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Regulated by B7-1 and B7-2 Costimulation in Autoantibody Responses and Pathology in MRL/lpr Lupus

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Autoantibody Responses and Pathology Regulated by B7-1 and B7-2 Costimulation in MRL/lpr Lupus

Bailin Liang,* Michael J. Kashgarian, † Arlene H. Sharpe, ‡ and Mark J. Mamula2*

The activation of T lymphocytes requires both Ag-mediated signaling through the TCR as well as costimulatory signals transmitted through B7-1 and/or B7-2 with CD28. The interference of B7-mediated costimulatory signals has been proposed as one immunotherapeutic intervention for the prevention autoimmune disease. This study has examined autoantibody responses and autoimmune pathology in a murine model of human systemic lupus erythematosus (SLE), the MRL-lpr/lpr mouse, genetically deficient in B7-1 or B7-2, or in mice treated with B7-1/B7-2 blocking Abs. In contrast to other studies of murine models of SLE, MRL-lpr/lpr mice treated with B7 blocking Abs exhibit strong anti-small nuclear ribonucleoprotein (snRNP) and anti-DNA autoantibody responses with some changes in isotype switching as compared with untreated animals. All MRL-lpr/lpr mice deficient in B7-1 or B7-2 produce anti-snRNP and anti-DNA titers with isotypes virtually identical with wild-type animals. However, the absence of B7-2 costimulation did interfere with the spontaneous activation and the accumulation of memory CD4+ or CD8+ T lymphocytes characteristic of wild-type MRL-lpr/lpr mice. IgG and C3 complement deposition was less pronounced in the kidneys of B7-2 deficient MRL-lpr/lpr mice, reflecting their lesser degree of glomerulonephritis. By comparison, B7-1-deficient MRL-lpr/lpr mice had more severe IgG and C3 deposits in glomeruli. The Journal of Immunology, 2000, 165: 3436–3443.

Mice of the MRL lpr/lpr strain uniquely develop an autoimmune syndrome nearly indistinguishable from the human disease characterized by immune complex glomerulonephritis and a similar spectrum of autoantibodies, including anti-dsDNA and anti-small nuclear ribonucleoprotein (snRNP)1 (1). The spontaneous production of autoantibodies specific for snRNP and chromatin (DNA and histone) reflects the abnormal regulation of B cells in MRL-lpr/lpr mice (1, 2). These specific autoantibodies are accompanied by a polyclonal increase in serum Ig, particularly of the IgG2a isotype, which implies a T cell-dependent response in a manner analogous to immunity to foreign Ags (3). Anti-DNA Ab of the IgG2a isotype plays an important role in both vasculitis and glomerulonephritis (4).

A large number of investigations have established that CD4+ T cells are required for the development of autoantibodies and end organ disease in MRL lupus. Such studies have included treatment by neonatal thymectomy, anti-Thy-1.2 Ab, anti-CD4 Ab, anti-CD3 Ab, FK506, and cyclosporin A, as well as the genetic elimination of CD4 T cells via MHC class II deficiency and CD4 deficiency: T cell activation status.

Activation of CD4+ T cells requires two signals, an Ag-specific signal through the TCR and a second costimulatory signal between the T cell and APC. TCR ligation in the absence of a costimulatory signal can result in anergy or cell death (12). The major costimulatory molecules expressed by APCs are B7-1 and B7-2, which bind to CD28 and/or CTLA-4 expressed on T cells (12). A requirement for T cell costimulation in the development of autoimmunity has been observed in several animal models. For example, treatment with CTLA-4-Ig, a fusion protein which blocks both B7-1 and B7-2, suppresses the development of spontaneous lupus autoimmunity in NZB/NZW F1 mice, nonobese diabetic (NOD) mice, as well as experimentally induced autoimmune encephalomyelitis (EAE) (13–15).

However, the role of B7-1 or B7-2 as individual costimulatory ligands in the development of autoimmunity is not as clear. For example, the treatment of animals with anti-B7-1 inhibited the development of EAE, whereas anti-B7-2 exacerbated the severity of disease (16). In contrast, anti-B7-2 suppressed and anti-B7-1 accelerated the development of diabetes in NOD mice (14). Autoimmunity in the NZB/NZW mouse in unaffected by inhibitors of either B7-1 or B7-2 individually (17).

The current studies were designed to investigate the role of B7 costimulation in a murine model of human systemic lupus erythematosus (SLE). We used two approaches to interfere with the biological functions of the B7 molecule in MRL-lpr/lpr mice. First, mice were treated with anti-B7-1 and/or anti-B7-2 blocking Abs throughout the development of disease. Second, parallel studies were performed with B7-1- or B7-2-deficient mice backcrossed into the MRL-lpr/lpr background. Mice were examined for the presence of autoantibody specificity, titers, and isotypes as well as T cell activation status.

Our previous data demonstrated that the blocking of B7-1 alone by Ab treatment or the genetic deletion of either B7-1 or B7-2 did not significantly ameliorate the course of spontaneous lupus autoimmunity, however, blocking B7-1 alone significantly inhibited anti-dsDNA autoantibody while having no effect on anti-snRNP production (18). Moreover, blocking both B7-1 and B7-2 at the...
same time by Ab treatment significantly suppressed anti-snRNP and anti-dsDNA autoantibody production but it did not completely eliminate autoantibodies (18). In the present studies, we have demonstrated that Ig isotype switching occurs in anti-B7-1/B7-2-treated and in B7-1- or B7-2-deficient mice. However, significantly different immunologic and pathologic outcomes were observed between anti-B7-1/B7-2-treated and between B7-1 and B7-2 genetic-deficient mice. Combined Ab treatment reduced but did not eliminate the production of anti-snDNA IgG1, IgG2a, and IgG3 isotypes. In contrast, B7-1- or B7-2-deficient mice made anti-snDNA autoantibodies with isotypes similar to wild-type animals. Furthermore, our observations suggest that B7-1 and B7-2 have unique roles relative to the autoimmune pathology observed in MRL-lpr/lpr mice. An understanding of the inductive phase of autoimmunity and the breakdown of immunologic tolerance will be crucial for understanding both the genesis and the therapeutic intervention of autoimmune diseases such as SLE.

Materials and Methods

Anti-B7-1/B7-2 Ab treatment

Anti-B7-1/B7-2 Ab treatment protocols for MRL-lpr/lpr mice were based on previous studies for which efficacy in the treatment of EAE and diabetes was observed (13, 14, 16). The duration of Ab treatment lasted from 10–20 wk, with a minimum of 10 mice in each treatment group. MRL-lpr/lpr mice (5–8 wk old; The Jackson Laboratory, Bar Harbor, ME) were injected with: 1) control mAb (2–4A1), 2) rat anti-B7-1 (1G10; IgG2a), 3) rat anti-B7-2 (2D10, IgG1), and 4) anti-B7-1 together with anti-B7-2 mAbs three times a week at the dose of 100 µg/mouse, i.p., over the entire course of the study. Serum samples were collected every 2–3 wk, and examined for the presence of autoantibodies in indirect immuno-fluorescence (ANA) and by ELISA for anti-snDNA and anti-snRNP as described below.

Genetic deficient mice

B7-1- and B7-2-deficient mice on a 129 and BALB/c background, respectively, were derived as previously reported (19) and backcrossed to the third generation with MRL-lpr/lpr mice (The Jackson Laboratory, Bar Harbor, ME). The intercrossed F2 generation was used in the present experiments. PCR analysis was performed to confirm B7-1-deficient, B7-2-deficient, and lpr/lpr genotypes. As controls, heterozygous B7-1-deficient or B7-2-deficient, MRL-lpr/lpr F2 mice, and wild-type MRL-lpr/lpr mice were examined in parallel studies. Serum samples were collected every 2 wk starting from week 5 to 6, and analyzed for the presence of anti-snRNP and anti-snDNA autoantibodies. Individual Ig isotype analysis of anti-snRNP and anti-snDNA was additionally performed on the samples as described in detail below. Kidney pathology was also examined at the indicated time points as detailed below.

Indirect immuno-fluorescence (ANA)

Indirect immuno-fluorescence assays were performed using commercially available cell substrates (Quidel, San Diego, CA). In brief, 30 µl of a 1:40 dilution of serum were placed on slides coated with human epithelial cells (HEp-2) and incubated at room temperature for 1 h. After a 5-min wash in PBS-Tween (0.1%), FITC-conjugated anti-mouse IgG (Sigma) was applied to individual wells and incubated in the dark at room temperature for 1 h. After another 5-min wash, wells were examined by UV-fluorescence microscopy.

Anti-snRNP and anti-snDNA Ig isotype analysis

The spontaneous production of autoantibodies may reflect the abnormal function of B cells in MRL-lpr/lpr mice. These specific autoantibodies are accompanied by a polyclonal increase in serum Ig, particularly of the IgG2a isotype. To determine the dominant subclass of autoantibody in MRL-lpr/lpr mice, anti-snRNP and anti-dsDNA IgM and four anti-snRNP and anti-dsDNA IgG isotypes, IgG1, IgG2a, IgG2b, and IgG3 were measured by ELISA (using the commercially available ImmunoPure Monoclonal Ab Isotyping Kit; Pierce, Rockford, IL). In brief, native snRNP Ag was coated on U-shape vinyl plate (Costar, Cambridge, MA) overnight at 4°C. Plates were incubated with 5% BSA/PBS followed by a 10–2 dilution of serum samples incubated at room temperature for 2 h. Rabbit anti-mouse IgM, IgG1, IgG2a, IgG2b, and IgG3 Abs were then added to the plate followed by alkaline phosphatase-conjugated goat anti-rabbit Ab. Plates were finally incubated with pNPP substrate (Sigma, St. Louis, MO), and OD (405 nm) was measured at various time points (Tiettek Multiskan, Tiettek, Finland). Experimental values from separate experiments were normalized to a single MRL-lpr/lpr positive control serum used in every assay (arbitrarily defined as 100 U).

Flow cytometry analysis of T cell activation status

Mice were sacrificed at indicated time points (see figure legends) and spleens were removed to cold Click’s medium supplemented with 5% FBS (Gemini Bio-Products, Calabasas, CA), 10 mg/ml gentamicin, 2 mM l-glutamine, 0.1 mM 2-ME. RBCs were lysed in buffer (Sigma) on ice for 5 min. Splenocytes were stained with optimal concentrations of fluorescent-conjugated mAbs (2×10^6 cells in 200 µl of PBS, 1% BSA, and 0.1% sodium azide in U-shaped microtiter plates at 4°C for 30 min), and fixed with 1% paraformaldehyde. Samples were analyzed on a FACScaliber instrument (Becton Dickinson, Mountain View, CA). The following commercially available mAbs were used for analysis of T cell activation markers: anti-CD62L PE, anti-CD44 Cy-Chrome, anti-CD4 FITC, and anti-CD8 FITC (PharMingen, San Diego, CA).

Immunofluorescent evaluation of IgG, IgM, and C3 depositions in kidney

Kidney halves were fixed in a 0.7% paraformaldehyde-lysine-periodate (PLP) solution overnight at 4°C. Then, each sample was incubated in a 30% sucrose phosphate buffer at room temperature for ≥2 h and frozen in OCT compound (Sakura Finetek USA, Torrance, CA). Thereafter, kidney sections were stained by ELISA (using the commercially available ImmunoPure Monoclonal Ab Isotyping Kit; Pierce, Rockford, IL). In brief, native snRNP Ag was coated on U-shape vinyl plate (Costar, Cambridge, MA) overnight at 4°C. Plates were incubated with 5% BSA/PBS followed by a 10–2 dilution of serum samples incubated at room temperature for 2 h. Rabbit anti-mouse IgM, IgG1, IgG2a, IgG2b, and IgG3 Abs were added to the plate followed by alkaline phosphatase-conjugated goat anti-rabbit Ab. Plates were finally incubated with pNPP substrate (Sigma), and OD (405 nm) was measured at various time points (Tiettek Multiskan). Experimental values from separate experiments were normalized to a single MRL-lpr/lpr positive control serum used in every assay (arbitrarily defined as 100 U).

Statistical analysis

Student’s t test was performed using statistic analysis tools in MS EXCEL program to compare the significant difference between two groups. p < 0.05 is considered significant.

Results

Antinuclear Ab (ANA) production

After 5–14 wk of anti-B7-1, anti-B7-2, or both anti-B7-1/B7-2 treatment (three treatments a week; minimum 10 mice in each

Table 1. ANA response in anti-B7-treated and B7-deficient MRL-lpr/lpr mice

<table>
<thead>
<tr>
<th>Wild-type MRL-lpr/lpr</th>
<th>Ab Treatment</th>
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* Assayed at age 5–6 wk (prior to Ab treatment) or at the same age in knockout mice.
* Frequencies determined at endpoint, 5–14 wk for Ab-treated mice and 22 wk for B7-1- or B7-2-deficient mice.
(treatment group), all of the control Ab-treated and anti-B7-1- and/or anti-B7-2 Ab-treated mice showed positive antinuclear Ab (ANA) responses by immunofluorescence (Table I). The mice treated with both anti-B7-1 and anti-B7-2 Abs also showed positive ANA responses, suggesting that Ab treatment did not completely eliminate autoantibody production (Table I). The spectrum of ANA patterns were primarily of a homogeneous nuclear pattern, consistent with anti-DNA autoantibodies, and a speckled nuclear pattern, typically associated with autoantibodies to the snRNP complex. A smaller frequency of sera revealed a nucleolar-staining
pattern although all three patterns were observed in anti-B7-1/B7-2-treated mice or in wild-type MRL-lpr/lpr mice (18). B7-1- or B7-2-deficient MRL-lpr/lpr mice followed a virtually identical progression of autoantibody response throughout their lifetime. All groups of mice showed negative or very weak ANA response by immunofluorescence at early ages (5–6 wk old), but progressed to a stronger positive response by 9 wk of age with the highest ANA titers observed as the mice reached older ages (22 wk old; Table I). There was no significant difference in the onset or development of ANA between the homozygous and heterozygous B7-1- or B7-2-deficient MRL-lpr/lpr mice. These results indicate that blocking both B7-1 and B7-2 molecules with specific Ab or completely eliminating either B7 molecule did not significantly alter the overall titers of autoantibodies in MRL-lpr/lpr mice although the resulting pathologies were significantly different as described below.

**Anti-snRNP and anti-dsDNA Ig isotypes**

An extensive analysis of anti-snRNP and anti-dsDNA autoantibody isotypes in anti-B7-1/B7-2-treated mice and B7-1/B7-2-deficient mice was performed since studies in nonautoimmune mice have demonstrated that the absence of B7 molecules greatly affects the formation of germinal centers and Ab class switching (20).

Our first Ab experiment showed that IgG1, IgG2a, IgG2b, IgG3, and IgM autoantibodies specific for snRNPs were significantly elevated over time by anti-B7-1 treatment (Fig. 1A). Simultaneous anti-B7-1 and anti-B7-2 Ab treatment significantly suppressed the production of IgG1, IgG2a, and IgG2b autoantibodies specific for dsDNA, and IgG2b autoantibody specific for snRNP as compared with control Ab treatment (Fig. 1, B and C). Coadministration of anti-B7-1/B7-2 Abs also significantly lowered the production of IgG1 and IgG2b autoantibodies specific for snRNP as compared with control Ab treatment (Fig. 1, B and C). Anti-B7-2 Ab treatment significantly lowered the levels of IgG2a, IgG3, and IgM autoantibodies specific for snRNP as compared with anti-B7-1 Ab treatment (Fig. 1C).

B7-1- or B7-2-deficient mice developed a very similar pattern of Ig isotypes specific for snRNP as compared with heterozygous B7-1- or B7-2-deficient MRL-lpr/lpr mice. However, B7-2-deficient mice had significantly higher anti-snRNP IgM level than B7-1-deficient mice (Fig. 2A). Anti-dsDNA IgG2a, IgG2b, and IgG3 autoantibodies in B7-2-deficient mice were surprisingly higher
than that in B7-1-deficient mice, despite the fact that the B7-2-deficient mice had much milder kidney pathology (Fig. 2B). These observations imply that pathogenic anti-dsDNA specificities may differ between the groups (i.e., B7-2-deficient vs wild-type mice) although the overall titers of anti-dsDNA Abs may be similar.

Surface expression of T and B cell activation markers

Surface expression of CD62L, CD44 on CD4 and CD8 T cells were examined by flow cytometry to determine the T cell activation status in B7-1- or B7-2-deficient mice. We examined the mice during 8–16 wk old, MRL-lpr/lpr wild-type mice possess large population of splenic T lymphocytes of an activated (CD44high, L-selectinlow) and memory (CD44highCD62low) phenotype, although the role of the B7 costimulatory molecules in this phenotype is not known. CD4 and CD8 T cells from B7-1-deficient mice demonstrated similar populations of activation markers as compared with that of the wild-type mice at all ages (data not shown). However, B7-2-deficient mice expressed significantly lesser populations of activated T cells (13% and 17% in CD4 and CD8 T cells, respectively) and of memory T cells (2% in both CD4 and CD8 T cells) as compared with that of the wild-type mice (23% and 29% activated T cells and 76% and 69% memory T cells in CD4 and CD8 T cells, respectively) (Fig. 3). B7-2-deficient mice showed fewer cells counted due to the tremendous amount of CD4+CD8+ cell population (data not shown). Furthermore, surface expression of the B7 molecules was also examined to investigate whether the expression of B7-2 is altered in the absence of the B7-1 molecules, (or, conversely, if B7-1 levels are altered in the absence of B7-2). The data indicated that splenocytes of some homozygous B7-1-deficient mice may express B7-2 at a higher level as compared with heterozygous mice (Fig. 4). Conversely, splenocytes from some homozygous B7-2-deficient mice also expressed higher level of B7-1 as compared with heterozygous mice (Fig. 5). This difference may indicate that there is alternative compensatory mechanisms for B7-1 and B7-2 expression at the cell surface. B7 expression was not examined on T cell populations although some possibility exists for their role in regulating immune responses.

IgG, IgM, IgG isotypes, and complement C3 deposition in kidney

As the kidney pathology in B7-1 and B7-2 single-deficient mice was so distinctively different despite of their similar levels of anti-snRNP and anti-dsDNA autoantibodies (18), we next examined specific Ab deposition in the kidneys. Immunofluorescence staining revealed that MRL-lpr/lpr wild-type mice had significant granular peritubular, peripheral capillary wall, and mesangial IgG and IgM deposition in the kidney (Fig. 6, Table II). B7-1-deficient mice also had granular mesangial and peripheral capillary wall IgG and IgM deposition. The B7-2-deficient mice only had short linear mesangial IgG and IgM deposition and lacked peritubular and peripheral capillary wall deposition with less severe Ab deposition than the B7-1-deficient mice. We also stained the kidneys for IgG1, IgG2a, IgG2b, IgG3, and complement C3 deposition. Mesangial and peripheral capillary wall deposition with IgG1, IgG2a, IgG2b, and IgG3 were found in wild-type MRL-lpr/lpr mice, whereas only peripheral capillary wall and mesangial IgG1 and IgG2a deposition in B7-1-deficient mice and mesangial IgG2a and IgG3 deposition were found in B7-2-deficient mice. Complement C3 was found in wild-type, B7-1-deficient, and B7-2-deficient MRL-lpr/lpr mice, however, the wild-type and B7-1-deficient mice had similar peripheral capillary wall and mesangial patterns and more severe deposition of IgG2a and C3 as compared with the B7-2-deficient mice (Fig. 6, Table II). These observations are consistent with the more severe glomerulonephritis found in B7-1-deficient mice.

Discussion

The requirement for costimulation in the development of autoimmune diseases has been demonstrated in several experimental models using CTLA-4-Ig or a combination of anti-B7-1 and anti-B7-2 to block the B7-1 and B7-2 costimulatory molecules (13, 17). However, treatment with anti-B7-1 or anti-B7-2 Ab alone resulted in either exacerbation or amelioration of disease severity, depending upon the model of autoimmunity studied (14–17). In the current studies, blocking both B7-1 and B7-2 using Abs significantly inhibited anti-snRNP and anti-dsDNA autoantibody production in MRL-lpr/lpr mice, whereas anti-B7-2 Ab significantly reduced anti-dsDNA autoantibody production. Importantly, Ab treatments did not completely eliminate autoantibody production. B7-1 or B7-2 single-deficient mice developed high titers of autoantibodies over time, although the B7-2-deficient mice exhibited less severe kidney pathology. This observation raised a question as to why the B7-2-deficient mice developed higher overall titers of autoantibodies but less severe kidney damage? It is likely that autoantibody specificity plays an important role in the development of pathology and follow-up studies of clonal autoantibody populations are underway to try to address this question.

The results from this study are in contrast to those from a previous study in lupus-prone NZB/NZW F1 mice in which both anti-B7-1 and anti-B7-2 mAbs were able to prevent the development and progression of lupus (17). B7-2 was hypothesized to provide a more critical role in Th2-mediated cytokine production (17). In another study, CTLA-4-Ig suppressed the lupus-like illness in the
NZB/NZW F1 mouse model and prolonged life even when the treatment was administered late in disease (13). Mice treated with CTLA-4-Ig did not make Abs to dsDNA at any time during the course of treatment, including the 3 mo of observation after treatment was stopped. Suppression of autoantibody production was accompanied by a significant reduction in the severity of lupus nephritis. The distinct difference among these studies and our current studies suggests that B7 costimulatory signals (or the absence thereof) are unique in MRL-\(lpr/lpr\) spontaneous lupus. Furthermore, B7-1 and B7-2 probably play distinct roles in autoantibody specificity and severity of kidney pathology. Clearly, the generation of autoantibodies in MRL-\(lpr/lpr\) mice is unaffected by the absence of either B7-1 or B7-2.

Non-autoimmune mice deficient in both B7-1 and B7-2 fail to generate Ag-specific IgG1 and IgG2a responses and lack germinal centers under all conditions of immunization, including immunization in CFA (20). In addition, nonautoimmune B7-2-deficient mice immunized intraperitoneally without adjuvant showed a delay in the kinetics of IgG responses. When immunized i.v. without adjuvant, there was a complete failure to switch Ab isotypes, a 90% reduction in the number of Ab producing cells, and no germinal center formation. In our studies with MRL-\(lpr/lpr\) mice, treatment with anti-B7-1 or anti-B7-1 alone or together did not prohibit Ig class switching. However, anti-B7-2 treatment did suppress the levels of IgG1, IgG2b, and IgG3 specific for snRNP while having no effect on the production of IgG2a and IgM specific for the snRNP autoantigen. Anti-B7-1 plus anti-B7-2 treatment significantly suppressed IgG1, IgG2a, and IgG2b specific for dsDNA whereas not affecting the production of IgG3 specific for dsDNA. These data suggest that Th1 mediated IgG2a responses may be inhibited only if both B7-1 and B7-2 are blocked. Recall that IgG2a isotypes of anti-DNA autoantibodies have previously been shown to elicit pathology in MRL-\(lpr/lpr\) models of SLE (4).

The different outcomes observed between nonautoimmune and MRL-\(lpr/lpr\) mice indicate that there must be fundamental differences in the immune responses or regulation in the MRL-\(lpr/lpr\) strain. One possible explanation could be that the autoreactive T and B cells in MRL-\(lpr/lpr\) mice have a lower threshold for activation and therefore could be stimulated by weaker or delayed alternative costimulatory signals in the presence of autoantigen. Historically, B7-CD28 costimulation has been considered an essential component of the signaling process required for T cell proliferation and IL-2 production. Subsequently, evidence has emerged which suggests that the requirement for B7-CD28 costimulation may not be absolute in all immune responses. Ags with high affinity for TCR or in high concentration on the surface of APCs may require little or no costimulation. For example, TCR transgenic CD4\(^+\) T cells from CD28-deficient mice are able to proliferate and produce IL-2 at high concentrations of antigenic peptide (21). Furthermore, one study demonstrated that the number of IL-2-producing cells increased as the concentration of stimulatory peptide increased within a clonal Th1 population (22). These results demonstrated a quantitative requirement of B7-mediated costimulation on a T cell clone (12). A less number of TCRs had to be triggered for a response to be generated by a given concentration of Ag (12).

The fact that anti-snRNP and anti-dsDNA autoantibodies were significantly inhibited in the anti-B7-1- and anti-B7-2-treated mice but not in B7-1- or B7-2-deficient mice may indicate that B7-1 or B7-2 has the ability of compensate for each other in the absence of the other B7 molecule. Previous studies showed that optimal concentrations of either B7-1- or B7-2-deficient APCs stimulated OVA-transgenic T cells to proliferate and produce cytokines (23). Our studies also showed an elevation of B7-2 and B7-1 in the

![FIGURE 4](http://www.jimmunol.org/)

**FIGURE 4.** Splenic B7-1 and B7-2 expression in wild-type, B7-1-deficient homozygous, and heterozygous MRL-\(lpr/lpr\) mice. Mice (8–16 wk of age) of each group were examined. Total splenocytes were size-gated on lymphocytes before displaying B7-1 and B7-2 profiles. Homozygous B7-1-deficient mice exhibit higher B7-2 expression as compared with heterozygous mice. Data are representative of the analysis of 10 mice ages 8–16 wk.

![FIGURE 5](http://www.jimmunol.org/)

**FIGURE 5.** Splenic B7-1 and B7-2 expression in wild-type, B7-2-deficient homozygous, and heterozygous MRL-\(lpr/lpr\) mice. Mice (8–16 wk of age) of each group were examined. Total splenocytes were size-gated on lymphocytes before displaying B7-1 and B7-2 profiles. Homozygous B7-2-deficient mice have higher B7-1 expression as compared with heterozygous mice. Data are representative of the analysis of 10 mice aged 8–16 wk.
B7-1- and B7-2-deficient mice, respectively, as compared with control mice.

In addition, there may be other alternative costimulation pathways for T cell activation in the MRL mouse model. One possible pathway that may bypass or compensate for the B7-1/7-2-CD28/CTLA-4 pathway is CD40-CD40L pathway. Mice deficient in CD40L fail to develop normal T cell-dependent humoral immune responses, Ig isotype switching, and germinal center formation. In murine lupus and autoimmune oophoritis, complete long-term suppression of disease was only observed with the inhibition of both the CD28-B7 and CD40-CD40L pathways (24). However, in experimental autoimmune thyroiditis, treatment of recipients with a combination of anti-B7-1, anti-B7-2, and anti-CD40L did not further suppress disease development as compared with the treatment of anti-B7-1 and anti-B7-2 without anti-CD40L (25). This indicates that there may be an alternative pathway that could compensate for the lack of B7-CD28 or CD40-CD40L costimulation.

Two recent studies identified new members of the costimulatory molecule family, inducible costimulator (ICOS), which resembles CD28 in potency, and B7 h, which is a close homologue of B7-1 and B7-2 (26, 27). ICOS enhances all basic T cell responses to a foreign Ag, namely proliferation, secretion of lymphokines, up-

-regulation of molecules that mediate cell-cell interaction, and effective help for Ab secretion by B cells. Unlike the constitutively expressed CD28, ICOS has to be de novo induced on the T cell surface, does not up-regulate the production of IL-2, but superinduces the synthesis of IL-10, a B cell differentiation factor. B7 h shares only 20% amino acid identity with B7-1 and B7-2. It is a transmembrane molecule highly expressed in B cells, peripheral lymphoid and nonlymphoid tissues but not in T cells. This molecule costimulates T cell proliferation via a CD28-independent mechanism and it can be rapidly induced and up-regulated by TNF-α through activation of proinflammatory NF-κB/Rel transcription factors. It is plausible that ICOS and B7 h may be major regulators of the adaptive immune response and might contribute to the autoantibody production in MRL-lpr/lpr mice.

Based on prior studies, we anticipated that interfering with the biological function of B7-2 should impede isotype switching of autoantibodies in spontaneous disease (20). This notion is based on the absence of isotype switching or germinal center formation after i.v. immunization of B7-2-deficient mice or in mice treated with anti-B7-2 blocking Abs (20, 23). We have found that isotype switching of anti-DNA and anti-snRNP indeed occurred in both B7-1-deficient and B7-2-deficient MRL-lpr/lpr mice. The ability to develop mature autoantibody subsets in these mice may be due to the strength of TCR signaling although the source of endogenous autoantigen and its avidity for TCR in MRL-lpr/lpr mice is not known.

Although titers and isotypes of autoantibodies from B7-2-deficient mice resembled those of wild-type mice, overall kidney pathology, IgG and IgM deposition were generally less severe in the absence of B7-2. Studies are underway to examine the fine-specificity (via sequencing of variable regions) of anti-dsDNA Abs from wild-type and B7-deficient mice. Cationic, complement fixing anti-DNA isotypes may be essential for tissue damage (4, 29, 30).

Table II. Ig and complement C3 deposition in B7-deficient MRL-lpr/lpr mice

<table>
<thead>
<tr>
<th>MRL-lpr/lpr Mice</th>
<th>IgG</th>
<th>IgM</th>
<th>IgG2a</th>
<th>C3 Complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>B7-1 -/-</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>B7-2 -/-</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
</tbody>
</table>

Quantitation was based on a score of 0 as observed in nonautoimmune wild-type animal to 3+ as found in fulminant kidney disease in end-stage MRL-lpr/lpr mice.
Autoreactive CD4+ T cells can execute a number of direct effector functions to promote pathology such as mediating direct cytotoxicity to target organs (31), secreting inflammatory cytokines (33, 34), and forming memory T cells (35). Activated CD4+ T cells can be cytotoxic both via perforin and granzyme release (36, 37) and through Fas ligand expression (38). In addition, this cytokine and subsequent chemokine production (40, 41) also recruits secondary inflammatory cells (42), composed of CTL, dendritic cells, granulocytes, macrophages, and NK cells, which all are observed in the inflammatory infiltrate of diseased MRL kidneys (43). Indeed, T cells can initiate some forms of kidney pathology in B cell deficient MRL-lpr/lpr mice (43).

Whether B7-1 and B7-2 have identical, overlapping, or distinct functions still needs to be further investigated. Some investigators have reported differences in CD4+ T cell cytokine profiles in the selective presence of one B7 molecule vs the other, whereas other investigators find no differences (12). Putative differences in function between B7-1 and B7-2 could potentially be due to differences in the timing and level of expression as well as differences in the strength or quality of the signal delivered upon binding to CD28 and CTLA-4. It is clear from our studies that a greater understanding of the role of B7-mediated costimulation in lupus autoimmunity may be required to fully appreciate the potential therapeutic benefits of manipulating costimulatory pathways.

Acknowledgments
We thank Renelle J. Gee for technical assistance and Drs. Denise Faherty and Nasrin Nabavi of Hoffmann-La Roche for the generous gift of anti-B7.1 and anti-B 7.2 Abs.

References
6. Seaman, W. E., D. Wofsy, J. S. Greespan, and J. A. Ledbetter. 1983. Treatment of MRL/lpr/lpr mice treated with nonmitogenic anti-CD3 antibody functions to promote pathology such as mediating direct tissue damage directly (39). In addition, this cytokine and subsequent chemokine production (40, 41) also recruits secondary inflammatory cells (42), composed of CTL, dendritic cells, granulocytes, macrophages, and NK cells, which all are observed in the inflammatory infiltrate of diseased MRL kidneys (43). Indeed, T cells can initiate some forms of kidney pathology in B cell deficient MRL-lpr/lpr mice (43).

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