A NEW ALLELE OF THE lpr LOCUS, lpr9, 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 x brother mating (10). In 1983, the specific pathogen-free (SPF)† 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 x 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-+), C3H/HeJms (C3H-+), C57BL/6Jms (B6-+), DDD/nu/nu (DDD-nu), SWR/Jms (SWR-+), and NZW/NJms (NZW-+)

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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.** F1, F2, 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 5 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 x C3H-lpr)F1, and (CBA-m x C3H-gld)F1 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% NaN3, 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 (A405) (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 F1, F2, 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 F1 Progeny.** 30, 86, 21, 4, 26, 37, and 32 male and female F1 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-+)F1 mice. None of the total number of 236 F1 mice showed any sign of lymphoid hyperplasia in support of the recessive nature of the mutation.

**Lymphadenopathy in F2 Progeny.** F2 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.](image-url)
with massive lymphadenopathy and that of normal mice were 39 and 127 in (CBA-
m x B6-+)F2, 40 and 112 in (CBA-m x CBA-+)F2, 13 and 26 in (CBA-m x
C3H-+)F2, 16 and 52 in (CBA-m x MRL-+)F2, and 13 and 39 in (CBA-m x
NZW-+)F2, respectively. When these results were combined, 121 F2 mice were
affected by the hereditary disease, but 356 were normal. The ratio of the diseased to
diseased 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 x B6-+)F1 and (CBA-m x
CBA-+)F1 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 con-
clusion, the single autosomal recessive gene control.

### Table I

| Crosses                  | Sex   | No. of mice observed | No. with lymphadenopathy |
|-------------------------|-------|----------------------|-------------------------|
| (CBA-m x B6-+)F2        | Male  | 82                   | 19 (23.2)               |
|                         | Female| 84                   | 20 (23.8)               |
| (CBA-m x CBA-+)F2       | Male  | 70                   | 17 (24.3)               |
|                         | Female| 82                   | 23 (28.0)               |
| (CBA-m x C3H-+)F2       | Male  | 26                   | 9 (34.6)                |
|                         | Female| 13                   | 4 (30.8)                |
| (CBA-m x MRL-+)F2       | Male  | 45                   | 11 (24.4)               |
|                         | Female| 23                   | 5 (21.7)                |
| (CBA-m x NZW-+)F2       | Male  | 29                   | 9 (31.0)                |
|                         | Female| 23                   | 4 (17.4)                |
| Total                   |       | 477                  | 121 (25.4)              |

### Table II

| Crosses                  | Sex   | No. of mice observed | No. with lymphadenopathy |
|-------------------------|-------|----------------------|-------------------------|

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

| Crosses                  | Sex   | No. of mice observed | No. with lymphadenopathy |
|-------------------------|-------|----------------------|-------------------------|
| (CBA-m x CBA-+)F1       | Male  | 108                  | 67 (62.0)               |
| x CBA-m                 | Female| 92                   | 41 (44.5)               |
| (CBA-m x B6-+)F1        | Male  | 44                   | 21 (47.7)               |
| x CBA-m                 | 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 x C3H-gld)F1, 77 (CBA-m x C3H-lpr)F1, and 30 (CBA-m x MRL-lpr)F1 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 x C3H-gld)F1 mice. All (C3H-gld x C3H-lpr)F1 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 x C3H-gld)F1, and between CBA-+ or CBA-m and (CBA-m x C3H-lpr)F1 mice (Table IV). 30 of 137 (21.9%) and 7 of 39 (17.9%) mice developed moderate lymphadenopathy in the ([CBA-m x C3H-gld]F1 x CBA-+) and ([CBA-m x C3H-gld]F1 x 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 x C3H-gld]F1 x CBA-m) and ([CBA-m x C3H-gld]F1 x C3H-gld) backcross populations, respectively. Very significantly, 26, 11, and 10 ([CBA-m x C3H-gld]F1 x 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 x C3H-gld)F1 mice and that of nondiseased mice in the populations from crosses of the F1 to CBA-m and

**Table III**

| Crosses | No. of mice observed | No. with lymphadenopathy |
|---------|----------------------|--------------------------|
| CBA-m x C3H-+ | 21 | 0 (0) |
| CBA-+ x C3H-gld | 21 | 0 (0) |
| CBA-m x C3H-gld | 101 | 101 (100) |
| CBA-+ x C3H-lpr | 25 | 0 (0) |
| C3H-gld x C3H-lpr | 24 | 0 (0) |
| CBA-m x C3H-lpr | 77 | 77 (100) |
| CBA-m x MRL-+ | 26 | 0 (0) |
| CBA-m x MRL-lpr | 30 | 30 (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^g (lpr complementing gld).

**Comparison of Lymphoproliferation among gld/gld, lpr/lpr, lpr^g/lpr^g, lpr/lpr^g, and +/gld^+/+lpr^g (gld-lpr^g) 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-
plasia and splenomegaly were of significantly lesser severity in (CBA-m × C3H-gld)F1 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)F1 mice (data not shown), it became far less severe at 10 and 12 mo of age in (CBA-m × C3H-gld)F1. 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)F1, but abnormally higher in the other mice (data not shown). These findings support the conclusion of the genetic studies that the mutant gene, lpr\(^{g}\), is allelic with lpr but nonallelic with gld.

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

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### 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)\(^{a}\) | Spleen weight |
|----------------------|-----|-----|----------------------|----------------------------------------|--------------|
|                      |     |     |                      | Combined superficial lymph nodes       | Internal Mesenteric lymph nodes |
|                      |     |     |                      | mg                                     | mg           |
|                      |     |     |                      | Superficial internal Mesenteric         |
|                      |     |     |                      | lymph nodes                             | lymph nodes |
|                      |     |     |                      | mg                                     | mg           |
| CBA-m                | 2   | Male| 5                    | <72 ± 2\(^{1}\)                        | <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 | 430 ± 28 |
|                      | 5   | Male| 8                    | 2,596 ± 329                            | 422 ± 66     | 49 ± 10 | 693 ± 190 |
|                      | 5   | Female| 8                   | 4,320 ± 432                            | 1,185 ± 104  | 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)F1  | 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)F1  | 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 ± 51 |
|                      | 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 |

\(^{a}\) 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.

\(^{1}\) Mean ± SE. The value with or without ± SE means that some or all lymph nodes were normal, respectively.
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The 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 slg, Ly-5(B220), Thy-1, Lyt-2, and L3T4 were essentially the same in gld/gld, lpr/lpr, lpr'g/lpr'g, lpr/lpr'g, and lpr-ldr'g 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'g can induce the anomalous differentiation of T cells as do gld/gld, lpr/lpr, and lpr'g/lpr'g.

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)F1, C3H-lpr, and C3H-gld mice, which developed massive lymphoid hyperplasia but remained at insignificant or very low levels in (CBA-m × C3H-gld)F1, with slighter lymphadenopathy and the other normal or hybrid mice completely free from the disease. This supports the genetic conclusion that lpr'g 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'g mice considered to have 75% or more MRL genetic background (data not shown). This also supports the conclusion that lpr'g 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).

| Lymph node cells from: | Genotype | No. of mice observed | slg | Ly-5(B220) | Thy-1 | Lyt-2 | L3T4 |
|------------------------|----------|----------------------|-----|------------|-------|-------|------|
| C3H-+                  | +/+      | 3                    | 15* | 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'g/lpr'g | 6             | 2   | 90         | 96    | 4     | 8    |
| (CBA-m × C3H-lpr)F1   | lpr'g/lpr | 5               | 6   | 83         | 91    | 6     | 12   |
| (CBA-m × C3H-gld)F1    | +/gld +/lpr'g | 5          | 13  | 73         | 87    | 8     | 14   |

* Mean percent positive cells.
| Mouse | IgG | IgG | Anti-dsDNA | Anti-ssDNA |
|-------|-----|-----|------------|------------|
|       | n   | mg/ml | A<sub>405</sub> | A<sub>405</sub> |
| CBA-+ | 6 | 0.65 ± 0.03* | 6.28 ± 1.00 | 11 | 0.023 ± 0.002 | 0.001 ± 0.001 |
| CBA-m | 8 | 1.21 ± 0.11 | 29.40 ± 2.88 | 20 | 0.194 ± 0.022 | 0.226 ± 0.030 |

* Mean ± SE.
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**Table VIII**

\textit{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 |
|----------------|-----------------------|-------------------------------|
| CBA-+          | +/+                   | 11 0.042 ± 0.006*             |
| (CBA-+ × CBA-+)F\textsubscript{1} | +/lpr\textsuperscript{a} | 7 0.047 ± 0.009               |
| CBA-+          | lpr\textsuperscript{b}/lpr\textsuperscript{b} | 12 0.420 ± 0.061             |
| (CBA-+ × C3H-lpr)F\textsubscript{1} | lpr\textsuperscript{b}/lpr | 14 0.531 ± 0.047             |
| CBA-+          | +/lpr\textsuperscript{a} | 10 0.066 ± 0.003             |
| C3H-lpr\textsuperscript{a} | lpr/lpr\textsuperscript{b} | 14 0.301 ± 0.043             |
| (CBA-+ × C3H-gld)F\textsubscript{1} | +/gld +/lpr\textsuperscript{c} | 12 0.034 ± 0.008             |
| (C3H-gld × C3H-lpr)F\textsubscript{1} | +/gld | 9 0.021 ± 0.004             |
| C3H-gld\textsuperscript{a} | gld/gld | 8 0.278 ± 0.025             |
| (C3H-gld × C3H-lpr)F\textsubscript{1} | +/gld +/lpr | 12 0.004 ± 0.001             |

* Mean ± SE.

**Discussion**

Autoimmune mice homozygous for \textit{lpr} or \textit{gld} develop massive lymphoproliferation and associated autoimmune processes leading to autoantibody production and autoimmune kidney disease (2, 3, 19). Although \textit{gld} and \textit{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\textsuperscript{+}, Lyt-2\textsuperscript{+}, L3T4\textsuperscript{+}, Ly-5\textsuperscript{b}+, Ly-22\textsuperscript{-}, Ly-24\textsuperscript{-}, sIg\textsuperscript{-}, ThB\textsuperscript{-}, Ia\textsuperscript{+}, HSA\textsuperscript{-/+}, and PC.1\textsuperscript{-} (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-\gamma (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 \textit{sid} 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 \textit{gld} and \textit{lpr} may represent alterations in two different enzymes that act in a common metabolic pathway of major importance to T cell differentiation and function (7, 20).

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

The conclusion of the genetic studies has been supported by many phenotypic features common to \textit{gld/gld}, \textit{lpr/lpr}, \textit{lpr\textsuperscript{a}/lpr\textsuperscript{a}}, \textit{lpr\textsuperscript{a}/lpr\textsuperscript{b}}, and \textit{gld-lpr\textsuperscript{a}} genotypes. Lym-
phoid cells from enlarged lymph nodes of C3H-gld, C3H-lpr, CBA-m, (CBA-m × C3H-gld)F1, and (CBA-m × C3H-lpr)F1 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)F1, and (CBA-m × C3H-gld)F1, 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)F1, and (CBA-m × C3H-lpr)F1, 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)F1 than in (CBA-m × C3H-gld)F1 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+ with gld, and they suggest that the cooperation between lpr+ and gld may be sufficient to develop anomalous 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+ 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+ 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+ 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+ 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/KIJms (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 F1, F2, 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/Mp-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 F1 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+, 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/lpr, lpr/lpr, lpr/lpr, lpr/lpr, and +/+/+/+/+ genotypes, as well as by massive lymph node hyperplasia and high titers of autoantibodies in the first four genotypes, but slight hyperplasia and insignificant antibody production in the last. The discovery of lpr provided strong genetic evidence for the parallels between anomalous phenotypes of gld and lpr, and CBA/Jms-lpr/lpr 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. Kyogoku 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. Wolpowicz, 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/KJJms- lpr/lpr. 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 suggest a common pathway 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. 100:249.

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, F. Fonteneau, and F. Loor. 1988. Partial expression of the lpr locus in the heterozygous state: presence of autoantibodies. Immunology. 64:31.