Differences Defined by Bone Marrow Transplantation Suggest that *lpr* and *gld* Are Mutations of Genes Encoding an Interacting Pair of Molecules

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Summary

Homozygosity for either of the lymphoproliferation (*lpr*) or generalized lymphoproliferative disease (*gld*) mutations of mice causes the development of systemic lupus erythematosus–like autoimmune syndromes that are characterized by severe lymphadenopathy and highly elevated serum immunoglobulin levels. Although the mutations are nonallelic, analysis of homozygous *lpr/lpr* and *gld/gld* mice on the same strain background has indicated that the pathology and severity of the autoimmune syndromes induced by these mutations are indistinguishable. To explain this, it has previously been suggested that *lpr* and *gld* may represent mutations in molecules involved in sequential steps of an intracellular metabolic pathway of T cells. We have now investigated the behavior of both *lpr* and *gld* in a variety of bone marrow chimeras and have found that functional differences between *lpr* and *gld* become apparent after bone marrow transfer. Transfer of *lpr/lpr* bone marrow to irradiated congenic +/+ recipients caused the development of a graft-vs.-host–like lymphoid wasting syndrome, whereas transfer of *gld/gld* bone marrow to +/+ recipients resulted in development of a *gld*-like autoimmune syndrome. Additionally, *gld/gld* hosts behaved like +/+ hosts irrespective of the genotype of the donor bone marrow, whereas *lpr/lpr* hosts behaved unlike +/+ hosts when reconstituted with either *lpr/lpr*, *gld/gld*, or +/+ bone marrow. These are the first clear differences between these two mutations yet described. Our studies indicate that the molecule altered by the *gld* mutation is expressed only by bone marrow–derived cells, whereas the molecule altered by the *lpr* mutation is expressed by both bone marrow–derived cells and by one or more peripheral radioresistant cell populations. To reconcile these differences with the fact that homozygous *lpr/lpr* and *gld/gld* mice are indistinguishable, we suggest an alternative model for the relationship between the *lpr* and *gld* mutations in which the two molecules affected represent an interacting ligand-receptor pair expressed by different cells.

Homozygosity for the recessive mutation lymphoproliferation (*lpr*) causes an SLE-like autoimmune syndrome in mice characterized by high serum Ig levels, development of autoantibodies and proliferation of a $CD4^-CD8^-$ T cell population (1–4). This mutation first arose during derivation of the MRL/MpJ (MRL) strain and was subsequently transferred by repeated backcrossing to a number of other inbred strain backgrounds including C3H/HeJ (C3H). Although the fundamental phenotype described above develops in *lpr/lpr* mice of all strains, there are differences in the severity and associated pathology of the *lpr*-induced autoimmune syndrome in different strains (3). This suggests that "background" genes can modify phenotypic characteristics of the *lpr*-induced autoimmune syndrome.

Another recessive mutation, designated generalized lymphoproliferative disease (*gld*) occurred in the C3H strain and produces phenotypic features very similar to those induced by *lpr* (3–7). It has been reported that the autoimmune syndromes induced by these two genes differ significantly (8). However, this conclusion was drawn by comparison of homozygous *lpr/lpr* and *gld/gld* mice of different strain backgrounds. When studied on the same strain background (e.g., C3H-*gld/gld* vs. C3H-*lpr/lpr*), no differences in longevity, degree or rate of lymphoproliferation, serum Ig levels, anti-DNA autoantibody levels, or phenotype of the abnormally expanded T cell population have been found (Sidman et al., unpublished data; see also references 7 and 9). Thus, it is likely that the previously noted differences in the effects of these two mutations were due to differences in modifying "background" genes and not to differences between the *lpr* and *gld* mutations.

The *gld* mutation has been mapped to chromosome 1 (5), while the location of the *lpr* mutation has not yet been determined. However, it has been demonstrated that *gld* and *lpr* are nonallelic (5). These two mutations therefore identify dis-

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/90/11/1367/09 $2.00
Volume 172 November 1990 1367–1375

tinct loci but result in indistinguishable autoimmune syndromes. In view of this, it has been suggested that *lpr* and *gld* are mutations in sequential enzymes in an intracellular metabolic pathway of T cells (6).

Bone marrow from homozygous *lpr/lpr* mice demonstrates unusual behavior upon transfer to irradiated congenic +/+ recipients. Rather than recreating an *lpr*-like autoimmune syndrome in +/+ hosts, *lpr/lpr* bone marrow induces a graft-vs.-host (GVH)-like syndrome (10, 11) consisting of an early inflammatory response with transient splenomegaly and lymphadenopathy (12) followed by progressive lymphoid atrophy (13). This GVH-like syndrome in *lpr/lpr* → +/+ bone marrow chimeras appears to be due to a functional defect associated with the *lpr* mutation rather than to an antigenic difference between congenic *lpr/lpr* and +/+ mice (12).

In the current report, we compare the behavior of cells from homozygous *gld/gld* and *lpr/lpr* mice in a series of bone marrow transfer experiments. The results obtained suggest that these two mutations are expressed by different cell compartments and thus argue against the previous hypothesis that these mutations affect sequential steps of a single intracellular metabolic pathway. We therefore propose an alternative model for the relationship between the *lpr* and *gld* mutations, in which they affect an interacting pair of molecules.

Materials and Methods

Mice. All experiments described were performed using mice of the C3H/HeJ (C3H) genetic background. Essential observations (absence of early splenomegaly in *gld/gld* → +/+ chimeras, development of lymphadenopathy rather than lymphoid wasting in *gld/gld* → +/+ chimeras, and development of a lymphoid wasting syndrome in *lpr/lpr* → *gld/gld* chimeras) were confirmed using mice of the C57BL/6J genetic background in order to verify that the observed differences in the behavior of the *lpr* and *gld* mutations were not due to strain-specific effects of background genes.

The *lpr* mutation arose during derivation of the MRL/MpJ strain and was subsequently transferred onto the C3H background by 10 cycles of cross-intercross matings (1–3). The *gld* mutation initially arose in the C3H strain (5). Donor and recipient mice in all bone marrow transfer experiments were 4–11 wk of age at transfer. Bone marrow chimeras are indicated as donor genotype → host genotype. Although *lpr* and *gld* represent mutations at separate loci, by convention, only altered loci are indicated in chimera designations (e.g., *gld/gld* → *lpr/lpr* is actually *gld/gld*, +/+ → +/+, *lpr/lpr*).

Bone Marrow Transfer. Bone marrow cells were prepared by flushing the femurs and tibias of donor mice with Earle's Balanced Salt Solution (Gibco Laboratories, Grand Island, NY) supplemented with 2% FCS (Biofluids Inc., Rockville, MD) (EBSS/2% FCS). Cells were harvested by centrifugation at 200 *g* for 10 min at 4°C, washed twice in EBSS/2% FCS under the same conditions, and then resuspended in 0.9% NaCl to a concentration of 2×10^7 viable cells/ml. Recipient mice received 1,000 rad of irradiation from a ^{137}Cs source at a rate of 186–190 rad/min, and then received 5×10^6 donor marrow cells via the tail vein.

Necropsy of Mice. Upon necropsy, the spleen, as well as the left and right axillary, brachial, and inguinal lymph nodes, were dissected free of adjacent tissue and weighed. The term "lymph node weight" used throughout Results refers to the combined weight of these six lymph nodes.

Determination of Immunoglobulin Levels by ELISA. All Ig levels were determined by means of an ELISA method, as previously described (14).

Results

Differences between Bone Marrow Cells from lpr/lpr and gld/gld Mice

We have previously demonstrated that transfer of *lpr/lpr* bone marrow to irradiated congenic +/+ recipients results in an early phase of lymphadenopathy and splenomegaly that begins at ~3 wk, peaks at 5 wk, and declines by 9 wk post-transfer (12). This phase of splenomegaly and lymphadenopathy is accompanied by a sharp rise in serum IgG1 and (to a lesser extent) IgG2 levels, which also peak at ~5 wk and return to normal by 9 wk post-transfer.

To determine whether the *gld* mutation causes the same effects after marrow transfer as the *lpr* mutation, we analyzed C3H-+/+ mice reconstituted with congenic C3H-+/+, -*lpr/lpr*, or -*gld/gld* bone marrow. Although transfer of *lpr/lpr* bone marrow to +/+ recipients resulted in significant splenomegaly by 40 d post-transfer, *gld/gld* → +/+ chimeras demonstrated no comparable increase in spleen weight at this time (Table 1, groups 2a vs. 3a). This indicates a functional difference between *lpr/lpr* and *gld/gld* bone marrow.

We also compared the effects of *lpr/lpr* and *gld/gld* bone marrow cells on serum Ig levels in congenic +/+ recipient mice. Although *lpr/lpr* bone marrow caused a significant increase in serum IgG1 and IgG2b in congenic +/+ recipients by 5 wk post-transfer (as expected from previous results [12]), *gld/gld* bone marrow did not induce an early rise in IgG levels (Table 2). We have previously demonstrated that the early increase in serum IgG1 levels also occurs in *lpr/lpr* → *lpr/lpr* chimeras and is thus a functional effect of the *lpr* mutation per se, rather than of the interaction of *lpr/lpr* bone marrow cells with the +/+ host environment (12). The inability of *gld/gld* bone marrow to induce the transient IgG response therefore represents anther functional difference between the *lpr* and *gld* mutations.

Differences between the lpr/lpr and gld/gld Host Environments

Recipients of lpr/lpr Bone Marrow Cells. Although *lpr/lpr* → *lpr/lpr* chimeras did not demonstrate significant early splenomegaly (Table 1, group 2b vs. 1b), *lpr/lpr* → *gld/gld* chimeras did demonstrate this phenomenon (Table 1, group 2c vs. 1c). Histological analysis indicated that the splenomegaly occurring in *lpr/lpr* → *gld/gld* chimeras was due to the same type of inflammatory response as was observed in *lpr/lpr* → +/+ chimeras (not shown). This indicates that donor cells of the *lpr/lpr* genotype recognize the *gld/gld* host environment as +/+-like rather than as *lpr/lpr*-like, and thus, that the *gld/gld* and *lpr/lpr* host environments differ in some functional parameter.

To further investigate this difference between the *lpr/lpr* and *gld/gld* host environments, we assessed the long-term development of chimeras constructed from all nine possible donor

Table 1. *Lymphoid Organ Weights in Chimeras 5–6 wk after Transfer*

Chimera	*n*	Spleen weight	Lymph node weight*
		mg	*mg*
1. +/+ Donor			
a. +/+ → +/+	6	93.7 ± 7.7	20.8 ± 1.9
b. +/+ → *lpr/lpr*	7	109 ± 5	22.8 ± 2.0
c. +/+ → *gld/gld*	8	103 ± 5	22.4 ± 1.1
2. *lpr/lpr* Donor			
a. *lpr/lpr* → +/+	7	153 ± 16↑	27.4 ± 2.9↑
b. *lpr/lpr* → *lpr/lpr*	7	112 ± 5	25.6 ± 2.3
c. *lpr/lpr* → *gld/gld*	8	164 ± 16↑↑	31.2 ± 3.2↑↑
3. *gld/gld* Donor			
a. *gld/gld* → +/+	7	83.6 ± 3.5	17.9 ± 0.8
b. *gld/gld* → *lpr/lpr*	7	91.1 ± 21.3	25.7 ± 2.6
c. *gld/gld* → *gld/gld*	8	114 ± 8	23.9 ± 3.1

Statistically significant ([↑] $p \leq 0.05$; [↑↑] $p \leq 0.01$ by Mann-Whitney analysis) increases in comparison with +/+ donor → same recipient group.
* Lymph node weight refers to the combined weights of the left and right axillary, brachial, and inguinal lymph nodes. Results are expressed to three significant figures and indicated as mean ± SE.

→ host combinations of the *lpr/lpr*, *gld/gld*, and +/+ genotypes. Sets of the nine groups of chimeras were necropsied at 5 and 12 mo post-transfer and assessed for the degree of lymph node and spleen enlargement or atrophy. As described below, significant differences emerged in the behavior of all marrow types in the *lpr/lpr* vs. the *gld/gld* host environments.

Confirming earlier studies (10–12), *lpr/lpr* → +/+ chimeras (Table 3, group 2a) developed a lymphoid wasting syndrome characterized by decreased spleen weights and atrophic lymph nodes. Additionally, *lpr/lpr* → *gld/gld* chimeras (Table 3, group 2c) developed a similar lymphoid wasting syndrome, consistent with the occurrence of early splenomegaly in these mice (Table 1). However, the *lpr/lpr* → *lpr/lpr* chimeras (Table 3, group 2b) developed lymphadenopathy and splenomegaly typical of homozygous *lpr/lpr* mice.

Analysis of Ig levels at 5 mo post-transfer supported these findings. As shown in Table 4, Ig levels in *lpr/lpr* → +/+ (group 2a) and *lpr/lpr* → *gld/gld* (group 2c) chimeras showed little elevation, with several isotypes being significantly depressed rather than increased. This was presumably because of the development of the lymphoid wasting syndrome in these mice. However, Ig levels in *lpr/lpr* → *lpr/lpr* chimeras (group 2b) were highly elevated.

In a separate experiment, we performed a kinetic analysis

Table 2. *Serum Ig Levels in Chimeras 5–6 wk after Transfer*

Isotype	+/+ → +/+	*lpr/lpr* → +/+	*gld/gld* → +/+
	μg/ml	*μg/ml*	*μg/ml*
IgM	680 ± 40*	750 ± 90	640 ± 80
IgG1	240 ± 70	590 ± 10↑	170 ± 10
IgG2a	190 ± 60	410 ± 90	190 ± 80
IgG2b	130 ± 20	300 ± 60↑	130 ± 20
IgG3	270 ± 80	900 ± 540	270 ± 20
IgA	310 ± 50	500 ± 130	380 ± 140
Total‡	1,800 ± 140	3,500 ± 850↑	1,800 ± 100

(↑) Statistically significant ($p \leq 0.05$ by Mann-Whitney analysis) increases in comparison with +/+ → +/+ group.
* Results are expressed to two significant figures and indicated as mean ± SE (three mice per group).
‡ Total refers to the sum of the six Ig classes measured.

Table 3. *Lymphoid Organ Weights in Chimeras 5 and 12 mo after Transfer*

Chimera	Spleen weight		Lymph node weight	
	5 mo	12 mo	5 mo	12 mo
	mg		*mg*	
1. +/+ Donor				
a. +/+ → +/+	77.6 ± 6.8 (4)*	88.0 ± 24.0 (3)	17.8 ± 0.4 (4)	12.0 ± 3.8 (3)
b. +/+ → *lpr/lpr*	144 ± 16 (3)↑	132 ± 23 (6)	27.4 ± 1.6 (3)↑	21.9 ± 3.1 (6)
c. +/+ → *gld/gld*	85.7 ± 4.7 (9)	80.0 ± 2.0 (2)	18.5 ± 1.0 (9)	12.2 ± 3.1 (2)
2. *lpr/lpr* Donor				
a. *lpr/lpr* → +/+	57.9 ± 8.1 (6)	45.3 ± 6.4 (5)↓	Nodes atrophic	Nodes atrophic
b. *lpr/lpr* → *lpr/lpr*	195 ± 3 (3)↑↑↑	397 ± 151 (6)↑↑↑↑	231 ± 34 (3)↑	150 ± 43 (6)↑↑
c. *lpr/lpr* → *gld/gld*	53.2 ± 3.8 (8)↓↓↓	43.1 ± 10.5 (3)↓	Nodes atrophic	Nodes atrophic
3. *gld/gld* Donor				
a. *gld/gld* → +/+	122 ± 18 (6)↑	272 ± 59 (5)↑	100 ± 20 (6)↑↑	644 ± 160 (5)↑
b. *gld/gld* → *lpr/lpr*	136 ± 31 (3)	170 ± 27 (8)	28.3 ± 2.8 (3)↓↓	96.1 ± 32.6 (8)↓↓↑↑
c. *gld/gld* → *gld/gld*	164 ± 17 (8)↑↑↑	Dead	199 ± 39 (8)↑↑↑↑	Dead

* All values are expressed to three significant figures and are indicated as mean ± SE. The number of mice in each group is indicated in parentheses. Open arrows indicate statistically significant ([↑] $p \leq 0.05$; [↑↑] $p \leq 0.01$ by Mann-Whitney analysis) increases (↑) or decreases (↓) in comparison with same donor → +/+ recipient.
Closed arrows indicate statistically significant ([↑] $p \leq 0.05$; [↑↑] $p \leq 0.01$; [↑↑↑] $p \leq 0.001$ by Mann-Whitney analysis) increases (↑) or decreases (↓) in comparison with +/+ donor → same recipient.

of a similar set of C3H chimeras, in which individual mice were bled at intervals until 300 d post-transfer and serum IgG2b levels were determined. As shown in Fig. 1, IgG2b levels increased steadily in *lpr/lpr* → *lpr/lpr* chimeras, but showed no increase in *lpr/lpr* → +/+ or *lpr/lpr* → *gld/gld* chimeras.

These results indicate that *lpr/lpr* bone marrow behaves differently in the *lpr/lpr* vs. the *gld/gld* (and +/+) host en-

Table 4. *Serum Ig Levels in Chimeras 5 mo after Transfer*

Chimera	Isotype						
	IgM	IgG1	IgG2a	IgG2b	IgG3	IgA	Total
				μg/ml			
1. +/+ Donor							
a. +/+ → +/+	460 ± 40*	500 ± 30	980 ± 120	340 ± 60	260 ± 70	180 ± 20	2,700 ± 70
b. +/+ → *lpr/lpr*	850 ± 280	410 ± 70	1,500 ± 590	370 ± 170	350 ± 90	110 ± 20↓	3,600 ± 900
c. +/+ → *gld/gld*	530 ± 40	560 ± 110	1,300 ± 170	300 ± 60	250 ± 50	120 ± 10↓	3,000 ± 330
2. *lpr/lpr* Donor							
a. *lpr/lpr* → +/+	530 ± 70	390 ± 80↓	1,900 ± 410	180 ± 40↓	600 ± 130↑	110 ± 20↓	3,400 ± 460
b. *lpr/lpr* → *lpr/lpr*	1,500 ± 480↑	1,600 ± 180↑↑↑	4,500 ± 550↑↑↑	5,200 ± 2,900↑↑↑	1,200 ± 350↑	160 ± 20↑	14,000 ± 2,900↑↑
c. *lpr/lpr* → *gld/gld*	490 ± 30	500 ± 70	1,100 ± 170↓	170 ± 20↓	1,000 ± 460	90 ± 10↓	3,400 ± 650
3. *gld/gld* Donor							
a. *gld/gld* → +/+	720 ± 90↑	1,300 ± 260↑	3,800 ± 880↑↑	2,700 ± 1,400↑↑	840 ± 190↑	210 ± 30	9,500 ± 1,600↑↑
b. *gld/gld* → *lpr/lpr*	1,300 ± 300	750 ± 170	2,000 ± 570	530 ± 100↓↓	240 ± 60↓	180 ± 40	5,000 ± 1,000↓↓
c. *gld/gld* → *gld/gld*	960 ± 140↑↑↑	1,200 ± 310	3,200 ± 560↑↑	3,400 ± 1,000↑↑↑	610 ± 140↑	200 ± 30↑↑	9,600 ± 1,000↑↑↑

* Results are expressed to two significant figures and indicated as mean ± SE (three to eight mice per group). Total refers to the sum of the six Ig classes measured. Symbols are used as defined in Table 3.

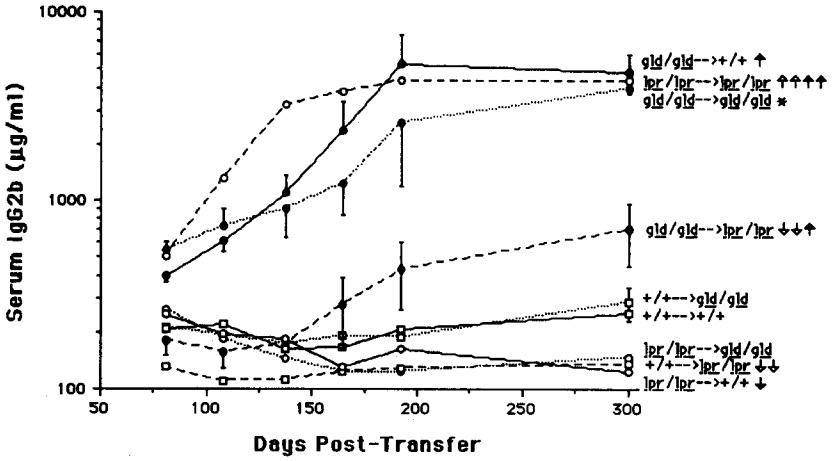

Figure 1. Kinetic analysis of changes in serum IgG2b levels of bone marrow chimeras constructed using all nine possible congenic C3H-+/+, -*lpr*/*lpr*, and -*gld*/*gld* donor → host combinations. Each point represents the mean of two to eight animals. Arrow symbols refer to statistical comparison of serum IgG2b levels at 300 d post-transfer and were used as defined in Table 3. Error bars represent 1 SE above and/or below the mean. (*) The *gld*/*gld* → *gld*/*gld* group consisted of only two animals at 300 d, resulting in a low significance level (0.061 by Mann-Whitney analysis when compared with the +/+ → *gld*/*gld* group) despite the obvious elevation in IgG2b levels in this group.

vironments, and therefore shows that the *lpr*/*lpr* and *gld*/*gld* host environments are functionally distinguishable.

Recipients of gld/gld Bone Marrow Cells. Recipients of *gld*/*gld* bone marrow did not develop an *lpr*/*lpr* → +/+-like lymphoid wasting syndrome. In fact, significant lymphadenopathy and splenomegaly were observed in *gld*/*gld* → +/+ and *gld*/*gld* → *gld*/*gld* chimeras necropsied 5 or 12 mo post-transfer (Table 3, groups 3a and 3c). However, *gld*/*gld* → *lpr*/*lpr* chimeras (Table 3, group 3b) demonstrated far less lymphadenopathy than *gld*/*gld* → +/+ (or *gld*/*gld* → *gld*/*gld*) chimeras. This effect was not due to an inability of the *lpr*/*lpr* host environment to support lymphoproliferation, since *lpr*/*lpr* → *lpr*/*lpr* chimeras (Table 3, group 2b) demonstrated splenomegaly and lymphadenopathy comparable with those observed in *gld*/*gld* → +/+ (Table 3, group 3a) and *gld*/*gld* → *gld*/*gld* (Table 3, group 3c) chimeras. Thus, the slow rate of development of lymphadenopathy in *gld*/*gld* → *lpr*/*lpr* chimeras appears to be due to an abnormal interaction occurring between *gld*/*gld* marrow-derived cells and the *lpr*/*lpr* host environment.

Ig levels in *gld*/*gld* → *lpr*/*lpr* chimeras (Table 4, group 3b) were not significantly elevated at 5 mo post-transfer, and were in fact much lower than those in *gld*/*gld* → *gld*/*gld* (Table 4, group 3c) or *gld*/*gld* → +/+ (Table 4, group 3a) chimeras at this time. This finding was supported by the kinetic analysis of IgG2b levels (Fig. 1), which increased steadily in *gld*/*gld* → *gld*/*gld* and *gld*/*gld* → +/+ chimeras, but much more slowly and with a later time of onset in *gld*/*gld* → *lpr*/*lpr* chimeras. Even after 1 yr, IgG2b levels in *gld*/*gld* → *lpr*/*lpr* chimeras were ~10 times lower than levels in *gld*/*gld* → +/+ or *gld*/*gld* → *gld*/*gld* chimeras.

These findings with *gld*/*gld* bone marrow cells confirm the existence of a functional difference between the *lpr*/*lpr* and *gld*/*gld* host environments.

Recipients of +/+ Bone Marrow Cells. Throughout these studies, +/+ → *lpr*/*lpr* chimeras demonstrated slight abnormalities when compared with +/+ → +/+ or +/+ → *gld*/*gld* chimeras. Whereas organ weights from +/+ → *gld*/*gld* chimeras were indistinguishable from those in +/+ → +/+ chimeras, +/+ → *lpr*/*lpr* chimeras developed a small degree of lymphadenopathy (Table 3). Serum IgG2b levels in +/+ → *lpr*/*lpr* chimeras also tended to be depressed (Fig. 1), being >1 SD below mean levels of +/+ → +/+ chimeras in 23/33 sera obtained at 100 or more days post-transfer, whereas only 3/18 +/+ → *gld*/*gld* sera obtained at comparable times fell in this category (assuming a normal distribution, it would be expected that one in six sera would be >1 SD below the mean). Thus, even +/+ bone marrow behaves differently in the *lpr*/*lpr* vs. the *gld*/*gld* host environments.

Discussion

Although the *lpr* and *gld* mutations cause indistinguishable autoimmune syndromes in homozygous mice, they behave very differently after bone marrow transfer in two ways. First, *gld*/*gld* bone marrow does not induce a lymphoid wasting syndrome in irradiated congenic +/+ recipients analogous to that induced by *lpr*/*lpr* bone marrow. Second, there are fundamental differences in the behavior of all three marrow types studied when transferred to *lpr*/*lpr* vs. *gld*/*gld* (or +/+) host environments. Thus, *lpr*/*lpr* bone marrow causes a lymphoid wasting syndrome in *gld*/*gld* (and +/+) recipients, but a lymphoproliferative syndrome in *lpr*/*lpr* recipients; *gld*/*gld* bone marrow causes a lymphoproliferative syndrome in *gld*/*gld* (and +/+) recipients, but this syndrome is significantly retarded in *lpr*/*lpr* recipients; and +/+ bone marrow completely normalizes *gld*/*gld* (and +/+) recipients, but fails to do so in *lpr*/*lpr* recipients. Two possible explanations for these different behaviors are: (*a*) the *lpr* mutation may be linked to (or give rise to) a histocompatibility antigen that causes the effects noted above, whereas the *gld* mutation is not associated with any antigenic alteration; or (*b*) the *lpr* and *gld* mutations may be functionally different with respect both to donor bone marrow and host environment effects.

An antigenic basis for these differences is unlikely for several reasons. First, the GVH-like reaction induced by *lpr*/*lpr* bone marrow has several notable differences from a typical antigen-induced GVH-syndrome. One such difference is that the GVH-like reaction here is not initiated by mature T cells present in the bone marrow inoculum (10–12). Second, al-

though the reaction is T cell dependent, we have previously demonstrated that either + /+ or *lpr/lpr* T cells will mediate it (12), suggesting that their role is not in recognition of foreign antigen. Third, the increase in IgG1 levels that parallels the early inflammatory response occurring in *lpr/lpr* → + /+ chimeras also occurs in *lpr/lpr* → *lpr/lpr* chimeras, and thus cannot be caused by an antigenic disparity (12). The most likely explanation of the results reported here is that there is a functional difference between the effects of the *lpr* and *gld* mutations that becomes apparent after bone marrow transfer.

In addition to this putative functional difference, the results reported here suggest that the *lpr* and *gld* mutations are expressed by different cell lineages or developmental stages. Bone marrow transfer allows splitting of the immune system into two cellular compartments. One of these consists of radiation-sensitive, bone marrow–derived cells and is donor-derived in the resultant chimera. The other compartment consists of radiation-resistant stromal tissue and long-lived, bone marrow–derived cells, both of which are host derived in the resultant chimera. We will refer to these two cellular subsets as the bone marrow compartment and the peripheral compartment, respectively. Construction of the chimeras described here allowed us to compare the behavior of these two separate compartments in *lpr/lpr* and *gld/gld* mice.

The bone marrow compartment of *lpr/lpr* mice is abnormal in that it induces a GVH-like reaction in both + /+ and *gld/gld* recipients. The *lpr/lpr* peripheral compartment is also abnormal as it behaves unlike the + /+ and *gld/gld* peripheral compartments when reconstituted with any of the three marrow types studied. These facts indicate that the molecule altered by the *lpr* mutation is expressed by cells in both the bone marrow and the peripheral compartments. The *gld* mutation, on the other hand, appears to affect only the bone marrow compartment, since the *gld/gld* peripheral compartment behaved similarly to that of + /+ mice in all cases studied. Thus, the molecule altered by the *gld* mutation is expressed by a population of cells that is completely contained within the bone marrow compartment. This differential localization of functional expression of the *lpr* and *gld* mutations suggests that the molecules affected are expressed by different cells.

If the *lpr*- and *gld*-affected genes are expressed by different cells, and cause functional differences on both the donor and recipient sides of bone marrow transfers, the question arises of how the *lpr* and *gld* mutations can give rise to near identical autoimmune syndromes. In view of the finding that *lpr* and *gld* are expressed by different cell subsets, it seems unlikely that the genes affected by these mutations are involved in sequential steps of a single metabolic pathway of T cells, as has been suggested previously (6). An alternate possibility that may fit the present observations better is that *lpr* and *gld* may be mutations in interacting molecules (e.g., a cytokine and its receptor or two interacting cell surface molecules expressed by different cell populations) (Fig. 2). In such a model, the phenotype of *lpr/lpr* and *gld/gld* homozygotes would be identical, since whether the receptor or ligand is impaired, the interaction between the two molecules would fail and the end result would be the same.

Since the molecule altered by the *lpr* mutation is hypothesized to be expressed by both the bone marrow compartment and by the peripheral compartment, whereas the molecule altered by the *gld* mutation is thought to be expressed only by the bone marrow compartment, the differential expression of these molecules in the various chimeras studied in this paper can be derived. As indicated in Table 5, the development of *lpr*- or *gld*-like autoimmune syndromes characterized by lymphadenopathy and hypergammaglobulinemia is associated with situations in which either the *gld*-associated molecule is replaced by the mutant *gld* variant or the *lpr*-associated molecule is entirely replaced by the mutant *lpr* variant (i.e., in both the bone marrow and peripheral compartments). Partial expression of the *lpr* variant (i.e., in the bone marrow compartment but not the peripheral compartment or vice versa) would be expected to occur in some of the chimeras constructed (*lpr/lpr* → + /+, *lpr/lpr* → *gld/gld*, + /+ → *lpr/lpr*, and *gld/gld* → *lpr/lpr*), and may provide a basis for

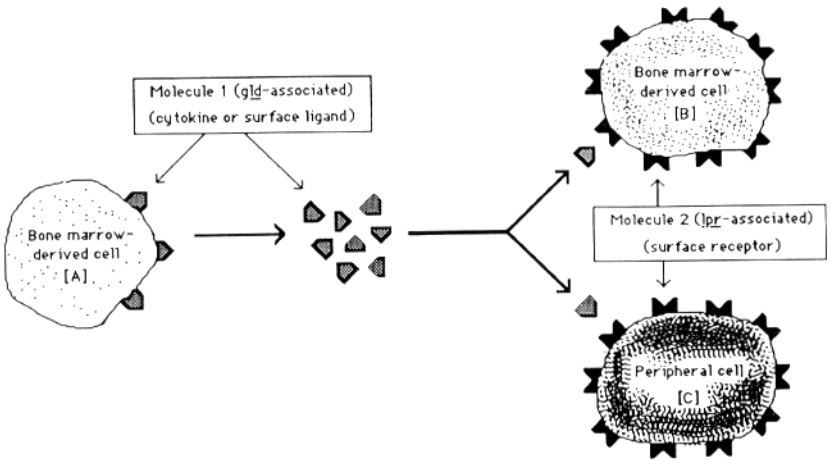

Figure 2. Suggested model of the relationship between the *lpr*- and *gld*-associated molecules. A bone marrow–derived cell (*A*) secretes a cytokine, or expresses a cell surface molecule (Molecule 1), that interacts with a surface molecule (cytokine receptor?) (Molecule 2) expressed by both bone marrow–derived cells (*B*) and peripheral cells (either peripheral lymphoid tissue or long-lived, radiation-resistant, marrow-derived cells) (*C*). The *gld* mutation results in loss of function of Molecule 1. The *lpr* mutation results in loss of function of Molecule 2. Loss of function of either molecule prevents the same interaction from occurring, and autoimmunity develops as a result of a cascade of events initiated by failure of this interaction.

Table 5. *Hypothetical Expression of lpr- and gld-associated Molecules in the Chimeras Studied*

Chimera	Hypothetical expression of molecules			Effects observed
	Molecule 1 (*gld*) marrow*	Molecule 2 (*lpr*)		
		Marrow‡	Peripheral§	
A. Normal form of both molecules				
Unmanipulated +/+	Normal	Normal	Normal	No abnormalities
+/+ → +/+	Normal	Normal	Normal	No abnormalities
+/+ → *gld/gld*	Normal	Normal	Normal	No abnormalities
B. Altered (*gld*) form of Molecule 1				
Homozygous *gld/gld*	*gld*	Normal	Normal	Lymphadenopathy/elevated Ig
gld/gld → *gld/gld*	*gld*	Normal	Normal	Lymphadenopathy/elevated Ig
gld/gld → +/+	*gld*	Normal	Normal	Lymphadenopathy/elevated Ig
C. Altered (*lpr*) form of Molecule 2 (complete expression)				
Homozygous *lpr/lpr*	Normal	*lpr*	*lpr*	Lymphadenopathy/elevated Ig
lpr/lpr → *lpr/lpr*	Normal	*lpr*	*lpr*	Lymphadenopathy/elevated Ig
D. Altered (*lpr*) form of Molecule 2 (partial expression)				
lpr/lpr → +/+	Normal	*lpr*	Normal	GVH-like syndrome
lpr/lpr → *gld/gld*	Normal	*lpr*	Normal	GVH-like syndrome
+/+ → *lpr/lpr*	Normal	Normal	*lpr*	Decreased Ig levels
gld/gld → *lpr/lpr*	*gld*	Normal	*lpr*	Impaired *gld*-like syndrome

Considering that *gld* is a mutation in a hypothetical molecule (Molecule 1) that is expressed only in the bone marrow compartment, while *lpr* is a mutation in a second hypothetical molecule (Molecule 2) that is expressed in both the bone marrow and peripheral compartments (Fig. 2), the expected expression of either the normal (i.e., wild-type) or mutant (*gld* in the case of Molecule 1; *lpr* in the case of Molecule 2) forms of each molecule in the various chimeras studied is indicated.

* The *gld* variant of Molecule 1 would be expressed in the bone marrow compartment only when donor marrow was of the *gld/gld* genotype.

‡ The *lpr* variant of Molecule 2 would be expressed in the bone marrow compartment only when donor marrow was of the *lpr/lpr* genotype.

§ The *lpr* variant of Molecule 2 would be expressed in the peripheral compartment only when the host was of the *lpr/lpr* genotype.

explaining the novel biological consequences of these transfers (Table 5, group D). This situation would not occur in any of the other chimeras studied (Table 5, groups A–C).

Considering the model proposed above in which *gld* and *lpr* may represent mutations in a cytokine-receptor pair, the aberrant effects associated with partial expression of the *lpr* variant could be explained by hypothesizing that production of this cytokine involves feedback regulation via its receptor. Thus, when cells with nonfunctional cytokine receptors (i.e., cells expressing the *lpr* variant) are present, cytokine production would be dysregulated, leading to higher than normal levels of cytokine. Partial expression of the functional cytokine receptor would mean some cells would be present that can respond to the cytokine while other cells present (those expressing the nonfunctional *lpr* variant) may produce it in a dysregulated manner. Thus, the aberrant effects observed in the chimeras expected to have partial expression of the nonfunctional (*lpr*) variant of the receptor could be due to the response of a cell population bearing normal cytokine receptor to high levels of the cytokine produced by a cell population bearing nonfunctional (*lpr*) cytokine receptor. Specifically, the GVH-like syndrome occurring in *lpr/lpr* → +/+ and *lpr/lpr* → *gld/gld* chimeras vs. the lymphoproliferative syndrome in *lpr/lpr* homozygotes and *lpr/lpr* → *lpr/lpr* chimeras may result from the normal responses of peripheral +/+ or *gld/gld* cells but not of peripheral *lpr/lpr* cells to the involved cytokine. Similarly, the impaired responses of +/+ → *lpr/lpr* and *gld/gld* → *lpr/lpr* chimeras compared with those of +/+ → +/+ and *gld/gld* → +/+ chimeras may reflect the inability of *lpr/lpr* peripheral cells to respond to the cytokine.

The novel effects observed in chimeras expected to have partial expression of the *lpr*-associated gene product are perhaps a clue to the normal function(s) of this molecule. Partial expression by peripheral tissue (in *lpr/lpr* → +/+ and *lpr/lpr* → *gld/gld* chimeras) causes the development of an early inflammatory response characterized by neutrophil influx into the spleen and lymph nodes and a subsequent lymphoid wasting syndrome. This suggests that the molecules altered by the *lpr* and *gld* mutations may be involved in mediating inflammatory responses.

Recently, a new mutation at the *lpr* locus, lpr^{cg}, has been described (15). Unlike the original *lpr* allele, the lpr^{cg} allele "complements" the *gld* mutation; i.e., $lpr^{cg}/+$, *gld*/+ double heterozygotes develop lymphadenopathy similar to what occurs in *lpr*/*lpr* or *gld*/*gld* homozygous mice, whereas *lpr*/+, *gld*/+ double heterozygotes appear normal. Our model could explain this difference by proposing that the *lpr* mutation results in a nonfunctional, non–ligand-binding receptor, whereas the lpr^{cg} mutation results in a nonfunctional receptor that nevertheless binds ligand. In $lpr^{cg}/+$ heterozygotes, the nonfunctional receptor would compete for ligand with the functional receptor, thus lowering the effective ligand concentration. In $lpr^{cg}/+$, *gld*/+ double heterozygotes, the effective ligand concentration would first be decreased as a result of the presence of one dose of the *gld* allele as transcripts from this allele would be ineffective. Additionally, the presence of the lpr^{cg} allele would further lower the effective ligand concentration as nonfunctional receptor would compete for available ligand (unlike the situation in *lpr*/+, *gld*/+ heterozygotes). This double effect could cause effective ligand-receptor interactions to be reduced to such a degree that lymphadenopathy analogous to that observed in homozygous *lpr*/*lpr* and *gld*/*gld* mice develops. Unlike homozygous mice, however, the double heterozygote would have the capacity to both produce the cytokine and respond to it, and could "adjust" by producing increasingly greater amounts of cytokine. This may explain why the lymphadenopathy becomes less severe as these mice age, whereas it becomes progressively more severe with age in homozygous *lpr*/*lpr* or *gld*/*gld* mice (15).

The model that we have proposed for the relationship between the *lpr* and *gld* mutations is therefore able to accommodate all of the observations made to date concerning the behavior and interaction of these two loci. Further studies are underway to delineate the cell populations expressing the *lpr*- and *gld*-associated molecules and to determine whether these molecules represent a known ligand-receptor pair.

We thank Drs. D. C. Roopenian and R. Evans for critical review of this manuscript.

These studies were supported by National Institutes of Health grants AI-20232, AI-25765, and CA-35845. R. Allen was supported by a Francis M. Sherwin Endowment Fellowship and by funds provided by The Jackson Laboratory.

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Received for publication 26 June 1990 and in revised form 16 August 1990.

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