

An Intrinsic B Cell Defect Is Required for the Production of Autoantibodies in the *lpr* Model of Murine Systemic Autoimmunity

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Summary

Mice homozygous for the gene *lpr* develop marked lymphadenopathy and a spectrum of autoantibodies closely resembling that of human systemic lupus erythematosus. The unusual T cell phenotype of the expanded lymphocyte population and the T-dependence of several antibodies in this strain have suggested that primary T cell abnormalities underlie the autoimmune syndrome. Using double chimeras, we now show that expression of the *lpr* gene in B cells is absolutely necessary for autoantibody production. Combinations of anti-Thy 1.2 + C' treated bone marrow from congenic strains of C57BL/6 mice, differing only at the immunoglobulin heavy chain (*Igh*) and *lpr* loci, were transferred into lethally irradiated B6/*lpr* mice. Double chimerism was documented by allotype-specific surface IgD and IgM immunofluorescence assay of peripheral blood and by allotype-specific enzyme-linked immunosorbent assay for total IgM in serum. Despite the presence of both +/+ and *lpr* B cells, IgM and IgG2a anti-chromatin as well as IgM anti-IgG were entirely the products of *lpr* B cells. Total serum IgG2a and IgG1 were also dominated by the *lpr* phenotype but not to the same extent. A similar experiment using B6/*lpr*-*Igh*^a recipients confirmed these findings. Additional experiments in which B6/*lpr* recipients were infused with ratios of donor bone marrow favoring B6.C20 +/+ over B6/*lpr* showed that even though +/+ B cells were overrepresented, autoantibodies were only of the *lpr* allotype. In addition, in the presence of *lpr* B cells, normal B cells showed little response to an exogenous, T cell-dependent antigen. The data thus indicate that *lpr* B cells manifest an intrinsic abnormality which is essential for autoantibody production in the *lpr* model.

Mice homozygous for the autosomal recessive gene *lpr* develop a broad spectrum of autoantibodies and a clinical syndrome resembling human SLE. One of the most striking features of these mice is the marked accumulation of abnormal lymphocytes in lymph nodes and, to a lesser extent, in the spleen. The expanded population appears to be of T cell lineage, since the cells bear T cell receptors (1, 2) and have a germline immunoglobulin gene configuration (2). However, most are of an atypical phenotype, positive for Thy 1 but negative for CD4 and CD8 (3, 4). These "double negative" T cells (DNTs)¹ also bear unusual markers, such as Ly-6 (5), Ly24 (6), PC.1 (7), and H1.2F3 (8). Despite their

massively expanded numbers in vivo, these cells have been surprisingly inert in vitro (9, 10).

Since the T cells in *lpr* mice seem so obviously deranged, this population has been the primary focus of a large body of research (11). In vivo treatment with anti-T cell mAb (12) or neonatal thymectomy (13–15) greatly diminished adenopathy and autoantibody production, while thymectomy followed by transplantation of a normal thymus reconstituted disease (16). More recently, we have demonstrated that the characteristically abnormal T cells in *lpr* mice are intrinsically defective (17). Lethally irradiated C57BL/6-*lpr/lpr* (B6/*lpr*) mice reconstituted with equal amounts of bone marrow from normal congenic mice (C57BL/6-Ly1.1) and from B6/*lpr* (Ly 1.2⁺) mice developed massive lymphadenopathy as well as autoantibodies comparable in titer to mice reconstituted with B6/*lpr* marrow alone. Strikingly, the lymphadenopathy was due almost entirely to cells derived from the *lpr* marrow, indicating that normal T cells were not undergoing abnormal differentiation in an *lpr* environment.

B cells from *lpr* mice, in contrast, are not as obviously ab-

¹ Abbreviations used in this paper: BBS, borate buffered saline; BMC, bone marrow cell; B6, C57BL/6ByJ; B6/*lpr*, C57BL/6-*lpr/lpr*; B6/*lpr*-*Igh*^a, C57BL/6-*lpr/lpr*-*Igh*^a/*Igh*^a; DEA, diethanolamine; DNT, double negative T cell; DTT, dithiothreitol; EDF, equivalent dilution factor; *Igh*, immunoglobulin heavy chain; MB/*lpr*, (MRL/*lpr* × B6/*lpr*)F1; MRL/*lpr*, MRL/Mp-*lpr/lpr*; MRL/+, MRL/Mp-+/+; PNP, paranitrophenyl phosphate; SS, single-stranded; TNP-HSA, trinitrophenyl-human serum albumin.

normal; yet certain defects have been identified. B cells from older *lpr* mice hyperexpress Ia (18), but otherwise have no known aberrant markers. Polyclonal activation at an early age has been demonstrated (19), and B cell tolerance can be difficult to induce (20, 21). The C57BL/6-*nu/nu*,*lpr/lpr* strain develops some autoantibodies in the absence of functional T cells (22), suggesting that the *lpr* B cell is intrinsically abnormal. Anti-IgM treatment of B6/*lpr* mice depleted B cells, as expected, but more interestingly, resulted in a moderate reduction in adenopathy (23, 24) and a marked decrease in glomerulitis (23). Recently, Perkins et al. (25) have shown that neonatal MRL-Mp-+/+ (MRL/+) mice engrafted with allotype-congenic MRL/Mp-*lpr/lpr*-Igh^c bone marrow preferentially developed total IgG2a and IgG2a anti-single stranded (ss)DNA antibodies of donor *lpr* allotype. This occurred despite indirect evidence that most of the splenic B cells were of host origin.

In the present work, we have tested critically whether autoantibody production in the *lpr* model of SLE depends on *lpr*-determined B cell abnormalities. Using congenic strains on the B6 background, we have created double chimeras so that normal and *lpr* B cells, marked by allotype, could develop together in an *lpr* environment. Although the resultant mice contained large numbers of both normal and *lpr* B cells, they produced IgM and IgG2a autoantibodies almost exclusively of the *lpr* donor allotype. Therefore, *lpr* B cells are intrinsically prone to produce autoantibodies and are not merely passive recipients of abnormal signals from aberrant *lpr* T cells. Furthermore, the striking autoantibody production of the *lpr* mouse absolutely requires this intrinsic B cell abnormality.

Materials and Methods

Mice. MRL/Mp-+/+ (MRL/+), MRL/Mp-*lpr/lpr* (MRL/*lpr*), C57BL/6ByJ (B6), B6.C20 (B6-Igh^a), C57BL/6-*lpr/lpr* (B6/*lpr*), C57BL/6-*lpr/lpr*-Igh^b/Igh^a (B6/*lpr*-Igh^a), and (MRL/*lpr* × B6/*lpr*)F1 were maintained in our breeding facility. The MRL/*lpr*, MRL/+, B6, and B6/*lpr* strains were originally obtained from The Jackson Laboratory (Bar Harbor, ME), and the B6.C20 was obtained from Gayle Bosma (Institute for Cancer Research, Philadelphia, PA). The B6/*lpr*-Igh^a strain was developed in our laboratory by crossing B6.C20 and B6/*lpr*. Homozygosity for Igh^a and *lpr* was tested at the F2 generation, and the strain was established from a single breeding pair. B6/*lpr*-Igh^a characteristically develop significant lymphadenopathy, splenomegaly, and autoantibodies to chromatin and IgG by 5 mo-of-age.

Production of Double Chimeras. 1 wk before cell transfer, 2-3-mo-old mice were bled and transferred to autoclaved cages in an isolation cubicle and provided with neomycin (0.2% w/v)-treated water. On the day of bone marrow transfer, the mice received 900 rad of γ -radiation in a Gammacell 40 ¹³⁷Cs apparatus (Atomic Energy of Canada, Ltd., Ottawa, Canada). Bone marrow cells (BMCs) were prepared from the femurs and tibias of age- and sex-matched congenic mice. After extensive washing in RPMI 1640 buffered with 15 mM HEPES and supplemented with penicillin and streptomycin, BMCs were resuspended at 20 × 10⁶/ml and treated with saturating concentrations of monoclonal rat anti-Thy 1.2 (NEN Research Products, Boston, MA) for 30 min at 4°C, washed again, and incubated with Low-Tox-M rabbit complement

(Cedarlane Laboratories Limited, Ontario, Canada) for 45 min at 37°C. For each donor group, equal numbers of cells from the appropriate suspensions were mixed together. In some cases, where noted (see Results), B6/*lpr* recipients were infused with ratios of donor marrow favoring B6.C20 (+/+) over B6/*lpr*. Each mouse received at least 10⁷ BMCs by tail vein injection. Control mice received no injection and died within 2 wk of irradiation.

Immunization of Chimeras with Trinitrophenyl-Human Serum Albumin (TNP-HSA). Human serum albumin was trinitrophenylated using 2,4,6 trinitrobenzenesulfonic acid (Eastman Kodak Co., Rochester, NY). Each mouse was injected subcutaneously with 0.1 mg TNP-HSA in CFA (0.5 ml), followed, at 20-d intervals, by two i.p. booster injections of 0.05 mg TNP-HSA in PBS. Mice were bled midway between injections and 10 d after the second boost.

Enzyme-linked Immunosorbent Assay (ELISA)

Total Serum IgM^a and IgM^b. This assay is a modification of a previously described protocol (26). Polyvinylchloride microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated with 100 μ l/well of Bet 2, a non-allotype-specific rat anti-mouse IgM mAb (27), at 1.5 μ g/ml in borate buffered saline (BBS). The plates were then washed in BBS and incubated with 200 μ l/well of coating buffer (BBS, 0.5% BSA, 0.4% Tween 80, and 0.1% NaN₃) for at least 1 h. After aspiration, serum samples or standard positive sera diluted 1:2000 to 1:5000 in coating buffer were added and allowed to bind overnight in the cold. Standard curves for calibration of the assay consisted of two-fold serial dilutions of TEPC 183 (mouse IgM^a; Organon Teknika, Durham, NC) and CBPC 112 (mouse IgM^b; [26]). The assay was developed with biotinylated HB100 (rat IgG1 anti-mouse IgM^a; [27]) and AF6-78.25.2 (mouse IgG1 anti-IgM^b; [28]). In addition, standard curves were compared to each other by development with biotinylated Bet 2. Plates were then incubated with avidin-alkaline phosphatase and developed with paranitrophenyl phosphate (PNP; Sigma Chemical Co., St. Louis, MO) at 1 mg/ml in 0.01 M diethanolamine (DEA), pH 9.8. The plates were read on an automated ELISA reader (Emax; Molecular Devices Corporation, Menlo Park, CA) at timed intervals. OD results were converted to μ g/ml based on the concentration of TEPC 183.

Total Serum IgG2a^a and IgG2a^b. Microtiter plates were coated with 100 μ l/well affinity-purified goat anti-mouse Fab' (3 μ g/ml) in BBS as first step. Sera were diluted at 1:100,000 or 1:1,000,000 in coating buffer, and standard curves consisted of serial dilutions of HB63 (IgG2a^a; [26]) and CBPC 101 (IgG2a^b; [26]). The plates were washed, and previously standardized affinity-purified rabbit anti-mouse IgG2a^a or IgG2a^b were added to replicate plates. Standard curves were also developed with affinity-purified biotinylated goat anti-mouse pFc' as an allotype nonspecific reagent. The plates were then treated as above, and results are expressed as μ g/ml based on the concentration of HB63.

Total IgG1^a and IgG1^b. This assay was performed as an allotype-specific competitive inhibition ELISA. Microtiter plates were coated with 20-9.10 (mAb anti-mouse IgG1^a; [29]) or 412-49.7.20 (mAb anti-mouse IgG1^b; a gift of Dr. Fred Finkelman, Uniformed Services University of the Health Sciences, Bethesda, MD) at 3 μ g/ml (100 μ l/well) in BBS. After washing, plates were incubated with 200 μ l/well of coating buffer. To inactivate IgM rheumatoid factor, serum samples were reduced by being diluted 1:250 into 0.005 M dithiothreitol in BBS (BBS-DTT). Two-fold serial dilutions of HB57 (mouse IgG1^a; [26]) and MOPC 245 (mouse IgG1^b; [26]) were also prepared in BBS-DTT. After reduction for 1 h at room temperature, samples were alkylated with an

equal volume of 0.011 M iodoacetamide in BBS. After 15 min, each sample was divided into two equal aliquots, and an equal volume of a 2 µg/ml solution of either HB57-alkaline phosphatase (HB57-AP) or MOPC 245-alkaline phosphatase (MOPC 245-AP) was added. Those samples containing HB57-AP were added to plates coated with 20-9-10 and those with MOPC 245-AP were added to plates coated with 412-49.7.20. After 2 h at 4°C, the plates were washed and PNP substrate in DEA was added. Plates were read at intervals as above. Standards and control sera of one allotype could not inhibit the other in this assay.

Allotype-specific IgM and IgG2a Anti-Chromatin. These assays paralleled those described above for allotype-specific total IgM and IgG2a, except that chromatin, purified from chicken erythrocyte nuclei (30), was used as the first step (3 µg/ml protein; 100 µl/well), and samples were diluted 1:500. Total immunoglobulin isotype levels and anti-chromatin were generally determined in the same assay. Results are reported based on the concentration of TEPC 183 (for IgM anti-chromatin) or HB63 (for IgG2a anti-chromatin).

Allotype-specific IgM Anti-IgG1^a and -IgG2b^b Rheumatoid Factor. Myeloma proteins HB57 (mouse IgG1^a) and BPC4 (mouse IgG2b^b) were absorbed with a Bet 2-Sepharose 4B gel to reduce background, and used to coat microtiter plates at a concentration of 3 µg/ml. After washing, plates were incubated with coating buffer, followed by 1:500 dilutions of sera. Standard curves were developed using serial dilutions of serum from reference B6/lpr and B6/lpr-Igh^a mice. Following overnight incubation, previously standardized dilutions of biotinylated F(ab')₂ fragments of HB100 (anti-IgM^a) or AF6-78.25.2 (anti-IgM^b) were added to replicate plates. In addition, serial dilutions of reference sera were also developed with biotinylated F(ab')₂ fragments of Bet 2 to allow standardization to each other. The rest of the assay was handled as above, except for data analysis, where results are reported in equivalent dilution factors (EDF). This is defined by the formula: EDF = (Dilution of standard reference sera which gives the equivalent OD of the test serum) × 10⁶ (30).

Immunofluorescence. Allotype-specific two-color IgD and IgM immunofluorescence was routinely performed on PBMCs and selectively on splenocytes to assess chimerism. Approximately 200 µl of tail vein blood was collected into heparinized tubes, and mononuclear cells were isolated using Lympholyte M (Cedarlane, Hornby, Ontario) density gradients. White cells were collected into buffered HBSS, supplemented with 3% FCS and 0.1% NaN₃. For IgD staining, saturating amounts of AF3-33.3.2 (IgG2a anti-IgD^b; [28]) and biotinylated Hδ^a/1 (IgG2b anti-IgD^a; a generous gift of Dr. F. Finkelman) were added for 30 min at 4°C. The second step consisted of incubation with saturating amounts of fluoresceinated goat anti-mouse IgG2a (Southern Biotechnology Associates, Birmingham, AL) and PE-coupled avidin (Southern Biotechnology Associates). The cells were washed three times in PBS and fixed with an equal volume of 2% paraformaldehyde in PBS. The IgM protocol followed that for IgD except that AF6-78.25.2 (IgG1 anti-IgM^b) was used as the first step, followed, after washing, by saturating amounts of fluoresceinated goat anti-mouse IgG1 (Southern Biotechnology Associates). After more washing, more AF6-78.25.2 was added to saturate any unoccupied second-step binding sites. Biotinylated DS1 (IgG1 anti-IgM^a; [31]) was added and followed, after washing, by PE-coupled avidin. In all cases, B6.C20, B6, and (B6.C20 × B6)F1 PBLs were prepared concurrently as controls. Analysis was performed on an Epics V flow cytometer (Coulter Electronics Inc., Hialeah, FL) with size gating on the lymphocyte population. Data were plotted on a three-decade logarithmic scale. At least 5,000 events were collected for each sample.

Results

Initial Experiments in (MRL/lpr × B6/lpr)F1 Chimeras Suggested an Essential Role for the lpr B Cell. Cohorts of 3-mo-old (MRL/lpr × B6/lpr)F1 (MB/lpr) mice were lethally irradiated and reconstituted with five combinations of T cell-depleted bone marrow (4–5 mice per group): I. MRL/+ & B6; II. MRL/lpr & B6; III. MRL/+ & B6/lpr; IV. MRL/lpr & B6/lpr; and V. MB/lpr. 4 mo after transfer, double chimerism in Groups I–IV was verified by immunofluorescence of PBMCs with mAbs to H-2^b (3-25.1) and H-2^k (16-1-2), both a gift of Dr. Jeff Frelinger (University of North Carolina, Chapel Hill, NC) (32, 33). Consistent with our earlier results (17), the peripheral blood of mice from each group showed substantial numbers of cells from each donor type, although in Groups II and III, the lpr cells predominated over the +/+ cells by an average ratio of 2:1. 6–7 mo after transfer, the mice were sacrificed. Groups II–V had marked lymphadenopathy, and the expanded populations mainly expressed the aberrant DNT phenotype, Thy 1⁺ CD4⁻ CD8⁻ 9F3⁺, typical of lpr T cells. Groups II and III had a marked predominance of cells of the lpr vs +/+ phenotype in the lymph nodes (80% vs 5%), but the thymus was equally reconstituted, again in agreement with earlier results. Examination of splenic B cells showed I-A^b to I-A^k ratios similar to those seen for H-2 expression in the peripheral blood (data not shown).

Serum total IgM and IgG2a levels were determined by allotype-specific ELISA. Group II and III showed strong skewing towards the allotype of the lpr donor. All mice were also tested for IgM rheumatoid factor, IgG2a anti-ssDNA, and IgG2a anti-chromatin in allotype-specific ELISAs. In Groups II and III, autoantibodies were mainly of the lpr donor allotype (data not shown). These data suggested two alternative hypotheses: (a) The production of autoantibodies in lpr mice may require expression of the lpr gene in the B cells themselves. (b) The lpr T cells that may provide abnormal help cannot cooperate effectively with nonsyngeneic B cells, even though the T cells have differentiated in an F1 host. To distinguish between these two possibilities, we conducted more extensive chimera experiments in histocompatible congenic strains of mice on the B6 background.

Double Chimerism in B6/lpr Recipients Was Achieved at the Level of Peripheral Blood Lymphocytes. 2–3-mo-old B6/lpr male mice were lethally irradiated and reconstituted as double chimeras with four combinations of T cell depleted bone marrow: I. B6.C20 (Igh^a) & B6 (Igh^b); II. B6/lpr-Igh^a & B6; III. B6.C20 & B6/lpr; and IV. B6/lpr-Igh^a & B6/lpr. Chimerism was assessed at 5 mo after reconstitution using two-color, allotype-specific IgD and IgM immunofluorescence of peripheral blood B cells. The data for one of two similar sets of experiments are summarized in the top half of Table 1. Chimeras reconstituted with two normal (Group I) or two lpr marrows (Group IV) showed nearly equivalent numbers of both IgM- and IgD-bearing cells derived from each donor. Groups II and III, reconstituted with combinations of lpr and +/+ marrow, had B cells of both donors, although the lpr allotype tended to dominate.

Table 1. Allotype Distribution in B6/lpr and B6/lpr-Igh^a Double Chimeras

Group	Immunofluorescence of PBLs				Serum immunoglobulin*										Serum autoantibody levels*			
					Serum immunoglobulin*					Anti-chromatin isotype					IgM RF specificity			
	IgD		IgM		IgM		IgG2a		IgG1		IgM		IgG2a		IgG1 (EDF)		IgG2b ^b (EDF)	
	a [†]	b [†]	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
(%)		(%)		(μg/ml)		(μg/ml)		(μg/ml)		(μg/ml)		(ng/ml)						
B6/lpr recipients[§]																		
I	17(3)	21(5)	28(6)	11(2)	260	110	540	1,150	215	1,400	4	3	<5	14	3	<1	3	<2
II	18(7)	18(6)	32(5)	3(1)	1,800	110	3,200	570	980	280	95	2	1,005	7	20	<1	250	3
III	4(1)	24(5)	12(3)	21(5)	100	740	52	3,300	100	3,800	1	75	<5	7,000	3	31	3	71
IV	10(2)	21(5)	21(3)	17(3)	720	300	1,690	1,670	825	2,200	24	15	500	1,200	16	8	35	28
B6/lpr-Igh^a recipients[¶]																		
I	24(9)	13(3)	35(6)	16(2)	532	233	1,430	80	490	700	<2	<1	100	<10	1	<0.5	6	<0.5
II	13(4)	6(2)	22(5)	7(1)	1,870	125	4,900	100	635	591	35	<1	2,300	10	30	<0.5	68	<0.5
III	6(2)	24(5)	15(5)	12(5)	240	2,100	993	1,200	604	2,000	3	30	190	1,750	3	22	3	33
IV	30(3)	21(5)	31(3)	14(3)	610	990	1,780	800	825	1,690	16	4	750	410	11	3	27	11

B6/lpr or B6/lpr-Igh^a mice were lethally irradiated and given an equal number of bone marrow cells of each allotype in four combinations: I. B6.C20 & B6; II. B6/lpr-Igh^a & B6; III. B6.C20 & B6/lpr; and IV. B6/lpr-Igh^a & B6/lpr. The data shown were obtained from mice bled 4 mo after reconstitution.

* Geometric means.

† Igh allotype.

§ Seven mice per group.

|| Mean (SD).

¶ Five mice per group, except for Group II, which had six.

Serum Ig in Double Chimeric Mice Came from both Bone Marrow Donors. IgM, IgG2a, and IgG1 of the a and b allotypes were detected in all double chimeric mice tested at 4 mo after reconstitution (Table 1). In Groups II and III, the lpr donor allotype (a and b, respectively) dominated in all cases, particularly for IgG2a and IgG1 in Group III. In Groups I and IV neither allotype was consistently favored, although the b allotype was increased in the IgG1 and IgG2a isotypes in Group I.

Autoantibodies Were Derived from lpr Donor Marrow. IgM and IgG2a anti-chromatin autoantibody levels were determined by allotype-specific ELISAs 4 mo after reconstitution (Table 1). Strikingly, in Groups II and III, IgM and IgG2a anti-chromatin autoantibodies were wholly of the lpr donor allotype. The double lpr chimeras in Group IV had substantial amounts of anti-chromatin of both allotypes, while Group I showed only very low levels of b allotype autoantibody (probably due to recipient marrow recovery; see Discussion).

Rheumatoid factors reactive with murine IgG1^a and IgG2b^b were also assayed (Table 1). Again, autoantibodies in mice of Groups II and III were derived entirely from the lpr donor B cells. Trace levels of rheumatoid factor were present in Group I mice, while Group IV mice had RF of both allotypes.

Results Were Similar with B6/lpr-Igh^a Hosts. Parallel experiments were performed with B6/lpr-Igh^a recipients, and data from one of two similar experiments are summarized in the bottom half of Table 1. Immunofluorescence of PBMCs again showed mixed chimerism, although Groups I and IV showed some excess of B cells of the a allotype. Serum total IgM, IgG2a, and IgG1 were generally balanced in Groups I and IV (except IgG2a in Group I), while these isotypes were mostly skewed toward the lpr donor allotype in Groups II and III. As in the B6/lpr-recipient chimeras, autoantibodies in Groups II and III were entirely of the lpr donor allotype, with the exception of a single Group III mouse, in which there was some IgG2a^a anti-chromatin in the presence of a larger amount of IgG2a^b anti-chromatin.

Double Chimeras Reconstituted with Excess Normal Marrow Still Produced Autoantibodies Bearing Only the Allotype Derived from the lpr Marrow. An additional experiment was performed in which B6/lpr mice were reconstituted at ratios 2:1, 5:1, and 10:1 of B6.C20 to B6/lpr bone marrow in order to produce chimeras that favored the normal B cells. 1 mo after reconstitution, peripheral blood B cells were predominantly of the normal a allotype, as shown by both IgD and IgM immunofluorescence (data not shown). Over the next 6 mo, the percent of cells bearing the lpr phenotype increased, par-

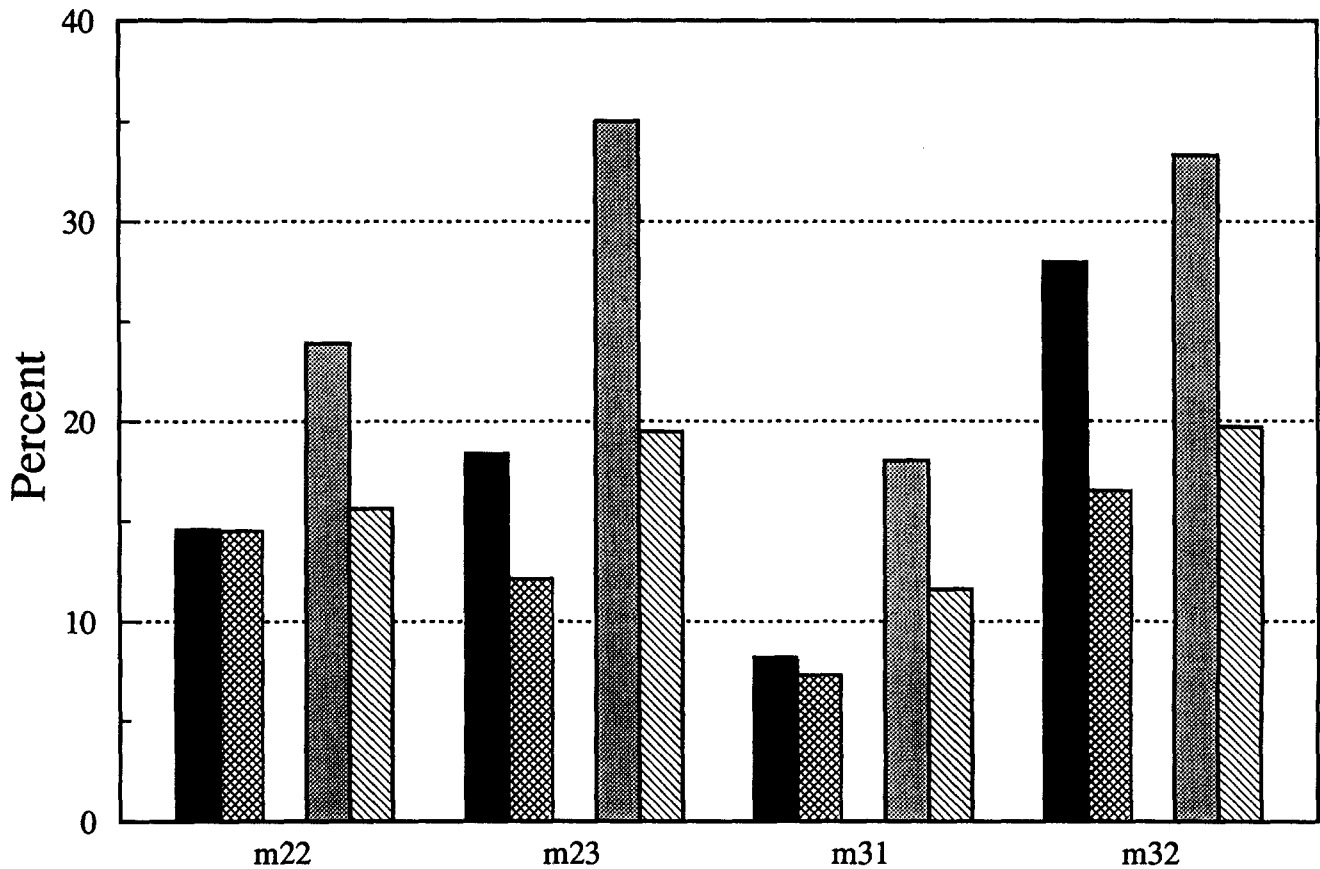


Figure 1. Allotype distribution of splenic B cells in unequally reconstituted B6.C20 & B6/lpr \rightarrow B6/lpr Group III chimeras. 4 mo after reconstitution, four mice were sacrificed and splenocytes examined by immunofluorescence. IgD^a (■). IgD^b (▨). IgM^a (▩). IgM^b (▧).

ticularly as detected by anti-IgM, although the normal B cell predominance persisted in most cases. At 4 mo, four representative mice were sacrificed, and their spleens were examined by immunofluorescence (Fig. 1). The relative proportions of cells expressing the a and b allotypes were comparable to that seen in peripheral blood and still favored the normal a allotype.

Table 2 lists the serological data for individual mice 3–4 mo after reconstitution. In spite of the cellular bias towards the normal +/+ B cells, total serum IgM was relatively evenly balanced at this time. In contrast, serum total IgG2a and IgG1 were strongly biased towards the b allotype of the *lpr* donor. Autoantibodies were detected in most mice and were derived solely from the *lpr* donor.

In Presence of lpr, Normal B Cells Showed Little Response to Exogenous Antigen. To determine whether the +/+ B cells present in +/+-*lpr* double chimeras were potentially functional, approximately 7 mo after reconstitution, mice from each of the four groups shown in the top half of Table 1 were immunized with TNP-HSA. Group I mice showed low preimmunization levels of anti-TNP antibody and a strong IgG2a response of both allotypes (Fig. 2). In contrast, *lpr* B cells in Groups II, III and IV showed high preimmunization titers of anti-TNP, but had little additional response to immuniza-

tion. +/+ B cells in Groups II and III had low preimmunization titers, but produced only a blunted response to antigen. IgM anti-TNP had a decreased but parallel response (data not shown). This experiment was repeated at an earlier time following reconstitution (4 mo) using some of the B6/lpr recipients given an excess of +/+ marrow, again with similar results (data not shown).

Discussion

The present results indicate that the production of antibodies in the *lpr* model of SLE requires expression of the *lpr* gene in B cells. Preliminary experiments were performed by transferring combinations of MRL and B6 bone marrow into irradiated (MRL/*lpr* \times B6/*lpr*)F1 hosts. We confirmed our earlier results demonstrating an intrinsic T cell abnormality (17). Surprisingly, autoantibodies appeared to be made preferentially by cells from the *lpr* donor. Although this result suggested that an *lpr*-specific abnormality had to be expressed in the B cells that produced autoantibodies, we could not exclude the possibility that abnormal *lpr* T cells preferentially interacted with the syngeneic (i.e., H-2 compatible) *lpr* B cells. We therefore performed additional experiments with chimeras constructed using Igh-allotype congenic strains of B6 mice.

Table 2. Allotype Distribution in Unequally Reconstituted Group III Mice

Mouse number	Serum autoantibody levels																	
	Immunofluorescence of PBLs				Serum immunoglobulin						Anti-chromatin isotype				IgM RF specificity			
	IgD		IgM		IgM		IgG2a		IgG1		IgM		IgG2a		IgG1 (EDF)		IgG2b ^b (EDF)	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
	(%)		(%)		($\mu\text{g/ml}$)		($\mu\text{g/ml}$)		($\mu\text{g/ml}$)		($\mu\text{g/ml}$)							
24 (2:1)*	7	5	12	5	100	450	100	1,740	<50	4,700	<1	46	<10	250	<1	<1	<1	14
29 (2:1)	33	12	5	5	570	590	80	1,130	70	5,500	<1	10	<10	<10	<1	10	<1	21
30 (2:1)	28	11	9	6	345	710	85	1,205	140	6,050	<1	5	<10	1,200	<1	8	<1	26
21 (5:1)	22	6	16	3	805	905	125	750	90	1,500	<1	15	<10	575	<1	54	<1	76
22 (5:1)	20	7	21	5	605	550	130	1,325	100	4,300	<1	36	<10	650	<1	18	<1	32
23 (5:1)	21	8	27	8	570	685	115	1,350	70	3,600	<1	9	<10	275	<1	10	<1	16
32 (5:1)	32	9	8	3	360	470	100	1,570	60	5,600	<1	67	<10	550	<1	6	<1	9
33 (5:1)	16	3	8	3	290	370	75	560	140	3,000	<1	35	<10	<10	<1	<1	<1	5
27 (10:)	24	5	20	5	435	710	125	1,120	<50	4,100	<1	20	<10	350	<1	<1	<1	48
31 (10:1)	18	4	11	4	1,040	1,775	145	2,250	95	3,200	<1	6	<10	575	<1	16	<1	27
34 (10:1)	27	5	13	6	895	1,200	120	1,620	330	6,600	<1	6	<10	<10	<1	53	<1	55

B6/lpr mice were lethally irradiated and given a combination of thy 1-treated bone marrow from B6.C20 and B6/lpr donors at the indicated ratios. Mice were bled at monthly intervals. Immunofluorescence data are from 3 mo after reconstitution, while serologic data are from 3.5 to 4 mo. * Ratio of B6.C20:B6/lpr donor marrow.

In these reconstituted mice, *lpr* and +/+ B cells differed only by Igh allotype. The results of five separate experiments indicated that *lpr* B cells were the sole source of autoantibodies, despite the presence of +/+ -derived cells as well.

To exclude the possibility of systematic assay errors and to assess better the potential differences in autoantibody production afforded by different Igh genes, both B6/lpr and B6/lpr-Igh^a hosts were tested. An additional possible confounding factor was host bone marrow recovery, despite lethal irradiation (34). In this regard, for B6/lpr hosts, data from Group III, a mixture of B6.C20 and B6/lpr donor marrow, allowed the clearest assessment of the contribution of B cells derived from infused normal marrow, since, in these cases, host bone marrow recovery could only add to the antibody bearing the *lpr* donor allotype. Likewise, for B6/lpr-Igh^a hosts, Group II, was the most critical. In both of these groups, serum total IgM and IgG antibody were mainly of the *lpr* donor allotype, yet B cells and immunoglobulin from the +/+ donor were clearly present. Nevertheless, autoantibodies derived from normal donor cells were never seen, even though anti-chromatin and rheumatoid factor of the *lpr* allotype were usually present. Recipient bone marrow recovery probably accounted for the increased amounts of b and a allotype IgG in B6/lpr and B6/lpr-Igh^a, respectively, in Group I of Table 1; for the low levels of autoantibody seen in some Group I mice; and for the single Group III B6/lpr-

Igh^a recipient which made appreciable levels of a allotype IgG2a anti-chromatin. Finally, the crossover design of our experiments also excluded the possibility that the preferential use of the b allotype for autoantibody production, which we have previously shown (35), could obscure the interpretation of our results.

The ability to construct *bona fide* +/+ -*lpr* double chimeras was critical to our approach. It has been reported that reconstitution of *lpr* mice with a mixture of *lpr* and +/+ marrow results in complete takeover by *lpr* marrow within 1 mo (34), although experience in our laboratory has been different (17). In the current work, recipients of a 1:1 ratio of +/+ :*lpr* showed a predominance of the *lpr*-derived B cells in the peripheral blood; however, +/+ B cells were always present in significant numbers (Table 1). In those double chimeras reconstituted with excess normal bone marrow, the number of +/+ phenotype B cells usually exceeded that of *lpr* origin, in both the peripheral blood and the spleen (Table 2). In these same animals, serum IgM was nearly balanced, while serum IgG2a and IgG1 was dominated by the allotype derived from the *lpr* donor. As in the donor-balanced chimeras, autoantibodies of the IgM and IgG2a isotypes were entirely of *lpr* origin.

Other work has suggested a role for cells not of bone marrow origin in the development of *lpr* lymphadenopathy (36-38). When *lpr* bone marrow is introduced into a lethally irradiated congenic +/+ mouse, a curious wasting syndrome

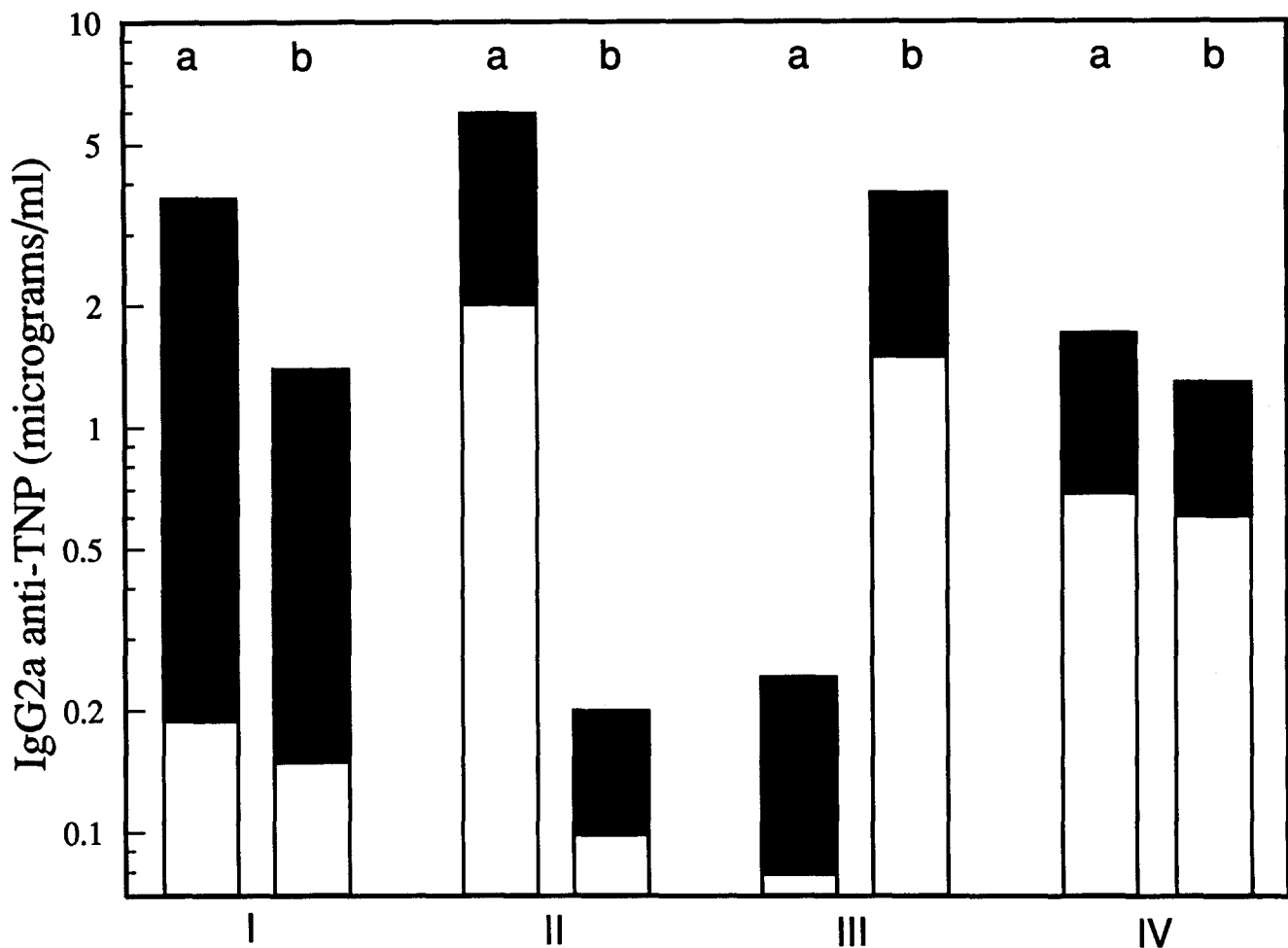


Figure 2. IgG2a anti-TNP response of chimeric mice. Mice were immunized subcutaneously with 100 μ g TNP-HSA in CFA and boosted twice at 20-d intervals with 50 μ g given intraperitoneally. IgG2a anti-TNP antibodies were quantitated by allotype-specific ELISA. Pre-immunization levels (open bars) and final titers 10 d after the last injection (closed bars) are shown for each group as geometric means.

ensues, leading to lymphoid depletion and early mortality (39, 40). If, however, an *lpr* lymph node is also transplanted, these mice will develop adenopathy in that transplanted node (38), suggesting that nonhemopoietic cells may be necessary for full expression of the T cell defect. Perkins et al. (25) have demonstrated that neonatal $+/+$ mice given *lpr* bone marrow produce preferentially *lpr*-allotype IgG2a and IgG2a anti-ssDNA. These results indicated an intrinsic *lpr* B cell defect, as shown in our current studies. Our present data extend these observations to show that a complete *lpr* environment, including the expanded population of *lpr* T cells, is still not sufficient to stimulate normal B lymphocytes to secrete autoantibodies, thus indicating that the *lpr* B cell is an absolute requirement for autoantibody production in *lpr* mice.

An additional finding in the current study was that normal lymphoid cells appeared to be immunosuppressed in an *lpr* environment. This result parallels the impaired responses *lpr* mice are known to have to exogenous antigens (41, 42). In double chimeric mice immunized with the T cell dependent antigen, TNP-HSA, the normal B cells responded poorly in

the presence of *lpr* donor marrow (Groups II and III; Fig. 2). This immunosuppression was not due to the non-bone-marrow-derived *lpr* host environment, since *lpr* mice reconstituted with normal marrows (Group I) had a good IgG2a response to TNP. Therefore, we assume that either the *lpr* T cells or the *lpr* B cells exerted a strong downregulatory effect in vivo. We are currently investigating the mechanism of this suppression with further double chimera experiments.

The observed results cannot easily be explained by transfer of *lpr* B cells already committed to autoantibody production. Autoantibodies did not become evident until at least 2 mo after transfer. In addition, examination by immunofluorescence of bone marrow cells from *lpr* and normal mice failed to show any appreciable staining for mouse κ light chains (data not shown). Furthermore, allophenic mice, made by the fusion of *lpr* and normal embryos, also showed autoantibodies only of the *lpr* phenotype (T. Katagiri, S. Azuma, Y. Toyoda, S. Mori, K. Kano, P.L. Cohen, and R.A. Eisenberg, manuscript in preparation). The present study thus suggests that either the *lpr* cells were specifically driven to IgG and

autoantibody production or that the +/+ cells were inhibited from doing so. In any case, the presence of the *lpr* gene was essential for the production of autoantibodies by the B cells.

The finding that *lpr* B cells are intrinsically abnormal raises questions as to the relative role of T cell and B cell defects in the *lpr* model of autoimmune disease. Although T cells are clearly required for autoantibody production in *lpr* mice,

it is unknown whether the *lpr* T cell is uniquely required. We are currently testing the possibility that normal T cells can stimulate *lpr* B cells to secrete autoantibodies in vivo. In any case, primary B cell abnormalities have now been shown to play a fundamental role in the immune dysregulation of *lpr* disease.

We thank Rebecca Rapoport for breeding the B6/*lpr*-Igh^a strain and Robert L. Cheek for helping with the ELISAs. We acknowledge NCI-CB-25584 for the four Igh^b myelomas obtained from Dr. Michael Potter.

This work was supported by USPHS Grants AR-40620, AR-26574, AR-34156, AR-33887, and T32 AR-07416. Dr. Sobel is a Postdoctoral Fellow of the Arthritis Foundation.

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Received for publication 19 November 1990 and in revised form 27 February 1991.

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