Costimulation by B7-1 and B7-2 Is Required for Autoimmune Disease in MRL- FasLR Mice

Koji Kinoshita, Greg Tesch, Andreas Schwarting, Ruth Maron, Arlene H. Sharpe and Vicki Rubin Kelley

J Immunol 2000; 164:6046-6056; doi: 10.4049/jimmunol.164.11.6046
http://www.jimmunol.org/content/164/11/6046

Why The JI?
- Rapid Reviews! 30 days* from submission to initial decision
- No Triage! Every submission reviewed by practicing scientists
- Speedy Publication! 4 weeks from acceptance to publication

References This article cites 46 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/164/11/6046.full#ref-list-1

Subscription Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Costimulation by B7-1 and B7-2 Is Required for Autoimmune Disease in MRL-Fas<sup>lpr</sup> Mice<sup>1</sup>

Koji Kinoshita,* Greg Tesch,* Andreas Schwarting,* Ruth Maron,† Arlene H. Sharpe,‡ and Vicki Rubin Kelley<sup>2</sup>

Autoimmune lupus nephritis is dependent on infiltrating autoreactive leukocytes and Igs. B7 costimulatory molecules (B7-1 and B7-2) provide signals essential for T cell activation and Ig class switching. In MRL-Fas<sup>lpr</sup> mice, a model of human lupus, although multiple tissues are targeted for autoimmune injury, nephritis is fatal. We identified intrarenal B7-1 and B7-2 expression, restricted to kidney-infiltrating leukocytes, before and increasing with progressive nephritis in MRL-Fas<sup>lpr</sup> mice. Thus, we hypothesized that the B7 pathway is required for autoimmune disease in MRL-Fas<sup>lpr</sup> mice. To investigate the role of B7 costimulatory molecules in this autoimmune disease, we generated a MRL-Fas<sup>lpr</sup> strain deficient in B7-1 and B7-2. Strikingly, MRL-Fas<sup>lpr</sup> mice lacking both B7 costimulators do not develop kidney (glomerular, tubular, interstitial, vascular) pathology, or proteinuria, and survive far longer. Intrarenal downstream effector transcripts (IFN-γ, IL-12, monocyte chemoattractant protein-1, CSF-1) linked to nephritis remained at normal levels compared with wild-type mice. Skin lesions and lymphoid enlargement characteristic of MRL-Fas<sup>lpr</sup> mice were diminished in B7-1/B7-2-deficient MRL-Fas<sup>lpr</sup> mice. B7-1/B7-2-deficient MRL-Fas<sup>lpr</sup> mice did not develop leukocytic infiltrates, elevated serum IgG and isotypes (G1,G2b,G3), autoantibodies, and intrarenal IgG deposits. Our findings demonstrate that B7-1 and B7-2 costimulatory pathways are critical to the pathogenesis of autoimmune lupus. The Journal of Immunology, 2000, 164: 6046–6056.

MRL-Fas<sup>lpr</sup> mice develop a systemic autoimmune disease sharing features with human lupus (1). Although pathology is evident in the skin, lungs, salivary and mandibular glands, and joints, kidney disease is the usual cause of death (2). Massive lymphadenopathy and splenomegaly result from the trafficking and accumulation of T cells within these lymphoid tissues, and T cell infiltrates are prominent in most vital tissues. Furthermore, there are notably high levels of circulating Igs, including a multitude of autoantibodies. The rapid tempo (50% mortality at 5.5 mo of age) and the predictability of these pathologic hallmarks makes this an attractive model in which to study the pathogenesis of autoimmune disease.

Kidney disease in the MRL-Fas<sup>lpr</sup> mouse is complex (3, 4). Renal pathology involves glomerular, tubular, interstitial, and vascular components. Each component is infiltrated by leukocytes, including T cells and macrophages. The T cells that accumulate in the kidney include CD4, CD8, and CD4<sup>+</sup> CD8<sup>+</sup>, B220 double negative (DN) T cells. Based on evidence using “knockouts” that deplete these T cell populations, it is clear that T cells are required for autoimmune disease (5–7). The T cell-mediated mechanism responsible for inciting kidney disease requires IFN-γ (8). IFN-γ, released from activated T cells within the kidney, triggers a cascade of cytokines (IL-12, CSF-1, TNF-α) culminating in tissue destruction (8–12). Thus, identifying the signals responsible for T cell activation and clonal expansion within tissues, such as the kidney, offers potential therapeutic targets for combating autoimmune tissue destruction.

The B7 family of costimulatory molecules, B7-1 (CD80) and B7-2 (CD86), regulates T cell activation, differentiation, and peripheral tolerance. The B7-1 and B7-2 costimulators have dual specificity for their ligands CD28 and CTL-associated molecule-4 (CTLA-4). The B7-CD28 interactions promote T cell growth, survival, and differentiation, while B7-CTLA-4 interactions provide a down-regulatory signal for T cell activation with the potential for regulating autoreactive T cells in the periphery (13). The kinetics of B7-1 and B7-2 expression are distinct: B7-2 is constitutively expressed at low levels on APC (i.e., macrophages, dendritic cells, and B cells) and is rapidly up-regulated on T cells and APCs in response to cytokines, activation signals, and infections. B7-1 expression is induced on activated APCs and T cells and is up-regulated later than B7-2. B7-1 and B7-2 have critical overlapping functions in regulating T cell cytokines and B cell Igs (14, 15). This overlap in function suggests that blocking both B7 costimulators (B7-1 and B7-2) may be a potential therapeutic strategy for progressive autoimmune diseases.

Blockade of the B7 pathway using receptor antagonists and Abs has provided insight into the importance of these costimulatory molecules in autoimmune disease (16–19). B7 costimulation may contribute to the development of systemic autoimmune disease in the MRL-Fas<sup>lpr</sup> mouse in several ways. B7 costimulation may be critical for activation of self-reactive T cells and the production of pathogenic cytokines and chemokines. B7 costimulation may also be essential for generating autoantibodies because B7 costimulators have an obligatory role in Ig class switching and germinal center formation (15). In this report, we investigate the importance of B7 costimulation in the pathogenesis of autoimmune disease in

*Laboratory of Molecular Autoimmune Disease, Renal Division, Department of Medicine, †Center for Neurological Disease, and ‡Immunology Research Division, Department of Pathology, Brigham and Women’s Hospital, Boston, MA 02115

Received for publication October 8, 1999. Accepted for publication March 16, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported in part by the National Institutes of Health Grant DK 36149 (to V.R.K.) and DK52369 (to V.R.K.) and AI 38310 (to A.H.S.).

2 Address correspondence and reprint requests to Dr. Vicki Rubin Kelley, Brigham and Women’s Hospital, Harvard Institutes of Medicine, 77 Avenue Louis Pasteur, Boston, MA 02115. E-mail address: VKelley@rics.bwh.harvard.edu

3 Abbreviations used in this paper: DN, double negative; gcs, glomerular cross-section; MCP, macrophage chemoattractant protein; TEC, tubular epithelial cell; ANA, antinuclear Ab.

Copyright © 2000 by The American Association of Immunologists
MRL-Faslpr mice through the generation and analysis of a B7-1/B7-2-deficient (−/−) MRL-Faslpr mouse strain. We investigated whether 1) B7-1/B7-2 is necessary for progressive, fatal autoimmune disease; 2) B7-1/B7-2 molecules mediate pathology in the kidney, lung, liver, skin, lymph nodes, and spleen; and 3) B7-1/B7-2 expression is required for T cell infiltration into the kidney and the other tissues targeted for destruction; and 4) B7-1/B7-2 molecules are required for the production of nephritogenic Igs.

We now report that B7 costimulatory molecules provide essential and proximal signals that are required for the development of autoimmune disease in MRL-Faslpr mice. B7-1 and B7-2 expressed by kidney-infiltrating leukocytes are detected before overt pathology and increase with progressive nephritis. This intrarenal B7 expression is critical for the activation of self-reactive T cells because B7-1/B7-2-deficient mice were entirely protected from fatal kidney disease. In the absence of B7 molecules, leukocytes did not accumulate in kidney and lungs and were reduced in lymphoid tissues. In addition, B7-1/B7-2-deficient MRL-Faslpr mice did not develop elevated IgG isotypes, nor autoantibodies characteristic of MRL-Faslpr mice. Thus, the B7 costimulatory pathways are critical in the pathogenesis of autoimmune lupus.

Materials and Methods

**Mice**

MRL/MpJ-Faslpr/Faslpr (MRL-Faslpr), MRL/MpJ +/+ (MRL +/+), and C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). B7-1/B7-2-deficient mice (129/SvS4Jae) were constructed as previously reported and maintained in our pathogen-free animal facility (15).

**Generating B7-1/B7-2-deficient MRL-Faslpr mice**

We constructed a B7-1/B7-2-deficient MRL-Faslpr strain using a backcross-intercross breeding scheme (8). MRL-Faslpr mice were mated with B7-1/B7-2-deficient mice to yield heterozygous F1 offspring. We crossed F1 mice and screened the progeny for the Faslpr (+/+) mutation and B7-1/B7-2 genes the progeny using tail genomic DNA amplified by PCR and identified with specific oligonucleotide primers. Specifically, we extracted DNA from mouse tails using the QIAamp tissue kit (Qiagen, Hilden, Germany). Tail DNA was assessed with primers recognizing the B7.1 gene (antisense, 5′-TGG GCC CCG TGA AAG GAC-3′; sense, 5′-TGA ACA ACT GTC CAA GTC AGT-3′) and B7.2 gene (antisense, 5′-CGA TCA CTG ACA GGT TTA-3′; sense, 5′-ACA TAA GCA TGT AGG TG-3′) (15). The Faslpr mutation was identified as previously reported (20). The progeny were backcrossed with the MRL-Faslpr strain. After three generations of backcross-intercross matings, we generated B7-1/B7-2-deficient MRL-Faslpr mice (94% MRL background). We analyzed B7-1/B7-2-deficient and intact MRL-Faslpr strains at three generations of backcross-intercross matings, because we have established that the tempo and expression of autoimmune disease and survival is similar to the wild-type MRL-Faslpr strain by the third backcross generation (8, 47). This was verified by comparing B7-1/B7-2−/− wild-type and B7-1B7-2−/− intact MRL-Faslpr mice on the third backcross generation for survival, kidney disease, and autoantibodies (see below). The B7-1/B7-2-deficient and intact MRL-Faslpr of the third generation are referred to hereafter as B7-1/B7-2−/− MRL-Faslpr and B7-1/B7-2−/− MRL-Faslpr, respectively.

**Proteinuria**

We assessed urinary protein levels monthly using albumin reagent strips (Albustix; Bayer Diagnostic Division, Elkhart, IN), and graded them semi-quantitatively (0, none; 1, 30–100 mg/dl; 2, 101–300 mg/dl; 3, 301–1000 mg/dl; 4, >1000 mg/dl). Each monthly value was determined by sampling and measuring urine on sequential days. In the event that these values differed (>5%), we repeated this process.

**Gross pathology**

We scored gross skin pathology in B7-1/B7-2−/− and B7-1/B7-2+/− MRL-Faslpr mice monthly. We evaluated lymphadenopathy comparing B7-1/B7-2−/− and B7-1/B7-2−/− MRL-Faslpr mice monthly using a scale of 0–3 assessing the number and size of palpable nodes (0, none; 1, one; 2, small to moderate; 3, three or more, moderate to large). Skin lesions, which consist of alopecia and scab formation, were scored from 0 to 3 based on the number of lesions and area (0, none; 1, one, <0.5 cm; 2, two or more, <0.5 cm; 3, multiple, >0.5 cm). Spleen enlargement was assessed at the time of sacrifice or death. We compared the spleen weights in the B7-1/B7-2−/− and B7-1/B7-2−/− MRL-Faslpr mice.

**Histopathology**

Kidneys were either snap-frozen in OCT compound (Miles Scientific, Naperville, IL) for cryostat sectioning or fixed in 10% neutral-buffered formalin. Formalin-fixed tissue was embedded in paraffin, and 4-μm sections were stained with hematoxylin and periodic acid Schiff and evaluated by light microscopy (21). We evaluated the glomerular, tubular, interstitial, and perivascular pathology morphometrically. The glomeruli were assessed by counting 50 glomerular cross-sections (gcs) per kidney and scoring each glomerulus on a semiquantitative scale: 0, normal (35–40 cells/gcs); 1, mild (few lesions with slight proliferative changes and hypercellularity) (41–50 cells/gcs); 2, moderate (moderate hypercellularity) (51–60 cells/gcs) segmental and/or diffuse proliferative changes, hyalinosis, and moderate exudate; 3, severe hypercellularity (>60 cells/gcs) with segmental or global sclerosis and/or severe necrosis, crescent formation, and heavy exudation. We evaluated tubular pathology by counting the percentage tubules that were damaged (dilation and/or atrophy and/or necrosis in 200 randomly selected tubules (magnification, ×400). We evaluated the interstitial pathology by counting the number of infiltrating cells in 20 random inter and intralobular arteries (magnification, ×400). The perivascular cell accumulation was determined by scoring the number of cell layers surrounding 10 random inter and intralobular arteries (score: 0, none; 1, <5 layers surrounding more than half of the vessel; 2, 5–10 layers surrounding less than half of the vessel; 3, >10 layers surrounding less than half the vessel). Scoring was evaluated using coded slides.

Lungs were fixed in formalin, sectioned (4 μm), stained with hematoxylin and eosin, and evaluated by light microscopy (22). The perivascular leukocyte infiltration was determined using a morphometric analysis. We measured the leukocyte infiltrates surrounding 10 random vessels (score: 0, none; 1, less than three layers surrounding <50% of more than six layers). Peribronchiolar leukocyte infiltration was determined by semiquantitative scoring the cells surrounding 10 random bronchii (score: 0, none; 1, less than three layers surrounding >50% bronchi; 2, three to six cell layers surrounding >50% bronchi; 3, more than six layers surrounding >50% bronchi).

**Identifying B7-1/B7-2 in kidney sections**

To evaluate the presence of B7-1 and B7-2, cryostat sectioned kidneys (4 μm) were fixed in ice-cold acetone for 10 min. We detected B7-1 and B7-2 in kidney sections by an indirect immunoperoxidase procedure using hamster anti-mouse B7.1 Ab (Teclon, TX) and rat antimouse B7.2 Ab (PharMingen) (5 μg/ml) as previously described (23). The specificity of the B7-1 and B7-2 Abs has been previously established (24). As negative controls we used B7-1/B7-2−/− kidneys and nonreactive Abs consisting of polyclonal hamster IgG (PharMingen) for B7-1 and rat anti-mouse IgG2a (PharMingen) for B7-2.

**Identifying kidney leukocytic infiltrates**

Cryostat-sectioned kidneys were stained for the presence of 1) macrophages with F4/80 hybridoma culture supernatant (HB1/18; American Type Culture Collection, Manassas, VA); 2) T cells with Abs to CD4, CD8, and Thy1.1; and 3) B cells with Abs to CD21/35 rat anti-mouse mAb (PharMingen) using immunoperoxidase procedures. We replaced the primary Ab with normal rat IgG as a specificity control. Macrophages and T cells within the kidney were enumerated and reported as cells/gglomeruli or cells/interstitial field as previously described (8).

**Identifying B7-1/B7-2 and cytokine/chemokine transcripts in the kidney**

Total RNA was extracted from the snap-frozen renal cortex of half a kidney using RNAzol B (Tel-Test, Friendswood, TX). A reverse transcription (RT) reaction was performed on this RNA using oligo(dT) and the Superscript II DNA preamplification kit (Life Technologies, Grand Island, NY). The resulting RT product was used as a cDNA template for PCR with

G. Tesch. Submitted for publication.
analysis. B7-1/B7-2 transcripts were measured by semiquantitative RT-PCR (15). Similarly, IFN-γ, macrophage chemoattractant protein (MCP)-1, IL-12, and CSF-1 expression were detected as 400-, 350-, 350-, and 245-bp PCR products, respectively (8, 11, 25, 26). GAPDH expression, the housekeeping gene, was detected as a 500-bp product resulting from PCR using specific oligonucleotide primers.

**B7-1/B7-2 in tubular epithelial cell (TEC)**

To determine whether TEC express B7-1/B7-2, TEC were isolated from MRL-\textit{Fas}^{lpr} and MRL-++ mice as previously described (27). TEC were grown on collagen-coated plates, cultured for 5–10 passages, and stimulated with IFN-γ (50 U/ml) or LPS (5 μg/ml) for 24 h. Total RNA was

![Figure 1](http://www.jimmunol.org/)

**FIGURE 1.** B7-1 and B7-2 costimulatory molecules are up-regulated during the progressive kidney disease in MRL-\textit{Fas}^{lpr} mice. A, B7-1 and B7-2 transcripts were increased in MRL-\textit{Fas}^{lpr} mice at 2, 4, and 6 mo of age and were increased as compared with C57BL/6 strain as determined by RT-PCR (*, \(p < 0.001; **, \(p < 0.05; n = 3/\text{group})). \text{Values are mean} ± \text{SD.} \text{B, B7-1 and B7-2 protein increased within the kidneys of MRL-\textit{Fas}^{lpr} mice from 2, 4, and 6 mo of age and were increased in comparison to the C57BL/6 strain as evaluated by immunostaining (grade: 0 (none) to 3 (maximum)) (*, \(p < 0.001; **, \(p < 0.005; n = 3/\text{group}). \text{Values are mean} ± \text{SD.} \text{C, B7-1 and B7-2 expression are most prominent in the areas surrounding glomeruli (long thick arrow), and it also was detected in the interstitium, and within glomeruli (long thin arrow) and is notably absent in TEC (arrowhead) in MRL-\textit{Fas}^{lpr} kidneys (magnification, ×400).}
isolated from stimulated and unstimulated TEC, and B7-1/B7-2 transcripts were measured by semiquantitative RT-PCR according to previously reported methods (15).

**Serum Igs**

To assess the serum Igs, we collected serum from B7-1/B7-2-deficient and intact MRL-Fas<sup>br</sup> mice at 5 mo of age (n = 8/group). We used an ELISA to measure total IgG and IgM and IgG isotypes including IgG1, IgG2a, IgG2b, and IgG3. Plates coated with goat anti-mouse Ig Ab (Southern Biotechnology Associates, Birmingham, AL) were developed with alkaline phosphatase-conjugated isotype-specific anti-Ig Abs (Southern Biotechnology Associates). We diluted the sera from 1:100 to 1:72,900 and analyzed using a fluorescence microscope and scored for intensity on a scale of 0 – 4 (0, none; 1, weak; 2, moderate; 3, strong; 4, very strong).

**IgG deposits within renal glomeruli**

Kidney cryostat cross-sections (4 μm thick) were incubated with fluorescein anti-mouse IgG (ICN Biomedicals, Costa Mesa, CA) and tited at dilutions of 1:1000, 1:5000, and 1:25000. Slides were analyzed using a fluorescence microscope at the lowest positive dilution (1:1000). The fluorescence intensity within the glomerular capillary walls were scored on a scale of 0 – 4 (0, none; 1, weak; 2, moderate; 3, strong). At least 10 glomeruli per section were analyzed. Values are recorded as mean/group ± SD.

**Results**

B7-1/B7-2 is expressed before renal injury in wild-type MRL-Fas<sup>br</sup> kidneys and rises with advancing disease

To determine whether B7-1 and B7-2 were up-regulated in wild-type MRL-Fas<sup>br</sup> kidneys, we evaluated MRL-Fas<sup>br</sup> mice during progressive renal injury (2, 4, and 6 mo of age). B7-1 and B7-2 transcripts were expressed before nephritis in MRL-Fas<sup>br</sup> mice. In contrast, neither B7-1 nor B7-2 were detectable in age- and sex-matched normal C57BL/6 kidneys (Fig. 1A). B7-1 and B7-2 transcripts continued to increase in MRL-Fas<sup>br</sup> mice with advancing age (Fig. 1A). Because the severity of renal pathology and loss of function in MRL-Fas<sup>br</sup> mice progressively increases from 2 to 6 mo of age, we conclude that B7-1 and B7-2 transcripts increased in proportion to the severity of kidney disease (28). In addition, B7-1 and B7-2 proteins increased within the MRL-Fas<sup>br</sup> kidneys at 4 and 6 mo of age, paralleling the rise in B7-1 and B7-2 transcripts (Fig. 1B). In contrast, normal C57BL/6 kidneys did not express B7-1/B7-2 protein (Fig. 1B). Using immunostaining, we localized B7-1 and B7-2 protein expression to glomerular (intra and peri), interstitial, and perivascular areas (Fig. 1C). B7-1 and B7-2 proteins were notably absent in kidney intrinsic cells, including TEC in MRL-Fas<sup>br</sup> mice (immunostaining). Furthermore, B7-1/B7-2 transcripts were not detected in isolated unstimulated or stimulated (LPS or IFN-γ) TEC derived from MRL-Fas<sup>br</sup> and congenic MRL++ mice using RT-PCR (Table I). It should be noted that B7-1 and B7-2 were not detected within the kidneys of the B7-1/B7-2<sup>−/−</sup> MRL-Fas<sup>br</sup> kidneys (negative control), nor using control Abs for B7-1 and B7-2, hamster anti-IgG and rat anti-mouse IgG2a, respectively. Thus, we concluded that the B7-1 and B7-2 in MRL-Fas<sup>br</sup> kidneys is restricted to kidney-infiltrating leukocytes.

**B7-1/B7-2<sup>−/−</sup> MRL-Fas<sup>br</sup> mice survive far longer than B7-1/B7-2<sup>−/−</sup> MRL-Fas<sup>br</sup> mice**

Survival in the B7-1/B7-2<sup>−/−</sup> MRL-Fas<sup>br</sup> female and male mice was dramatically extended as compared with B7-1/B7-2<sup>−/−</sup> MRL-

---

**Table 1. B7-1/B7-2 transcripts are not detected in isolated TEC of MRL mice**

<table>
<thead>
<tr>
<th>Strain of TEC</th>
<th>Stimulation</th>
<th>B7-1/GAPDH</th>
<th>B7-2/GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRL-Fas&lt;sup&gt;br&lt;/sup&gt;</td>
<td>none</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MRL-Fas&lt;sup&gt;br&lt;/sup&gt;</td>
<td>IFN-γ</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MRL-Fas&lt;sup&gt;br&lt;/sup&gt;</td>
<td>LPS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MRL++</td>
<td>none</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Evaluated by semiquantitative PCR. TEC were isolated and transferred to collagen-coated plates, cultured between the fifth and tenth passage, and stimulated with IFN-γ (50 U/ml) or LPS (5 μg/ml) for 24 h (n = 3). The entire kidney from the MRL-Fas<sup>br</sup> served as a positive control.*

---

![FIGURE 2.](http://www.jimmunol.org/...)

*FIGURE 2. B7-1/B7-2<sup>−/−</sup> MRL-Fas<sup>br</sup> mice survive longer than B7-1/B7-2<sup>−/−</sup> MRL-Fas<sup>br</sup> mice. At 8 mo of age, 60% of B7-1/B7-2<sup>−/−</sup> MRL-Fas<sup>br</sup> strain (n = 43) have not survived. By comparison, nearly all (91%) of the B7-1/B7-2<sup>−/−</sup> MRL-Fas<sup>br</sup> mice (n = 21) remained alive (p > 0.05).*
Furthermore, the urinary protein levels in the B7-1/B7-2−/− MRL-Faslopr mice that remained alive at 12–13 mo of age did not increase (1.2 ± 0.5, n = 12) as compared with earlier time points (Fig. 3A). Thus, the B7-1/B7-2−/− MRL-Faslopr strain is protected from proteinuria.

Gross pathology is reduced in B7-1/B7-2−/− MRL-Faslopr mice
Skin lesions, lymphadenopathy, and splenomegaly are gross pathologic features characteristic of systemic autoimmune disease in MRL-Faslopr mice. Gross pathologic skin lesions were reduced 2-fold in B7-1/B7-2−/− MRL-Faslopr mice at 4, 6, and 8 mo of age (⁎*, p < 0.05) but not normal mice (⁎, p < 0.001). In addition, lymphadenopathy was similarly 2-fold less in B7-1/B7-2−/− (n = 31–46) vs B7-1/B7-2−/− (n = 40–47) MRL-Faslopr mice at 4, 6, and 8 mo of age (⁎⁎⁎, p < 0.005). Finally, splenomegaly, determined by the spleen weight, was less in B7-1/B7-2−/− (n = 31–46) vs B7-1/B7-2−/− (n = 40–47) MRL-Faslopr strain at 5 mo of age (⁎⁎⁎, p < 0.05). However, the B7-1/B7-2−/− MRL-Faslopr spleens were larger than normal spleens (C57BL/6; #, p < 0.001; n = 4). Values are mean ± SD.
B7-1/B7-2^{−/−} MRL-Fas^{−/−} mice (n = 31–46/group) as compared with B7-1/B7-2^{+/−} MRL-Fas^{−/−} mice (n = 40–47) evaluated at 4, 6, and 8 mo of age (p < 0.001) (Fig. 3B). Similarly, lymphadenopathy was 2-fold less in B7-1/B7-2^{−/−} MRL-Fas^{−/−} mice (n = 40–47) as compared with B7-1/B7-2^{+/−} MRL-Fas^{−/−} mice (n = 31–46) at 6 or 8 mo of age, respectively (p < 0.005) (Fig. 3C). And the spleen size was 2-fold smaller in B7-1/B7-2^{−/−} MRL-Fas^{−/−} mice (n = 8) as compared with the B7-1/B7-2^{+/−} MRL-Fas^{−/−} mice (n = 8; p < 0.05). However, B7-1/B7-2^{−/−} MRL-Fas^{−/−} mice were not totally protected from an increase in lymphocytes accumulating in the lymph nodes and spleens (histologic and flow cytometric analysis, data not shown), because these lymphoid tissues were larger than normal (C57BL/6 strain) (n = 8; p < 0.01; Fig. 3D).

B7-1/B7-2-deficient MRL-Fas^{−/−} are protected from renal disease

Renal disease including glomerular, tubular, and vascular pathology was entirely prevented in B7-1/B7-2^{−/−} MRL-Fas^{−/−} mice. We compared renal disease in groups of B7-1/B7-2^{−/−} and B7-1/B7-2^{+/−} MRL-Fas^{−/−} strains at 5 mo of age (Figs. 4A and 5). B7-1/B7-2^{−/−} MRL-Fas^{−/−} mice at 5 mo of age had 1) glomerular pathology consisting of hypercellularity, hyalinosis, and moderate exudates; 2) tubular pathology consisting of dilation, atrophy, and/or necrosis in 20% of tubules; 3) leukocytes in the interstitium (80 cells/field); and 4) leukocytes in the surrounding vessels (5–10 layers surrounding majority of vessel). By comparison, renal glomeruli, tubules, and the interstitium and vasculature in the B7-1/B7-2^{−/−} MRL-Fas^{−/−} mice remained normal. Magnification, ×400, periodic acid Schiffs staining.

FIGURE 5. Photomicrographs of B7-1/B7-2^{−/−} MRL-Fas^{−/−} mice that are protected from kidney disease. B7-1/B7-2^{+/−} MRL-Fas^{−/−} mice had sclerotic glomeruli (short, wide arrow), interstitial leukocytic infiltrates (long arrow), and tubular atrophy and casts (star). By comparison, the kidneys of the B7-1/B7-2^{−/−} MRL-Fas^{−/−} mice remained normal. Magnification, ×400, periodic acid Schiffs staining.
to this age (Fig. 4A). As an aside, the extent of renal pathology declined in B7-1/B7-2 intact MRL-Fas\(^{br}\) groups at 13 mo as compared with 5 mo of age. This decline in renal pathology is a result of selecting for mice that survive longer (>28%) and hence have a milder disease. We also examined four of the six B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice that did not survive, and their kidneys remained histologically normal (data not shown). Thus, MRL-Fas\(^{br}\) mice lacking B7-1/B7-2 are totally protected from renal disease. In fact, other than a mild increase in the spleen and lymph nodes size, tissues (lung, liver) of these mice were spared from the pathological changes characteristic of MRL-Fas\(^{br}\) mice. Therefore, we were unable to determine the exact cause of death of the few B7-1/B7-2-deficient MRL-Fas\(^{br}\) mice that did not survive.

**Interrenal cytokines/chemokines that are up-regulated and promote kidney disease in MRL-Fas\(^{br}\) kidneys are not increased in B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice**

We previously established that CSF-1, IFN-\(γ\), MCP-1, and IL-12 are up-regulated in the kidney in advance of injury and increase with progressive renal damage in MRL-Fas\(^{br}\) mice (8, 11, 31, 32).

In addition, we have established that provision of CSF-1, IFN-\(γ\), or IL-12 via gene transfer fosters an influx of distinct leukocytic phenotypes that incite kidney injury (11, 32, 33), while removing CSF-1, IFN-\(γ\), IL-12, or MCP-1 are not up-regulated in the B7-1/B7-2 intact MRL-Fas\(^{br}\) kidney at 5 mo of age (Fig. 6, A–D). In contrast, CSF-1, IFN-\(γ\), IL-12, and MCP-1 are not up-regulated in B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice and remain similar to age- and sex-matched normal C57BL/6 kidneys (Fig. 6, A–D). Taken together, we conclude the B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice are protected from kidney disease mediated by nephritogenic cytokines.

**An influx of leukocytes into the lungs is prevented in B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice**

In addition to the kidney, the lungs in MRL-Fas\(^{br}\) have a progressive influx of leukocytes surrounding the vasculature. Similar to the kidney, B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice did not have an accumulation of leukocytes in the perivascula, nor peribronchiolar areas at 5 mo of age as compared with B7-1/B7-2\(^{+/+}\) MRL-Fas\(^{br}\) mice (n = 8/group; *, p < 0.001) and remained similar to age- and sex-matched MRL++ mice with normal lungs (Fig. 7). Thus, the B7-1/B7-2 lungs are spared from leukocytic infiltrates.

**B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice do not develop elevated serum IgG levels (IgG1, IgG2b, IgG3)**

To determine whether B7-1/B7-2 are required for the increase in serum IgG, which is notable in the MRL-Fas\(^{br}\) serum, we evaluated serum of B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice and B7-1/B7-2\(^{+/+}\) MRL-Fas\(^{br}\) mice at 5 mo of age (Fig. 8). Total IgG, IgG1, IgG2b, IgG2a, and IgG3, and IgM were substantially elevated in the serum of B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice and were similar to age- and sex-matched wild-type MRL-Fas\(^{br}\) mice (n = 8/group; Fig. 8). In contrast, total IgG and IgG1, IgG2b, and IgG3 in B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice remained similar to C57BL/6 normal serum levels (n = 8/group; *, p < 0.01 and **, p < 0.005; Fig. 8). Notably, the levels of IgG2a in B7-1/B7-2\(^{−/−}\) and B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice were similar, and serum IgM was augmented in MRL-Fas\(^{br}\) mice lacking both B7-1 and B7-2 (p < 0.01; Fig. 8).

**ANA levels are drastically lower in the B7-1/B7-2\(^{−/−}\) vs B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) strain**

Elevated ANA are characteristic of wild-type MRL-Fas\(^{br}\) mice (5 mo of age) and are not detected in normal C3H/FeJ mice (n = 4; p < 0.025; Fig. 9). The B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice have a increase in serum ANA at 3 and 5 mo of age (n = 5 and 6, respectively) as compared with normal serum levels (C3H/FeJ mice; n = 3; p < 0.01). Serum ANA levels in B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) mice at 5 mo of age are similar to wild-type MRL-Fas\(^{br}\) mice (n = 4; Fig. 9). In contrast, the B7-1/B7-2\(^{−/−}\) MRL-Fas\(^{br}\) strain at 3 and 5 mo of age (n = 5 and 6, respectively) has...
4-fold less serum ANA than the B7-1/B7-2+/− MRL-Fas<sup>bm</sup> strain (n = 6/group; p < 0.01; Fig. 9). B7-1/B7-2+/− MRL-Fas<sup>bm</sup> serum ANA do not remain totally normal but rise modestly by 5 mo of age as compared with age- and sex-matched C3H-FeJ strain (p < 0.01; Fig. 9).

**Ig(IgG) deposition was prevented in the B7-1/B7-2+/− MRL-Fas<sup>bm</sup> kidneys**

IgG deposits in glomeruli of the wild-type MRL-Fas<sup>bm</sup> strain are notable at 5 mo of age as compared with normal glomeruli (C3H/FeJ) and reflects the extent of glomerular damage (n = 3/strain; p < 0.05). IgG deposits in the glomeruli of wild-type MRL-Fas<sup>bm</sup> mice and B7-1/B7-2+/− MRL-Fas<sup>bm</sup> mice 5 mo of age were similar (n = 6 and 3, respectively; Fig. 10). In contrast, the IgG deposits in the B7-1/B7-2+/− MRL-Fas<sup>bm</sup> glomeruli did not increase (n = 6; p < 0.01; Fig. 10) and remained similar to normal age- and sex-matched C57BL/6 glomeruli (n = 3).

**FIGURE 8.** The increase in serum total IgG, IgG1, IgG2a, and IgG3 is prevented in B7-1/B7-2+/− MRL-Fas<sup>bm</sup> mice. B7-1/B7-2+/− MRL-Fas<sup>bm</sup> have an increase in total IgG, IgG1, IgG2a, IgG3, and IgM, similar to the wild-type MRL-Fas<sup>bm</sup> strain, as compared with normal C57BL/6 mice (n = 8/group; *, p < 0.01; **, p < 0.005). Of note, the level of IgG2a in the B7-1/B7-2+/− MRL-Fas<sup>bm</sup> mice was not reduced, while the level of IgM was higher in the B7-1/B7-2+/− MRL-Fas<sup>bm</sup> strain as compared with the B7-1/B7-2+− MRL-Fas<sup>bm</sup> or wild-type MRL-Fas<sup>bm</sup> strains (+, p < 0.01). Ig levels measured by ELISA. Values are mean ± SD. Wild type+, MRL-Fas<sup>bm</sup> mice that have not been crossed with another strain.

**FIGURE 9.** ANA are prevented in the B7-1/B7-2+/− MRL-Fas<sup>bm</sup> mice. ANA in the B7-1/B7-2+/− MRL-Fas<sup>bm</sup> serum at 3 and 5 mo of age was prevented from rising as compared with B7-1/B7-2+/− MRL-Fas<sup>bm</sup> mice (n = 5 and 6, respectively; *, p < 0.01). By comparison, the B7-1/B7-2 intact MRL-Fas<sup>bm</sup> and the age- and sex-matched wild-type MRL-Fas<sup>bm</sup> mice had similar levels of serum ANA. It should be noted that there was a modest increase in serum ANA in B7-1/B7-2+/− MRL-Fas<sup>bm</sup> mice at 5, but not 3, mo of age, as compared with normal mice (n = 6 and 4, respectively; p < 0.01). Fluorescence was scored on a scale of 0 (none) to 4 (very high). Values are mean ± SD; *, p < 0.01; **, p < 0.025; (wild type)+, MRL-Fas<sup>bm</sup> mice that have not been crossed with another strain. The Mann-Whitney U test was used for statistical analysis.

**FIGURE 10.** Intrarenal deposition of IgG is prevented in B7-1/B7-2+/− MRL-Fas<sup>bm</sup> mice. There is a reduction in IgG in the B7-1/B7-2+/− MRL-Fas<sup>bm</sup> glomeruli at 5 mo of age as compared with B7-1/B7-2+/− MRL-Fas<sup>bm</sup> glomeruli (n = 6/group, p < 0.01). The amount of IgG in the kidneys of B7-1/B7-2+/− MRL-Fas<sup>bm</sup> glomeruli is similar to age- and sex-matched normal mice (C57BL/6; n = 3). The amount of IgG in the glomeruli of B7-1/B7-2+/− MRL-Fas<sup>bm</sup> mice is similar to the wild-type, standard MRL-Fas<sup>bm</sup> strain. IgG in the kidneys was detected using fluorescein-conjugated anti-mouse IgG. Fluorescence intensity was scored on a scale of 0–4 (0, none; 1, weak; 2, moderate; 3, strong). Values are mean ± SD; *, p < 0.01; **, p < 0.05; (wild type)+, MRL-Fas<sup>bm</sup> that have not been crossed with another strain. The Mann-Whitney U test was used for statistical analysis.
Discussion

Using B7-1/B7-2-deficient MRL-Fas<sup>lpr</sup> mice, we have investigated the role of B7 costimulation in autoimmune disease. We have established that B7-1/B7-2 expression is up-regulated before overt pathology, continues to increase with progressive disease, and is detected on kidney-infiltrating leukocytes, and not in intrinsic cells in the MRL-Fas<sup>lpr</sup> kidney. To examine the function of the B7 costimulation in autoimmune disease, we have generated a B7-1/B7-2-deficient MRL-Fas<sup>lpr</sup> strain. Eliminating B7-1 together with B7-2 in MRL-Fas<sup>lpr</sup> mice confers enduring and complete protection from kidney (glomerular, tubular, interstitial, and vascular) and lung pathology, proteinuria, and diminishes the skin lesions and lymphoid enlargement characteristic of the MRL-Fas<sup>lpr</sup> strain. MRL-Fas<sup>lpr</sup> mice deficient in both costimulatory molecules are spared from multiple pathogenic components including leukocytic infiltrates, rising intrarenal cytokines/chemokines (IFN-γ, IL-12, CSF-1, MCP-1), elevated serum total IgG and IgG isotypes (G1,G2b,G3), and autoantibodies, as well as IgG deposits within glomeruli. Thus, we demonstrate that B7-1 and B7-2 together have a critical role in the pathogenesis of systemic autoimmune disease in MRL-Fas<sup>lpr</sup>.

B7 costimulatory molecules are responsible for the accumulation of leukocytes in tissues targeted for autoimmune destruction in MRL-Fas<sup>lpr</sup> mice. Massive accumulation of leukocytes (CD4, CD8, DN T cells, and macrophages) within multiple tissues is a hallmark of autoimmune disease in the MRL-Fas<sup>lpr</sup> strain (34, 35). Leukocytic infiltrates are prominent within the kidney and lung and are responsible for lymphadenopathy and splenomegaly. In the absence of B7 costimulatory molecules, leukocytes do not accumulate in the kidney and lungs and are dramatically reduced in lymphoid tissues. There are many possible explanations linked to the requirement for B7-1 and B7-2 in T cell activation. The absence of B7 molecules may limit T cell expansion, homing to the requirement for B7-1 and B7-2 in T cell activation. The absence of B7 molecules may limit T cell expansion, homing to the requirement for B7-1 and B7-2 in T cell activation. The absence of B7 molecules may limit T cell expansion, homing to the requirement for B7-1 and B7-2 in T cell activation. The absence of B7 molecules may limit T cell expansion, homing to the requirement for B7-1 and B7-2 in T cell activation.
**References**


