A NEW ALLELE OF THE *lpr* LOCUS, lpr^{cg}, THAT COMPLEMENTS THE *gld* GENE IN INDUCTION OF LYMPHADENOPATHY IN THE MOUSE

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Three strains of autoimmune mice, MRL/Mp-*lpr/lpr*, C3H/HeJ-*gld/gld*, and BXSB/Mp-*Yaa*, have been established from spontaneous mutant mice (1–5). They have served as models for pathological, immunological, and molecular biological studies on autoimmune diseases and proliferation of abnormal lymphocytes (6–9). Several mice with massive lymph node hyperplasia were found in the CBA/KlJms colony maintained at the Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo. The CBA/Kl mice were originally introduced from the Karolinska Institute in Sweden in 1969 and have been maintained by sister × brother mating (10). In 1983, the specific pathogen-free (SPF)[1] colony was established by Caesarean section. We discovered these diseased mice in this colony in 1985. They were mated with each other to investigate the development of lymphadenopathy in their offspring. As a result, they all developed massive lymphadenopathy composed of clearly enlarged superficial and internal lymph nodes and palpable splenomegaly before 5 mo of age. These mice have been maintained as a mutant strain by brother × sister mating and confirmed to transmit this mutation stably. Thus, genetic studies were conducted by crossing them with various strains of mice. As presented in this paper, the mutant strain of mice has been confirmed to have a new allele of the *lpr* locus that interacts with the *gld* gene to induce lymphoid hyperplasia. In support of the genetic conclusion, the serological and immunopathological studies demonstrated that CBA/KlJms mutants were very similar to C3H/HeJ-*lpr/lpr* and C3H/HeJ-*gld/gld* mice in anomalous phenotypes, including hypergammaglobulinemia, high titers of anti-DNA antibodies, and surface markers of lymphoid cells from enlarged lymph nodes.

Materials and Methods

Mice. CBA/KlJms (CBA-+), mutant CBA/KlJms (CBS-*m*), C3H/HeJms (C3H-+), C57BL/6Jms (B6-+), DDD/1-*nu/nu* (DDD-*nu*), SWR/JJms (SWR-+), and NZW/NJms (NZW-+)

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[1] *Abbreviation used in this paper:* SPF, specific pathogen free.

mice maintained at the Laboratory Animal Research Center (10) were used. These strains of mice have not developed lymphadenopathy. MRL/MpJ (MRL-+), MRL/MpJ-*lpr/lpr* (MRL-*lpr*), C3H/HeJ-*lpr/lpr* (C3H-*lpr*), and C3H/HeJ-*gld/gld* (C3H-*gld*) mice were obtained from The Jackson Laboratory (Bar Harbor, ME), bred at our center, and used. Most mice were kept under SPF conditions in a light cycle (12 h light and 12 h dark)- and temperature-controlled room.

Observation of Lymphadenopathy. F_1, F_2, and backcross mice from crosses between CBA-*m* and another strain of mice were examined by palpation for enlargement of superficial lymph nodes and spleens weekly after 2 mo of age. Most mice were killed at the age of 5–6 mo, since all CBA-*m* mice had shown the first signs of lymphadenopathy before 3 mo and had visible enlarged lymph nodes at 4 mo of age. Some mice were observed up to 1 yr of age for the survival, development of lymphadenopathy, and progress of the disease. Especially, CBA-*m*, C3H-*gld*, C3H-*lpr*, (CBA-*m* × C3H-*lpr*)F_1, and (CBA-*m* × C3H-*gld*)F_1 mice were killed by chloroform overdose for weight determinations of lymph nodes and spleens at 2, 3, 5, 10, or 12 mo of age. Lymph nodes and spleens were excised, cleared of the surrounding tissue, and weighed wet separately. As all lymph nodes except the mesenteric lymph nodes never exceeded 5 mg in weight in CBA-+ mice, those under this weight, or missed because of their impossible discrimination from the surrounding tissue at excision, were expressed as <5 mg in weight for calculation of the means. The weights of the cervical, axillary, brachial, and inguinal lymph nodes were added and presented as the combined superficial lymph node weight, and those of the mediastinal, renal, lumbar, and sciatic lymph nodes were also added and presented as the combined internal lymph node weight. The mesenteric lymph node weight was presented separately, since its determination was not so accurate because of difficulty in distinguishing the nodes from the surrounding fat tissue unless enlarged, and additionally because they are far larger than the other internal lymph nodes.

Antibodies. A panel of rat mAbs was used as culture supernatants. Both AT83 specific for Thy-1.2 (11) and GK-1.5 directed against L3T4 (12) were originally supplied by F. Fitch (University of Chicago, Chicago, IL). The 53-6.7 was directed against Lyt-2 (13). The hybridoma that secretes mAb against B220(3A1) was purchased from the American Type Culture Collection (Rockville, MD). FITC-conjugated goat $F(ab')_2$ anti-mouse IgM and FITC-conjugated goat anti-rat IgG were purchased from Tago Inc. (Burlingame, CA). Alkaline phosphatase-conjugated anti-mouse IgM and IgG, specific for μ and γ chains, respectively, were obtained from Cappel Laboratories (Malvern, PA).

Preparation of Cell Suspensions. Lymph nodes were excised aseptically from normal, mutant, and hybrid mice aged 5–6 mo, and single cell suspensions were prepared in MEM containing 3% FCS. Lymph node cells were from a pool of cervical, axillary, inguinal, and mesenteric nodes. Their viability as determined by trypan blue exclusion was >90%.

Immunofluorescence Staining and Flow Cytometry. Direct and indirect methods were used for immunofluorescent staining of cells with FITC-conjugated polyclonal antibodies. For direct assay, 10^6 cells were suspended in 100 μl of PBS containing 3% FCS and 0.1% NaN_3, and incubated with FITC-conjugated goat anti-mouse IgM for 30 min at 4°C. The cells were washed three times with the medium. For indirect assay, 10^6 cells were incubated in the same medium for 30 min at 4°C with hybridoma supernatants containing mAbs specific for Thy-1.2, L3T4, Lyt-2, and Ly-5(B220). After washing twice, the cells were incubated with FITC-conjugated anti-rat IgG in 100 μl of the medium for 30 min at 4°C. Control cells were treated with FITC-conjugated reagent alone. After washing an additional three times, the cells were analyzed by flow cytometry (Spectrum III; Ortho Diagnostics Systems, Inc., Westwood, MA), and the data were collected using a logarithmic amplification.

Serum Ig and Anti-DNA Antibody Determinations. Blood was collected by heart puncture from normal, mutant, and hybrid mice aged 6 mo, and serum was separated for assays. IgM and IgG concentrations were determined by single radial immunodiffusions (The Binding Site, Birmingham, UK). Anti-ssDNA and anti-dsDNA antibodies were determined by ELISA, described by Kanai et al. (14). Briefly, 96-well microtiter plates were first coated with poly-L-lysine and subsequently with purified nucleic acids. They were blocked with Tris-buffered saline (TBS; 25 mM Tris, 140 mM NaCl, pH 7.4) containing 5% FCS and 0.05% Tween 20. Sera were 50-fold diluted with TBS containing FCS alone and assayed. After each incuba-

tion, the plates were washed extensively with TBS containing Tween alone. Bound antibodies were detected with alkaline phosphatase-conjugated anti-mouse IgM or IgG using *p*-nitrophenylphosphate (Sigma Chemical Co., St. Louis, MO) as a substrate. Antibody levels were expressed as the absorbance at 405 nm (A_{405}) (ImmunoReader; Nippon InterMed, Tokyo, Japan).

Histology. Main organs from 6-mo-old CBA-m mice were fixed in 10% formalin in PBS, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin for histologic examination.

Results

1 yr Follow-up of CBA-m Mice. 36 males and 26 females from the CBA-*m* colony under SPF conditions were observed for the development of lymphadenopathy and mortality up to 1 yr of age. In all mice, the enlargement of superficial lymph nodes commenced at ~2.5 mo of age with a tendency of earlier onset in cervical than in inguinal lymph nodes, and splenomegaly was clearly palpable after 3 mo of age. The first death was recorded at 19 and 29 wk of age, and the survival rate at 1 yr of age was 61.1 and 46.2% in males and females, respectively (Fig. 1). All nonmutant counterparts survive >1 yr under similar conditions.

CBA-*m* mice with the above macroscopic pathological characters were used in genetic studies. Breeding tests involving F_1, F_2, and backcross mice were conducted in order to clarify the genetic control of the mutant trait. Practically the same results were obtained with regard to the development and progression of lymphadenopathy in the crosses of mutant males with normal females, and in the reverse crosses, demonstrating the autosomal inheritance of the disease. Thus, the pooled results from the reciprocal crosses are presented in the tables.

Lymphadenopathy in F_1 Progeny. 30, 86, 21, 4, 26, 37, and 32 male and female F_1 mice were obtained by mating CBA-*m* to B6-+, CBA-+, C3H-+, DDD-*nu*, MRL-+, NZW-+, or SWR-+ mice, respectively, and observed for the presence or absence of enlarged lymph nodes and splenomegaly by palpation for a 5–6-mo period and by autopsy at the end of this period, since the prolonged observation up to 1 yr of age had been confirmed to have no influence on the outcome in (CBA-*m* × CBA-+)F_1 mice. None of the total number of 236 F_1 mice showed any sign of lymphoid hyperplasia in support of the recessive nature of the mutation.

Lymphadenopathy in F_2 Progeny. F_2 mice derived from the combinations of CBA-*m* × B6-+, CBA-*m* × CBA-+, CBA-*m* × C3H-+, CBA-*m* × MRL-+, and CBA-*m* × NZW-+ were observed as mentioned above (Table I). The number of mice

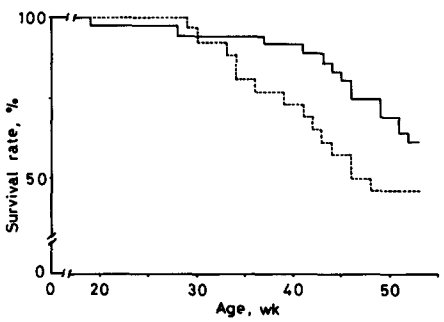

FIGURE 1. Survival of CBA-*m* male (*solid line*) and female (*dotted line*) mice during 1-yr observation.

TABLE I

Incidence of Lymphadenopathy in F_2 Populations Arising from Crosses between CBA-m and B6-+, CBA-+, C3H-+, MRL-+, or NZW-+ Mice

Crosses	Sex	No. of mice observed	No. with lymphadenopathy
			%
(CBA-*m* × B6-+)F_2	Male	82	19 (23.2)
	Female	84	20 (23.8)
(CBA-*m* × CBA-+)F_2	Male	70	17 (24.3)
	Female	82	23 (28.0)
(CBA-*m* × C3H-+)F_2	Male	26	9 (34.6)
	Female	13	4 (30.8)
(CBA-*m* × MRL-+)F_2	Male	45	11 (24.4)
	Female	23	5 (21.7)
(CBA-*m* × NZW-+)F_2	Male	29	9 (31.0)
	Female	23	4 (17.4)
Total		477	121 (25.4)

with massive lymphadenopathy and that of normal mice were 39 and 127 in (CBA-*m* × B6-+)F_2, 40 and 112 in (CBA-*m* × CBA-+)F_2, 13 and 26 in (CBA-*m* × C3H-+)F_2, 16 and 52 in (CBA-*m* × MRL-+)F_2, and 13 and 39 in (CBA-*m* × NZW-+)F_2, respectively. When these results were combined, 121 F_2 mice were affected by the hereditary disease, but 356 were normal. The ratio of the diseased to nondiseased mice was 1:2.94. Therefore, the hereditary disease was verified to be transmitted by a single autosomal recessive gene in accordance with the mendelian law.

Lymphadenopathy in Backcross Progeny. (CBA-*m* × B6-+)F_1 and (CBA-*m* × CBA-+)F_1 were backcrossed to CBA-*m* mice, and their offspring were observed as mentioned above (Table II). In the former backcross, 108, but not 92, mice developed obvious lymphadenopathy. The latter gave a similar result: 50, but not 53, mice had enlarged lymph nodes and spleens. Collectively, 158, but not 145, backcross mice were hereditarily diseased. Their ratio was 1:0.92. This result also supports the above conclusion, the single autosomal recessive gene control.

TABLE II

Incidence of Lymphadenopathy in Backcross Populations Arising from Crosses between CBA-m and CBA-+ or B6-+ Mice

Crosses	Sex	No. of mice observed	No. with lymphadenopathy
			%
(CBA-*m* × CBA-+)F_1 × CBA-*m*	Male	108	67 (62.0)
	Female	92	41 (44.6)
(CBA-*m* × B6-+)F_1 × CBA-*m*	Male	44	21 (47.7)
	Female	59	29 (49.2)
Total		303	158 (52.1)

Allelism of the Mutant Gene with gld, lpr, and Yaa. So far, three mutant genes, *gld*, *lpr*, and *Yaa*, have been reported to be involved in lymphadenopathy with autoimmune disease in mice (1–5). Since the *Yaa* gene is linked to Y chromosome (1, 4, 5), the new mutant gene is clearly considered to be different from it. Both *gld* and *lpr* are autosomal recessive genes (1). The former is mapped on chromosome 1 (3, 15), but the genetic linkage of the latter has not been established, despite the fact that 47% of the autosomal genomes has been tested (2, 16). Lymph node and spleen enlargements in mice homozygous for either gene progressed in a similar course as in the mutant mice. Therefore, a question arose as to whether the mutant gene is allelic with *gld* or *lpr*, or is different from both. To answer the question, 101 (CBA-*m* × C3H-*gld*)F_1, 77 (CBA-*m* × C3H-*lpr*)F_1, and 30 (CBA-*m* × MRL-*lpr*)F_1 mice were observed for the development of lymphadenopathy as mentioned above. Contrary to our expectations, all these hybrids developed palpable and visible lymphadenopathy (Table III), although the lymph node and spleen enlargements were smaller in severity in (CBA-*m* × C3H-*gld*)F_1 mice. All (C3H-*gld* × C3H-*lpr*)F_1 mice were completely free from illness in palpation and at autopsy, in accord with the different allelism of *gld* and *lpr* (2). All other control hybrids were negative for lymphadenopathy. To further analyze the allelism of the mutant gene with *gld* or *lpr*, backcrossing tests were conducted between CBA-+, C3H-+, CBA-*m*, or C3H-*gld* and (CBA-*m* × C3H-*gld*)F_1, and between CBA-+ or CBA-*m* and (CBA-*m* × C3H-*lpr*)F_1 mice (Table IV). 30 of 137 (21.9%) and 7 of 39 (17.9%) mice developed moderate lymphadenopathy in the ([CBA-*m* × C3H-*gld*]F_1 × CBA-+) and ([CBA-*m* × C3H-*gld*]F_1 × C3H-+) backcross populations, respectively. In addition, 89 of 120 (74.2%) and 37 of 47 (78.7%) mice were affected with lymphadenopathy in the ([CBA-*m* × C3H-*gld*]F_1 × CBA-*m*) and ([CBA-*m* × C3H-*gld*]F_1 × C3H-*gld*) backcross populations, respectively. Very significantly, 26, 11, and 10 ([CBA-*m* × C3H-*gld*]F_1 × C3H-*gld*) backcross mice had massively enlarged, moderately enlarged, and normal lymph nodes, respectively, and were therefore considered to be homozygous for *gld*, heterozygous for both *gld* and *m*, and wild type, respectively. The presence of diseased mice in the populations obtained by mating normal to (CBA-*m* × C3H-*gld*)F_1 mice and that of nondiseased mice in the populations from crosses of the F_1 to CBA-*m* and

TABLE III

Incidence of Lymphadenopathy in Various Hybrids Originating from Crosses between Two of CBA-m, CBA-+, C3H-gld, C3H-lpr, C3H-+, MRL-lpr, and MRL-+ Mice

Crosses	No. of mice observed	No. with lymphadenopathy
		%
CBA-*m* × C3H-+	21	0 (0)
CBA-+ × C3H-*gld*	21	0 (0)
CBA-*m* × C3H-*gld*	101	101 (100)
CBA-+ × C3H-*lpr*	25	0 (0)
C3H-*gld* × C3H-*lpr*	24	0 (0)
CBA-*m* × C3H-*lpr*	77	77 (100)
CBA-*m* × MRL-+	26	0 (0)
CBA-*m* × MRL-*lpr*	30	30 (100)

TABLE IV

Tests of allelism of the Mutant Gene, m, with the gld or lpr Gene by Observation of Lymphadenopathy in Backcross Populations Obtained by Mating (CBA-m × C3H-gld)F1 to CBA-+, CBA-m, C3H-+, or C3H-gld and (CBA-m × C3H-lpr)F1 to CBA-+ or CBA-m Mice

Backcross population	Sex	No. of mice observed	No. with lymphadenopathy
			%
(CBA-*m* × C3H-*gld*)F1 × CBA-+	Male	71	16 (22.5)
	Female	66	14 (21.2)
(CBA-*m* × C3H-*gld*)F1 × C3H-+	Male	20	3 (15.0)
	Female	19	4 (21.1)
	Total	176	37 (21.0)
(CBA-*m* × C3H-*gld*)F1 × CBA-*m*	Male	62	45 (72.6)
	Female	58	44 (75.9)
(CBA-*m* × C3H-*gld*)F1 × C3H-*gld*	Male	28	23 (82.1)
	Female	19	14 (73.7)
	Total	167	126 (75.4)
(CBA-*m* × C3H-*lpr*)F1 × CBA-+	Male	55	0 (0)
	Female	41	0 (0)
	Total	96	0 (0)
(CBA-*m* × C3H-*lpr*)F1 × CBA-*m*	Male	116	116 (100)
	Female	110	110 (100)
	Total	226	226 (100)

C3H-*gld* mice clearly demonstrates that the mutant gene is not allelic with *gld*. In contrast, all of 226 ([CBA-*m* × C3H-*lpr*]F1 × CBA-*m*) but none of 96 ([CBA-*m* × C3H-*lpr*]F1 × CBA-+) backcross mice developed lymphadenopathy (Table IV). It is, therefore, very reasonable to conclude that the mutant gene may be allelic with or lie on the same chromosome in close proximity to *lpr*. The former possibility is more likely, since the mutant gene and *lpr* can be estimated to exist within 0.62 cM from the absence of crossing over in the sum total of 322 backcross mice.

In conclusion, the new mutant gene is considered to be allelic with *lpr*, but able to complement *gld* in induction of lymphadenopathy, and therefore is named *lpr^{cg}* (*lpr* complementing *gld*).

Comparison of Lymphoproliferation among gld/gld, lpr/lpr, lpr^{cg}/lpr^{cg}, lpr/lpr^{cg}, and +/gld +/lpr^{cg} (gld-lpr^{cg}) Genotypes. The course of lymphoproliferation was investigated by weight measurements of lymph nodes and spleens in CBA-*m*, C3H-*gld*, C3H-*lpr*, (CBA-*m* × C3H-*gld*)F1, and (CBA-*m* × C3H-*lpr*)F1 mice (Table V). In CBA-*m* mice, the superficial lymph nodes and spleens commenced to enlarge at 2 mo of age, and the internal lymph nodes did so at 3 mo of age. Lymphadenopathy became more severe with age. However, the mesenteric lymph nodes did not show marked hyperplasia. At 5 mo of age, the profile of lymphoproliferation was practically the same in CBA-*m*, C3H-*gld*, C3H-*lpr*, and (CBA-*m* × C3H-*lpr*)F1 mice, except for the normal size of mesenteric lymph nodes in the first. In contrast, lymph node hyper-

TABLE V

Lymph Node and Spleen Weights in CBA-m, C3H-gld, C3H-lpr, (CBA-m × C3H-lpr)F1, and (CBA-m × C3H-gld)F1 Mice

Strain	Age	Sex	No. of mice observed	Combined lymphnode weight (mg)* Superficial lymph nodes	Internal lymph nodes	Mesenteric lymph nodes	Spleen weight
	mo				*mg*		
CBA-*m*	2	Male	5	<72 ± 2‡	<40	47 ± 6	103 ± 5
	2	Female	6	<75 ± 4	<40	48 ± 3	135 ± 11
	3	Male	9	436 ± 86	<59 ± 10	121 ± 40	414 ± 49
	3	Female	9	350 ± 41	<54 ± 7	51 ± 5	450 ± 28
	5	Male	8	2,596 ± 329	422 ± 66	49 ± 10	693 ± 190
	5	Female	8	4,320 ± 432	1,185 ± 184	86 ± 14	1,306 ± 181
C3H-*gld*	5	Male	5	4,138 ± 279	1,292 ± 165	286 ± 78	649 ± 87
	5	Female	5	4,321 ± 246	1,072 ± 136	243 ± 49	754 ± 99
C3H-*lpr*	5	Male	5	2,507 ± 408	461 ± 61	183 ± 41	403 ± 20
	5	Female	5	2,697 ± 277	522 ± 75	212 ± 25	629 ± 55
(CBA-*m* × C3H-*lpr*)F_1	5	Male	5	3,873 ± 433	755 ± 51	137 ± 33	621 ± 106
	5	Female	6	4,787 ± 574	1,098 ± 264	132 ± 37	1,387 ± 325
(CBA-*m* × C3H-*gld*)F_1	3	Male	4	285 ± 60	<40	60 ± 7	128 ± 11
	3	Female	4	308 ± 70	<42 ± 1	80 ± 11	153 ± 23
	5	Male	7	929 ± 53	<140 ± 38	77 ± 9	225 ± 38
	5	Female	7	763 ± 87	<119 ± 34	44 ± 6	199 ± 31
	10	Male	5	<351 ± 81	<40	41 ± 2	133 ± 9
	10	Female	5	<282 ± 62	<41 ± 1	91 ± 44	116 ± 11
	12	Male	5	<313 ± 52	<44 ± 4	38 ± 2	117 ± 8
	12	Female	5	<277 ± 61	<104 ± 64	32 ± 2	176 ± 60

* The weight of a lymph node was expressed as <5 mg when it was normal (see the text). Therefore, the combined superficial and internal lymph node weights are <50 and <40 mg, respectively, when all lymph nodes are normal in size.

‡ Mean ± SE. The value with or without ± SE means that some or all lymph nodes were normal, respectively.

plasia and splenomegaly were of significantly lesser severity in (CBA-*m* × C3H-*gld*)F_1 mice. The superficial lymph nodes were >5 and >15 times heavier than the normal ones at 3 and 5 mo of age, respectively, but the internal lymph nodes and spleen were practically normal and sporadically hyperplastic, respectively. More interestingly, although lymphoproliferation was generally progressive after 5 mo of age in CBA-*m*, C3H-*gld*, C3H-*lpr*, and (CBA-*m* × C3H-*lpr*)F_1 mice (data not shown), it became far less severe at 10 and 12 mo of age in (CBA-*m* × C3H-*gld*)F_1. Hyperplasia was sporadic even in the superficial lymph nodes, and all internal lymph nodes were normal in size in many mice, suggesting regression of lymphadenopathy. In addition, the peripheral leukocyte count at 5 mo of age was in the normal range in (CBA-*m* × C3H-*gld*)F_1, but abnormally higher in the other mice (data not shown). These findings support the conclusion of the genetic studies that the mutant gene, *lpr*^{cg}, is allelic with *lpr* but nonallelic with *gld*.

Comparison of Surface Antigens of Lymph Node Cells among gld/gld, lpr/lpr, lpr^{cg}*/lpr*^{cg}*, lpr/lpr*^{cg}*, and gld-lpr*^{cg} *Genotypes.* Lymph node cells from 5–6-mo-old mice with these

genotypes were examined for their reactivity to a panel of antibodies (Table VI). As expected from the genetic studies, the proportions of cells positive for sIg, Ly-5(B220), Thy-1, Lyt-2, and L3T4 were essentially the same in *gld/gld*, *lpr/lpr*, *lpr^{cg}/lpr^{cg}*, *lpr/lpr^{cg}*, and *gld-lpr^{cg}* mice. As already reported in C3H-*gld* and C3H-*lpr* (7), CBA-*m* mice were also characterized by the major population of Thy-1$^+$, Ly-5(B220)$^+$, Lyt-2$^-$, L3T4$^-$ cells in enlarged lymph nodes, as compared with CBA-+ and C3H-+ normal mice. The presence of such anomalous lymphoid cells was further confirmed by two-color flow cytometric analyses (data not shown). The results indicate that the combination of *gld-lpr^{cg}* can induce the anomalous differentiation of T cells as do *gld/gld*, *lpr/lpr*, and *lpr^{cg}/lpr^{cg}*.

Serum Ig and Anti-DNA Antibody Levels. Hyperimmunoglobulinemia and antinuclear antibodies are the important characters of mice homozygous for *lpr* or *gld*. As expected from the genetic studies, serum IgM and IgG levels, and anti-ssDNA and anti-dsDNA antibody titers, were abnormally higher in CBA-*m* (Table VII), as in C3H-*gld* (3) and MRL-*lpr* mice (4). Moreover, the anti-ssDNA antibody titer was compared among normal, mutant, and hybrid mice (Table VIII). It was significantly higher in CBA-*m*, (CBA-*m* × C3H-*lpr*)F$_1$, C3H-*lpr*, and C3H-*gld* mice, which developed massive lymphoid hyperplasia but remained at insignificant or very low levels in (CBA-*m* × C3H-*gld*)F$_1$, with slighter lymphadenopathy and the other normal or hybrid mice completely free from the disease. This supports the genetic conclusion that *lpr^{cg}* is allelic with *lpr* but not with *gld*.

Histological Examination of CBA-m Mice. Infiltration of lymphoid cells were frequently seen in the livers, lungs, and kidneys from 6-mo-old CBA-*m* mice. However, these organs had no pathologic lesions characteristic of autoimmune disease, and were especially free from interstitial pneumonitis reported in C3H-*gld* (3), and glomerulonephritis and vasculitis reported in MRL-*lpr* (17). The absence of renal pathologic lesions might be due to the CBA background genes, since renal and vascular diseases were found in some of *lpr^{cg}* mice considered to have 75% or more MRL genetic background (data not shown). This also supports the conclusion that *lpr^{cg}* is a new allele of the *lpr* locus. The basic histopathological and immunopathological features of CBA-*m* mice are reported in greater detail elsewhere (18).

TABLE VI

Expression of Cell Surface Antigens by Lymph Node Cells from Normal, Mutant, and Hybrid Mice Aged 5–6 mo

Lymph node cells from:	Genotype	No. of mice observed	Cell surface antigens				
			sIg	Ly-5(B220)	Thy-1	Lyt-2	L3T4
C3H-+	+/+	3	16*	15	81	21	56
C3H-*lpr*	*lpr/lpr*	6	8	86	81	4	10
C3H-*gld*	*gld/gld*	5	5	86	83	4	9
CBA-+	+/+	3	15	12	84	20	63
CBA-*m*	*lpr^{cg}/lpr^{cg}*	6	2	90	96	4	8
(CBA-*m* × C3H-*lpr*)F$_1$	*lpr^{cg}/lpr*	5	6	83	91	6	12
(CBA-*m* × C3H-*gld*)F$_1$	*+/gld +/lpr^{cg}*	5	13	73	87	8	14

* Mean percent positive cells.

TABLE VII

Serum Ig and Anti-DNA Antibody Levels in CBA-+ and CBA-m Mice Aged 5–6 mo

	Igs			Anti-dsDNA			Anti-ssDNA		
Mouse	*n*	IgM	IgG	*n*	IgM	IgG	*n*	IgM	IgG
		mg/ml			A_{405}			A_{405}	
CBA-+	6	0.65 ± 0.03*	6.28 ± 1.00	11	0.023 ± 0.002	0.001 ± 0.001	11	0.093 ± 0.006	0.033 ± 0.008
CBA-*m*	8	1.21 ± 0.11	29.40 ± 2.88	20	0.194 ± 0.022	0.226 ± 0.030	20	0.339 ± 0.022	0.298 ± 0.027

* Mean ± SE.

TABLE VIII

IgG Anti-ssDNA Antibody Levels in Normal, Mutant, and Hybrid Mice Aged 5–6 mo

Mouse	Genotype	No. of mice observed	IgG anti-ssDNA antibody level
			A_{405}
CBA-+	+/+	11	0.042 ± 0.006*
(CBA-+ × CBA-*m*)F_1	+/lpr^{cg}	7	0.047 ± 0.009
CBA-*m*	lpr^{cg}/lpr^{cg}	12	0.420 ± 0.061
(CBA-*m* × C3H-*lpr*)F_1	lpr^{cg}/lpr	14	0.531 ± 0.047
(CBA-+ × C3H-*lpr*)F_1	+/*lpr*	10	0.006 ± 0.003
C3H-*lpr*	*lpr/lpr*	14	0.301 ± 0.043
(CBA-*m* × C3H-*gld*)F_1	+/*gld* +/lpr^{cg}	12	0.034 ± 0.008
(CBA-+ × C3H-*gld*)F_1	+/*gld*	9	0.021 ± 0.004
C3H-*gld*	*gld/gld*	8	0.278 ± 0.025
(C3H-*gld* × C3H-*lpr*)F_1	+/*gld* +/*lpr*	12	0.004 ± 0.001

* Mean ± SE.

Discussion

Autoimmune mice homozygous for *lpr* or *gld* develop massive lymphoproliferation and associated autoimmune processes leading to autoantibody production and autoimmune kidney disease (2, 3, 19). Although *gld* and *lpr* are not allelic (1–3), a large body of evidence has accumulated to demonstrate that both genes have many anomalous phenotypic manifestations in common: (*a*) most lymphoid cells from enlarged lymph nodes are Thy-1^+, Ly-1^+, Lyt-2^-, L3T4^-, Ly-5(B220)$^+$, Ly-6^+, Ly-22^+, Ly-24^+, sIg$^-$, ThB$^-$, Ia$^-$, HSA$^{-/+}$, and PC.1^+ (7, 20, 21); (*b*) the anomalous cells show the same profile of binding lectins (7); (*c*) they are refractory to stimulation with antigen or mitogen and do not produce IL-2 or IFN-γ (7, 22–24); (*d*) spleen and lymph node cells produce high levels of *c-myb* RNA (22, 23); and (*e*) serum IgM, IgG, and IgA levels and anti-ssDNA and anti-dsDNA antibody titers are elevated (2, 3, 24). In addition, the *xid* gene has similar modifying effects on both genes (25). Based on these striking parallels between phenotypes of the two nonallelic genes, it has been suggested that *gld* and *lpr* may represent alterations in two different enzymes that act in a common metabolic pathway of major inportance to T cell differentiation and function (7, 20).

The mutant mice (CBA-*m*) reported here also develop massive lymphadenopathy similar in severity and profile of lymph node hyperplasia and splenomegaly to that in *gld* or *lpr* homozygotes (Table V). Genetic studies have provided evidence that the mutation is a single autosomal recessive gene like *gld* and *lpr*, which are not allelic with each other (Tables I and II). To our surprise, however, this gene interacted with either *gld* or *lpr* to induce lymphoproliferation (Table III). Further genetic analyses demonstrated that the mutant gene is not allelic with *gld* but exists within 0.62 cM on the same chromosome or is allelic with *lpr* (Table IV). Thus, the mutant gene was named lpr^{cg}, an *lpr* gene complementing *gld* in induction of lymphoproliferation.

The conclusion of the genetic studies has been supported by many phenotypic features common to *gld/gld*, *lpr/lpr*, lpr^{cg}/lpr^{cg}, *lpr*/lpr^{cg}, and *gld*-lpr^{cg} genotypes. Lym-

phoid cells from enlarged lymph nodes of C3H-*gld*, C3H-*lpr*, CBA-*m*, (CBA-*m* × C3H-*gld*)F_1, and (CBA-*m* × C3H-*lpr*)F_1 mice showed the same profile of surface markers: Thy-1$^+$, Ly-1$^+$, Lyt-2$^-$, L3T4$^-$, Ly-5(B220)$^+$, Ly-6$^+$, Ly-24$^+$, sIg$^-$, and Ia$^-$ (Table VI and unpublished data). Expression of the TCR protein on these abnormal cells was diminished in CBA-*m*, (CBA-*m* × C3H-*lpr*)F_1, and (CBA-*m* × C3H-*gld*)F_1, as in MRL-*lpr* mice (26) (unpublished data). However, Southern blot analysis of lymph node cell-derived DNA revealed polyclonal lymphoproliferation with TCR-β gene rearrangements in C3H-*gld*, C3H-*lpr*, CBA-*m*, (CBA-*m* × C3H-*gld*)F_1, and (CBA-*m* × C3H-*lpr*)F_1, as reported in C3H-*gld* mice (27) (unpublished data). These results clearly support the idea that both *gld* and *lpr* cause abnormal differentiation of T cells through the same mechanism. On the other hand, lymphadenopathy was far more massive in C3H-*gld*, C3H-*lpr*, CBA-*m*, and (CBA-*m* × C3H-*lpr*)F_1 than in (CBA-*m* × C3H-*gld*)F_1 mice (Table V), and antinuclear and anti-DNA antibody levels were abnormally high in the first four strains of mice, but in the normal range in the last (18) (Table VIII). These findings are reasonable in the light of the distinct allelism of lpr^{cg} with *gld*, and they suggest that the cooperation between lpr^{cg} and *gld* may be sufficient to develop anomalaous T cells but insufficient to induce autoantibodies, and that the anomalous lymphocytes in massively enlarged lymph nodes may have an important role in autoantibody formation. In terms of *gld*-lpr^{cg} interaction, it is of great interest that *lpr* has been shown not to be totally recessive, since some B cell hyperactivity is expressed in a heterozygous state (28). It may be possible that lpr^{cg} functions in a heterozygous state to produce a protein that may be slightly different from the product of *lpr* and can effect *gld*.

The discovery of the lpr^{cg} gene in CBA mice has provided strong evidence for the similarities between the syndromes induced by *gld* and *lpr*, and strongly suggests that both genes may influence the same point of a common metabolic pathway of major importance to the differentiation and function of T cells. We believe that CBA-lpr^{cg} mice will provide an experimental material vital to elucidation at the molecular and gene levels of the mechanism by which *gld* and *lpr* induce the abnormal differentiation and functions of lymphocytes in mice.

Summary

Several mice with generalized lymphadenopathy were found in the CBA/KlJms (CBA) colony maintained at our institute. A new mutant strain of mice that develop massive lymphoid hyperplasia at 100% incidence within 5 mo after birth was established by crossing these diseased mice. Genetic studies on lymphadenopathy were conducted in F_1, F_2, and backcross populations from crosses between mutant CBA (CBA-*m*) and various inbred strains of mice. The results supported the control of lymphadenopathy by a single autosomal recessive gene. Since C3H/He-*gld*/*gld* (C3H-*gld*), MRL/MpJ-*lpr*/*lpr* (MRL-*lpr*), and C3H/HeJ-*lpr*/*lpr* (C3H-*lpr*) mice develop the same type of lymphoid hyperplasia, allelism of the mutant gene with *gld* or *lpr* was tested by investigating lymphadenopathy in F_1 and backcross populations from crosses between CBA-*m* and C3H-*gld*, MRL-*lpr*, or C3H-*lpr* mice. The gene was confirmed to be allelic with *lpr* but not with *gld*. Interestingly, however, the mutant gene interacted with *gld* to induce less severe lymphadenopathy. Thus, the mutant gene was named lpr^{cg}, an *lpr* gene complementing *gld* in induction of lymphoproliferation. The genetic conclusion was supported by the same profile of surface markers

of lymphoid cells with *gld/gld*, *lpr/lpr*, *lpr*^{cg}/*lpr*^{cg}, *lpr*^{cg}/*lpr*, and +/*gld* +/*lpr*^{cg} genotypes, as well as by massive lymph node hyperplasia and high titers of autoantibodies in the first four genotypes, but slight hyperplasia and insignificant autoantibody production in the last. The discovery of *lpr*^{cg} provided strong genetic evidence for the parallels between anomalous phenotypes of *gld* and *lpr*, and CBA/KlJms-*lpr*^{cg}/*lpr*^{cg} mice will contribute to elucidation of the mechanism of induction of the same abnormal differentiation and functions of lymphocytes by *gld* and *lpr*.

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References

1. Roths, J. B. 1987. Differential expression of murine autoimmunity and lymphoid hyperplasia determined by single genes. *In* New Horizons in Animal Models for Autoimmune Disease. M. Kyougoku and H. Wigzell, editors. Academic Press, Tokyo. 21–33.
2. Theofilopoulos, A. N., and F. J. Dixon. 1985. Murine models of systemic lupus erythematosus. *Adv. Immunol.* 37:269.
3. Roths, J. B., E. D. Murphy, and E. M. Eicher. 1984. A new mutation, *gld*, that produces lymphoproliferation and autoimmunity in C3H/HeJ mice. *J. Exp. Med.* 159:1.
4. Murphy, E. D. 1981. Lymphoproliferation (lpr) and other single-locus models for murine lupus. *In* Immunologic Defects in Laboratory Animals 2. M. E. Gershwin and B. Merchant, editors. Plenum Publishing Corp., New York. 143–173.
5. Murphy, E. D., and J. B. Roths. 1978. Autoimmunity and lymphoproliferation: induction by mutant gene *lpr*, and acceleration by a male-associated factor in strain BXSB mice. *In* Genetic Control of Autoimmune Disease. N. R. Rose, P. E. Bigazzi, and N. L. Warner, editors. Elsevier Science Publishing, Inc., New York. 207–221.
6. Singer, P. A., R. J. McEvilly, D. J. Noonan, F. J. Dixon, and A. N. Theofilopoulos. 1986. Clonal diversity and T-cell receptor α-chain variable gene expression in enlarged lymph nodes of MRL-*lpr/lpr* lupus mice. *Proc. Natl. Acad. Sci. USA.* 83:7018.
7. Davidson, W. F., F. J. Dumont, H. G. Bedigian, B. J. Fowlkes, and H. C. Morse III. 1986. Phenotypic, functional, and molecular genetic comparisons of the abnormal lymphoid cells of C3H-*lpr/lpr* and C3H-*gld/gld* mice. *J. Immunol.* 136:4075.
8. Yui, K., S. Wadsworth, A. Yellen, Y. Hashimoto, Y. Kokai, and M. I. Greene. 1988. Molecular and functional properties of novel T cell subsets in C3H-*gld/gld* and nude mice. Implications for thymic and extrathymic maturation. *Immunol. Rev.* 104:121.
9. Shlomchik, M. J., A. M. Rothstein, C. B. Wolfowicz, T. L. Rothstein, and M. G. Weight. 1987. The role of clonal selection and somatic mutation in autoimmunity. *Nature (Lond.).* 328:805.
10. Tanaka, S., A. Matsuzawa, H. Kato, K. Esaki, K. Sudo, and K. Yamanouchi. 1987. Inbred strains of mice maintained at the Institute of Medical Science, University of Tokyo. *Jpn. J. Exp. Med.* 57:241.
11. Glasebrook, A. L., M. Sarmiento, M. R. Loken, D. P. Dialynas, J. Quintas, L. Eisenberg, C. T. Lutze, D. Wilde, and F. W. Fitch. 1981. Murine T lymphocyte clones with distinct immunological functions. *Immunol. Rev.* 54:225.
12. Dialynas, D. P., D. B. Wilde, P. Marrack, A. Pierres, K. A. Wall, W. Havran, G. Otten, M. R. Loken, M. Pierres, J. Kappler, and F. W. Fitch. 1983. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK 1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. *Immunol. Rev.* 74:29.
13. Ledbetter, J. A., and L. A. Herzenberg. 1979. Xenogenic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* 47:63.

14. Kanai, Y., M. Tauchi, S. Aotsuka, and R. Yokohari. 1982. A simple and rapid microenzyme-linked immunosorbent assay for antibodies to poly(ADP-ribose) in systemic lupus erythematosus. *J. Immunol. Methods.* 53:355.
15. Seldin, M. F., H. C. Morse III, J. P. Reeves, C. L. Scribner, R. C. LeBoeuf, and A. D. Steinberg. 1988. Genetic analysis of autoimmune *gld* mice. I. Identification of a restriction fragment length polymorphism closely linked to the *gld* mutation within a conserved linkage group. *J. Exp. Med.* 167:688.
16. Smith, H. R., and A. D. Steinberg. 1983. Autoimmunity perspective. *Annu. Rev. Immunol.* 1:175.
17. Andrews, B. S., R. A. Eisenberg, A. N. Theofilopoulos, S. Izui, C. W. Wilson, P. J. McConahey, E. D. Murphy, J. B. Roths, and F. J. Dixon. 1978. Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. *J. Exp. Med.* 148:1198.
18. Kimura, M., H. Mohri, K. Shimada, T. Wakabayashi, Y. Kanai, and A. Matsuzawa. 1990. Serological and histological characterization of the new mutant strain of *lpr* mice, CBA/KlJms-*lpr*^{g}/*lpr*^{g}. *Clin. Exp. Immunol.* In press.
19. Altman, A., A. B. Theofilopoulos, R. Weiner, D. H. Katza, and F. J. Dixon. 1981. Analysis of T cell function in autoimmune murine strains. *J. Exp. Med.* 154:791.
20. Davidson, W. F., K. L. Holmes, J. B. Roths, and H. C. Morse III. 1985. Immunologic abnormalities of mice bearing the gld mutation suggests a common pathwav for murine nonmalignant lymphoproliferative disorders with autoimmunity. *Proc. Natl. Acad. Sci. USA.* 82:1219.
21. Dumont, F. J., L. Z. Coker, R. C. Habbersett, and J. A. Treffinger. 1985. Xenogeneic monoclonal antibody to an Ly-6-linked murine cell surface antigen: differential reactivity with T cell subpopulations and bone marrow cells. *J. Immunol.* 134:2357.
22. Mountz, J. D., A. D. Steinberg, D. M. Kleinman, H. R. Smith, and J. F. Mushinski. 1984. Autoimmunity and increased c-myb transcription. *Science (Wash. DC).* 226:1087.
23. Mountz, J. D., J. F. Mushinski, G. E. Mark, and A. D. Steinberg. 1985. Oncogene expression in autoimmune mice. *J. Mol. Cell. Immunol.* 2:121.
24. Mountz, J. D., K. E. Huppi, M. F. Seldin, J. F. Mushinski, and A. D. Steinberg. 1986. T cell receptor gene expression in autoimmune mice. *J. Immunol.* 137:1029.
25. Seldin, M. F., J. P. Reeves, C. L. Scribner, J. B. Roths, W. F. Davidson, H. C. Morse III, and A. D. Steinberg. 1987. Effect of xid on autoimmune C3H-*gld*/*gld* mice. *Cell. Immunol.* 107:249.
26. Davignon, J.-L., P. L. Cohen, and R. A. Eisenberg. 1988. Rapid T cell receptor modulation accompanies lack of in vitro mitogenic responsiveness of double negative T cells to anti-CD3 monoclonal antibody in MRL-*lpr*/*lpr* mice. *J. Immunol.* 141:1848.
27. Hashimoto, Y., A. M. Maxan, and M. I. Greene. 1986. T-cell antigen-receptor genes in autoimmune mice. *Proc. Natl. Acad. Sci. USA.* 83:7865.
28. Jachez, B., E. M. Rodoriguez, P. Fonteneau, and F. Loor. 1988. Partial expression of the lpr locus in the heterozygous state: presence of autoantibodies. *Immunology.* 64:31.