

# A Novel Mouse with B Cells but Lacking Serum Antibody Reveals an Antibody-independent Role for B Cells in Murine Lupus

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## Summary

The precise role of B cells in systemic autoimmunity is incompletely understood. Although B cells are necessary for expression of disease (Chan, O., and M.J. Shlomchik. 1998. *J. Immunol.* 160:51–59, and Shlomchik, M.J., M.P. Madaio, D. Ni, M. Trounstein, and D. Huszar. 1994. *J. Exp. Med.* 180:1295–1306), it is unclear whether autoantibody production, antigen presentation, and/or other B cell functions are required for the complete pathologic phenotype. To address this issue, two experimental approaches were used. In the first, the individual contributions of circulating antibodies and B cells were analyzed using MRL/MpJ-*Fas*<sup>pr</sup> (MRL/*lpr*) mice that expressed a mutant transgene encoding surface immunoglobulin (Ig), but which did not permit the secretion of circulating Ig. These mice developed nephritis, characterized by cellular infiltration within the kidney, indicating that B cells themselves, without soluble autoantibody production, exert a pathogenic role. The results indicate that, independent of serum autoantibody, functional B cells expressing surface Ig are essential for disease expression, either by serving as antigen-presenting cells for antigen-specific, autoreactive T cells, or by contributing directly to local inflammation.

Key words: nephritis • transgenic • T cell • vasculitis • antigen presentation

Systemic lupus erythematosus (SLE) is considered to be an immune disease in which the deposition of immune complexes or direct autoantibody deposition leads to the activation of complement, ligation of FcRs, and subsequent inflammation (1). Nevertheless, mononuclear infiltrates along with autoantibodies are usually present in affected tissues, including vessels, salivary glands, skin, and kidneys (2–10). In these situations, infiltrating T cells, macrophages, and other cells reside within destructive lesions (4–6). In particular, interstitial nephritis (IN)<sup>1</sup> is relatively common in human SLE; and this finding is associated with a poor prognosis (7, 9, 11, 12). These observations raise questions about the relative roles and connections between humoral and cellular autoimmunity. On the one hand, immune complex formation may lead to cellular recruitment. Alternatively, T cell infiltration may occur directly through antigen-specific events involving either macrophages or parenchymal cells.

Deciphering the contributions of these events in tissue injury has both mechanistic and therapeutic implications.

To develop a better understanding of the role of B cells during systemic autoimmunity, we created the J<sub>H</sub>D-MRL/MpJ-*Fas*<sup>pr</sup> mouse strain (J<sub>H</sub>D), a strain of MRL/*lpr* that lacks B cells (13, 14). The MRL/*lpr* strain develops a spectrum of disease manifestations that is quite similar to fulminant human SLE, including severe nephritis, vasculitis, sialoadenitis, and skin disease (15). When rendered B cell deficient, these animals lacked not only the classic immune-deposit manifestations of nephritis, such as glomerulonephritis (GN), but also failed to develop cellular infiltrates within organs, including IN, vasculitis (13), and skin disease (Chan, O.T.M., J. McNiff, and M.J. Shlomchik, manuscript in preparation). Furthermore, the absence of B cells substantially blocked the accumulation of activated and memory T cells (16).

Although these results clearly define a critical role for a B cell function(s) in the development of pathogenic lesions, they do not distinguish the precise roles of autoantibodies and B cells (i.e., independent from soluble antibodies). Here, we present a novel system to distinguish these mechanisms by developing an MRL/*lpr* mouse with B cells but no circulating antibodies. These animals demonstrate T cell

<sup>1</sup>Abbreviations used in this paper: dsDNA, double stranded DNA; GN, glomerulonephritis; IN, interstitial nephritis; mIgM, membrane IgM; NOD, nonobese diabetic; NP, (4-hydroxy-3-nitro-phenyl) acetyl; Tg, transgene; Tgic, transgenic.

activation, cellular infiltration including vasculitis and IN, and increased mortality, indicating that autoreactive B cells promote the development of pathogenic T cells in this disease. The results support a novel and important role for B cells in the pathogenesis of systemic autoimmunity.

## Materials and Methods

**Transgene Construction.** The mIgM construct: a VDJ region containing the canonical anti-(4-hydroxy-3-nitro-phenyl) acetyl membrane (NP) V<sub>H</sub>186.2 was cut from plasmid C<sub>μ</sub>B1-8.24, containing the V region of the hybridoma B1-8 (17), using EcoRI and XbaI. It was then ligated to an XbaI-EcoRI fragment containing the IgH intronic enhancer region from plasmid C<sub>μ</sub>19.7.1 (17). The BglII-XhoI fragment of pICEM-C<sub>μ</sub> (containing C<sub>μ</sub>1-4, and membrane and secreted exons) was replaced by the corresponding region from pSV5-C<sub>μ</sub>m, from which the secreted exon and polyadenylation site had been deleted (18). The EcoRI-XhoI fragment of the resulting plasmid, pICEM-C<sub>μ</sub>m, was then inserted into pBKS-II. The VDJ-enhancer unit was then excised as an EcoRI fragment and inserted at the EcoRI site of calf intestinal phosphatase (CIP)-treated pBKS-C<sub>μ</sub>m; correct orientation was confirmed by restriction digest. The resulting V<sub>H</sub>186.2-mIgM construct (termed mIgM hereon) encodes membrane-bound, but not secreted, IgM<sup>a</sup>.

The mIgM construct was tested by transfection into the CH1 cell line, which expresses IgM<sup>b</sup> using λ light chain. Although FACS<sup>®</sup> analysis confirmed the presence of NP-binding IgM<sup>a</sup> on transfectants, no IgM<sup>a</sup> was detectable by ELISA in culture supernatant. Transgenic (Tgic) mice resulting from microinjection of the purified DNA construct were initially identified by Southern blot analysis.

**Mice.** Mice bearing the mIgM transgene (Tg) were initially backcrossed with MRL/*lpr* mice four times, fixing homozygosity for *lpr*. The resultant Tg-positive mice were then backcrossed to the J<sub>H</sub>D/*lpr* strain to fix homozygosity of the J<sub>H</sub>D mutation, a *neo* insertion in the J<sub>H</sub> locus (fixation occurred after backcross one). This eliminated the expression of endogenous Ig heavy chains. All subsequent Tg-positive mice were generated by backcrossing to the J<sub>H</sub>D/*lpr* strain. Through this breeding strategy, all the Tgic animals used in this study had >98.9% MRL genes. The resulting mIgM.MRL/MpJ-*Fas*<sup>lpr</sup> mice (termed mIgM mice hereon) have functional B cells that do not secrete appreciable Ig (see below). All animals used in this study were obtained from our colony at the Yale University School of Medicine (New Haven, CT) and were housed under specific pathogen-free conditions. All Tgic mice and controls analyzed in this study were aged 24 wk or more.

A PCR detecting the rearranged V<sub>H</sub>186.2 V(D)J segment of the mIgM Tg was used to identify the Tg-positive mice. The oligonucleotides used for this PCR were V<sub>H</sub>186.2 5' (5'-TGCTCTTCTTGCCAGCAAC-3' [5' primer]) and V<sub>H</sub>186.2 3' (5'-TGAGGAGACTGTGAGAGTG-3' [3' primer]). Amplification conditions were 94°C for 2 min and 35 cycles of 30 s each at 94°C, 54°C, and 72°C, followed by a 7-min incubation at 72°C. Homozygosity for *lpr* was detected by PCR as previously described (13).

**Reagents and Antibodies.** The following mAbs were used as FACS<sup>®</sup> reagents in this study: CD19 (1D3-biotin; PharMingen); B220 (RA3-6B2-FITC); CD4 (H129.19-CyChrome; PharMingen); CD44 (Pgp-1-FITC); CD62L (Mel-14-biotin); and anti-mouse Fcγ receptor (2.4G2). Streptavidin-conjugated PE (Molecular Probes) was added as a secondary step for the biotinylated reagents. Pgp-1, Mel-14, and RA3-6B2 were purified from hybridoma supernatants on protein G columns (Amersham Pharmacia

Biotech) after ammonium sulfate precipitation, and were conjugated as described (19). The antibodies were verified by comparison with commercially available antibodies with the same specificities.

The following mAbs were used as standard controls for the ELISAs: T183 (IgM; Sigma Chemical Co.), 4G7 (IgG1; reference 19), Hy1.2 (IgG2a; reference 20), P19-10 (IgG2b), P19-11 (IgG3), P19-10 (anti-double stranded [ds]DNA; reference 21), 400tμ.23 (RF; reference 19), and LG4-1 (anti-chromatin; reference 22). 23.3 culture supernatant was used as the source of anti-NP IgG2a for the rheumatoid factor ELISAs (19). Polyclonal anti-mouse κ conjugated to FITC (Southern Biotechnology Associates, Inc.) was used in the immunofluorescence assays to detect glomerular Ig deposition.

**Cell Preparation and Flow Cytometry.** FACS<sup>®</sup> analysis was conducted as previously described (16).

**ELISA.** For anti-Ig ELISA, Falcon 96-well, flat-bottomed plates (Becton Dickinson) were coated overnight at 4°C with the primary antibody (antiisotype). Plates were then blocked with 1% BSA in PBS for 1 h at room temperature. Serum dilutions along with the standard were allowed to incubate for 2 h. The secondary antibody (antiisotype conjugated to alkaline phosphatase; Southern Biotechnology Associates, Inc.) was then incubated in the plate for 1 h at room temperature.

For autoantibody ELISA, the assays were conducted in the same manner as indicated above. However, the following were used to coat the plates with autoantigen: poly-1-lysine and purified calf thymus DNA (anti-dsDNA), NP/anti-NP IgG2a complexes (RF), and purified calf thymus chromatin (antichromatin). The secondary antibody was goat polyclonal anti-mouse κ conjugated to biotin, followed by streptavidin-alkaline phosphatase (Southern Biotechnology Associates, Inc.). The colorimetric data from the pNPP substrate was quantified using Bio Kinetics Reader EL 340 (Bio-Tek Instruments). DeltaSoftIII software (BioMetallics, Inc.) was used to analyze this data.

**Tissue Preparation.** Kidneys were bisected and fixed in 10% buffered formalin and embedded in paraffin, and sections were stained with hematoxylin and eosin. For immunofluorescent evaluation, kidney halves were lightly fixed in a 0.7% paraformaldehyde-lysine-periodate 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 (Tissue-Tek). 7-μm sections were cut using a Cryocut 1800 cryostat (Reichert-Jung) and stained overnight with an anti-mouse κ conjugated with FITC (Southern Biotechnology Associates, Inc.). Fluorescence was visualized using a Nikon Optiphot microscope (Nikon, Japan) at 200× or 400× and was photographed with an exposure time of 5–6 s on Elite ASA 400 film (Eastman Kodak Co.).

**Renal Scoring.** The severity of nephritis and vasculitis was graded based on a semiquantitative scale using the parameters described in Table I of reference 23. In brief, a 0–4+ scale was used for each compartment (glomerular, interstitial, and vascular) with the pathology graded according to specified criteria as absent, mild, moderate, or severe. For comparative purposes, all of the tissue sections were scored by one observer (M.P.M.), who was blinded to their origin, in three sessions for the unmanipulated mice. A proportion of samples was reread a second time, with generally excellent concordance (<1 point average difference). In rare cases of disagreement, the sections were evaluated a third and final time and the average of the scores was used.

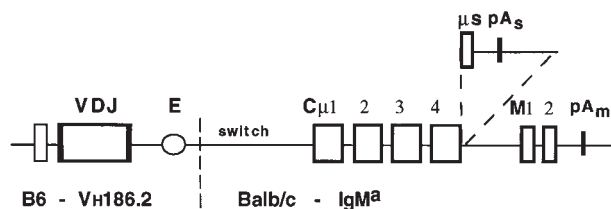
**Statistics.** Mortality curves were plotted using the Kaplan-Meier method and examined for significance using the Mantel-Cox logrank test. All other statistical tests used the nonparametric Mann-Whitney U statistic. The statistical analyses were con-

ducted using StatView 4.5 (Abacus Software) for the Macintosh.  $P < 0.05$  was considered significant.

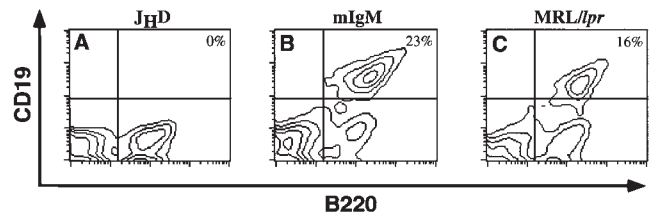
## Results

**B Cells Are Restored in Tgic mIgM Mice.** Tgic mice with the mIgM construct, which lacked the secreted exons, were created as described in Materials and Methods. A diagram of the Tg is shown in Fig. 1. Mice bearing the mIgM Tg were backcrossed at least seven times to the B cell-deficient  $J_H D$  strain, placing the Tgs onto the autoimmune MRL/*lpr* background. This also established homozygosity of the  $J_H D$  mutation, which prevented the development of B cells expressing endogenous Ig. As shown in Fig. 2, the mIgM Tg restored B cell maturation. The percentages of B cells in the Tg mice were comparable with those in wild-type animals (average spleen percentage: B cell-intact –  $15 \pm 7\%$ , mIgM –  $16 \pm 8\%$ ).

**mIgM Mice Have Greater Splenomegaly than  $J_H D$  Mice.**  $J_H D$  mice at 4–6 mo of age have reduced lymphoid organ weights and cell numbers compared with B cell-intact animals (13, 16). Table I lists the cell numbers and organ weights of the mIgM Tgic animals along with age-matched B cell-intact and B cell-deficient MRL/*lpr* mice. Splenic weight and cell number were greater in mIgM mice than in B cell-deficient animals (weight: 2.5-fold increase,  $P < 0.02$ ; cell number: 2.2-fold increase,  $P < 0.002$ ). Since B cells generally comprise only 15–20% of splenocytes in older MRL/*lpr* mice, this 2.2-fold difference in cell number cannot be accounted for simply by B cells per se. Most of the increased cell number is attributable, instead, to T cells, consistent with our previous reports on the effect of B cells on T cell activation and expansion. Although significantly greater than  $J_H D/lpr$  mice, splenomegaly in the mIgM Tgics was decreased when compared with control MRL/*lpr* mice. Similarly, in the lymph nodes restoration of B cells in the Tgic mice had no effect on weight and cell number. The phenotype of partial restoration of T cell accumulation mediated by the Tgic mice most likely reflects the restricted repertoire enforced by a single  $V_H$  in the Tg. A partial or reduced disease phenotype has been found in several other conventional Ig or TCR Tgic strains associated with the partial repertoire restriction imposed by allelic exclusion by the Tgs (24, 25).



**Figure 1.** Tg schematic. The mIgM heavy chain Tg is depicted. The DNA directing secretion ( $\mu s$ ) and the transcription termination site ( $pA_m$ ) have been deleted.  $V_H 186.2$  is the rearranged heavy chain V(D)J segment. E indicates the heavy chain intronic enhancer. Much of the switch region has been deleted; a small region of residual switch region is indicated.



**Figure 2.** The Tg restores splenic B cells in  $J_H D$  mice. Spleen cells from  $J_H D$  (A), mIgM (B), and MRL/*lpr* (C) mice were analyzed via flow cytometry. Live (propidium iodide-negative) cells were analyzed for CD19 (PE) and B220 (FITC) expression. B cells are  $B220^+/CD19^+$  (upper right quadrant). Percentages of B cells among live splenocytes are shown. In MRL/*lpr* mice,  $B220^+/CD19^-$  cells are T cells.

**Spontaneous T Cell Activation in mIgM Mice Occurs in the Absence of Circulating Ig.** The majority of T cells in MRL/*lpr* mice have an activated/memory phenotype (16, 26). However, in B cell-deficient MRL/*lpr* animals, the percentage of naive cells is increased, whereas the percentage of memory T cells is decreased (16). Furthermore, B cells are required for the accumulation of most memory T cells since there are 5–10 times as many memory T cells in B cell-intact mice compared with B cell-deficient mice. In principle, B cells could be exerting this effect on memory T cell development either directly (e.g., via presentation of [auto]antigens) or indirectly (e.g., via autoantibody-mediated inflammation causing the release of autoantigens).

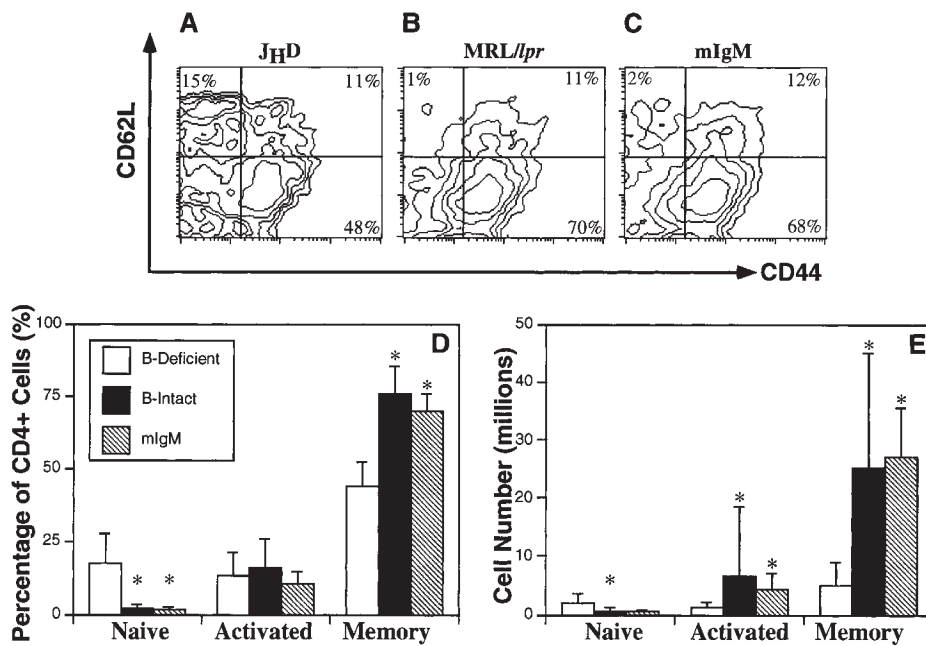
To distinguish between these possibilities and to determine the mechanism by which B cells promote spontaneous T cell activation, we analyzed  $CD4^+$  T cells from the spleens of mIgM mice for expression of CD44 and CD62L (Fig. 3). The T cell activation profiles of mIgM animals resembled B cell-intact mice rather than B cell-deficient mice. When compared with  $J_H D$  animals, the percentages of memory cells ( $CD44^{high}$ ,  $CD62L^{low}$ ) were significantly greater (1.6-fold increase,  $P < 0.0005$ ) in mIgM mice (Fig. 3 D). Furthermore, percentages of naive cells ( $CD44^{low}$ ,

**Table I.** Lymphoid Organ Weight and Total Cell Numbers of mIgM, MRL/*lpr*, and  $J_H D$  Mice

Genotype	Spleen		Lymph node	
	Weight	Cell No.	Weight	Cell No.
	mg	$\times 10^6$	mg	$\times 10^6$
mIgM	$366 \pm 107^*$	$181 \pm 43^*$	$11 \pm 5$	$4 \pm 3$
MRL/ <i>lpr</i>	$696 \pm 358^*$	$224 \pm 162^*$	$175 \pm 150^*$	$59 \pm 67^*$
$J_H D$	$148 \pm 80$	$82 \pm 72$	$32 \pm 37$	$12 \pm 17$

Splenic and inguinal lymph node organ weights and total cell numbers were obtained from mIgM, MRL/*lpr* (B cell-intact), and  $J_H D$  (B cell-deficient) mice. Averages and one standard deviation were calculated from samples consisting of 6–8-mo-old animals. Sample sizes for spleen and lymph nodes are as follows: mIgM weight and cell number ( $n = 6$ ), MRL/*lpr* weight and cell number ( $n = 25$ ),  $J_H D$  weight ( $n = 9$ ), and  $J_H D$  cell number ( $n = 8$ ).

\* $P < 0.05$  for comparisons to B cell-deficient  $J_H D$  mice.



**Figure 3.** Spontaneous splenic CD4<sup>+</sup> T cell activation is restored by the mIgM Tg. Three-color FACS<sup>®</sup> analysis was conducted on splenocytes. Representative FACS<sup>®</sup> contour plots are shown for J<sub>H</sub>D (A), MRL/*lpr* (B), and mIgM (C) mice. CD4<sup>high</sup> gated cells were analyzed for CD44 (FITC) and CD62L (PE) expression. Percentages of total CD4<sup>+</sup> T cells (D) were obtained for the naive (CD44<sup>low</sup>CD62L<sup>high</sup>), activated (CD44<sup>high</sup>CD62L<sup>high</sup>), and memory (CD44<sup>high</sup>CD62L<sup>low</sup>) subsets as depicted in parts A–C. Cell numbers (E) were calculated by multiplying total cell percentages of each activation subset by the total spleen cell number for each mouse. Spleens show the averages of percentages (D) or cell numbers (E) of a cohort of mice. Error bars represent one standard deviation. Sample sizes for cell percentages are: J<sub>H</sub>D (*n* = 17), MRL/*lpr* (*n* = 26), mIgM (*n* = 6) mice. Sample sizes for cell numbers are: J<sub>H</sub>D (*n* = 9), MRL/*lpr* (*n* = 25), mIgM (*n* = 5). All mice were 24–32 wk of age. Asterisks indicate significant differences of *P* < 0.05 with the B cell-deficient group, as determined by the two-tailed Mann-Whitney U test.

CD62L<sup>high</sup>) were reduced by 90% (*P* < 0.002). The number of memory CD4<sup>+</sup> T cells in the mIgM mice was greater (5.5-fold increase, *P* < 0.003) than that of B cell-deficient animals and was similar to the B cell-intact, control MRL/*lpr* mice (Fig. 3 E). As in the spleen, the percentage of naive cells in the lymph nodes was decreased and the percentage of memory cells was increased in mIgM Tgic mice compared with B cell-deficient animals (data not shown). However, there was no statistical difference in memory CD4<sup>+</sup> cell number since lymph nodes were not generally enlarged in the mIgM mice. For CD8<sup>+</sup> T cells, a similar pattern of naive cell reduction and memory cell augmentation was observed (data not shown). Since the accumulation of memory and activated T cells proceeded efficiently in the absence of secreted antibody (see below) in the mIgM mice, we conclude that B cells directly promote T cell activation and accumulation, rather than indirectly via antibody-mediated tissue damage.

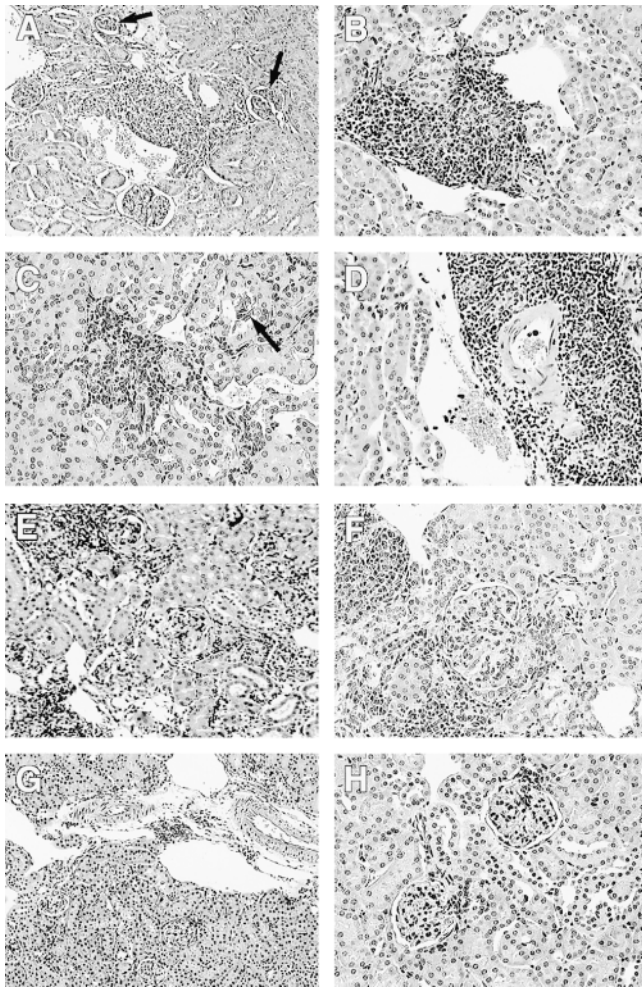
**mIgM Mice Develop Renal Lesions in the Absence of Circulating Ig.** MRL/*lpr* mice characteristically develop spontaneous nephritis (Fig. 4, E and F), whereas B cell-deficient J<sub>H</sub>D mice do not (Fig. 4, G and H) (13). mIgM mice, on the other hand, have significant cellular infiltrates in the renal interstitium and around the vessels, despite the inhibition of secreted Ig (Fig. 4, A–D). These infiltrates were predominantly composed of T cells (data not shown).

Renal disease in the glomeruli, interstitium, and vessels was scored blindly to assess severity (Fig. 5, A–C). IN and vasculitis scores of the mIgM mice were greater than those of the J<sub>H</sub>D animals (interstitium, *P* < 0.03; vessels, *P* < 0.03) and comparable to those of the MRL/*lpr* mice. There were no statistically significant differences in glomerular

scores between the J<sub>H</sub>D and mIgM strains, both of which were lower than MRL/*lpr* mice (*P* < 0.02). Nevertheless, focal lesions that resembled glomerular atrophy were observed in some glomeruli of the mIgM mice (Fig. 4, A and C), whereas these lesions were never observed in the J<sub>H</sub>D strain (Fig. 4, G and H). This picture is different than the typical proliferative GN picture seen in mild to moderate MRL/*lpr* disease and may reflect a previously unappreciated antibody-independent mode of glomerular disease perhaps related to adjacent interstitial or vascular disease.

**mIgM Mice Have Greater Mortality than J<sub>H</sub>D Mice.** In our colony, 50% mortality for B cell-intact MRL/*lpr* mice occurred at 32 wk (*n* = 203) (Fig. 5 D). The J<sub>H</sub>D strain, with markedly reduced nephritis, vasculitis, and T cell activation, had a significantly greater life span (*n* = 267, *P* < 0.0001). The mortality of mIgM mice (50% at 56 weeks, *n* = 71) was accelerated when compared with the J<sub>H</sub>D mice (*P* < 0.0001), demonstrating that the restoration of B cells in the absence of circulating, soluble autoantibody has a direct and relevant effect on disease expression. However, in keeping with the fact that restoration of B cells with a restricted repertoire leads to milder nephritis and somewhat less T cell activation than in wild-type MRL/*lpr* animals, mIgM mice also had prolonged survival compared with the control MRL/*lpr* strain (*P* < 0.0007).

**mIgM Mice Do Not Have Significant Serum Antibody.** To confirm the absence of secreted antibody in the mIgM strain, serum Ig and autoantibody levels were measured (Fig. 6). Some animals had no detectable Ig as expected (baseline dots in Fig. 6). However, others had trace quantities of some Ig isotypes; these concentrations were 100–1,000 times lower than MRL/*lpr* controls. Notably, there



**Figure 4.** Lupus nephritis occurs in the absence of circulating Ig. Kidneys were fixed in formalin, and sections were stained with hematoxylin and eosin: (A) 23 wk mIgM, (B) 30 wk mIgM, (C and D) 42 wk mIgM, (E) 31 wk MRL/*lpr*, (F) 30 wk MRL/*lpr*, and (G and H) 45 wk J<sub>H</sub>D. Cellular interstitial and perivascular infiltrates are observed in mIgM (A–D) and MRL/*lpr* (E and F), but not in J<sub>H</sub>D mice, even up to 45 wk of age (G and H). Black arrows point to glomerular lesions in the mIgM mice (A and C).

were mIgM animals that did not have any detectable serum Ig, yet developed renal disease. Finally, there was no significant total anti-dsDNA, RF, or antichromatin detected in the serum for most of the mIgM mice (Fig. 6 B).

*Ig Deposition Is Not Detected in the Kidneys of mIgM Mice.* Using immunofluorescence, we examined the kidneys of two mIgM mice that had detectable circulating Ig. In both, there were no observable Ig deposits in the glomeruli, interstitium, or vessels, consistent with the low to absent levels of serum Ig and autoantibodies. These mIgM kidneys were indistinguishable in this regard from kidneys of the B cell-deficient, negative controls, whereas MRL/*lpr* mice showed intense staining, as expected (Fig. 7).

## Discussion

SLE is a complex disease that may have multiple pathogenic manifestations. In addition to the classic GN, vasculitis, IN,

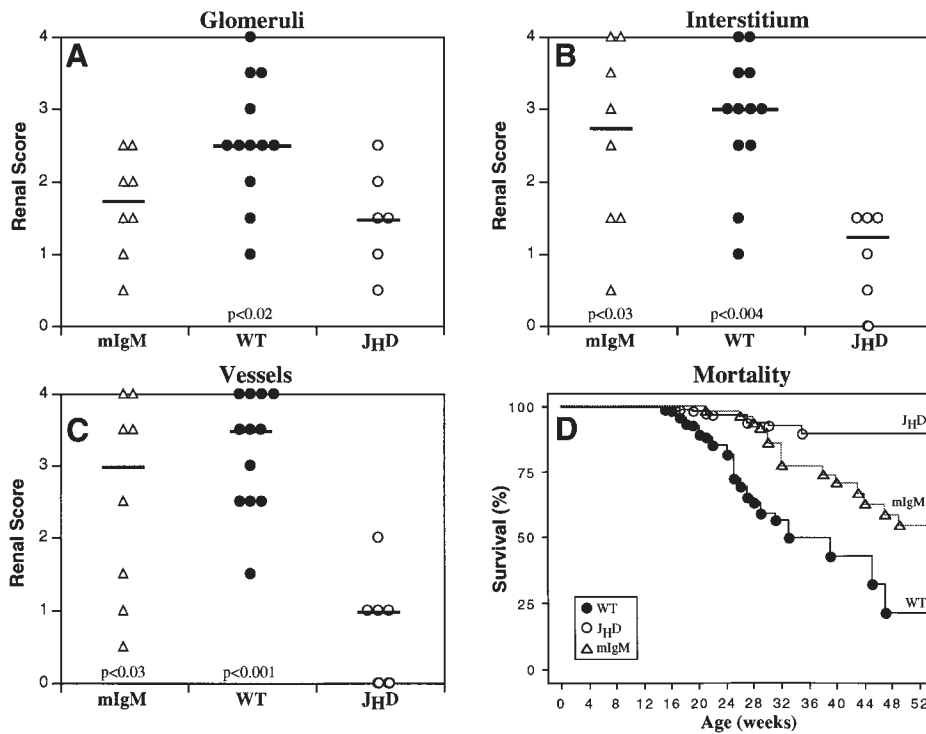
arthritis, and skin disease are often seen. The key finding of this work is that in the absence of circulating Ig and renal antibody deposition, the mIgM strain developed IN, vasculitis, and focal glomerular atrophy. Thus, these studies demonstrate for the first time an antibody-independent mechanism for renal and vascular disease in a murine model of SLE. Disease promoted by B cells in the absence of Ab is biologically relevant in that IN, vasculitis, and, importantly, mortality were all enhanced by B cells alone. To the extent that these results are generalizable to other murine models and to human SLE, the data suggest a wider view of lupus pathogenesis, which is generally thought to be solely antibody mediated (1).

Renal cellular infiltrates in MRL/*lpr* mice, which are not observed in the J<sub>H</sub>D strain, contain significant numbers of T cells (Chan, O.T.M., and M.J. Shlomchik, unpublished observation, and references 6, 27). These cells are restored in mIgM mice (Fig. 4 and immunocytochemistry data not shown). It is important to emphasize that interstitial disease and vasculitis are prominent features in diseased kidneys of many SLE patients (7, 9, 12). Indeed, interstitial injury may correlate best with overall outcome. Moreover, cellular infiltration is a prominent feature in other SLE manifestations, such as some skin lesions and sialoadenitis (10, 28). It is worth noting that cellular infiltration in the MRL/*lpr* model is not due to the *Fas<sup>lpr</sup>* mutation since Fas-intact MRL/MpJ-*+ Fas-lpr/+ Fas-lpr* mice also develop cellular infiltrates (references 3 and 5, and Chan, O.T.M., unpublished data).

Although overall statistical analysis of blind readings in a system that emphasized generalized disease did not show a difference in glomerular disease, this masks the fact that focal glomerular lesions were seen in mIgM mice (Fig. 4). Focal glomerular lesions have never been noted in J<sub>H</sub>D mice (13). The nature of these lesions has not yet been defined; they could be due to adjacent vascular damage, periglomerular infiltrates, and/or local release of toxic cytokines (8, 29). However, it seems that in the absence of marked glomerular disease, an antibody-mediated component is missing from the mIgM mice, as might have been predicted. The relationship of IN and GN has been controversial and the subject of speculation in the literature (30); but, until now, linkage could not be addressed in any experimental way in either humans or murine models. The present data show that IN and vasculitis can proceed without severe GN, but that some elements of GN might be exacerbated or even caused by surrounding cellular infiltrates as suggested by focal glomerular necrosis observed. In fact, several recent studies of experimental GN models that were thought to be solely antibody mediated have now demonstrated an important role for T cells (31–34). From all these studies, one could propose that there is synergy and cooperation among B and T cells to mediate a variety of pathogenic outcomes and that T cells do play a direct role in mediating disease that was previously thought to be only antibody dependent.

The mIgM T<sub>gic</sub> mice accumulated memory T cells in their spleens at levels similar to that of wild-type animals, in contrast to B cell-deficient mice. Thus, T cell activation is antibody-independent, but exactly how B cells promote T cell activation and cellular infiltration into tissues such as



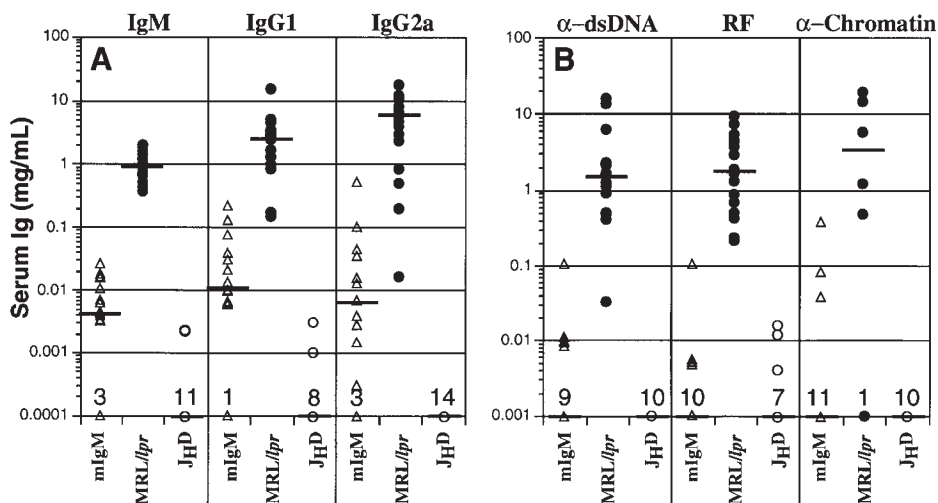


**Figure 5.** Kidney disease scores and mortality in MRL/*lpr* mice. Kidney samples were examined via light microscopy and scored blindly for disease on a semiquantitative scale from 0 (normal) to 4 (maximum). Scores for the glomeruli (A), interstitium (B), and vessels (C) are shown above. Each point represents an individual animal. Black horizontal bars indicate the median score. All animals were 24–30 wk of age. Comparisons to J<sub>H</sub>D mice were conducted, and statistical significance is indicated in each graph. Sample sizes for the mortality graph (D) are: MRL/*lpr* ( $n = 203$ ), J<sub>H</sub>D ( $n = 267$ ), and mIgM ( $n = 71$ ). The J<sub>H</sub>D mice had greater survival than either MRL/*lpr* ( $P < 0.0001$ ) or mIgM ( $P < 0.0001$ ) mice. Survival curves were generated as described in Materials and Methods using the Kaplan-Meier method and examined for statistical significance using the Mantel-Cox logrank tests.

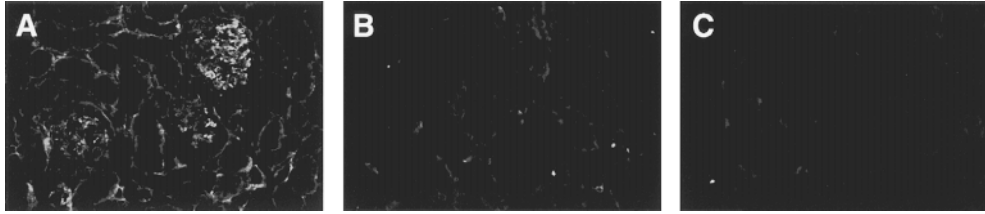
kidney and vessels is not completely clear. However, it is likely that B cells primarily act as autoantigen-presenting cells for the amplification of autoreactive T cells. In this scenario, autoreactive T cells may initially be activated by other APCs, such as dendritic cells; however, as B cell autoimmunity progresses and expanded clones of self-reactive B cells accumulate (35, 36), these cells become increasingly important APCs for T cells (37–41). B cells are known to be extraordinarily efficient APCs for antigens that can bind to their surface Ig (42–44). Indeed, B cells that accumulate in MRL/*lpr* mice chiefly have specificity for self-IgG (RFs) or DNA/histone (36). Such B cells bind particles (either immune complexes or nucleosomes) that are likely to contain multiple proteins, which could stimulate self-reactive T cells. Thus, these B cells are well suited to obtain T cell

help. As certain autoreactive B cells expand and dominate the B cell repertoire, their relative importance in promoting disease via T cell activation (possibly including breaking peripheral T cell tolerance) (45, 46) and autoantibody secretion would escalate. This might represent an element of a positive feedback circuit that leads to fulminant disease.

Mamula, Janeway, and colleagues first suggested that B cells might be important autoantigen-presenting cells and might promote the breakdown of peripheral T cell tolerance (45–47). Using a cross-immunization scheme in normal mice, they were able to elicit autoreactive T cells in a fashion that was likely to be B cell dependent. They also suggested that this might be a mechanism for epitope spreading, a common phenomenon in both lupus and organ-specific autoimmunity. These studies were conducted



**Figure 6.** Serum Ig levels. Sera were obtained from mIgM, MRL/*lpr*, and J<sub>H</sub>D mice. Ig levels as determined by ELISA for isotypes IgM, IgG1, and IgG2a are shown on the logarithmic y axis in A. Anti-dsDNA, anti-IgG2a rheumatoid factor, and antichromatin Ig are shown in B. Each point represents one mouse. Horizontal bars indicate the median Ig level.



**Figure 7.** Lack of renal antibody deposition in mIgM mice. Mice were assayed for Ig deposition, as determined by anti-mouse  $\kappa$ -FITC: (A) MRL/*lpr*, (B) J<sub>H</sub>D, and (C) mIgM. Deposition was observed in MRL/*lpr* mice (A), whereas none was detected in J<sub>H</sub>D (B) and mIgM (C) mice. All animals were 29–36 wk of age.

in normal mice via immunization with heterologous proteins in Freund's adjuvant. Our work now lends support to the idea that these mechanisms are actually operating in spontaneously autoimmune animals.

Although we favor a role for B cells as APCs, we cannot formally rule out that B cells are also acting as producers of cytokines that promote T cell activation or pathology. B cells are known to produce cytokines under certain circumstances (48–51). However, since B cells are not considered major producers of cytokines, it is difficult to envision B cells as supplying rate-limiting quantities of cytokines, particularly in a scenario in which there is massive T cell activation. In addition, immunohistochemical analyses revealed few B cells in the proximity of the renal infiltrates of MRL/*lpr* mice (data not shown), making it very unlikely that B cells are the source of cytokines responsible for direct parenchymal injury.

Although IN and vasculitis were marked in mIgM mice compared with their B cell-deficient MRL/*lpr* counterparts, the median renal disease scores of the mIgM mice were not as high as those of the control MRL/*lpr* mice. Similarly, mIgM mice had substantially accelerated mortality compared with B cell-deficient MRL/*lpr* mice; but these Tgic mice had delayed mortality when compared with the wild-type. The lack of circulating autoantibody could certainly contribute to milder disease. Particularly for

IN and vasculitis, it is likely that milder disease in mIgM mice is also due to a reduced Ig repertoire resulting from the fixed heavy chain Tg. It is possible that this restriction limited the generation of autoreactive B cells in the Tgic animals, thereby inhibiting the full potential of the B cell compartment to recognize self-antigens, activate them, and present these autoantigens to T cells. We predict that disease would have been more severe had a full Ig repertoire been expressed, but thus far the technology to produce such a mouse is not readily at hand.

The importance in SLE of cell-mediated autoimmune pathogenesis revealed by our studies is reminiscent of present views of organ-specific autoimmune diseases such as diabetes. It is intriguing that B cell deletion also inhibited diabetes in the nonobese diabetic (NOD) mouse (52–54). This result was interpreted as due to lack of APC function, a point that was not actually proven but could potentially be demonstrated directly using our Tgs on the NOD background. In any case, the similarities suggest that organ-specific and systemic autoimmunity may not need to be distinguished as much on the basis of pathogenic mechanism, but rather in extent of autoimmune targeting. Our studies further support the rationale for directly targeting B cells in the therapy of SLE (13, 16) and, by extension of this logic, to other autoimmune diseases.

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