The Journal of Immunology

Immune Complexes Present in the Sera of Autoimmune Mice Activate Rheumatoid Factor B Cells

Ian R. Rifkin, Elizabeth A. Leadbetter, Britte C. Beaudette, Cornelia Kiani, Marc Monestier, Mark J. Shlomchik and Ann Marshak-Rothstein

J Immunol 2000; 165:1626-1633; ;
doi: 10.4049/jimmunol.165.3.1626
http://www.jimmunol.org/content/165/3/1626

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Immune Complexes Present in the Sera of Autoimmune Mice Activate Rheumatoid Factor B Cells[1]

Ian R. Rifkin,[2]* Elizabeth A. Leadbetter,[2†] Britte C. Beaudette,[†] Cornelia Kiani,[†] Marc Monestier,[‡] Mark J. Shlomchik,[§] and Ann Marshak-Rothstein[3†]

The fate of an autoreactive B cell is determined in part by the nature of the interaction of the B cell receptor with its autoantigen. In the *lpr* model of systemic autoimmunity, as well as in certain human diseases, autoreactive B cells expressing rheumatoid factor (RF) binding activity are prominent. A murine B cell transgenic model in which the B cell receptor is a RF that recognizes IgG2a of the j allotype (IgG2a^j), but not the b allotype, was used in this study to investigate how the form of the autoantigen influences its ability to activate B cells. We found that sera from autoimmune mice, but not from nonautoimmune mice, were able to induce the proliferation of these RF$^+$ B cells but did not stimulate B cells from RF$^-$ littermate controls. The stimulatory factor in serum was found to be IgG2a^j, but the IgG2a^j was stimulatory only when in the form of immune complexes. Monomeric IgG2a^j failed to stimulate. Immune complexes containing lupus-associated nuclear and cytoplasmic autoantigens were particularly potent B cell activators in this system. Appropriate manipulation of such autoantibody/autoantigen complexes may eventually provide a means for therapeutic intervention in patients with certain systemic autoimmune disorders. ***The Journal of Immunology,*** **2000, 165: 1626–1633.**

B cells reactive with self Ags are regulated at a number of levels. They can be removed from the repertoire by actual deletion (1–3) or by receptor editing (4, 5). Alternatively, potentially self-reactive B cells may persist in the circulation as anergic, partially anergic, or resting B cells (6–11). As quiescent cells, they pose no threat to the individual. However, under certain yet to be defined circumstances, these cells may become activated and then differentiate to secrete pathogenic autoantibodies. A number of animal models for systemic autoimmune disease have been identified and provide a means for further analysis of the factors that lead to the aberrant induction of autoreactive B cells.

One such model involves mice that inherit either the *lpr* or *gld* mutation and thus fail to express functional Fas or Fas ligand (FasL)[4] (12, 13). These mice spontaneously develop a systemic autoimmune disease associated with excessive autoantibody production and often develop extremely high rheumatoid factor (RF) serum titers (14, 15). Random sampling of B cell hybridoma cell lines derived from diseased MRL-*lpr* or C3H-*lpr* mice revealed that ~10–20% of the B cells spontaneously activated by the autoimmune disease process expressed RF-binding activity. The majority of these RF were of non-IgM isotypes and restricted in both their isotype and allotype specificity (16, 17). Subsequent sequence analysis demonstrated that many of these RF-producing B cells were derived from the extensive expansion of a limited number of clones and that members of these clones had undergone considerable somatic mutation (17, 18). Despite this somatic mutation, the extent of affinity maturation within a clone was limited and, in general, the affinity of RF for autologous IgG2a was relatively low (19). Many of these RF Abs were found to be IgG3 molecules with the capacity to cryoprecipitate. On adoptive transfer to normal mice, representative monoclonal RF cryoglobulins caused both skin vasculitis and glomerulonephritis, pathologies frequently associated with the *lpr* disease (20, 21). Thus, RF were found to be a prevalent prototypic autoantibody in Fas/FasL-deficient mice with the potential to contribute significantly to the effector phase of the disease process.

The allotype specificity of MRL-*lpr*-derived RF B cells made them particularly amenable to experimental analysis. The heavy and light chain genes from AM14, a typical MRL/*lpr*-derived RF$^+$ hybridoma cell line, were used to construct a B cell receptor (BCR) transgenic (Tg) mouse line designated AM14 (22). The AM14 RF is allotype restricted and recognizes IgG2a^j or IgG2a^a, but not IgG2a^b. This allotype specificity made it possible to study the regulation of a true autoreactive B cell in the presence (on an MRL IgG2a^j or BALB/c IgG2a^a background) or absence (on a B6 IgG2a^b background) of the corresponding autoantigen. In contrast to B cells from other Tg mice, where reactivity of the transgene encoded BCR with either natural (7, 8, 10) or experimental (1, 23) autoantigens led to deletion or anergy, B cells in nonautoimmune prone AM14 mice appeared remarkably unaffected by in vivo exposure to their cognate Ag. AM14 B cells were found to circulate with a normal life span in both IgG2a^b and IgG2a^a mice. In this state of "clonal ignorance," they were perfectly capable of responding in vivo to challenge with immune complexes (IC) of the appropriate allotype, as long as Ag-specific T cell help was available (9). Despite their clonally ignorant phenotype in wild-type

Departments of [†]Microbiology and *Medicine, Boston University School of Medicine, Boston, MA 02118; [‡]Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA 19140; and [§]Departments of Laboratory Medicine and Immunobiology, Yale University School of Medicine, New Haven, CT 06520

Received for publication February 14, 2000. Accepted for publication May 9, 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 by National Institutes of Health Research Grants AR35230 (A.M.R.), DK02597 (I.R.R.), T32-AI07309 (E.L.), AI26665 (M.M.), and AI36529 (M.J.S.)

[2] I.R.R. and E.A.L. contributed equally to this study.

[3] Address correspondence and reprint requests to Dr. Ann Marshak-Rothstein, Department of Microbiology, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118. E-mail address: amrothst@bu.edu

[4] Abbreviations used in this paper: FasL, Fas ligand; HA, heat-aggregated; IC, immune complexes; RF, rheumatoid factor; CD40L, CD40 ligand; SLE, systemic lupus erythematosus; sIgM, surface IgM; Tg, transgenic.

0022-1767/00/$02.00

mice, AM14 B cells can become spontaneously activated in IgG2a^a *lpr* mice (24).

A number of factors, not necessarily exclusive, could account for the distinct profiles of AM14 cells in Fas-sufficient and Fas-deficient mice. For example, it is possible that the essentially naive status of AM14 B cells in nonautoimmune mice simply reflects the absence of autoreactive T cell help; more help would be available in *lpr* mice due to the failure of such T cells to undergo activation-induced cell death (25–27). Another possibility is that activated AM14 B cells persist in *lpr* mice under conditions that, in normal mice, would normally lead to elimination by FasL-expressing T cells (28, 29). Alternatively, the level or form of IgG2a present in the sera of the normal mice may not be sufficient to effectively signal through the AM14 receptor, whereas the IgG2a in the sera of autoimmune mice has the capacity, either by virtue of titer or by valence, to reach an activation threshold.

To further address the latter possibility, we undertook an in vitro analysis of the parameters governing the response of AM14 B cells to serum and monoclonal IgG2a Abs. We found that AM14 B cells were effectively stimulated only by the sera of Fas/FasL-deficient mice that expressed the Igh^a and Igh^j allotypes, not by sera from wild-type mice or by sera from Fas/FasL-deficient mice of the inappropriate allotype. Additional studies with mAbs demonstrated that purified autoantibodies reactive with nuclear or cytoplasmic constituents could activate AM14 B cells, probably by complexing with ubiquitous nuclear components released into the media by dying cells. We speculate that similar complexes form in vivo, potentiating the activation of RF B cells. Such a pathway may explain the dominance of RF and anti-nucleosome specificities in *lpr* mice and in human systemic autoimmune disease.

Materials and Methods

Mice

BALB/c mice Tg for the AM14 heavy chain (9, 22) were backcrossed to MRL-+/+ mice and used after the eighth backcross generation. MRL mice homozygous for the AM14 Vκ8 light chain were provided by Dr. J. Erikson (Wistar Institute, Philadelphia, PA). Both lines were bred and maintained under pathogen-free conditions at the Boston University School of Medicine Laboratory Animal Sciences Center and crossed to produce RF$^+$ and RF$^-$ littermates. The RF$^+$ offspring were initially identified by PCR, and their identity was confirmed by flow cytometry using the 4-G7 monoclonal anti-Id (22). MRL/MpJ-*Fas*^{lpr} (MRL-*lpr*), MRL/MpJ (MRL-+/+), and B6.MRL-*Fas*^{lpr} (B6-*lpr*) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). MRL/MpJ-*Fas*^{gld} (MRL-*gld*) were derived from breeders originally provided by Dr. C. Sidman (University of Cincinnati, Cincinnati, OH). Double-mutant MRL-*lpr/gld* mice were bred and maintained at Boston University School of Medicine (30). MRL-*lpr*.Igh^b were originally obtained from Dr. R. Eisenberg (University of Pennsylvania Medical School, Philadelphia, PA); the MRL-*lpr*.Igh^b and light chain Tg MRL-*lpr* Vκ8 and MRL-*lpr*.Igh^b Vκ8 were bred at Yale University School of Medicine (New Haven, CT).

Serum samples and Abs

Blood samples collected by tail bleed or cardiac puncture were allowed to clot at room temperature for 1–2 h at which time the serum was removed and stored in aliquots at −80°C. The nucleosome-specific mAbs PR1-3, PL2-6, PL2-3, LG4-1, MRB-4, LG10-1, LG8-1, PL9-7, PL2-8, PL2-7, and MGC 23 were derived from MRL mice and purified by protein G affinity chromatography (31–34). The IgG2a anti-Sm mAb 2-12 (Igh-1^j) was provided by Dr. S. Clarke (University of North Carolina, Chapel Hill, NC) (35). The IgG2a anti-hapten mAbs 23 (Igh-1^j anti-NIP), 15G (Igh-1^b anti-NIP), Hy1.2 (Igh-1^a anti-TNP), and C4010 (Igh-1^b anti-TNP) (9, 16) were purified by protein A affinity chromatography.

Isolation of serum IgG2a

Serum IgG2a was isolated by anti-Ig affinity chromatography. An anti-IgG2a column was made by coupling the mAb TT1 (IgG1 anti-IgG2a) (36) to CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech, Uppsala, Sweden). The control column was coupled with the mAb 44-10 (IgG1 anti-Ars) (37). Aliquots of an MRL-*lpr*/*gld* serum pool, diluted to 4% in serum-free RPMI, were passed through either the anti-IgG2a column or the irrelevant IgG1 column. After the flowthrough was collected, the columns were washed extensively with PBS. Bound Ab was eluted with 0.1 M glycine-HCl, pH 2.8, and the eluate was neutralized immediately with 1 M NaOH. After neutralization, the eluate was filtered through a 0.2-μm pore size filter, and 50 μl of the filtered eluate were added to a total well volume of 200 μl in the experiments. Neutralized elution buffer alone was always included in the experiments as a control.

B cell activation

T-depleted spleen cell suspensions were obtained from MRL AM14 Tg (RF$^+$) mice or MRL nontransgenic (RF−) littermates by treatment with the mAbs GK1.5 (anti-CD4), 53.6.72 (anti-CD8) and 13-4 (anti-Thy-1.2) followed by rabbit C′ (Pel-freez Biologicals, Rogers, AR). In some experiments, the T-depleted spleen cells were preactivated through CD40 with a CD40 ligand (CD40L)-CD8 fusion protein and anti-CD8 mAb as previously described (28). Serum samples, mAbs, or F(ab′)$_2$ goat anti-mouse IgM (15 μg/ml, Jackson ImmunoResearch Laboratories, West Grove, PA) were diluted in RPMI 1640, 10% FCS, filter sterilized, and then added directly to the splenic B cells or added 24 h after the CD40L fusion protein. Cells were incubated at a final concentration of 2×10^6 cells/ml in RPMI 1640, 10% FCS for a total of 36–48 h in 96-well flat-bottom microtiter plates. ^{3}[H]Thymidine (New England Nuclear, Boston, MA) was added for the last 6–16 h at a final concentration of 25 μC/ml, DNA was isolated with a PHD Harvester (Cambridge Technology, Cambridge, MA), and thymidine incorporation was measured with an LKB Wallac 1212 Rackbeta counter (Perkin-Elmer, Wellsley, MA). Data are presented as the mean cpm of triplicate cultures or as a percentage of the anti-IgM response. For B cells pretreated with CD40L, the percentage anti-IgM response was calculated according to the formula [(cpm experimental condition − cpm CD40L alone)/(cpm anti-IgM − cpm CD40L alone)]. For B cells not pretreated with CD40L, the percentage anti-IgM response was calculated according to the formula [(cpm experimental condition − cpm medium alone)/(cpm anti-IgM − cpm medium alone)]. The response of the anti-IgM treated RF$^+$ B cells ranged from 23 to 42% of the response of the anti-IgM treated RF$^-$ B cells.

RIA and ELISA for detection of IgG2a, RFs, and ICs

Serum IgG2a titers were determined by competitive RIA as described previously (36). Circulating RF titers were determined by direct binding ELISA using a $\gamma_{2a}\lambda$ target Ab (23) and an anti-κ detecting Ab as described previously (16). IC were measured using a modified C1q binding assay (38). Costar Serocluster U vinyl plates (Costar, Cambridge, MA) were coated with 10 μg/ml human C1q (Calbiochem-Novabiochem, La Jolla, CA) in PBS. Plates were blocked with PBS, 5% nonfat milk for 2 h at room temperature. Experimental samples were titrated in PBS, 0.1% Tween 20, 0.2% BSA (PBS-Tween), added to the plates, and incubated at 37°C for 70 min. The plates were then washed, and biotin-conjugated F(ab′)$_2$ goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL) diluted 1:5000 was incubated on the plates overnight at 4°C. Plates were developed with a 1:7500 dilution of streptavidin-HRP (Southern Biotechnology Associates) followed by ABTS (Sigma, St. Louis, MO). Positive and negative controls consisted of unmanipulated and heat-aggregated (HA, 63°C for 25 min) mIgG (Sigma). Nonspecific binding was assessed on duplicate plates not coated with C1q.

Preparation of nucleosomes

Nucleosomes were isolated as described previously (39). Briefly, nucleosomes were purified from bovine thymus (Pel-Freez) by micrococcal nuclease (Worthington Biochemical, Freehold, NJ) digestion of disrupted nuclei and subsequent separation of mononucleosomes, dinucleosomes, and higher oligomers on a 5–30% sucrose gradient. Nucleosome fractions corresponding to mononucleosomes and dinucleosomes were then 5′-end labeled with [γ-^{33}P]ATP (New England Nuclear, Boston, MA) using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). The anti-nucleosome mAbs, PL2-3 and PR1-3, and an isotype-matched anti-NIP mAb, 23, were diluted to 20 μg/ml in PBS and added to a 96-well plate (Costar) for 16 h at 4°C. The plates were then blocked with 0.2% BSA in PBS for 2 h at room temperature and the γ-^{33}P-labeled nucleosomes were then added at 10,000 cpm/well for 90 min at room temperature. After extensive washing, the extent of nucleosome binding was determined by measuring β emission in a liquid scintillation counter.

Results

AM14 RF$^+$ B cells can be stimulated by sera from allotype-matched Fas/FasL-deficient mice

AM14 mice express a transgene encoded receptor specific for IgG2a$^{a/j}$ and the majority of B cells in BALB/c Tg$^+$ mice appear as naive RF (RF$^+$) B cells (9). However, on the B6/*lpr*.Igh^a, but not B6/*lpr*.Igh^b, background, these RF$^+$ B cells become spontaneously activated (24). We reasoned that this spontaneous activation resulted from the inability of autoreactive T cells to eliminate Fas-deficient B cells and/or unique features of the serum components in these mice. To further explore the potential role of serum components in the activation of such RF$^+$ B cells, it was important to determine whether any concentration or form of the IgG2a^j autoantigen could effectively engage the AM14 Ag receptor.

As a first approach, serum pools were collected from 4- to 5-mo-old MRL-+/+ and MRL-*lpr* mice for use as a source of IgG2a. Based on previous serological data, it was known that on the average the concentration of IgG2a in MRL-+/+ sera is higher than those in sera from AM 14 mice, and that the concentration of IgG2a in the MRL-*lpr* sera is even greater (9, 40). It was also expected that the IgG2a in the MRL-+/+ sera would be essentially monomeric, whereas a significant proportion of the IgG2a in the MRL-*lpr* sera would be in the form of IC (14).

The MRL sera were added to cultures of CD40L-preactivated B cells obtained from either RF$^+$ or RF$^-$ mice, and proliferation was assessed after 2 days of culture. CD40L was used as a source of pseudo-T cell help and under the conditions of these experiments routinely elicited only modest proliferation by itself. The B cells were also cultured with a combination of CD40L and F(ab′)$_2$ anti-IgM, to provide a direct comparison of the overall proliferative capacity of the RF$^+$ and RF$^-$ B cell preparations. As shown in Fig. 1*A*, both B cell populations proliferated moderately in response to CD40L alone and vigorously to the combination of CD40L and F(ab′)$_2$ anti-IgM. The RF$^+$ B cells could also be effectively stimulated by serum pools from either MRL-*lpr* or double mutant MRL-*lpr*/*gld* mice, but not by serum pools from MRL-+/+ mice. In contrast, the RF$^-$ B cells were not stimulated by any of the MRL sera, demonstrating that the effect of the autoimmune sera on RF$^+$ B cells could not be attributed to agonist-like autoantibodies (41) or excessive cytokine titers present in the sera. Because the responses of the RF$^+$ cells to CD40L alone as well as to the rabbit F(ab′)$_2$ anti-IgM was routinely lower than the corresponding response of the RF$^-$ B cells, the data were recalculated as the percentage of the F(ab′)$_2$ anti-IgM response to best reflect the inherent differences between the two populations (Fig. 1*B*). Dilutions of autoimmune serum pools were further tested on resting and CD40L-preactivated B cells. Although the extent of activation of the resting cells was significantly less than that of the CD40L-preactivated cells, the overall pattern remained the same (Fig. 2); the MRL-*lpr* and MRL-*lpr*/*gld* serum pools stimulated RF$^+$ B cells, and the MRL/+ sera did not.

Stimulation of RF$^+$ B cells requires IgG2a of the appropriate allotype

To stimulate, a serum sample needed to contain IgG2a of the appropriate allotype. Serum pools from B6-*lpr* mice (IgG2a^b) and allotype congenic MRL-*lpr*.Igh^b mice failed to stimulate RF$^+$ B cells, consistent with the allotype specificity of the AM14 RF receptor (Fig. 3). Moreover, when an MRL-*lpr* serum sample was passed through an affinity column coupled with monoclonal anti-IgG2a, the stimulatory material was depleted from the flowthrough and could be recovered in the eluate. No activity could be eluted from a column coupled with an irrelevant IgG1 (Fig. 4). Overall,

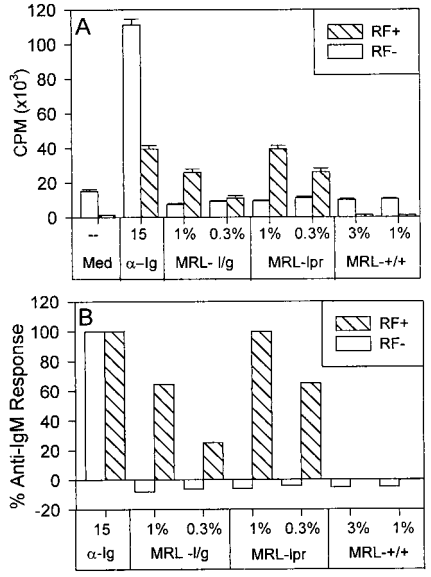

FIGURE 1. Serum pools from Fas/FasL-deficient mice stimulate AM14 RF B cells. Sera were collected from old MRL-*lpr/gld* (l/g), MRL-*lpr*, or MRL-+/+ mice. T-depleted spleen cells from RF$^+$ and RF$^-$ mice were preactivated with CD40L and then incubated at 37°C for 24 h in flat-bottom microtiter plates at a final concentration of 2×10^6 cells/ml. An additional 50 μl of culture medium, supplemented to bring the cultures to a final concentration of 15 μg/ml anti-IgM (α-Ig), or a final concentration of 3, 1, or 0.3% mouse serum, was then added to each of the wells. Control wells were given an additional 50 μl medium (Med). The cells were cultured for an additional 40 h; proliferation was assessed by [^{3}H]thymidine incorporation during the last 16 h of culture. Data are presented as the mean cpm of triplicate cultures ± SD (*A*) or as the percentage of the anti-IgM response (*B*).

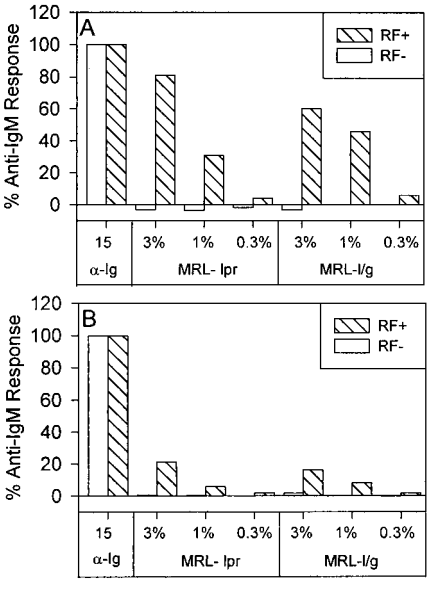

FIGURE 2. Serum pools from autoimmune mice can stimulate resting RF$^+$ B cells as well as CD40L-preactivated RF$^+$ B cells. A second set of sera were collected from 8- to 22-wk-old MRL-*lpr* and MRL-*lpr/gld* (l/g) mice and tested for their ability to stimulate both CD40L-preactivated (*A*) and unactivated (*B*) RF$^+$ and RF$^-$ B cells, as described in Fig. 1. For the unactivated B cells, serum dilutions were added to the B cells for the entire culture period.

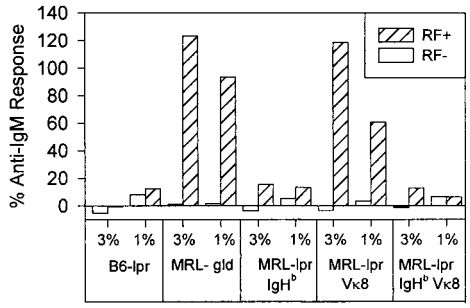

FIGURE 3. Stimulation of RF^+ B cells is allotype restricted. Serum pools were collected from diseased B6-*lpr* (IgH^b), MRL-*gld* (IgH^j), MRL-*lpr*.IgH^b (Igh^b), MRL-*lpr*.Vκ8 (IgH^j), and MRL-*lpr*.IgH^b.Vκ8 (IgH^b) mice and assayed as described in Fig. 1.

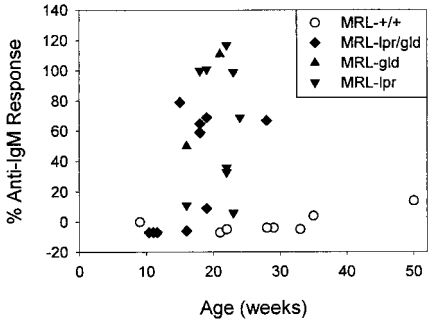

FIGURE 5. Stimulation of RF^+ B cells by sera from individual autoimmune or nonautoimmune mice. Sera were collected from individual MRL-+/+ (○), MRL-*lpr*/*gld* (◆), MRL-*gld* (▲), or MRL-*lpr* (▼) mice of various ages and tested for their ability to stimulate CD40L-pretreated RF^+ B cells when added to cultures at a final concentration of 1%.

these results were consistent with the assumption that IgG2a present in the MRL-*lpr* serum, but not the MRL-+/+ serum, was able to stimulate RF^+ B cells.

The ability of a serum sample to stimulate RF^+ B cells correlates with the titer of IC

The stimulatory capacity of the MRL-*lpr* serum pool in comparison with the MRL-+/+ serum pool was not simply due to a higher concentration of IgG2a in the MRL-*lpr* sample. When IgG2a titers of various MRL-*lpr* and MRL-+/+ serum pools were measured by RIA, it was found that wells supplemented with 0.3% MRL-*lpr* serum routinely contained less total IgG2a than the wells supplemented with 3.0% MRL-+/+ serum, even though RF^+ B cells cultured in the presence of 0.3% MRL-*lpr* cultures were effectively stimulated whereas RF^+ B cells cultured with 3.0% MRL-+/+ serum were not.

Sera from individual MRL-*lpr* mice vary widely with regard to IgG and specific autoantibody titers (14, 16). To determine whether the stimulatory activity apparent in the serum pools from the Fas/FasL-deficient mice reflected a general property of autoimmune sera, samples from 21 Fas/FasL-deficient (MRL-*lpr*/*gld*, MRL-*gld*, or MRL-*lpr*) mice and 8 individual MRL-+/+ mice, ranging in age from 2.5 to12 mo, were screened for their ability to stimulate RF^+ B cells. As shown in Fig. 5, sera from younger (11-wk) MRL-*lpr/gld* mice were not stimulatory. However, 14 of 18 serum samples from mice 4–6 mo of age, added to the cultures at a 1% final concentration, elicited responses that ranged from 33 to 117% of the anti-IgM response. By contrast, most of the MRL-+/+ sera had no detectable activity; only when MRL-+/+ sera were obtained from mice that were at least 9 mo old did they show any ability to stimulate, and even then the levels were low.

To better define the nature of the stimulatory activity in sera, a panel of stimulatory and nonstimulatory sera collected from 14 Fas/FasL-deficient mice were assayed for IgG2a, RF, and IC titers. Again, stimulatory activity did not correlate with the level of IgG2a, as detected by a standard competitive RIA. The possibility that a high level of serum RF could block stimulatory Abs and render a serum sample nonstimulatory was therefore also considered, however, we did not find any correlation between high RF titer and failure to stimulate. The same sera were also assayed for IC, as determined in a C1q binding assay. The IgG2a and IC titration curves for several representative serum samples are shown in Fig. 6, and the data from the entire panel are summarized in Table I. Overall, the results demonstrated a significant, although not absolute, correlation between stimulatory activity and C1q binding activity (Fig. 6 and Table I), implicating IC as the key element in RF activation. However, because the sera indicated by the open and closed inverted triangles in Fig. 6 have comparable IgG2a levels and C1q binding activity but differ in their ability to activate RF^+ B cells, the simple presence of IC cannot be the only factor. The extent to which particular features of IC, such as size, conformation, valency, or incorporation of complement components, may be relevant to activation of RF^+ B cells is under investigation.

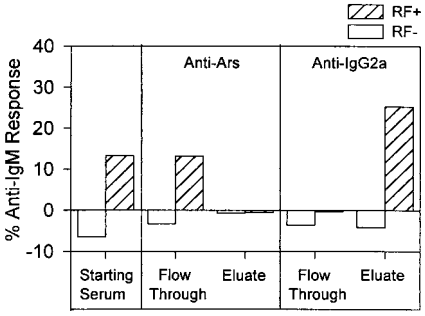

FIGURE 4. Stimulation of RF^+ B cells is IgG2a dependent. Serum from an MRL-*lpr*/*gld* mouse was diluted to a final concentration of 4% in serum-free medium, and 3 ml of the diluted serum were passed through affinity columns coupled with either a murine IgG1 anti-hapten mAb (44-10) or a murine IgG1 anti-IgG2a mAb (TT1). The starting material, as well as the flowthrough and eluate from both columns, were assayed on CD40L-preactivated RF^+ and RF^- B cells as described in Fig. 1.

RF^+ B cells can be stimulated directly by monoclonal anti-nucleosome/nucleosome IC

To further characterize the nature of the stimulatory Ab, we decided to examine the stimulatory capacity of specific monoclonal autoantibodies. Because nucleosomes are a common autoantigen in both murine and human systemic autoimmune disease (42, 43), initial studies were conducted with several MRL-*lpr*-derived IgG2a anti-nucleosome mAbs (31, 34). Nucleosomes were purified from bovine thymus as described previously (39). To demonstrate that the isolated material was in fact nucleosomes that could be bound by the mAbs, nucleosome fractions 4 and 7 (Fig. 7*A*) were labeled with [γ-^{33}P]ATP. Microtiter plates were then coated with the $IgG2a^j$ anti-nucleosome mAbs PL2-3 and PR1-3, or with an allotype-matched anti-NIP mAb, 23. The plates were blocked, and the radiolabeled nucleosomes were shown to specifically react with PL2-3 and PR1-3 (Fig. 7*B*). These anti-nucleosome mAbs, either alone or in combination with purified nucleosomes, were

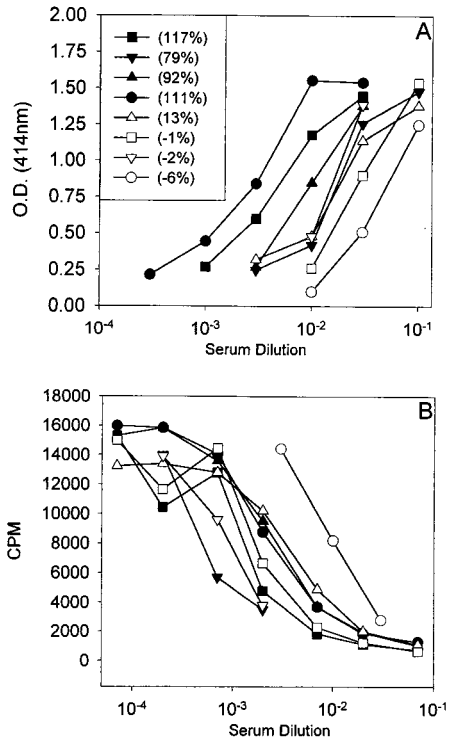

FIGURE 6. The ability of an autoimmune serum to stimulate RF^+ B cells corresponds to its ability to bind C1q. *A*, Representative serum samples diluted in PBS/Tween were added to C1q-coated and control (uncoated) plates. Bound Ab was detected with a biotinylated IgG-specific polyclonal antiserum, followed by HRP-streptavidin and substrate. Values from the control plate were subtracted from the values obtained from the C1q plate. Each line corresponds to an individual serum sample; solid symbols indicate stimulatory sera, and open symbols indicate nonstimulatory sera. The actual ability of each sample to stimulate RF^+ B cells is shown by the numbers in parentheses, which represent the percentage of the anti-IgM response elicited by a 1% final concentration of that particular serum. *B*, The same serum dilutions were assayed for IgG2a concentrations by competitive RIA.

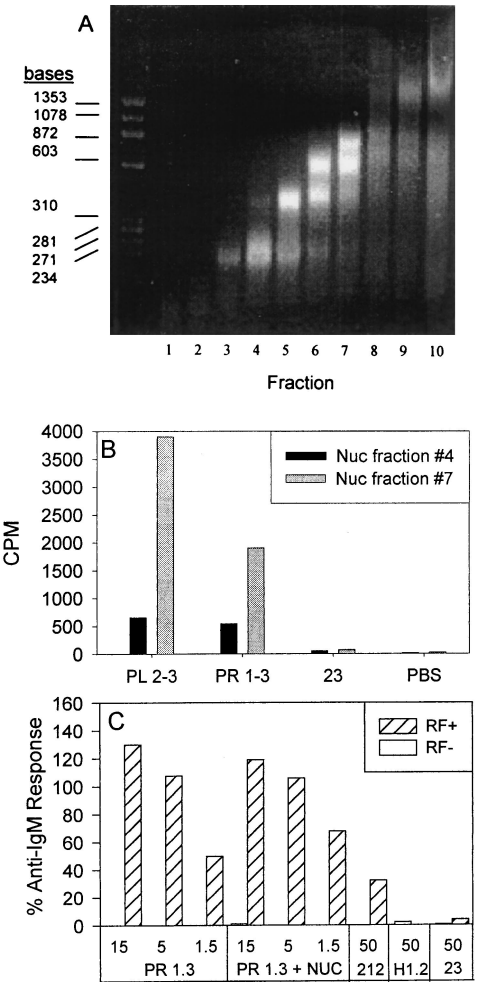

FIGURE 7. Nucleosome specific mAbs stimulate RF^+ B cells. *A*, Disrupted bovine thymus nuclei were digested with micrococcal nuclease, and the digested material was ultracentrifuged on a 5–30% sucrose gradient. Fractions were collected, and an aliquot from each fraction was run on a 2.5% agarose gel. Nucleosome-containing fractions with nucleosome monomers, dimers, and higher oligomers are shown. *B*, ^{33}P-labeled nucleosomes from fractions 4 and 7 above were added to wells precoated with the anti-nucleosome mAbs PL2-3 and PR1-3 or with the anti-NIP mAb 23 or to uncoated wells. The counts retained after washing were determined by measuring β emission in a liquid scintillation counter. *C*, The ability of anti-nucleosome (PR1-3), with and without the addition of nucleosome fraction 7 at 0.5 μg/ml, anti-Sm (2–12, Igh-1^j), anti-TNP (Hy1.2, Igh-1^a), and anti-NIP (23, Igh-1^j) mAbs to stimulate RF^+ B cells was determined by adding protein A-purified Abs, or in the case of 2-12, concentrated culture fluids, to CD40L-preactivated B cells. Ab concentrations in micrograms per ml are shown on the *x* axis.

then added to cultures containing the RF^+ and RF^- B cells. We expected the anti-nucleosome mAbs to stimulate only in the cultures supplemented with free nucleosomes (to allow for IC formation). Surprisingly, we found that the anti-nucleosome mAbs stimulated the RF^+ B cells more strongly than the $F(ab')_2$ anti-IgM, even in the cultures without added nucleosomes (Fig. 7*C*). This was true over a wide range of concentrations, even when non-CD40L-activated B cells were used in the assay (data not shown). These same mAbs had no effect on the RF^- B cells. A monoclonal IgG2a^j anti-Sm Ab, 2–12, was also found to specifically stimulate the RF^+ B cells under these conditions. In contrast, anti-hapten mAbs of the same isotype and allotype, purified in a comparable manner, failed to stimulate the RF^+ B cells. Presumably, the anti-nucleosome mAb bound nucleosomes released by dying cells present in the initial ascites fluids (44) and/or in the B cell cultures per se and formed IC even without the addition of the purified nucleosome preparation. This assumption was supported by data from a C1q binding assay where it was shown that anti-nucleosome mAbs bound to C1q better than the anti-hapten mAbs (Fig. 8*A*).

We needed to consider the possibility that a distinct physical feature of the anti-nucleosome mAbs rendered them particularly stimulatory for RF^+ B cells. To address this concern, an extensive panel of isotype-matched and -mismatched anti-nucleosome mAbs were screened for their ability to specifically stimulate the RF^+ B

Table I. *Correlation of serum characteristics with induction of proliferation*[a]

IC Titer vs Proliferation	RF Titer vs Proliferation	IgG2a Titer vs Proliferation	Age vs Proliferation
0.692 (n = 12)	0.002 (n = 13)	0.082 (n = 14)	0.246 (n = 14)

[a] Sera from 6- to 24-wk-old (mean age, 17.8 wk) MRL-*lpr* and MRL-*lpr/gld* mice were tested for their ability to induce proliferation of CD40L-preactivated RF^+ B cells. The IC, RF, and IgG2a titers of these same sera were measured in parallel, and r^2 values were determined.

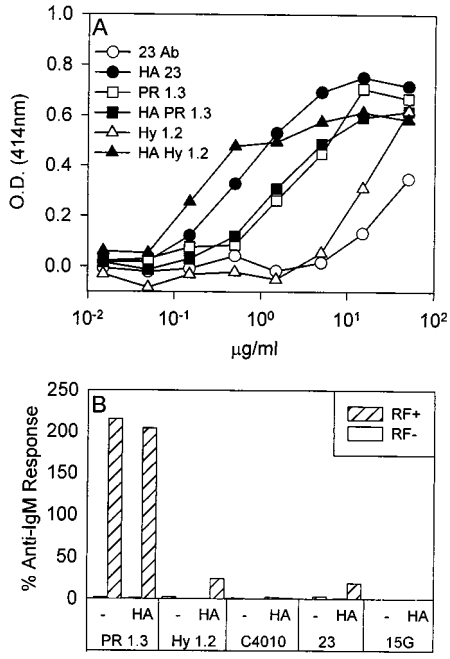

FIGURE 8. HA anti-hapten IgG2a$^{a/j}$ mAbs show enhanced binding to C1q and stimulate RF$^+$ B cells. Protein A-purified anti-TNP (Hy1.2, Igh-1^a; C4010, Igh-1^b) and anti-NIP (23, Igh-1^j; 15G, Igh-1^b) mAbs were HA at 63°C for 25 min and then compared with untreated Ab (−) with regard to C1q binding (*A*) and ability to stimulate CD40L-pretreated B cells (*B*).

cells. The panel included clonally related Abs, PL2-3 (γ2a), PL2-6 (γ2b), and PL2-8 (γ2b) that expressed comparable variable domains but differed with regard to heavy chain isotype. The IgG2a mAbs stimulated RF$^+$, but not RF$^-$, B cells (Table II), thereby demonstrating that anti-nucleosome Abs were not inherently mitogenic and that stimulation by anti-nucleosome mAb was dependent on specific recognition by the AM14 receptor.

Nonstimulatory anti-hapten Abs can be rendered stimulatory by heat aggregation

If the failure of the anti-hapten Abs to stimulate was simply due to their monomeric status, then IC of the same Abs would be expected to stimulate. The anti-hapten mAbs were therefore warmed to 63°C for 25 min to form heat aggregates. The IC status of the HA Abs was confirmed by C1q binding (Fig. 8*A*). When the HA preparations were compared with the nonaggregated Abs in an RF$^+$ B cell proliferation assay, the HA Abs were found to stimulate effectively, although not as well as the anti-nucleosome mAbs (Fig. 8*B*). The conclusion from these experiments is that AM14 RF$^+$ B cells can be effectively stimulated only by IgG2a in the form of IC, and not by monomeric IgG2a.

Table II. *Stimulation of RF$^+$ B cells by monoclonal anti-nucleosome Abs*

Ab	Isotype	Fine Specificity	% Anti-IgM Proliferation[a] RF$^+$	RF$^-$
PL 2-3	IgG2a^j	H2A-H2B-DNA	317	6
PR 1-3	IgG2a^j	H2A-H2B-DNA	312	−7
LG4-1	IgG2a^j	Nucleosome core particle	126	2
MRB4	IgG2a^j	H2A-H2B-DNA	138	11
LG10-1	IgG2a^j	H3-H4-DNA	154	9
LG8-1	IgG2a^j	H2A-H2B-DNA	48	9
PL 2-6	IgG2b^j	H2A-H2B-DNA	1	−6
PL 9-7	IgG3^j	H2A-H2B-DNA	−6	−16
PL 2-8	IgG2b^j	H2A-H2B-DNA	−1	−1
PL 2-7	IgG2b^j	H2A-H2B-DNA	−4	0
MGC 23	IgG2b^j	Nucleosome core particle	5	9

[a] CD40L-pretreated RF$^+$ or RF$^-$ B cells were cultured with 50 μg/ml designated mAbs, and proliferation was assessed at day 2.

Discussion

Potentially autoreactive B cells can persist in the periphery as quiescent cells. Such B cells may remain inactive because they are anergic and refractory to BCR-derived signals, because the appropriate T cell help is lacking, or because the corresponding autoantigen is sequestered or otherwise unavailable to the recirculating B lymphocyte pool. The current study demonstrates that the valence and/or configuration of the autoantigen may also be a key factor in determining its capacity to engage target B cells. Serum pools from Fas/FasL-deficient mice, containing IgG2a$^{a/j}$ in the form of IC, were found to very effectively stimulate RF$^+$ AM14 Tg B cells, whereas comparable levels of serum IgG2a from Fas/FasL-sufficient mice did not. These data implicate circulating IC as a key factor in the activation of autoreactive RF$^+$ B cells.

The main parameter assessed in the current study was proliferation; autoimmune sera were found to induce a vigorous proliferative response specifically in the AM14 RF$^+$ B cells. In that effective cross-linking of the BCR has been shown by others to up-regulate MHC class II Ag-processing mechanisms (45), it follows that IC stimulation of RF$^+$ B cells is likely to enhance the ability of these cells to present autoantigen epitopes contained within the complex. If the corresponding autoreactive T cells come in contact with the IC-stimulated B cells, the resulting cognate interactions could lead to autoantibody production, especially in Fas/FasL-deficient mice where activated autoreactive T and B lymphocytes are unaffected by signals that normally lead to FasL-induced apoptosis. Because RF$^+$ B cells can presumably be stimulated by IC containing a wide range of autoantigens, they have the potential to both activate and be stimulated by T cells with diverse autoantigen repertoires. This aspect of epitope spreading might explain why RF$^+$ B cells constitute such a high percentage of the MRL-*lpr* autoantibody repertoire (16, 17).

In contrast to the stimulatory capacity of the IC described in the current study, there is a considerable literature documenting the capacity of IC to very effectively block B cell activation by cross-linking the B cell surface IgM (sIgM) and FcγRIIB receptors (46, 47). The validity of these studies has been further supported by recent data demonstrating that FcγRII-deficient mice develop higher Ab titers than FcγRII-sufficient mice when challenged with either T-dependent or T-independent Ags (48). The results of the current study cannot simply be explained by failure of FcγRIIB receptor to bind Abs of the IgG2a subclass, given that IgG2a anti-SRBC and anti-hapten Abs have been shown to block B cell activation very effectively (49, 50). The enhancing effect of CD40L in this system may result from its ability to override FcR inhibition. It is also possible that the FcR-binding site on IgG2a molecules is blocked by the AM14 receptor. Another possibility is that not all IC are comparable and that the "stimulatory" IC present in autoimmune sera can effectively cross-link sIgM receptors with one another but cannot effectively cross-link sIgM and FcγRIIB receptors. "Inhibitory" IC, by comparison, would be able to effectively cross-link sIgM and FcγRIIB receptors.

The size and configuration of circulating IC have been extensively studied by Mannik and colleagues. Although in general, small-latticed IC (containing 1–2 Ab molecules) were shown to

persist in the circulation longer than soluble large-latticed IC (containing >2 Ab molecules), relatively high levels of large-latticed C1q-binding IC were detected in the sera of diseased MRL-*lpr* mice. Such large-latticed IC presumably circulate longer in autoimmune mice where the reticuloendothelial uptake system is saturated, thus slowing the IC clearance mechanisms (38, 51, 52). Because large-latticed IC are preferentially detected in the C1q assay (51), the data showing a correlation between the capacity of sera to bind C1q and stimulate RF^+ B cells strongly suggest that in fact large-latticed IC present in the Fas/FasL-deficient sera are responsible for the stimulatory activity. How these serum IC compare to the experimentally assembled IC used for the blocking studies cited above remains to be determined.

It is remarkable that Abs reactive with prototypic systemic lupus erythematosus (SLE)-associated nuclear and cytoplasmic autoantigens were found to activate RF^+ B cells particularly well, even without the addition of exogenous autoantigen. The high potency of the anti-nucleosome/nucleosome complexes that evidently form spontaneously also suggested that there is some aspect of the size, structure, or valence of these complexes that made them inherently unique with regard to their ability to stimulate B cells. Nuclear and/or cytoplasmic components released in vivo from dying cells may form complexes of comparable structure that effectively activate RF^+ B cells. Moreover, the non-Ig autoantigens in such IC may also serve to activate non-RF autoantigen-specific B cells. The formation of such complexes might explain why the injection of *lpr* mice with certain monoclonal anti-Sm Abs increases the percentage of *lpr* mice that go on to make anti-Sm autoantibodies (53). Perhaps these anti-Sm Abs form IC capable of stimulating either RF or Sm-specific B cells and thereby facilitate recognition by Sm-specific autoreactive T cells that then drive subsequent B cell expansion and differentiation.

The strongest responses in the current study resulted from costimulation of resting B cells with IC (or anti-IgM) together with CD40L, included as a substitute for a critical aspect of T cell help. Significant, albeit lower, responses were also induced in the absence of CD40L. Exactly how these in vitro responses reflect in vivo B cell status is not clear. In this regard, non-CD40L-pretreated AM14 B cells from both $IgG2a^{a/j}$ and $IgG2a^{b}$ mice could be activated by the anti-nucleosome Abs (data not shown), indicating that prior activation through the AM14 receptor in vivo was not a factor. Indeed, effective receptor engagement in vivo, in the absence of T cell help, often leads not to B cell expansion but rather to tolerance induction, either deletion, receptor editing, or anergy, as demonstrated in numerous BCR Tg models (1–8, 10). Ineffective engagement, as in the case of the $AM14/IgG2a^{a}$ mouse (9) or 3-83/soluble $H\text{-}2K^{k}$ mouse (54), may well have little impact. Based on the tolerance models alluded to above, it is reasonable to assume that in the absence of T cells, effective in vivo engagement of B cells by IC may also serve as a tolerogenic signal. Furthermore, it may well be that cytoplasmic and nuclear autoantigens routinely complex with "natural" Ab and that such complexes purge the repertoire of potentially hazardous autoreactive B cells. In this regard, Fas-deficient mice genetically altered to lack circulating IgM, and thus potentially deficient in tolerogenic natural IC, have higher autoantibody titers and shorter life spans than Fas-deficient mice with normal levels of circulating IgM (55).

We have shown that autoimmune sera contain IC that convert a relatively benign form of a genuine autoantigen (monomeric IgG2a) into a form (IgG2a IC) that can very effectively stimulate bona fide autoreactive (AM14 Tg RF^+) B cells. IC containing SLE-associated nuclear and cytoplasmic autoantigens were found to be particularly potent B cell activators in this system and are likely to contribute to the "spontaneous" activation of RF^+ B cells in AM14 Tg^+ IgG2a *lpr* mice. Appropriate manipulation of such autoantibody/autoantigen complexes may eventually provide a means for therapeutic intervention in patients with systemic autoimmune disorders.

Acknowlegments

We thank Kapil Bahl for expert technical assistance.

References

1. Nemazee, D. A., and K. Buerki. 1989. Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. *Nature 337:562.*
2. Hartley, S. B., J. Crosbie, R. Brink, A. A. Kantor, A. Basten, and C. G. Goodnow. 1991. Elimination from peripheral lymphoid tissues of self-reactive B lymphocytes recognizing membrane-bound antigens. *Nature 353:765.*
3. Chen, C., Z. Nagy, M. Z. Radic, R. R. Hardy, D. Huszar, S. A. Camper, and M. Weigert. 1995. The site and stage of anti-DNA B cell deletion. *Nature 373: 252.*
4. Gay, D., T. Saunders, S. Camper, and M. Weigert. 1993. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J. Exp. Med. 177:999.*
5. Tiegs, S. L., D. M. Russell, and D. Nemazee. 1993. Receptor editing in self-reactive bone marrow B cells. *J. Exp. Med. 177:1009.*
6. Goodnow, C. G., J. Crosbie, S. Adelstein, T. B. Lavoie, S. J. Smith-Gill, R. A. Brink, H. Pritchard-Briscoe, J. S. Wotherspoon, R. H. Loblay, K. Raphael, et al. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature 334:676.*
7. Erikson, J., M. Z. Radic, S. A. Camper, R. R. Hardy, C. Carmack, and M. Weigert. 1991. Expression of anti-DNA immunoglobulin transgenes in non-autoimmune mice. *Nature 349:331.*
8. Tsao, B. P., A. Chow, H. Cheroutre, Y. W. Song, M. E. McGrath, and M. Kronenberg. 1993. B cells are anergic in transgenic mice that express IgM anti-DNA antibodies. *Eur. J. Immunol. 23:2332.*
9. Hannum, L. G., D. Ni, A. M. Haberman, M. G. Weigert, and M. J. Shlomchik. 1996. A disease-related rheumatoid factor autoantibody is not tolerized in a normal mouse: implications for the origins of autoantibodies in autoimmune disease. *J. Exp. Med. 184:1269.*
10. Spatz, L., V. Saenko, A. Iliev, L. Jones, L. Geskin, and B. Diamond. 1997. Light chain usage in anti-double stranded DNA B cell subsets: role in cell fate determinations. *J. Exp. Med. 185:1317.*
11. Mandik-Nayak, L., S. J. Seo, A. Eaton-Bassiri, D. Allmand, R. R. Hardy, and J. Erikson. 2000. Functional consequences of the developmental arrest and follicular exclusion of anti-double-stranded DNA B cells. *J. Immunol. 164:1161.*
12. Watanabe-Fukunaga, R., C. Brannan, N. Copeland, N. Jenkins, and S. Nagata. 1992. Lymphoproliferative disorder in mice explained by defects in Fas antigen that mediate apoptosis. *Nature 356:314.*
13. Takahashi, T., M. Tanaka, C. Brannan, N. Jenkins, N. Copeland, T. Suda, and S. Nagata. 1994. Generalized lymphoproliferative disease in mice caused by a point mutation in the Fas ligand. *Cell 76:969.*
14. Izui, S., V. E. Kelley, K. Masuda, H. Yoshida, J. B. Roths, and E. D. Murphy. 1984. Induction of various autoantibodies by mutant gene *lpr* in several strains of mice. *J. Immunol. 133:227.*
15. Cohen, P. L., and R. A. Eisenberg. 1991. *lpr* and *gld*: single gene models of systemic autoimmunity and lymphoproliferative disease. *Annu. Rev. Immunol. 9:243.*
16. Wolfowicz, C. B., P. Sakorafas, T. L. Rothstein, and A. Marshak-Rothstein. 1988. Oligoclonality of rheumatoid factors arising spontaneously in *lpr/lpr* mice. *Clin. Immunol. Immunopathol. 46:382.*
17. Shan, H., M. J. Shlomchik, A. Marshak-Rothstein, D. P. Pisetsky, S. Litwin, and M. G. Weigert. 1994. The mechanism of autoantibody production in an autoimmune MRL/lpr mouse. *J. Immunol. 153:5104.*
18. Shlomchik, M. J., A. Marshak-Rothstein, C. B. Wolfowicz, T. L. Rothstein, and M. G. Weigert. 1987. The role of clonal selection and somatic mutation in autoimmunity. *Nature 328:805.*
19. Jacobson, B. A., J. Sharon, H. Shan, M. Shlomchik, M. G. Weigert, and A. Marshak-Rothstein. 1994. An isotype switched and somatically mutated rheumatoid factor clone isolated from a MRL-*lpr/lpr* mouse exhibits limited intraclonal affinity maturation. *J. Immunol. 152:4489.*
20. Reininger, L., T. Berney, T. Shibata, F. Spertini, R. Merino, and S. Izui. 1990. Cryoglobulinemia induced by a murine IgG3 rheumatoid factor: skin vasculitis and glomerulonephritis arise from distinct pathogenic mechanisms. *Proc. Natl. Acad. Sci. USA 87:10038.*
21. Berney, T., T. Fulpius, T. Shibata, L. Reininger, J. Van Snick, H. Shan, M. Weigert, A. Marshak-Rothstein, and S. Izui. 1992. Selective pathogenicity of murine rheumatoid factors of the cryoprecipitable IgG3 subclass. *Int. Immunol. 4:93.*
22. Shlomchik, M. J., D. Zharhary, S. Camper, T. Saunders, and M. Weigert. 1993. A rheumatoid factor transgenic mouse model for autoantibody regulation. *Int. Immunol. 5:1329.*
23. Rathmell, J. C., and C. G. Goodnow. 1994. Effects of the *lpr* mutation on elimination and inactivation of self-reactive B cells. *J. Immunol. 153:2831.*
24. Wang, H., and M. J. Shlomchik. 1999. Autoantigen-specific B cell activation in Fas-deficient rheumatoid factor immunoglobulin transgenic mice. *J. Exp. Med. 190:639.*
25. Dhein, J., H. Walczak, C. Baumier, K.-M. Debatim, and P. H. Krammer. 1995. Autocrine T-cell suicide mediated by APO-1/Fas/CD95). *Nature 373:438.*

26. Brunner, T., R. J. Mogil, D. LaFace, N. J. Yoo, A. Mahboubi, F. Echeverri, S. J. Martin, W. R. Force, D. H. Lynch, C. F. Ware, et al. 1995. Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. *Nature 373:441.*
27. Ju, S.-T., D. J. Panka, H. Cui, R. Ettinger, M. El-Khatib, D. H. Sherr, B. Z. Stanger, and A. Marshak-Rothstein. 1995. Fas (CD95)/FasL interactions required for programmed cell death after T-cell activation. *Nature 373:444.*
28. Rothstein, T. L., J. K. M. Wang, D. J. Panka, L. C. Foote, Z. Wang, B. Stanger, H. Cui, S.-T. Ju, and A. Marshak-Rothstein. 1995. Protection against Fas-dependent Th1-mediated apoptosis by antigen receptor engagement in B cells. *Nature 374:163.*
29. Schattner, E. J., K. B. Elkon, D. H. You, J. Tumang, P. H. Krammer, M. K. Crow, and S. M. Friedman. 1995. CD40L ligation induces Apo-1/Fas expression on human B lymphocytes and facilitates apoptosis through the Apo-1/Fas pathway. *J. Exp. Med. 182:1557.*
30. Wang, J. K. M., B. Zhu, S.-T. Ju, J. Tschopp, and A. Marshak-Rothstein. 1997. $CD4^+$ T cells reactivated with superantigen are both more sensitive to FasL-mediated killing and express a higher level of FasL. *Cell. Immunol. 179:153.*
31. Losman, M., T. M. Fasy, K. E. Novick, and M. Monestier. 1992. Monoclonal autoantibodies to subnucleosomes from a MRL/Mp-+/+ mouse. *J. Immunol. 148:1561.*
32. Losman, J. A., T. M. Fasy, K. E. Novick, M. Massa, and M. Monestier. 1993. Nucleosome-specific antibody from an autoimmune MRL/Mp-*lpr/lpr* mouse. *Arthritis Rheum. 36:552.*
33. Losman, J. A., T. M. Fasy, K. E. Novick, and M. Monestier. 1993. Relationships among antinuclear antibodies from autoimmune MRL mice reacting with histone H2A-H2B dimers and DNA. *Int. Immunol. 5:513.*
34. Monestier, M., and K. E. Novick. 1996. Specificities and genetic characteristics of nucleosome-reactive antibodies from autoimmune mice. *Mol. Immunol. 33:89.*
35. Bloom, D. D., J. L. Davignon, P. L. Cohen, R. A. Eisenberg, and S. H. Clarke. 1993. Overlap of the anti-Sm and anti-DNA responses of MRL/Mp-*lpr/lpr* mice. *J. Immunol. 150:1579.*
36. Perkins, D. L., J. Michaelson, and A. Marshak-Rothstein. 1987. The *lpr* gene is associated with resistance to engraftment by lymphoid but not erythroid stem cells from normal mice. *J. Immunol. 138:466.*
37. Marshak-Rothstein, A., M. Siekevitz, M. Margolies, M. Mudgett-Hunter, and M. Gefter. 1980. Hybridoma proteins expressing the predominant idiotype of the anti-azophenylarsonate response of A/J mice. *Proc. Natl. Acad. Sci. USA 77: 1120.*
38. Uwatoko, S., M. Mannik, I. R. Oppliger, M. Okawa-Takasuji, S. Aotsuka, R. Yokohari, G. Seki, S. Taniguchi, K. Suzuki, and K. Kurokawa. 1995. C1q-binding immunoglobulin G in MRL/l mice consists of immune complexes containing antibodies to DNA. *Clin. Immunol. Immunopathol. 75:140.*
39. Burlingame, R., and R. Rubin. 1990. Subnucleosome structures as substrates in enzyme-linked immunosorbent assays. *J. Immunol. Methods 134:187.*
40. Slack, J. H., L. Hang, J. Barkley, R. J. Fulton, R. D'Hoostelaere, A. Robinson, and F. J. Dixon. 1984. Isotype of spontaneous and mitogen-induced autoantibodies in SLE-prone mice. *J. Immunol. 132:1271.*
41. Weintraub, J. P., V. Godfrey, P. A. Wothusen, R. L. Cheek, R. A. Eisenberg, and P. L. Cohen. 1998. Immunological and pathological consequences of mutations in both Fas and Fas ligand. *Cell. Immunol. 186:8.*
42. Amoura, Z., H. Chabre, S. Koutouzov, C. Lotton, A. Cabrespines, J. F. Bach, and L. Jacob. 1994. Nucleosome-restricted antibodies are detected before anti-dsDNA and/or antihistone antibodies in serum of MRL-Mp *lpr/lpr* and +/+ mice and are present in kidney eluates of lupus mice with proteinuria. *Arthritis Rheum. 37:1684.*
43. Burlingame, R., M. L. Boey, G. Starkebaum, and R. Rubin. 1994. The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erythematosus. *J. Clin. Invest. 94:184.*
44. Bell, D. A., and B. Morrison. 1991. The spontaneous apoptotic death of normal human lymphocytes in vitro: the release of, and immunoproliferative response to, nucleosomes in vitro. *Clin. Immunol. Immunopathol. 60:1326.*
45. Xu, X., B. Press, N. M. Wagle, H. Cho, A. Wandinger-Ness, and S. K. Pierce. 1996. B cell antigen receptor signaling links biochemical changes in the class II peptide-loading compartment to enhanced processing. *Int. Immunol. 8:1867.*
46. Chan, P. L., and N. R. Sinclair. 1971. Regulation of the immune response. V. An analysis of the function of the Fc portion of antibody in suppression of an immune response with respect to interaction with components of the lymphoid system. *Immunology 21:967.*
47. Phillips, N. E., and D. C. Parker. 1984. Cross-linking of B lymphocyte Fc gamma receptors and membrane immunoglobulin inhibits anti-immunoglobulin-induced blastogenesis. *J. Immunol. 132:627.*
48. Takai, T., M. Ono, M. Hikada, H. Ohmori, and J. V. Ravetch. 1996. Augmented humoral and anaphylactic responses in FcγRII-deficient mice. *Nature 379:346.*
49. Milne, R. W., M. P. Chalon, and J. P. Vaerman. 1981. Suppression of the in vitro immune response by isolated mouse IgG subclasses. *Mol. Immunol. 17:1599.*
50. Phillips, N., and D. C. Parker. 1985. Subclass specificity of Fcγ receptor-mediated inhibition of mouse B cell activation. *J. Immunol. 134:2835.*
51. Wener, M. H., and M. Mannik. 1983. Influence of immune complex lattice on the C1q solid phase assay as determined with covalently cross-linked immune complexes. *Clin. Exp. Immunol. 52:543.*
52. Mannik, M. 1980. Physicochemical and functional relationships of immune complexes. *J. Invest. Dermatol. 74:333.*
53. Eisenberg, R. A., D. S. Pisetsky, S. Y. Craven, J. P. Grudier, M. A. O'Donnell, and P. L. Cohen. 1990. Regulation of the anti-Sm autoantibody response in systemic lupus erythematosus mice by monoclonal anti-Sm antibodies. *J. Clin. Invest. 85:86.*
54. Nemazee, D., D. Russell, B. Arnold, G. Haemmerling, J. Allison, J. F. A. P. Miller, G. Morahan, and K. Buerki. 1991. Clonal deletion of autospecific B lymphocytes. *Immunol. Rev. 122:117.*
55. Boes, M., T. Schmidt, K. Linkemann, B. Beaudette, A. Marshak-Rothstein, and J. Chen. 2000. Accelerated development of IgG autoantibodies and autoimmune disease in the absence of secreted IgM. *Proc. Natl. Acad. Sci. USA 97:1184.*