$ in each group) were treated with control reagents (C), anti-B7h mAb (•), or anti-ICOS mAb (▲) twice per week from 2 mo of age for 19 wk. Incidence of proteinuria (A) and survival rate (B) are shown. The data shown are representative of two independent experiments with similar results.
IL-4, and IL-10 were increased compared with those of the same age of BALB/c mice (IFN-γ, 0.8 \pm 0.04%; IL-4, 0.4 \pm 0.06%; IL-10, 0.2 \pm 0.04%), which were significantly reduced by the anti-B7h mAb treatment. The treatment with anti-ICOS mAb showed less inhibitory effects on IFN-γ and IL-10, whereas no inhibition was observed on IL-4. CD8+ T cells expressing IFN-γ, IL-4, and IL-10 in the control NZB/W F1 mice were 0.5 \pm 0.3%, 0.2 \pm 0.1%, and 0.2 \pm 0.1%, respectively, and these were not affected by the anti-B7h or anti-ICOS mAb treatment (data not shown). These results suggested that the anti-B7h mAb treatment inhibited the activation of both Th1 and Th2 cells, whereas the anti-ICOS mAb treatment had less inhibitory effect on T cell activation.

Therapeutic effect of anti-B7h mAb treatment after the onset of lupus nephritis

To investigate the effects of anti-B7h or anti-ICOS mAb treatment on ongoing lupus nephritis, we treated NZB/W F1 mice that had developed renal disease (proteinuria, >100 mg/dl) three times per week up to 12 mo of age. The mice treated with either control reagents or anti-ICOS mAb exhibited no significant improvement in proteinuria (not shown) and in survival rate, and all mice died within 2 mo after the initial treatment (Fig. 6A). The treatment with Table I. Effects of anti-B7h mAb treatment on peripheral blood

<table>
<thead>
<tr>
<th></th>
<th>Total Lymphocytes (×10^3/μl)</th>
<th>T Cells (×10^3/μl)</th>
<th>Conventional B Cells (×10^3/μl)</th>
<th>CD5+ B Cells (×10^3/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control reagents</td>
<td>6.6 \pm 0.4</td>
<td>4.0 \pm 0.4</td>
<td>1.2 \pm 0.0</td>
<td>0.3 \pm 0.0</td>
</tr>
<tr>
<td>Anti-B7h mAb</td>
<td>7.9 \pm 0.2^b</td>
<td>5.5 \pm 0.3^b</td>
<td>1.4 \pm 0.1</td>
<td>0.6 \pm 0.1^b</td>
</tr>
</tbody>
</table>

^a Absolute lymphocyte counts in peripheral blood from the mice at 7 mo of age were measured. Lymphocytes were stained with FITC-conjugated anti-CD45R mAb and PerCP-conjugated anti-CD3 mAb or PE-conjugated anti-CD5 mAb. CD3+ CD45R−, CD5+ CD45R−, and CD5− CD45R+ cell counts are presented as T cells, conventional B cells, and CD5+ B cells, respectively. Values are the means \pm SEMs from each group (n = 10).

^b Statistically different from the control reagent-treated group (p < 0.05).

FIGURE 3. Evaluation of renal disease by histopathology. Kidney sections from the control reagents or anti-B7h mAb-treated mice at 7 mo of age were examined by PAS staining and immunofluorescence for IgG and C3 (green). Propidium iodide (red) was used for counterstain. A. Representative staining of PAS (a and b) and IgG deposition (c and d) from control reagent (a and c) - or anti-B7h mAb (b and d)-treated groups. Original magnification, ×100. B. Hypercellularity of glomeruli (a), and IgG (b) and C3 (c) deposition were quantitatively evaluated as described in Materials and Methods. The mean scores \pm SEM of four mice in the control reagent-treated (□) or anti-B7h mAb (■)-treated group are shown. *, Statistically different (p < 0.05).

FIGURE 4. Effect of anti-B7h or anti-ICOS mAb treatment on serum anti-dsDNA IgG Ab. Serum samples from the control reagent (Capital), anti-B7h mAb (●), or anti-ICOS mAb (□)-treated mice were obtained at 3, 5, and 7 mo of age. Serum levels of anti-dsDNA total IgG, IgG1, IgG2a, and IgG2b Abs were measured by ELISA. Data shown are the mean \pm SEM of 10 mice from two independent experiments in each group. *, Statistically different (p < 0.05).
anti-ICOS mAb treatment significantly increased the percentage of CD25+ cells within KJ1-26+ T cells. In addition, LN cells from the anti-ICOS mAb-treated SCID mice produced higher amounts of IFN-γ, IL-4, and IL-10 after stimulation with OVA peptide. These results indicate that the treatment with anti-ICOS mAb promoted Ag-specific T cell activation and apoptosis/activation induced cell death, suggesting an agonistic activity of the anti-ICOS mAb.

**Discussion**

An array of costimulatory molecules has been implicated in the pathogenesis of lupus nephritis in NZB/W F1 mice (9–11, 14, 41). In this study, we first demonstrated a critical contribution of the ICOS-B7h pathway as well. The blockade of B7h by administration of anti-B7h mAb inhibited the onset and the progression of glomerulonephritis and prolonged the survival. The improvement in clinical manifestations was correlated with the inhibition of cellular and humoral immune responses mediated by both Th1 and Th2 cells and histopathological amelioration.

ICOS is expressed on naïve T cells only after activation (18). Surprisingly, most T cells expressed ICOS at a high level, and considerable percentages of T cells expressed activation markers, CD69 and CD25, at 7 mo of age in NZB/W F1 mice, suggesting aberrant activation of T cells with disease progression. In contrast to the inducible expression of ICOS on T cells, its ligand B7h is constitutively expressed on naïve B cells (19, 23). A recent report demonstrated that B7h on B cells was extinguished after activation through surface Ig- and IL-4-mediated signaling, and a costimulation through CD40 was capable of restoring the surface expression of B7h (42). In NZB/W F1 mice, we also observed the down-regulation of B7h on splenic B cells with disease progression (Fig.

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**Table II. Effects of anti-B7h mAb treatment on splenocytes**

<table>
<thead>
<tr>
<th>Total Lymphocytes ($\times 10^7$)</th>
<th>CD4+ T Cells ($\times 10^7$)</th>
<th>CD8+ T Cells ($\times 10^7$)</th>
<th>CD4/CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control reagents</td>
<td>17.6 ± 1.9</td>
<td>5.4 ± 0.6</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Anti-B7h mAb</td>
<td>8.0 ± 1.9*</td>
<td>2.8 ± 0.5*</td>
<td>0.8 ± 0.0*</td>
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* Statistically different from the control reagent-treated group ($p < 0.05$).

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**FIGURE 5.** Expression of activation markers and cytokines in splenic T cells. Splenocytes from the control reagent ( ), anti-B7h mAb ( ), or anti-ICOS mAb ( )-treated mice at 7 mo of age were obtained. Cells were stained with combinations of PE-conjugated anti-CD69 or anti-CD25 mAb and PerCP-conjugated anti-CD3 mAb, and PE-conjugated anti-IFN-γ, anti-IL-4, or anti-IL-10 mAb and PerCP-conjugated anti-CD4 mAb. An electronic gate was set on either CD3+ or CD4+ lymphocytes, and the percentages of CD69+ or CD25+ cells within CD3+ T cells and the percentages of IFN-γ+, IL-4+, or IL-10+ cells within CD4+ T cells were analyzed by flow cytometry. Data represent the mean percentages ± SEM of four mice in each group.

* Statistically different from the control group ($p < 0.05$).
representative of two separate experiments and expressed as the means ± SEM from each group of three mice. A). This might result from the cognate interaction with T cells during the process of disease progression.

In the present experiments, we have observed contradictory results between the anti-B7h and anti-ICOS mAb treatments. The treatment with anti-B7h mAb showed a consistent amelioration of both clinical and pathological manifestations, immunological abnormality including expression of T cell activation markers and cytokines, and all subclasses of anti-dsDNA autoantibody production. In contrast, the administration of anti-ICOS mAb failed to ameliorate clinical manifestations assessed by proteinuria and survival rate, although this treatment significantly inhibited IFN-γ and IL-10 production by splenic T cells and serum IgG2a and IgG2b anti-dsDNA Ab production. It should be noted that the anti-ICOS mAb treatment induced early production of IgG1 anti-dsDNA Ab, and this was further enhanced with age. In addition, this treatment did not significantly affect IL-4 production unlike the treatment with anti-B7h mAb. These results suggest that the anti-ICOS mAb treatment potentially has a reverse effect on Th2-mediated immune responses. In fact, the adoptive-transfer experiments using DO11.10 TCR-transgenic T cells demonstrated an agonistic property of the anti-ICOS mAb used in this study. In the CD4+ T cell responses against OVA, the treatment with anti-ICOS mAb apparently promoted activation of both Th1 and Th2 cells and apoptosis. Previous reports using the same anti-ICOS mAb (B10.5) demonstrated the opposing effect of this mAb on acute vs chronic graft-vs-host disease (43), and the ameliorating effect on a chronic colitis induced by transfer of CD4+CD45RBhigh T cells by enhancing Th2 cytokine production and apoptosis of infiltrating T cells (44). In addition, we also observed that treatment with various doses of anti-ICOS mAb (50, 100, 300 μg/body) persistently exacerbated the collagen-induced arthritis (H. Iwai and M. Azuma, unpublished observation), whereas the anti-B7h mAb treatment efficiently ameliorated the arthritis by inhibiting both Th1- and Th2-mediated immune responses (30). Taken together, it is likely that the binding of anti-ICOS mAb (B10.5) to T cells induced an agonistic signal to T cells, especially to Th2 cells in our NZB/W F1 model. Preferential expression of ICOS on Th2 cells has been reported (45–47). Therefore, the differential expression of ICOS between Th1 and Th2 cells and the differential contribution of Th1 and Th2 cells to the development of lupus may result in different outcomes of the binding of anti-ICOS mAb in this model.

It has been shown that the ICOS-B7h costimulatory pathway is preferentially involved in the effector phase of immune responses rather than the induction phase (47, 48). We previously examined the effects of anti-CD80 and -CD86 mAbs on the development and progression of lupus nephritis in NZB/W F1 mice with similar protocols (10). Based on the incidence of proteinuria and the survival rate at 10 mo of age, the inhibitory effect of the anti-B7h mAb treatment before the onset of disease seems to be inferior to
that of the anti-CD80/86 mAb treatment. Nevertheless, the survival rate at 12 mo of age in the therapeutic treatment was comparable with that of the CD80/86 mAb treatment. Consistent with our recent results in a collagen-induced arthritis model (30), the B7h blockade consistently showed an effectiveness against ongoing autoimmune diseases.

In conclusion, the blockade of B7h prevented the onset and progression of lupus nephritis in NZB/W F1 mice by efficiently inhibiting both cellular and humoral immune responses associated with disease. Our results demonstrated a critical role of the ICOS-B7h costimulatory pathway in the pathogenesis of lupus nephritis. Intervention of this pathway may become a novel strategy for the treatment of human SLE and possibly other autoimmune diseases.

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References