ASSOCIATION OF *lpr* GENE WITH GRAFT-VS.-HOST DISEASE–LIKE SYNDROME

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Transfer of autoimmune disease by implanting specific tissues from mice genetically predisposed to autoimmunity into nonautoimmune histocompatible recipients is an exceptionally good way to define the etiopathogenesis of such disorders. Although some autoimmune manifestations have been observed in normal mice transplanted with spleen or bone marrow (BM)[1] cells from NZB or NZB × NZW F_1 (NZB × W) mice, which are genetically prone to a systemic lupus erythematosus (SLE)-like disease (1–3), the results are questionable because the recipients usually differ from the donor at the major or several minor histocompatibility loci. Resulting allogeneic reactions may then induce autoimmune manifestations (4).

The recent availability of BXSB mice, the males and females of which differ substantially in mortality rates and expression of SLE-like syndromes (50% mortality at 5–6 mo for males, and beyond 18 mo for females), and of congenic MRL mice (MRL/Mp *lpr/lpr* [lymphoproliferative syndrome], 50% mortality at 5 mo, and MRL/Mp +/+ at 18 mo) (5, 6) provides the opportunity to perform well-controlled cell transfers to determine the importance of hemopoietic/lymphoid cell abnormalities in this disease. Bone marrow and spleen cell transfer studies in male and female BXSB mice have already shown that this disease is expressed by their hemopoietic stem cell population, and that nonlymphoid or hormonal influences are minimal (7, 8). We now report our experience with similar cell transfers between the *lpr/lpr* and +/+ lines of the MRL strain. The unexpected finding was that although these lines are considered congenic (<1% of their genomes differ) (5), unidirectional severe graft-vs.-host–like disease (GVHD) and lymphoid atrophy developed in the +/+ recipients of *lpr/lpr* lymphoid cells or

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[1] *Abbreviations used in this paper:* BM, bone marrow; BUN, blood urea nitrogen; C, complement; CFU, colony-forming unit; CML, cell-mediated lympholysis; Con A, Concanavalin A; GN, glomerulonephritis; GVHD, graft-vs.-host disease; IC, immune complexes; *lpr*, lymphoproliferation gene; LPS, lipopolysaccharide; MLR, mixed lymphocyte reaction; NK, natural killer; PNA, peanut agglutinin; SBA, soy bean agglutinin; SLE, systemic lupus erythematosus; SRBC, sheep red blood cell; ssDNA, single-stranded DNA.

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their precursors. On the other hand, *lpr/lpr* recipients of +/+ cells showed delayed autoimmunity.

Materials and Methods

Mice. MRL/Mp *lpr/lpr* and MRL/Mp +/+ mice (H-2^k), originally developed by Murphy and Roths (5) were bred and maintained at the Scripps Mouse Colony. MRL/Mp *lpr/lpr* mice develop a variety of autoantibodies, and massive Thy-1^+, Lyt-1^+ cell proliferation with lymph node hyperplasia and splenomegaly at 3–4 mo, and die with severe immune complex (IC) glomerulonephritis (GN) by 5 mo. The congenic +/+ mice do not contain the *lpr* gene, are genetically >99% identical to the *lpr/lpr* line, develop late SLE, and die in the latter half of the second year of life (5, 6). Additional strains used in these studies included three immunologically normal strains (C57BL/6, C3H/St, BALB/c), and one strain (C57BL/6 *lpr/lpr*) that, like the MRL *lpr/lpr*, expresses the *lpr* gene, develops lymphoid hyperplasia and autoantibodies, but sustains very mild GN (9–11). All mice used in this study were females and, unless otherwise stated, were 4 wk old at the time of cell transfers.

Preparation of Cells for Transfer. Cell populations used for transfers were: spleen, BM, and fetal liver cells. Spleen and fetal liver cell suspensions were made by passing the organs through a stainless steel screen in minimum essential medium (Gibco Laboratories, Grand Island, NY) supplemented with 0.02 M Hepes. BM cells were obtained by flushing the tibias and femurs with the above medium. Erythrocytes were lysed from these preparations with 0.17 M NH_4Cl. The cells were then washed three times with medium and resuspended to 10^8 viable spleen cells/ml, 4×10^7 BM cells/ml, and 1.6×10^7 fetal liver cells/ml. In some experiments, spleen or BM cells were treated with anti-Thy-1.2 (New England Nuclear, Boston, MA.) with or without complement (C) to lyse or sensitize, respectively, mature T cells. Anti-Thy-1.2 plus C–treated spleen cells were unresponsive to concanavalin A (Con A). Furthermore, in some experiments, BM cells were treated sequentially with soy bean agglutinin (SBA) and peanut agglutinin (PNA), as detailed by Reisner et al. (12), before transfer to eliminate mature and immature T and B cells from the stem cell inoculum.

Cell Transfers. Recipients of cell transfers were given puramycin (Ralston-Purina, St. Louis, MO) in their drinking water for at least 48 h, and fasted for 24 h before being given a lethal dose (850 rad) of irradiation on the morning of transfer. They were maintained on puramycin for 3 wk thereafter. 5×10^7 spleen cells, 2×10^7 BM cells, or 0.8×10^7 fetal liver cells were injected intravenously into each recipient. Some recipients were injected with mixtures of *lpr/lpr* and +/+ spleen or BM cells. Recipients were followed for evidence of clinical disease, especially enlarged lymph nodes, and sacrificed when moribund for complete autopsy and cellular studies. Serum samples were obtained at monthly intervals for serologic studies.

In some studies, +/+ mice were thymectomized (13) at birth and lethally irradiated at 1 mo for use as recipients of *lpr/lpr* spleen or BM cells. Effective thymic removal was ascertained 2 wk before irradiation by studying responsiveness to a thymus-dependent antigen, sheep red blood cells (SRBC), manifested as serum hemagglutinating antibodies.

Additionally, some *lpr/lpr* mice were thymectomized at birth and immediately given (under the skin at the back of the neck) whole thymus from 1-mo-old +/+ mice; 2 mo later, successfully transplanted animals, as judged by responsiveness to SRBC, were used as donors of spleen or BM cells. Conversely, other +/+ mice were thymectomized and transplanted with *lpr/lpr* thymuses; 2 mo later, these mice were lethally irradiated and reconstituted with BM cells from *lpr/lpr* mice.

Histologic Studies. Major organs from autopsied mice were weighed, sectioned, and stained with hematoxylin and eosin for microscopic examination. GN was quantitated on a 0–4 scale based on the intensity and extent of histologic involvement. A grade 3 or higher was considered contributory to death.

Serologic Studies. Blood urea nitrogen (BUN) was determined by Azostix strips, as recommended by the manufacturer (Miles Ames Division, Miles Laboratories, Inc., Elkhart, IN). IgG was estimated by single radial immunodiffusion. Anti–single-stranded

DNA (ssDNA) and anti–double-stranded DNA, as well as IC, were measured as described (6).

Cellular Studies. Primary mixed lymphocyte reactions (MLR), primary and secondary cell-mediated lympholytic reactions (CML) were performed as detailed elsewhere (14).

Suppression of proliferative responses of splenocytes from each MRL line to the T cell mitogen Con A, or a B cell mitogen, lipopolysaccharide (LPS) (*E. coli* 055:B5; Difco Laboratories, Detroit, MI), by splenocytes of the opposite line was tested by incubating each cell type separately with the mitogens (baseline value), or together at complementing proportions to maintain a constant total cell number in culture (2×10^5 cells in 0.2 ml). Con A was used at 4 μg/ml, and LPS at 2.5 μg/ml. After a 3-d incubation, proliferative responses were measured by the degree of [^{3}H]thymidine incorporation in the washed pellet (15). Recipients' total spleen and lymph node cells, and their T and B cell content were counted, at the time of sacrifice, as described (16).

Splenomegaly Assay for GVHD. With a modified Simonsen's assay (17), newborn +/+ mice were injected intraperitoneally with 5×10^7 splenocytes from 1-mo-old +/+ or *lpr/lpr* mice. Controls were injected with saline. 10 d later, litters were sacrificed, and spleen/body weight ratios were determined for both the inoculated recipients and control littermates. An index of spleen enlargement was computed by dividing the spleen/body weight ratios of the injected animals by that of their uninjected littermates.

Colony-forming Units (CFU). To determine the efficiency in hemopoietic precursors of BM in the two MRL lines, we counted hemopoietic stem cells by the spleen colony assay of Till and McCulloch (18). Enough viable nucleated BM cells (10^5) to yield 8–20 colonies were injected intravenously into groups of 10–20 lethally irradiated recipients. 11 d later, the mice were killed, their spleens fixed in Bouin's fluid, and the macroscopic surface colonies (CFU) counted.

Results

Spleen Cell Transfers. Fig. 1 depicts the mortality rates after spleen cell transfers between MRL *lpr/lpr* and MRL +/+ mice. MRL *lpr/lpr* recipients of MRL *lpr/lpr* spleen cells (MRL *lpr/lpr* → MRL *lpr/lpr*) had short life spans, similar to their unmanipulated counterparts, whether the donors were 1 mo old (prediseased) or 5 mo old (diseased) (50% mortality ~5.2 mo posttransfer). MRL *lpr/lpr* recipients of syngeneic splenocytes developed characteristic dermatitis and lymphadenopathy with enlarged submaxillary, inguinal, and axillary nodes

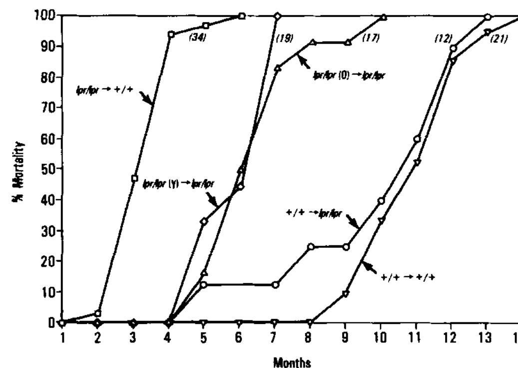

FIGURE 1. Mortality rates in spleen cell transfers between MRL *lpr/lpr* and MRL +/+ mice. The percentage of dead animals in each group is tabulated and graphed on a monthly basis, counting from the day of transplantation. In the case of MRL *lpr/lpr* → MRL *lpr/lpr* transfers, both 1-mo-old (young, *Y*) and 5-mo-old (old, *O*) donors were used. Numbers in parentheses indicate the size of each group.

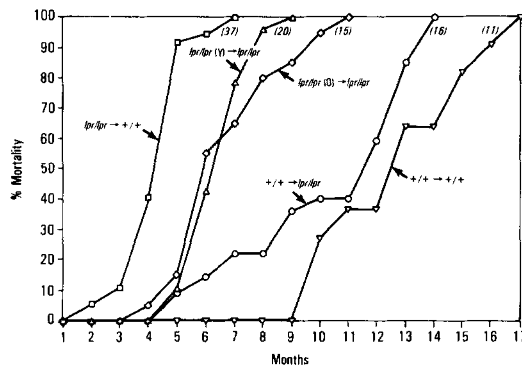

FIGURE 2. Mortality rates after BM cell transfers between MRL *lpr/lpr* and MRL +/+ mice, calculated as in Fig. 1. Numbers in parentheses indicate the size of each group.

by the fourth month posttransplantation, although this was not as apparent as that in the unmanipulated MRL *lpr/lpr* controls. In contrast, MRL *lpr/lpr* recipients of the MRL +/+ splenocytes had a longer 50% mortality rate (9.7 mo posttransfer) than the unmanipulated MRL *lpr/lpr* controls (5 mo), and developed minor lymphadenopathy. MRL +/+ → MRL +/+ recipients had a 50% mortality rate 10.2 mo posttransfer, compared to 17 mo for unmanipulated female +/+ mice. The shorter life span of the former group was attributed to some early deaths, apparently due to pulmonary infections. Unexpectedly, MRL +/+ recipients of MRL *lpr/lpr* splenocytes developed a severe wasting syndrome, and died 2–4 mo posttransfer (50% mortality 2.2 mo posttransfer) without the typical lymphadenopathy of the donor strain. In a group of such recipients followed closely, wasting was clinically apparent at ~1 mo posttransfer.

BM Cell Transfers. As Fig. 2 shows, the results of BM cell transfers closely resembled those observed with spleen cell transfers. MRL *lpr/lpr* recipients of MRL *lpr/lpr* BM cells derived from young (1 mo) or older (5 mo) donors had a 50% mortality at around 5 mo posttransfer, and developed severe lymphadenopathy. Furthermore, MRL *lpr/lpr* recipients of MRL +/+ BM cells outlived control MRL *lpr/lpr* mice that had been given MRL *lpr/lpr* transfers (50% mortality at 10.8 vs. 5 mo posttransfer). The 50% mortality for MRL +/+ recipients of MRL +/+ BM cells was around 12 mo posttransfer. As in the spleen cell transfers, MRL +/+ recipients of MRL *lpr/lpr* BM cells developed a severe wasting syndrome, and died 1–6 mo posttransfer (50% survival at 4.3 mo), without the clinical manifestations or lymphadenopathy of the MRL *lpr/lpr* donor strain. Even at 15 d, MRL +/+ recipients of MRL *lpr/lpr* cells had developed this wasting syndrome. Runt disease also developed by 3–4 mo posttransfer in a group of MRL +/+ mice given a mixture of 2×10^7 +/+ and 2×10^7 *lpr/lpr* BM cells. When 2×10^7 +/+ BM cells were given together with varying numbers of *lpr/lpr* BM cells, as few as 5×10^6 *lpr/lpr* cells in the inoculum (1:5 ratio of *lpr/lpr* to +/+ cells) caused the wasting syndrome in the +/+ recipients. Irradiating (250 rad) the *lpr/lpr* cells before inoculation delayed the wasting syndrome until about 5 mo of age, and irradiation of these cells with 500–1,000 rad completely eliminated it in +/+ recipients of cell mixtures.

Additional in vivo experiments showed that incremental injection of +/+ BM cells (2, 10, and 20 × 10^6) into +/+ mice transplanted 1 mo earlier with *lpr/lpr* cells failed to rescue the recipients from the wasting syndrome. Furthermore, injection of lethally irradiated (MRL *lpr/lpr* × MRL +/+)F_1 mice with MRL *lpr/lpr* BM cells resulted in wasting and death by 3–5 mo posttransfer, whereas no wasting developed even at 5 mo in F_1 mice reconstituted with MRL +/+ BM cells. Runt disease did afflict lethally irradiated C3H/St (H-2^k) mice transplanted with MRL *lpr/lpr* but not MRL +/+ BM cells.

Histologic Studies. MRL *lpr* recpients of +/+ lymphoid cells did not develop *lpr*-associated lymphoproliferation, although their lymphoid organs (spleen, thymus, and lymph nodes) showed varying degrees of immunoblastic changes, with increased numbers of plasmacytoid cells. Interestingly, +/+ recipients of *lpr* lymphoid cells not only failed to manifest lymphoproliferation, they showed signs of graft histoincompatibility. In the early stages (1–2 wk after engraftment), the +/+ recipients' splenic white pulp contained pycnotic cells, plasma cells, and macrophages (Fig. 3*a*), followed by progressive cellular depletion, complete hyalinization, and fibrosis of the splenic parenchyma by 4–5 mo (Fig. 3*b*). Their thymus, BM, and lymph nodes underwent similar stages of cellular depletion and hyalinization. Other organs showing the histologic changes associated with graft histoincompatibility were the liver, which showed focal fibrinoid necrosis of hepatocytes, and portal triaditis with inflammatory destruction of bile ducts (Fig. 3*c*), and the gastrointestinal tract (Fig. 3*d*) where there was inflammatory infiltration with destruction of the mucosal epithelium. Likewise, the bronchi and the inter- and intralobular ductal epithelium of salivary glands were infiltrated by mononuclear inflammatory cells. At autopsy, these affected organs show fibrosis and sclerosis.

Serologic Studies. The serologic characteristics of all splenic cell recipients and their respective controls are depicted in Table I. MRL +/+ → MRL +/+ and MRL *lpr/lpr* → MRL *lpr/lpr* recipients resembled their corresponding unmanipulated controls with respect to hypergammaglobulinemia, high levels of BUN, IC, and anti-DNA. In MRL *lpr/lpr* recipients of +/+ cells, these serologic values were reduced compared to those in unmanipulated *lpr/lpr* mice or to *lpr/lpr* → *lpr/lpr*. However, +/+ recipients of *lpr/lpr* cells failed to show any serologic characteristics of the donor strain; comparatively, their levels of IgG, anti-DNA, and IC were low.

Cellular Studies. The enlarged lymph nodes (median weight 460 mg) of *lpr/lpr* → *lpr/lpr* mice had >94% Thy-1^+, Lyt-1^+ cells, whereas +/+ → *lpr/lpr* chimeras (median weight 130 mg) contained 73% Thy-1^+, Lyt-1^+, and 22% surface Ig–positive cells. Spleens of +/+ mice given *lpr/lpr* splenocytes, which produced wasting and lymphoid atrophy, were also killed and examined for T and B cell composition at 15, 30, or 60 d posttransfer (Table II). At 30 d these mice had ~33% as many, and at 60 d ~0.3% as many splenocytes as at day 15 posttransfer, with a marked decline in Thy-1^+ cells. In contrast, the splenic cellularity and frequency of T and B cells remained unaltered in +/+ recipients of +/+ cells.

Histocompatibility Studies. Because of the consistent development of GVHD in +/+ recipients of *lpr/lpr* BM or spleen cells, possible histocompatibility

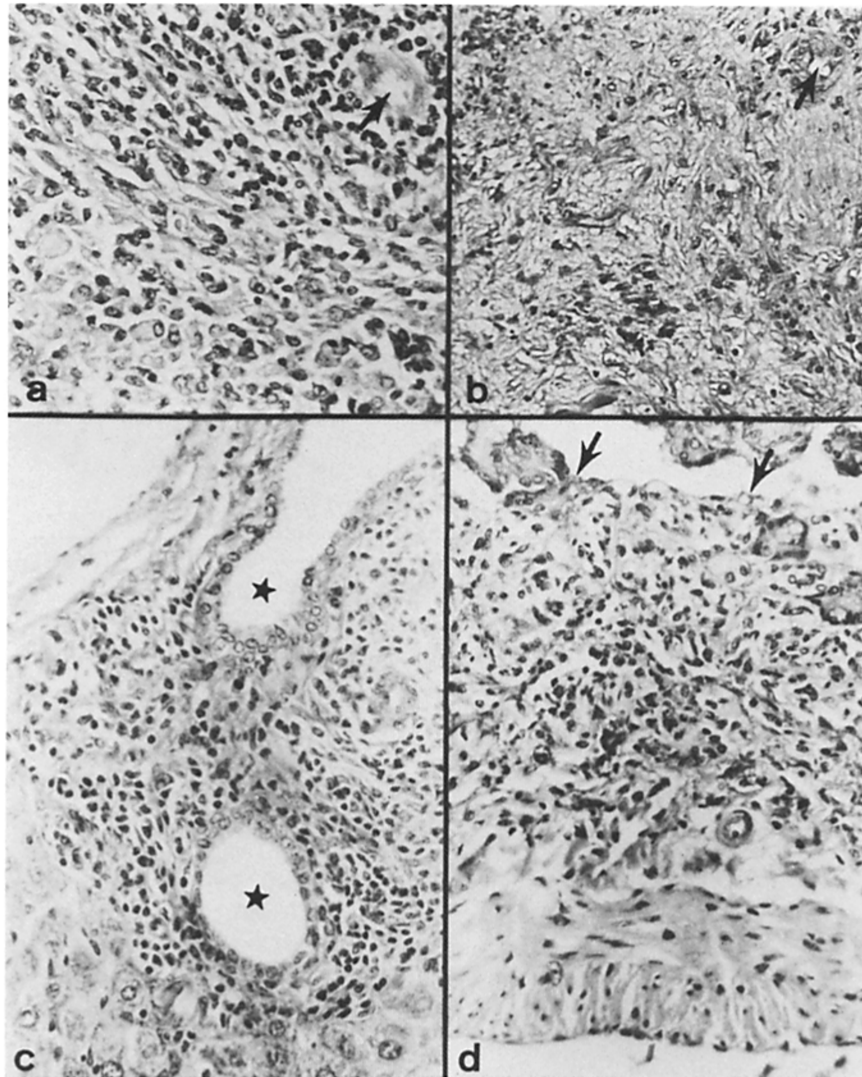

FIGURE 3. Histologic findings in MRL +/+ recipients of MRL *lpr/lpr* lymphoid cells. (*a*) +/+ spleens 2 wk after lymphoid cell transfer. Arrow indicates splenic arteriole, which is surrounded by scattered pycnotic lymphocytes. In the surrounding white pulp, the fibrous stroma contains plasma cells and macrophages. (*b*) +/+ spleens 4.5 mo after lymphoid cell transfer. Arrow marks splenic arteriole in upper right, which is surrounded by fibrous, largely acellular stroma remaining after depletion of most of the splenic cells. (*c*) Hepatic portal triad of +/+ recipients 2 mo after cell transfer. Stars indicate the bile ducts, which are surrounded by inflammatory lymphoid infiltrates. (*d*) Small bowel of +/+ recipients 2 mo after cell transfer. Arrows of upper part of photo mark the partially denuded mucosal surface. The entire submucosa is infiltrated by chronic inflammatory cells. The largely uninvolved muscularus and peritoneal surface are seen at the lower portion of the photo.

differences between these congenic lines were sought. As shown in Table III, *lpr/lpr* and +/+ cells exhibited normal MLR against H-2-incompatible cells (BALB/c, H-2^d), and no reactivity against themselves or H-2-compatible allogeneic cells (C3H/St, H-2^k). Similarly (Table IV), the primary CML against H-2-

TABLE I
Serologic Characteristics of Spleen Chimeras

Mice	IgG	Anti-ssDNA binding	BUN	IC
	mg/ml	*%*	*mg/ml*	*μg/ml*
+/+	13.4 ± 1.0	35.1 ± 6.6	15.1 ± 9.6	67.2 ± 32.0
lpr	31.0 ± 5.0	78.3 ± 6.4	>60.0	2,934.5 ± 793.5
+/+ → +/+	11.2 ± 1.4	33.9 ± 13.3	16.1 ± 10.1	19.9 ± 31.0
*lpr** → *lpr*	21.2 ± 8.0	72.0 ± 35.7	57.3 ± 12.7	2,569.1 ± 11,191.7
lpr‡ → *lpr*	35.5 ± 7.8	76.8 ± 14.3	>60.0	ND§
+/+ → *lpr*	21.0 ± 1.9	43.3 ± 20.9	35.0 ± 10.3	636.0 ± 153.8
lpr → +/+	7.9 ± 1.2	25.7 ± 20.0	11.0 ± 9.6	21.2 ± 24.9

* MRL *lpr/lpr* donors 1 mo old.
‡ MRL *lpr/lpr* donors 5 mo old.
§ ND, not determined.

TABLE II
Lymphoid Cell Numbers and Surface Characteristics in Spleen Cell Transfers

Type of transfer	Time posttransfer	Total spleen cells ($\times 10^{-6}$)	Thy-1$^+$	sIg$^+$
	d		%	%
lpr/lpr → +/+	15	48.7 ± 6.7	25.4 ± 3.2	46.9 ± 6.9
	30	16.0 ± 2.3	9.7 ± 1.8	35.8 ± 5.8
	60	0.15 ± 0.04	ND*	ND
+/+ → +/+	30	63.1 ± 8.8	44.3 ± 4.1	42.2 ± 7.7
	60	66.5 ± 2.4	42.3 ± 2.4	55.8 ± 3.9

Means ± SD of five individual mice for each time point.
* ND, not determined.

incompatible target cells and secondary responses against H-2-compatible allogeneic cells were normal, i.e., killing was substantial in both instances. Yet no primary, and only weak secondary CML between the two MRL lines was recorded; the secondary CML was in no way comparable to the secondary responses seen between these cells and C3H/St cells, which differ at minor histocompatibility loci.

Splenomegaly, tested neonatally, was unremarkable in +/+ recipients of +/+ or *lpr/lpr* cells. The spleen/body weight ratio between uninjected and injected mice was 0.991:1 for +/+ cells, and 0.994:1 for *lpr/lpr* cells.

Hemopoietic Precursor Efficiency of lpr/lpr. To determine whether the runt disease of +/+ recipients of *lpr/lpr* cells was caused by insufficient numbers of hemopoietic precursors in *lpr/lpr* BM inocula or by BM resistance phenomena (19), we counted CFU induced in reciprocal BM cell transfers between the two MRL lines. As shown in Table V, *lpr/lpr* BM inocula contained sufficient numbers of pluripotent cells; somewhat more, in fact, than in +/+ inocula.

Attempts to Overcome the Wasting Syndrome. We used several methods that reportedly (12, 20–22) prevent GVHD caused by major or minor histocompatibility differences. Treatment of the *lpr/lpr* BM inocula with anti-Thy-1.2 alone or in conjunction with C to opsonize or eliminate contaminating mature T cells,

TABLE III

Mixed Lymphocyte Reaction with MRL lpr/lpr and MRL +/+ Cells

Responder	Stimulator	[^{3}H]Thymidine uptake*	Stimulation index‡
		cpm	
lpr/lpr	—	14,829 ± 1,778	—
+/+	—	30,515 ± 1,772	—
C3H/St	—	30,127 ± 3,504	—
BALB/c	—	14,147 ± 3,504	—
lpr/lpr	*lpr/lpr*	16,688 ± 1,703	1.13
+/+		34,788 ± 3,795	1.14
C3H/St		28,111 ± 1,373	0.93
BALB/c		119,660 ± 4,100	8.46
lpr/lpr	+/+	22,910 ± 2,427	1.54
+/+		36,024 ± 3,482	1.18
C3H/St		24,908 ± 3,003	0.83
BALB/c		118,959 ± 1,962	8.41
lpr/lpr	BALB/c	168,458 ± 22,745	11.36
+/+		246,506 ± 23,495	8.08
C3H/St		231,827 ± 11,956	7.69
BALB/c		12,193 ± 2,110	0.86
lpr/lpr	C3H/St	15,488 ± 1,475	1.04
+/+		34,420 ± 5,204	1.13
C3H/St		23,244 ± 3,114	0.77
BALB/c		117,518 ± 6,462	8.31

* Mean ± SD of three experiments with spleen cells of 1-mo-old mice.

‡ Stimulation indices were calculated by dividing the mean cpm in a given experiment by the mean cpm in the responding cells' background control culture.

respectively, was unsuccessful. Similarly, treatment of *lpr/lpr* BM cells with PNA and SBA to remove mature and immature T and B cells, or reconstitution with *lpr/lpr* fetal liver cells devoid of T cells, failed to prevent the wasting syndrome or subsequent death of +/+ recipients.

Runt disease was also observed in the following combinations: (*a*) +/+ mice reconstituted with *lpr/lpr* splenocytes from 1-mo-old donors injected intraperitoneally at birth (<24 h old) with 5×10^7 +/+ splenocytes to render the donor "tolerant" to antigens expressed on +/+ cells; (*b*) +/+ recipients thymectomized at birth and lethally irradiated at 1 mo of age, before transplantation with *lpr/lpr* splenocytes or BM cells. This group's death rate and wasting syndrome were considerably retarded compared to nonthymectomized recipients (50% mortality rate at 7.2 mo posttransfer compared to 2–3 mo posttransfer, respectively); (*c*) +/+ recipients of splenocytes or BM cells from 1-mo-old *lpr/lpr* donors thymectomized at birth and transplanted with +/+ thymuses; and (*d*) +/+ recipients thymectomized and transplanted with *lpr/lpr* thymuses, then lethally irradiated at 2 mo of age and injected with *lpr/lpr* spleen or BM cells.

Mechanisms of the Wasting Syndrome. Although the wasting syndrome of +/+ mice receiving *lpr/lpr* cells resembled subacute GVHD, the causes and means of

TABLE IV

Primary and Secondary CML Responses Between MRL lpr/lpr and MRL +/+ Cells

Effector	Stimulator/Target*	Specific lysis[‡]	
		Primary[§]	Secondary[‖]
		%	
+/+	*lpr/lpr*	8.2 ± 2.1	14.7 ± 2.6
C3H/St		−2.6 ± 1.7	82.3 ± 9.9
BALB/c		76.8 ± 5.3	ND[¶]
lpr/lpr	+/+	4.6 ± 1.0	17.8 ± 0.4
C3H/St		−8.5 ± 3.0	74.0 ± 14.3
BALB/c		87.9 ± 5.5	ND
lpr/lpr	C3H/St	1.7 ± 0.8	60.5 ± 6.4
+/+		1.2 ± 0.3	65.6 ± 9.5
BALB/c		73.8 ± 7.1	ND
lpr/lpr	BALB/c	79.8 ± 4.8	ND
+/+		78.1 ± 11.1	ND
C3H/St		85.0 ± 7.9	ND

Results of five experiments with spleen cells from 1-mo-old mice.

* Stimulator splenocytes were irradiated with 3,000 rad. Target splenocytes were prepared by preincubation for 5 d with Con A.

‡ Specific lysis was determined by the formula: (experimental − background) × 100/(100 − background). Background lysis was determined as the ^{51}Cr release from target cells added to autologous effector and autologous stimulator cells.

§ Effector cells were obtained from unimmunized mice.

‖ Effector cells were obtained 5 d after in vivo immunization with irradiated stimulator target splenocytes.

¶ ND, not done.

TABLE V

Frequency of Hemopoietic Precursors in MRL lpr/lpr and MRL +/+ Mice

Bone marrow source*	Recipient[‡]	CFU[§]
lpr/lpr	*lpr/lpr*	15.44 ± 3.3
+/+	+/+	11.5 ± 3.08
lpr/lpr	+/+	17.1 ± 2.85
+/+	*lpr/lpr*	11.11 ± 2.36

* 10^5 BM cells in 0.5 ml i.v.

‡ Recipients were given 1,200 rad irradiation.

§ Counted 11 d after transfer. Donors and recipients were 1-mo-old females, six mice per group.

induction could not be defined with classical histocompatibility studies. Therefore, additional possibilities were examined, including: (*a*) potential activation of a latent virus by irradiation; (*b*) generation of cytotoxic or suppressor cells, and (*c*) BM resistance phenomena attributed to radioresistant natural killer (NK)-like cells of the host (19).

To examine the first possibility, 100 μl of serum, or a mixture of spleen,

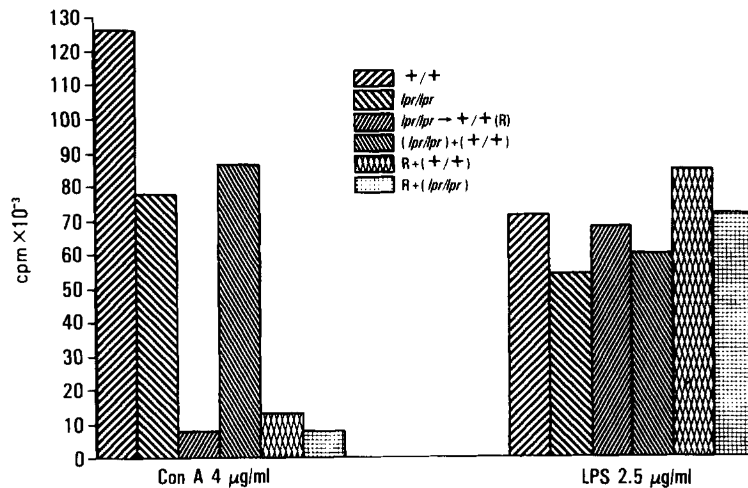

FIGURE 4. Effects of splenocytes from MRL *lpr/lpr* → MRL +/+ mice with runt disease on the proliferative response of T and B cells from unmanipulated MRL *lpr/lpr* and MRL +/+ mice. Splenocytes from unmanipulated MRL +/+, unmanipulated MRL *lpr/lpr* or MRL +/+ recipients of MRL *lpr/lpr* spleen cells with runt disease (*R*) were cultured either alone (2 × 10^5 cells in 0.2 ml), or in combination (10^5 cells from each group) in the presence of Con A (4 μg/ml) or LPS (2.5 μg/ml); 3 d later, [^{3}H]thymidine incorporation was measured.

thymus, and liver cells (1.2 × 10^8 cells irradiated at 3,000 rad) from 2-mo-old +/+ mice suffering from wasting syndrome were injected intraperitoneally into newborn +/+ mice. Additionally, 1-mo-old +/+ mice were lethally irradiated and reconstituted with +/+ BM cells (2 × 10^7) to which an equal number of cells (irradiated to 3,000 rad) from wasting +/+ mice had been added before transfer. Neither group developed clinical or histologic evidence of wasting, even at 5 mo posttransfer.

To examine the possible generation of cytotoxic or suppressor cells, two experiments were performed: First, spleen cells from mice with GVHD were tested for direct cytotoxicity on recipient and donor target cells in a 4-h ^{52}Cr-release assay. However, at effector/target cell ratios of 100:1, 50:1, and 25:1, spleen cells from mice with GVHD were not cytotoxic to either type of target cells (data not shown). Second, we examined the ability of cells from wasting mice to inhibit mitogenic responses of T and B cells from unmanipulated 2-mo-old *lpr/lpr* and +/+ mice (Fig. 4). Cells from unmanipulated 2-mo-old *lpr/lpr* mice had lower Con A responses than those from unmanipulated +/+ mice, as previously reported (23), but they showed no significant differences in LPS responsiveness. Cells from wasting +/+ mice had minimal Con A responses, but normal LPS responses. Mixing *lpr/lpr* with +/+ cells derived from unmanipulated mice had no effect on Con A or LPS responsiveness in either population, since the mixed response was equivalent to the expected sum of the responses by each cell population. In contrast, mixing cells from unmanipulated +/+ or *lpr/lpr* mice with cells from wasting +/+ mice considerably reduced Con A, but not LPS responsiveness in both cell populations.

With regard to BM resistance phenomena, since NK cell activity matures

ontogenically only after the third to fourth week of life (19), we reconstituted 1–2 wk, rather than 1-mo-old, +/+ mice with *lpr/lpr* BM cells, and found that wasting still developed.

Cell Transfers Between C57-lpr/lpr and C57BL/6 Mice. The *lpr* gene has recently been transferred into C57BL/6 and the C3H/HeJ mice (10, 11). To determine whether the *lpr* gene–related wasting syndrome was unique to MRL mice or was more general, we performed reciprocal BM transfers between C57BL/6 *lpr/lpr* and C57BL/6 mice. Like MRL mice, C57BL/6 mice given C57 *lpr/lpr* BM cells developed GVHD and wasting within 3–5 mo, whereas in the converse situation (C57BL/6 → C57 *lpr/lpr*) no runt disease developed within 5 mo.

Discussion

Establishing the means by which systemic autoimmune diseases develop led us to transplant hemopoietic cells reciprocally between two murine lines (MRL *lpr/lpr* and MRL +/+) with small differences in their genomes (<1%), but significant phenotypic differences in the degree of lymphoid hyperplasia and pace of SLE-like syndrome they develop. The present data show that (*a*) hemopoietic cells of such mice effectively induce the respective disease phenotypes in lethally irradiated syngeneic recipients; (*b*) cells of MRL +/+ mice maturing in an MRL *lpr/lpr* environment essentially retain the donor disease phenotype, i.e., they induce lupus relatively late in life and without lymphadenopathy, but (*c*) MRL *lpr/lpr* cells provoke an unexpected phenomenon when transplanted into irradiated MRL +/+ recipients; they induce not the early severe lupus and lymphadenopathy of the donor, but a severe wasting syndrome resembling subacute GVHD, as classically defined by Rappaport et al. (24).

The pace at which disease develops in irradiated MRL *lpr/lpr* mice transplanted with MRL *lpr/lpr* spleen or BM cells is the same regardless of whether the transferred cells originate from prediseased or diseased donors. Moreover, fetal liver cells induce the typical disease (data not shown). These findings suggest that the active cell being transferred is the stem cell, and that the age-dependent development of autoimmunity and lymphadenopathy does not result from an accumulation of defects at the stem cell level, i.e., stem cells from MRL *lpr/lpr* mice of any age are at the same stage of abnormality. Prolonged survival and absence of lymphoid hyperplasia in MRL *lpr/lpr* recipients of MRL +/+ hemopoietic cells indicate the irrelevance of the *lpr/lpr* nonlymphoid environment in this disease. In our previous thymic transplants between these two lines, the thymic genotype was of no consequence to the pace and characteristics of the disease (13), despite the need of a thymus for expression of the *lpr/lpr* phenotype (13, 25). Experiments with other types of lupus mice (NZB, BXSB) also showed that the origin of the transplanted cells and not the irradiated recipients' environment is the determining factor in disease development. Thus, in reciprocal spleen and BM cell transfers between the early-life disease-developing male and the late-life disease-developing female BXSB mice, the timing of disease onset is dependent on the sex of the donor and not of the recipient (7). Similarly, NZB hemopoietic cells transferred into H-2-compatible irradiated normal mice cause autoimmune disease, whereas normal cells transferred into irradiated NZB

mice do not (1–3).

The unexpected finding in the present study was the development of a severe wasting syndrome in MRL +/+ recipients of MRL *lpr/lpr* cells. Such mice failed to develop lymphoid hyperplasia and autoantibodies, as the origin of the donor cells would have dictated. Of course, the wasting syndrome may well preclude the development of the other characteristics. On the basis of their breeding history, MRL *lpr/lpr* and MRL +/+ mice are considered congenic except for the *lpr* locus (5). Therefore, this locus, or an extremely closely-linked genetic element(s), could be implicated in the observed phenomenon. A similar unidirectional wasting syndrome followed cell transfers between C57BL/6 *lpr/lpr* and C57BL/6 +/+ mice, reinforcing this gene's potential participation.

The *lpr* gene might promote the appearance of a GVHD-like disease in at least two ways. First, the *lpr* locus may directly control a cell surface antigen. If so, the *lpr* mutation could result in either a new or defective product or, more likely in light of the unidirectional nature of the GVHD, inability to express a product on the cell surface. Second, the *lpr* locus might be a regulatory gene that influences one or more other regulatory or structural genes, thereby interfering with, blocking, or altering the expression of their products. As a result, the MRL +/+ cells that do express this putative antigen(s) are recognized as foreign, and stimulate a graft-vs.-host reaction. The fact that MRL +/+ mice could not be rescued from the wasting syndrome by the simultaneous administration of *lpr/lpr* and +/+ cells would also tend to support this notion.

Genetic linkage of *lpr* has not yet been established, despite the fact that ~47% of the autosomal genome has been tested, and 27 linkage markers, including H-2, on various chromosomes have been analyzed (5). Nevertheless, interdependence or interaction between the *lpr* gene and the H-2 complex was suggested when Cronin et al. (26) found that certain monocytic-like cell lines derived from MRL *lpr/lpr* lymph nodes expressed low amounts of I-A^k, I-E^k (the MRL *lpr/lpr* mouse has been typed as H-2^k) and inappropriately high amounts of H-$2K^d$, H-$2D^d$, I-A^d and I-E^d. The authors concluded that a minute subset of monocytes in MRL *lpr/lpr* mice expressed inappropriate H-2^d–I-A^d antigens, perhaps derived from the LG/J (H-2^d) ancestor, which contributes 75% of the MRL *lpr/lpr* genome. Additionally, an increased frequency of Ia^k-positive peritoneal macrophages appeared at 3 mo of age; at or around the onset of lymphoproliferation in MRL *lpr/lpr* mice (27–29). Such increases have not been observed in MRL +/+ mice, nor in other *lpr* homozygous lines (C3H *lpr/lpr*, C57BL/6 *lpr/lpr*) of mice (28, 29). The relevance of these observations to the GVHD-like phenomenon is difficult to evaluate at present.

Attempts to identify *lpr/lpr*-associated lymphocyte surface antigens to account for the GVHD have met with little success. Thus, compared to the +/+ cells, MRL *lpr/lpr* lymphocytes possess a lower electronegative charge (30), a higher number of potassium channels (31), alterations of terminal carbohydrate structure of glycolipids and glycoproteins (32), and lower (Con A) or higher (*Helix pomatia*) number of receptor sites for certain lectins (30). Furthermore, the proliferating T cells of *lpr/lpr* mice express inappropriate alloantigens associated with pre-B/B cells (33–35). However, attempts to produce antibodies specific for the proliferating T cells were unsuccessful (A. N. Theofilopoulos, unpublished observations). The unidirectional nature of the wasting syndrome suggests that

the *lpr* gene does not encode a unique product on the lymphocyte surface, but rather deletes or alters a normal product (5).

Whatever modifications are introduced by the *lpr* gene on lymphocyte surfaces, compatibility testing does not reveal them, i.e., *lpr/lpr* cells do not evoke in the presence of +/+ cells significant primary or secondary MLR or CML reactions, nor a positive Simonsen splenomegaly assay. Clearly, minor histocompatibility differences cause minimal splenomegaly unless the donors are presensitized (36). Similarly, cytotoxic reactions to minor histocompatibility antigens are very weak except in secondary responses (37), but even secondary responses were weak between MRL *lpr/lpr* and MRL +/+ cells. At any rate, loci outside the H-2 complex have been cited in other examples of runt disease after cell transfers between certain combinations of mice (20, 21, 38).

GVHD observed after BM transplants between major or minor histocompatibility antigen-mismatched individuals possibly reflects alloaggression mediated not by stem cells or their progeny, but by mature T cells contaminating the spleen (30–35% T cells) or BM (2–7% T cells) inocula (20, 21). The basis of this view is the observation that GVHD does not develop if such inocula have been treated with anti-Thy-1 (22), anti-Thy-1 plus C (20, 21), or certain lectins that remove T cells (12). GVHD also does not appear, even across major histocompatibility complex barriers, if fetal liver cells devoid of T cells have been transferred (39, 40). In these instances, the T cell precursors maturing in the irradiated hosts' thymic environment are presumably tolerized. However, in the case of the MRL *lpr/lpr* → MRL +/+ transfers, none of these manipulations affected the associated GVHD, for unknown reasons. This runt syndrome may not have been mediated by T cells, considering that thymectomy of the MRL +/+ recipients, although delaying, was not preventative. Alternatively, T cells of the MRL *lpr/lpr* donor maturing in the MRL +/+ environment may be refractory to tolerance induction, as described in other systems (41, 42). Of particular interest, education of the MRL *lpr/lpr* cells in an MRL +/+ thymic environment before transfer to the MRL +/+ recipients, or transfer of MRL *lpr/lpr* cells into MRL +/+ mice that had been thymectomized and given an MRL *lpr/lpr* thymus just after birth, did not prevent the GVHD. Apparently, the MRL *lpr/lpr* thymic genotype has no unique property that allows the MRL *lpr/lpr* stem cells to acquire characteristics necessary for the initiation of the graft-vs.-host phenomenon. Furthermore, these data suggest an intrinsic defect in the tolerizability of *lpr/lpr* stem cells.

The possibility of BM resistance (19) was also considered here. In this phenomenon, grafts of hemopoietic cells fail or grow poorly in certain lethally irradiated allogeneic mice, including F_1 hybrid recipients of parental cells (43). Cudkowitz (44) suggested that special recessive, tissue-specific hemopoietic histocompatibility (Hh) genes, rather than classic histocompatibility genes, determine the fate of hemopoietic grafts. However, further consideration led others to conclude (45) that both class I and class II major histocompatibility genes, and not a special Hh gene, control hemopoietic resistance. The resistance is effected by an unusual host-vs.-graft reaction that is radioresistant, independent of the thymus, and is observed in F_1 mice injected with parental cells; these requirements were met in our MRL *lpr/lpr* → MRL +/+ and MRL *lpr/lpr* → (MRL *lpr/lpr* × MRL +/+)F_1 transfers. However, resistance to hemopoietic grafts matures only after

the 3–4 wk of life, concomitantly with the appearance of NK cells (19, 43). Yet, 1–2-wk-old MRL +/+ mice used as recipients of MRL *lpr/lpr* cells still developed the runt syndrome. Furthermore, BM inocula of MRL *lpr/lpr* mice induced sufficient numbers of CFU in irradiated MRL +/+ recipients. Both of these findings indicate that the runt disease in MRL *lpr/lpr* → MRL +/+ transfers was not mediated by a BM resistance phenomenon.

Because runt disease is lethal, and because donor T cells are required for its induction, it has been assumed that it is caused by donor cytotoxic T lymphocytes (46). However, runt disease also seems to develop via generation of suppressor T cells that inhibit physiologic cell proliferation in lymphohemopoietic tissues (38, 47). Such suppression caused by graft-vs.-host reactions is nonspecific, since it affects not only hemopoiesis of the recipients, but also that of the donor strain used for induction of the reaction (47–50). In the case of the MRL *lpr/lpr* and MRL +/+ mice, none of our assays showed significant cytotoxic T cells. However, cells of mice with wasting syndrome suppressed Con A-induced proliferative responses of MRL *lpr/lpr* and MRL +/+ splenocytes. These results, together with the severe lymphopenia and anemia of the MRL +/+ recipients of MRL *lpr/lpr* cells, suggest that, following the initial repopulation, the graft-vs.-host reaction instigates formation of nonspecific suppressor cells of hemopoiesis and lymphopoiesis.

The studies reported here indicate that a difference between two lines of mice at the *lpr* locus or closely-linked genetic elements results in a unidirectional GVHD-like reaction and runt disease. This type of GVHD may be mediated by a radiosensitive immature T cell in the spleen or BM inocula, since it is not abrogated by elimination of mature cells before transfer, and it can be induced by fetal liver cells. The model described here may be useful in further studies of the immunobiology of GVHD due to non-H-2 products, in understanding the means by which the *lpr* gene adversely affects lymphocyte regulation and homeostasis, and for identifying the modes of its actions.

Summary

Hemopoietic cells have been reciprocally transferred between two lines of mice (MRL *lpr/lpr* and MRL +/+) that are congenic, differing only at the *lpr* (lymphoproliferation) and possibly closely linked genes. The *lpr* strain develops a significantly more severe and fast-paced lupus-like syndrome than +/+ strain, along with a substantially larger lymphoid mass. The results showed that: (*a*) hemopoietic cells of such mice were sufficient to induce the respective disease phenotypes in lethally irradiated syngeneic recipients; (*b*) cells of MRL +/+ mice maturing in an MRL *lpr/lpr* environment essentially retained the disease-producing characteristics of the donor, i.e., they induced late-life lupus without lymphadenopathy; but (*c*) MRL *lpr/lpr* cells transferred into irradiated MRL +/+ recipients unexpectedly failed to induce the early-life severe lupus and lymphoid hyperplasia of the donor, instead they caused a severe wasting syndrome resembling, in many respects, graft-vs.-host disease (GVHD). This GVHD-like syndrome developed after transfer of MRL *lpr/lpr* fetal liver, bone marrow, or spleen cells, and was not abrogated by elimination of T cells from the inocula. Thymectomy of the MRL +/+ recipients retarded, but did not prevent, the

wasting disease. The unidirectional nature of this disease suggests that the *lpr* mutation conferred either a structural or regulatory defect that interferred, blocked, or altered the expression or structure of certain lymphocyte antigen(s). As a result, the MRL +/+ cells that did express this antigen(s) were recognized as foreign, and stimulated a graft-vs.-host reaction. These findings may allow definition of a new kind of rejection phenomenon caused by non-H-2 products, and may extend our understanding of the means by which the *lpr* gene adversely affects lymphocyte regulation and homeostasis.

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