THE *lpr* GENE CAUSES AN INTRINSIC T CELL ABNORMALITY THAT IS REQUIRED FOR HYPERPROLIFERATION

By TAKUYA KATAGIRI, PHILIP L. COHEN, AND ROBERT A. EISENBERG

From the Division of Rheumatology/Immunology, Department of Medicine, and the Department of Microbiology/Immunology, University of North Carolina, Chapel Hill, North Carolina 27599

The single autosomal recessive gene *lpr* (lymphoproliferation) causes massive lymphadenopathy and concomitant autoimmune disease when expressed in the context of the MRL (1) or even normal background genes (2, 3). Extensive studies have been performed regarding the expanded population of cells in *lpr* mice (4). These cells exhibit rearrangements of TCR β chain genes (5, 6), but not of Ig heavy chain genes (7), and therefore are probably of T cell origin. They manifest an immature T cell surface phenotype, i.e., Thy-1$^+$(dull), Ly-1$^+$(dull), Lyt-2$^-$ (8), L3T4$^-$ (9), and Paul-Bunnell-negative (P-B$^-$)[1] (10); yet they also have some B cell markers such as Ly-6 (11), 6B2, 3A1 (7), and PC-1 (12), as well as high levels of 9F3 antigen (13). Increased expression of the c-*myb* oncogene in these cells (14) further suggests their immaturity, since this proto-oncogene is characteristic of immature T cells and of the hematopoietic stem cells (15). However, the *lpr* cells express mature TCR α chain (TCR-α) message (5, 6, Croghan, T. W., J.-L. Davignon, J. Evans, J. P. Allison, R. A. Eisenberg, J. A. Frelinger, and P. L. Cohen, submitted for publication). Functional investigation of purified Thy-1$^+$,Lyt-2$^-$,L3T4$^-$ (double-negative) *lpr* T cells has indicated their inert (16) and immature nature (17). It has thus been impossible to define the differentiation stage of the abnormal *lpr* T cells, since they do not correspond to any known normal subpopulations.

Some information is now available concerning the mechanisms of proliferation of these T cells. The fact that neonatal thymectomy prevents the later development of lymphadenopathy indicates its thymus-dependent nature (18, 19). The operation of multiple factors, including some attributable to the spleen, was claimed by using a technique of whole spleen organ transplantation (20). Adoptive transfer experiments have shown that lymphadenopathy develops in lethally irradiated MRL/Mp-*lpr/lpr* mice given MRL/Mp-*lpr/lpr* bone marrow (*lpr/lpr* $\rightarrow$ *lpr/lpr*) but not in those mice given MRL/Mp-+/+ stem cells (+/+ $\rightarrow$ *lpr/lpr*) (21–23). These results suggest that hematopoietic precursor cells expressing the

This work was supported by U.S. Public Health Service grants AM-26574, AM-34156, and AM-33887 and by an Arthritis Foundation Biomedical Research Center Grant. Drs. Cohen and Eisenberg were Senior Investigators of the Arthritis Foundation.

[1] *Abbreviations used in this paper:* BMC, bone marrow cells; LNC, lymph node cells; P-B, Paul-Bunnell; PE, phycoerythrin.

lpr gene are essential for the massive lymphadenopathy. However, the absence of lymphadenopathy in (*lpr/lpr* → +/+) chimeras suggests that the *lpr* host environment is also important (22, 23).

Two different mechanisms of lymphoproliferation can be postulated: (*a*) the expanding *lpr* cell populations have an intrinsic defect(s) that causes unrestrained growth; or (*b*) one set of abnormal *lpr* cells may induce a minor population of normal cells to proliferate. The former hypothesis requires the *lpr* gene to be expressed in the expanding cell population itself, while according to the latter it may be expressed in other cells. To distinguish between these two potential mechanisms, we used Ly-1 congenic mice in an adoptive bone marrow transfer system. Bone marrow cell mixtures of B6-*lpr/lpr* (Ly-1.2) and B6-+/+ (Ly-1.1) were transferred into lethally irradiated B6-*lpr/lpr* mice. This system enabled us to trace the development of normal and *lpr*-derived stem cells in an *lpr* environment. Our results suggest that the *lpr* gene effect requires expression directly within the T cells that accumulate in large numbers in *lpr* mice.

Materials and Methods

Mice. C57BL/6-*lpr/lpr* (B6-*lpr/lpr*), C57BL/6-Ly-1.1 (B6/Ly-1.1), and C57BL/6Kh (B6/Kh) mice were obtained from The Jackson Laboratory, Bar Harbor, ME, and maintained in our mouse colony.

Antibodies. A panel of rat mAbs was used in the form of culture supernatants. AT83 (24), specific for Thy-1.2; GK-1.5 (25), directed against L3T4; and 53-6.7, directed against Lyt-2 (26), were originally supplied by Dr. Frank Fitch, University of Chicago, Chicago, IL. Hybridoma cells secreting 3A1 mAb (anti-B220) were obtained from The American Type Culture Collection, Rockville, MD. Culture supernatant containing 9F3 mAb was kindly donated by Dr. Francis Dumont, Merck Institute, Rahway, NJ. The hybridomas that secrete mAb against Ly-1.1 or Ly-1.2 were kindly donated by Dr. U. Hämmerling, Sloan-Kettering Institute, New York, NY. Serum-free culture supernatants were concentrated, and the mAbs were purified using protein A–coupled Sepharose 4B. These antibodies were then biotinylated. FITC-coupled MAR 18.5 (mouse IgG2a monoclonal anti-rat κ chain), phycoerythrin (PE)-coupled streptavidin, and FITC-coupled avidin were purchased from Becton Dickinson & Co., Mountain View, CA.

Bone Marrow Cell Transfer. 3–4-mo-old B6-*lpr/lpr* and B6/Ly-1.1 mice of both sexes were used as donors of bone marrow cells (BMCs). Femurs and tibias were removed aseptically, and bone marrow was expressed by syringe via a 23-G needle into cold Dulbecco's modified MEM buffered with 15 mM Hepes. 3-mo-old B6-*lpr/lpr* mice were used as recipients and received water containing 2 g/liter of antibiotic (Biosol 325; Upjohn Co., Kalamazoo, MI) from 1 wk before irradiation until they were killed. They received 850 rad in a Gamma-cell 40 apparatus (Atomic Energy of Canada, Ltd., Ottawa, Canada). 10 h after irradiation, they were injected with 10^7 B6-*lpr/lpr* (group I) or B6/Ly-1.1 (group II) BMCs or with a mixture of 10^7 B6-*lpr/lpr* and 10^7 B6/Ly-1.1 BMCs (group III). All the recipients were housed in sterilized cages in a room supplied with filtered air.

Assessment of Chimerism. The Ly-1 allotype of lymphocytes of group III chimeras was determined by cytotoxicity and immunofluorescence of mononuclear cells enriched from peripheral blood by flotation on LSM (Litton Bionetics, Kensington, MD). Cytotoxicity assays were performed according to the method of J. Klein et al. (27). Briefly, 2 μl of the cells (2×10^3) were incubated in a Microtest tissue culture plate (3034; Falcon Labware, Oxnard, CA) for 20 min at room temperature with 2 μl of 1/20 dilution of either the monoclonal anti-Ly-1.1 or the monoclonal anti-Ly-1.2. The mixture was washed and then incubated for 30 min at 37°C with a 1/15 dilution of Low Tox-M rabbit complement (Cedarlane, Willowdale, Ontario). The live and dead cells were distinguished through an inverted phase-contrast microscope. For immunofluorescence, $3–5 \times 10^5$ cells were incubated for 30 min at 4°C with a 1/20 dilution of biotinylated anti-Ly-1.1 or anti-Ly-

1.2. The mixture was washed and then incubated with a 1/500 dilution of FITC-labeled avidin. Cells were washed three times and preserved in 1% formalin until flow cytometric analysis.

Preparation of Cell Suspensions. Lymph nodes, spleens, and thymuses were removed aseptically, and single cell suspensions were prepared in sterile RPMI 1640 (University of North Carolina Cancer Center, Chapel Hill). Lymph node cells (LNCs) were from a pool of all axillary, cervical, and mesenteric nodes. Their viability, as determined by trypan blue exclusion, exceeded 90%.

Fluorescence Staining and Flow Microfluorometry Analysis. An indirect immunofluorescence protocol was used in all instances. HBSS (pH 7.2, UNC Cancer Center) containing 3% FCS (Hazelton Research Products, Denver, PA), 0.1% sodium azide, and 15 mM Hepes buffer was used as the staining medium. Samples of 10^6 cells were incubated in 100 μl of staining medium for 30 min at 4°C with hybridoma supernatants or with purified biotinylated anti-Ly-1.1 or anti-Lyt-1.2. After two washes, the cells were incubated in 100 μl of staining medium for 30 min at 4°C with FITC-coupled anti-rat κ, FITC-coupled avidin, or PE-coupled avidin, as appropriate. Control samples were treated only with FITC- and/or PE-coupled reagents. After an additional three washes, the cells were analyzed by flow cytometry (Epics V; Coulter Electronics, Hialeah, FL). For two-color immunofluorescence staining, both 9F3 or 3A1 and biotinylated anti-Ly-1.1 or anti-Ly-1.2 were added to the cells simultaneously, because none of these antibodies inhibited or enhanced the binding of the other antibodies to the cells. Fluorescence data were plotted logarithmically over a three-decade scale.

ELISA Assays. Serum levels of IgG antichromatin, IgG anti-ssDNA, and IgM rheumatoid factor were assayed in polyvinylchloride microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) coated with chromatin (10 μg/ml, reference 28), ssDNA (10 μg/ml), or IgG1 myeloma protein MOPC245 (10 μg/ml), respectively, for 5 h at room temperature. Plates were then washed with borate-buffered saline (BBS) and nonspecifically coated with coating buffer (BBS, 0.5% normal goat serum, 0.4% Tween-80, 0.5% BSA, 0.1% NaN_3) for 2 h. After the plates were washed, sera diluted at 1:250 in coating buffer were added and incubated overnight at 4°C. The plates were washed again, and affinity-purified biotinylated goat anti-mouse γ or goat anti-mouse μ was added for 2 h of incubation. After additional washing, the plates were incubated with avidin-linked alkaline phosphatase (Sigma Chemical Co., St. Louis, MO) for 2 h. After further washings, 104 phosphatase substrate (para-nitrophenylphosphate, 1 mg/ml; Sigma Chemical Co.) was added in 0.01 M diethanolamine, pH 9.8. The plates were read at appropriate intervals with a microELISA autoreader (Dynatech Laboratories, Inc.).

Results and Discussion

Selective Expansion of the lpr/lpr-derived T Cell Populations in (lpr/lpr plus +/+ → lpr/lpr) Chimeras. 3-mo-old B6-*lpr/lpr* (Ly-1.2) mice were lethally irradiated (850 rad) and reconstituted with 10^7 BMCs from B6-*lpr/lpr* (Ly-1.2) mice (group I, *lpr/lpr* → *lpr/lpr*); from B6/Ly-1.1 mice (group II, +/+ → *lpr/lpr*); or from both strains of mice (group III, *lpr/lpr* plus +/+ → *lpr/lpr*). Three control mice not given BMC died within 1 mo after irradiation. 4 mo after BMC transfer, the chimerism of 5 group III recipients was assessed by cytotoxicity and immunofluorescence of PBMC with monoclonal anti-Ly-1.1 and Ly-1.2. As shown in Table I, the PBMC of these mice contained nearly equivalent numbers of Ly-1^+ cells of each donor allotype (Ly-1.1^+/Ly-1.2^+ = 0.88).

7–8 mo after the transfer, all mice were killed for examination. One of group III had died just before sacrifice. Most mice in group I or group III had easily detected submandibular lymphadenopathy, while all of group II mice looked healthy at this time. To quantitate lymphoproliferation, the total numbers of

TABLE I

Chimerism of Group III Mice (lpr/lpr plus +/+ → lpr/lpr)

Assay	n*	Percent positive of PBM	
		Ly-1.1	Ly-1.2
cytotoxicity	3	34, 36, 33	36, 37, 39
immunofluorescence	2	30, 32	41, 38

* Number of mice tested. Five of eight mice in this group of chimeras were examined.

TABLE II

Lymphoid Organ Cell Numbers and Ly-1 Allotype of LN Cells in Bone Marrow Chimeras

Group	Type of transfer	n*	Number of cells		Percent positive of LN cells‡		
			Spleen	LN	Thy-1.2	Ly-1.1	Ly-1.2
			$\times 10^{-8}$	$\times 10^{-7}$			
I	*lpr/lpr → lpr/lpr*	5	2.2 ± 0.4§	20 ± 6.6	76 ± 3.2	1.8 ± 1.3	67 ± 2.2
II	*+/+ → lpr/lpr*	5	0.8 ± 0.1	2.2 ± 0.3	69 ± 4.0	59 ± 3.6	7.0 ± 3.2
III	*lpr/lpr* plus *+/+ → lpr/lpr*	7	1.9 ± 0.5	20 ± 6.9	76 ± 5.3	5.1 ± 1.1	69 ± 3.5
Unmanipulated							
	B6/Kh	4	1.0 ± 0.1	2.3 ± 0.3	70 ± 3.1	1.5 ± 1.7	60 ± 5.6
	B6/Ly-1.1	4	1.0 ± 0.1	2.4 ± 0.3	68 ± 2.1	57 ± 1.8	1.5 ± 1.2

* Number of mice examined.

‡ Percent positive of LN cells was determined by immunofluorescence and cytofluorometry.

§ Values represent means ± SD.

splenocytes and LNC, and the Ly-1 allotype of the LNC were determined. As shown in Table II, remarkable splenomegaly and lymphadenopathy were observed in both (*lpr/lpr → lpr/lpr*) and (*lpr/lpr* plus *+/+ → lpr/lpr*) chimeras. In contrast, the numbers of splenocytes and LNC of (*+/+ → lpr/lpr*) chimeras were similar to those of intact B6/Kh (Ly-1.2) or B6/Ly-1.1 mice. About 90% of the lymph node T cells were Ly-1.1$^+$ in group II mice, which confirmed their expected chimeric status. In group III chimeras, 70% of the cells in the expanded lymph nodes were Ly-1.2$^+$, while only 5% were Ly-1.1$^+$ (Ly-1.1$^+$/Ly-1.2$^+$ < 0.10; Tables II, III, and Fig. 1). Surprisingly, in contrast to the preferential reconstitution of the lymph nodes by Ly-1.2$^+$ cells, the thymuses of the group III chimeras were only moderately skewed toward the *lpr* phenotype (Ly-1.1$^+$/Ly-1.2$^+$ = 0.70; Table III, Fig. 2). This suggested either that lymphoproliferation occurred mainly after emigration to the peripheral organs, or that excessive emigration of *lpr* T cells from the thymus might result in massive peripheral accumulation (29).

From these results taken together, we conclude: (*a*) the *lpr* T cell population selectively expands in (*lpr/lpr* plus *+/+ → lpr/lpr*) chimeras; (*b*) in these mice, +/+ lymphocytes neither prevent the growth of *lpr* T cells, nor proliferate in the environment where the *lpr* gene is fully expressed; (*c*) +/+ lymphocytes cannot proliferate abnormally in *lpr/lpr* host mice even when they constitute 90% of the population of lymph node cells; and (*d*) the abnormal accumulation of T cells caused by the *lpr* gene occurs in the peripheral organ(s), but not in the thymus.

Phenotypic Similarity of the Expanded T Cell Population in (lpr/lpr plus +/+ →

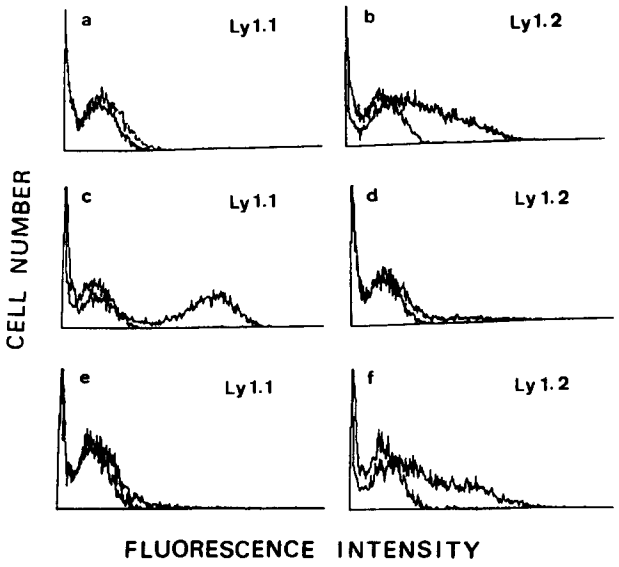

FIGURE 1. Fluorescence profiles of lymph node cells from (*lpr/lpr* → *lpr/lpr*) (*a* and *b*) (+/+ → *lpr/lpr* (*c* and *d*) or (*lpr/lpr* plus +/+ → *lpr/lpr*) (*e* and *f*) chimeras stained with monoclonal anti-Ly-1.1 (*a*, *c*, and *e*) or anti-Ly-1.2 (*b*, *d*, and *f*). Background staining with FITC-avidin alone is also shown in each profile.

TABLE III

Ly-1 Allotype of Thymocytes from Group III (lpr/lpr plus +/+ → lpr/lpr) Chimeras

Mouse Number	Number of		Percent positive*			
	LN cells	Thymocytes	LN cells		Thymocytes	
			Ly-1.1	Ly-1.2	Ly-1.1	Ly-1.2
	$\times 10^{-7}$	$\times 10^{-7}$				
1	30	1.3	5	71	38	55
2	19	1.7	6	65	32	46
3	16	1.2	6	69	35	50
4	21	1.8	5	73	40	58
5	27	ND	3	70	ND	ND
6	13	1.3	5	69	37	50
7	11	ND	6	63	ND	ND

* Ly-1 positive percent of the cells was determined by immunofluorescence.

lpr/lpr) Chimeras to that of Intact lpr Mice. Next, we investigated the surface differentiation antigens on the T cells of (*lpr/lpr* plus +/+ → *lpr/lpr*) chimeras to assess whether or not these cells were identical to those accumulating in intact *lpr* mice. As shown in Fig. 3, the majority of the cells in the chimeras was Thy-1$^+$ (Fig. 3*a*), Ly-1.2$^+$ (Fig. 3*b*), L3T4$^-$,Lyt-2$^-$ (Fig. 3*c*). The amounts of both Thy-1 and Ly-1 antigens on the surface of these cells were less than those on LNC of intact B6/Kh mice (Fig. 3, *d* and *e*). We also stained for the 9F3 and 3A1 (B-220) antigens, which are known to be expressed on the expanded T cell population of *lpr* mice (7, 13). As shown in Fig. 4, a remarkable increase in Ly-1.2$^+$, 9F3$^+$ (Fig. 4*a*) and Ly-1.2$^+$, 3A1$^+$ (Fig. 4*d*) cells was observed in (*lpr/lpr* plus +/+ → *lpr/lpr*) chimeras, as compared with (+/+ → *lpr/lpr*) chimeras (Fig. 4, *b* and *e*) or intact B6/Kh mice (Fig. 4, *c* and *f*). Neither 9F3 nor 3A1 antigens

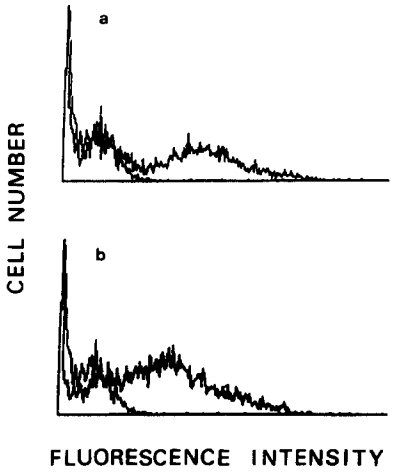

FIGURE 2. Fluorescence profiles of thymocytes from a representative (*lpr/lpr* plus +/+ → *lpr/lpr*) chimera stained with biotinylated monoclonal anti-Ly-1.1 (*a*) or anti-Ly-1.2 (*b*). Background staining with FITC-avidin alone is also shown in each profile.

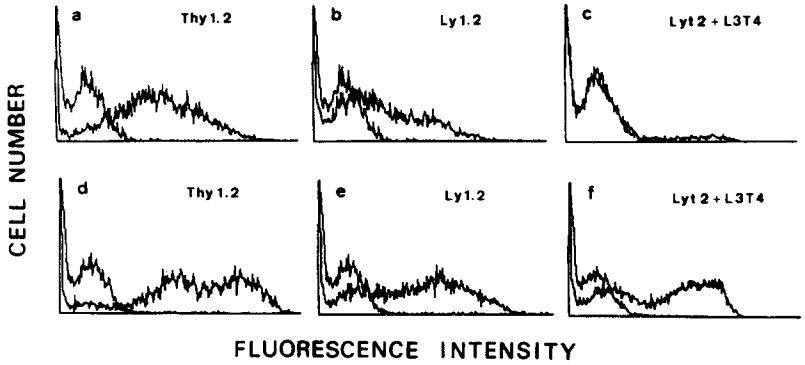

FIGURE 3. Comparison of the expression of Thy-1.2 (*a* and *d*) Ly-1.2 (*b* and *e*) or Lyt-2 plus L3T4 (*c* and *f*) on lymph node cells from an (*lpr/lpr* plus +/+ → *lpr/lpr*) chimera (*a*, *b*, and *c*) and an unmanipulated B6/Kh mouse (*d*, *e*, and *f*). Background staining with FITC-avidin alone is also shown in each profile. These data are representative of three separate experiments.

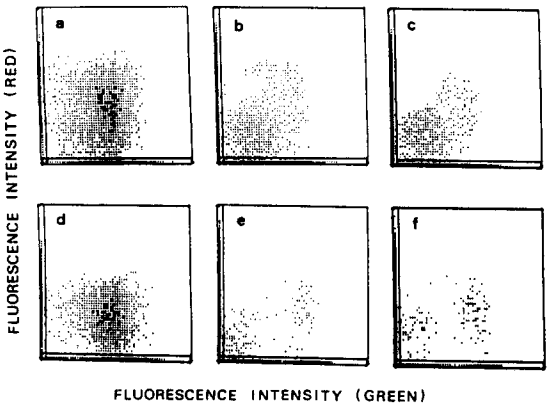

FIGURE 4. Two-color fluorocytometric analysis of 9F3 (*a*, *b*, and *c*; green) or 3A1 (*d*, *e*, and *f*; green) and Ly-1.2 (*a*, *c*, *d*, and *f*; red) or Ly-1.1 (*b*, and *e*; red) antigens on lymph node cells from an (*lpr/lpr* plus +/+ → *lpr/lpr*) chimera (*a* and *d*), a (+/+ → *lpr/lpr*) chimera (*b* and *e*), or an unmanipulated B6/Kh mouse (*c* and *f*). The levels that show <1% positive for both green and red fluorescence of lymph node cells treated with counterstains alone are delimited by the lines. These data are representative of three separate experiments.

were expressed on the minor population of Ly-1.1$^+$ LNC of group III chimeras (data not shown).

These data indicate that the major population of T cells in (*lpr/lpr* plus +/+ → *lpr/lpr*) chimeras is Thy-1$^+$(dull), Ly-1.2$^+$(dull), Lyt-2$^-$,L3T4$^-$, 9F3$^+$,3A1$^+$, which is identical to that seen in intact *lpr* mice.

Autoantibody Formation in Chimeras. Finally, the serum levels of three autoantibodies, IgG antichromatin, IgG anti-ssDNA, and IgM rheumatoid factor, were assessed in the three groups of chimeras and compared with those of unmanipulated B6-*lpr/lpr*, B6/Kh, and B6/Ly1.1 mice (Table IV). Both the group I (*lpr/lpr* → *lpr/lpr*) and the group III (*lpr/lpr* plus +/+ → *lpr/lpr*) sera had elevated levels of all three autoantibodies, while the group II (+/+ → *lpr/lpr*) sera exhibited no more activity than normal controls. These data confirm that the *lpr* internal milieu alone cannot induce autoantibody formation in normal lymphocytes (22, 23). Furthermore, the similar autoantibody levels of the intact B6-*lpr/lpr* mice compared with the group III chimeric mice (which presumably possess a full complement of normal T cells) indicate that autoantibody production in *lpr* mice cannot be suppressed by normal T cells and thus is not due to a lack of T suppressor cells.

Recently, Perkins et al. (30) demonstrated that MRL/*lpr* recipient mice were resistant to engraftment by normal A-Thy mouse lymphocytes and that (A-Thy → MRL/*lpr*) radiation chimeras were completely repopulated by MRL/*lpr* lymphocytes within 6 wk after transplantation. Our experimental system differs from theirs in that we have used as donors 99% genetically identical, congenic +/+ mice (instead of A-Thy mice). We found <10% repopulation by residual host bone marrow in (+/+ → *lpr/lpr*) chimeras 7 mo after the transplantation. However, Perkins' data suggest the existence of radioresistant effector cells that may attack and prevent the growth and differentiation of +/+ lymphocytes or precursors in the *lpr* host mice. Nevertheless, in our experiments we detected normal numbers of Lyt-2$^+$ and/or L3T4$^+$ T cells in (+/+ → *lpr/lpr*) chimeras (data not shown). In addition, Ly-1.1$^+$, +/+–derived cells constituted 33 and 36% of the cells in the peripheral blood and the thymus, respectively, of (*lpr/lpr*

TABLE IV

Serum Autoantibody Levels in Bone Marrow Chimeras

Group	Type of transfer	*n**	IgG antichromatin‡	IgG anti-ssDNA‡	IgM RF‡
I	*lpr/lpr* → *lpr/lpr*	5	0.362 ± 0.061§	0.434 ± 0.091§	0.384 ± 0.135§
II	+/+ → *lpr/lpr*	5	0.105 ± 0.039	0.089 ± 0.051	0.113 ± 0.010
III	*lpr/lpr* plus +/+ → *lpr/lpr*	7	0.504 ± 0.157§	0.659 ± 0.218§	0.416 ± 0.100§
Unmanipulated					
	B6-*lpr/lpr*	4	0.548 ± 0.059§	0.684 ± 0.132§	0.453 ± 0.149§
	B6/Kh	4	0.053 ± 0.010	0.074 ± 0.013	0.075 ± 0.024
	B6/Ly1.1	4	0.100 ± 0.022	0.089 ± 0.031	0.123 ± 0.033

* Size of each group.

‡ Values represent means ± SD of OD_{405} in ELISA.

§ These are significantly different from those of unmanipulated normal mice, $p < 0.05$ (Student's t test).

plus +/+ → *lpr/lpr*) chimeras. Therefore, marked suppression of the +/+ precursors in the *lpr* host did not occur in our system.

Our data support the hypothesis that the *lpr* gene causes intrinsic defect(s) in the T cells which hyperproliferate to form massive lymphadenopathy. We cannot exclude the possibility that an additional abnormality in the *lpr* internal milieu or in another subset of the cells, such as macrophages, might be associated with the triggering mechanism of the lymphadenopathy (31). However, such an abnormality alone is not sufficient, and the defect(s) in the expanding cell population itself is essential for the disease. An aberrant response of *lpr* T cells to growth-regulation lymphokine(s), or to autocrine growth mechanisms could explain the unrestrained growth of these cells, but confirmation of these possibilities is lacking (32). From the recent molecular approaches for the *lpr* T cells, we can speculate that the candidate defect(s) of *lpr* T cells could include: enhanced expression of c-*myb* and c-*raf* oncogenes (14); defects in regulation of c-*myc* oncogene expression (Katagiri, K., T. Katagiri, S. Yokota, R. A. Eisenberg, P. L. Cohen, and J. Ting, submitted for publication); altered K^+ channel expression (33); aberrant expression of surface glycoprotein and glycolipid (34); and abnormal phosphorylation of tyrosine residues of membrane protein (35; our unpublished observation). Although it is impossible to determine at this point which, if any, of these reported abnormalities are directly relevant to the *lpr* lymphoproliferation, our current data increase our confidence that the expanded, double-negative T cell population itself directly expresses the *lpr* gene defect.

Summary

The *lpr* gene induces marked lymphoproliferation characterized by the massive accumulation of T cells of an unusual phenotype and concomitant autoimmune disease. To clarify the mechanism of the *lpr* effect, bone marrow cells from B6-*lpr/lpr* (Ly-1.2) and B6-+/+ (Ly-1.1) mice were transferred into lethally irradiated B6-*lpr/lpr* mice. As has been previously reported, recipients of the B6-*lpr/lpr* bone marrow showed the typical *lpr* phenotype with marked lymphadenopathy, splenomegaly and increased levels of autoantibodies; while the recipients of B6-+/+ bone marrow had normal sized lymph nodes and spleen and no autoantibodies. A third group of mice received an equal mixture of bone marrow cells from the B6-*lpr/lpr* and B6-+/+ donors. These mice showed both lymphadenopathy and autoantibody production comparable to that of recipients of the B6-*lpr/lpr* marrow alone. Immunofluorocytometric analysis of the lymphoid populations in these mixed bone marrow recipients established that the T cells from the *lpr/lpr* and +/+ donors were equivalently represented in the peripheral blood and thymus. In striking contrast, the T cells that accumulated in abnormally large numbers in the lymph nodes were almost entirely from the *lpr* donor. Their surface phenotype was Thy-1^+(dull), Ly-1.2^+(dull), Lyt-2^-, L3T4^-, 9F3^+, and 3A1^+, which is consistent with that found in intact *lpr* mice. These results indicate that the *lpr* gene causes an intrinsic defect directly within the T cells that accumulate in large numbers in *lpr* mice. In addition, the presence of the +/+ T cells cannot prevent the expression of the *lpr* abnormalities.

We thank Ms. Katherine La Pan, Ms. Sylvia Craven, Mr. Bobby Cheek, and Ms. Rebecca Rapoport for their excellent technical assistance. We are grateful to Ms. Becky Ryder and Ms. Rebecca Rousseau for typing this manuscript.

Received for publication 10 July 1987 and in revised form 9 November 1987.

References

1. 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 Scientific Publishing Co., Amsterdam. 207–221.
2. Pisetsky, D. S., S. A. Caster, J. B. Roths, and E. D. Murphy. 1982. lpr gene control of the anti-DNA antibody response. *J. Immunol.* 128:2322.
3. Izui, S., V. E. Kelley, K. Masuda, H. Yoshida, J. B. Roths, and E. D. Murphy. 1984. Induction of various autoantibodies by mutant gene lpr in several strains of mice. *J. Immunol.* 133:2267.
4. Theofilopoulos, A. N., and F. J. Dixon. 1985. Murine models of systemic lupus erythematosus. *Adv. Immunol.* 37:269.
5. Davidson, W. F., F. 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.* 137:4075.
6. 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.
7. Morse, H. C., III, W. F. Davidson, R. A. Yetter, E. D. Murphy, J. B. Roths, and R. L. Coffman. 1982. Abnormalities induced by the mutant gene lpr: expansion of a unique lymphocyte subset. *J. Immunol.* 129:2612.
8. Lewis, D. E., J. V. Giorgi, and N. L. Warner. 1981. Flow cytometry analysis of T cells and continuous T cell lines from autoimmune MRL/l mice. *Nature* (*Lond.*). 289:298.
9. Wofsy, D., R. R. Hardy, and W. E. Seaman. 1984. The proliferating cells in autoimmune MRL/lpr mice lack L3T4, an antigen on "helper" T cells that is involved in the response to class II major histocompatibility antigens. *J. Immunol.* 132:2686.
10. Katagiri, T., H. Tomiyama, and K. Kano. 1986. Paul-Bunnell antigen in murine T cell differentiation: abnormal expression in MRL/Mp-lpr/lpr mice. *J. Immunol.* 136:913.
11. Melino, M. R., F. J. Dumont, R. C. Habbersett, and T. H. Hansen, 1983. Expression of a bone marrow-associated Ly-6 determinant on the T cell population expanding in the lymph nodes of the autoimmune mouse strain MRL/Mp-lpr/lpr. *J. Immunol.* 130:1843.
12. Dumont, F. J., R. C. Habbersett, L. Z. Coker, E. A. Nichols, and J. A. Treffinger. 1985. High level expression of the plasma cell antigen PC.1 on the T cell subset expanding in MRL/Mp-lpr/lpr mice: detection with a xenogenic monoclonal antibody and alloantisera. *Cell. Immunol.* 96:327.
13. Dumont, F. J., R. C. Habbersett, and E. A. Nichols. 1984. A new lymphocyte surface antigen defined by a monoclonal antibody (9F3) to the T cell population expanding in MRL/Mp-lpr/lpr mice. *J. Immunol.* 133:809.
14. 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.
15. Sheiness, D., and M. Gardinier. 1984. Expression of a proto-oncogene (proto-myb) in hematopoietic tissues of mice. *Mol. Cell. Biol.* 4:1206.
16. Davignon, J-L., R. C. Budd, R. Ceredig, P. F. Piquet, H. R. MacDonald, J. C.

Cerottini, P. Bassalli, and S. Izui. 1985. Functional analysis of T cell subsets from mice bearing the lpr gene. *J. Immunol.* 135:2423.

17. Katagiri, K., T. Katagiri, R. A. Eisenberg, J. Ting, and P. L. Cohen. 1987. Interleukin 2 responses of lpr and normal L3T4$^-$/Lyt-2$^-$ T cells induced by TPA plus A23187. *J. Immunol.* 138:149.
18. Steinberg, A. D., J. B. Roths, E. D. Murphy, R. T. Steinberg, and E. S. Raveche. 1980. Effects of thymectomy or androgen administration upon the disease of MRL/Mp-lpr/lpr mice. *J. Immunol.* 125:871.
19. Theofilopoulos, A. N., R. S. Balderas, D. L. Shawler, S. Lee, and F. J. Dixon. 1981. Influence of thymic genotype on the systemic lupus erythematosus-like disease and T cell proliferation of MRL/Mp-lpr/lpr mice. *J. Exp. Med.* 153:1405.
20. Smathers, P. A., T. J. Santoro, T. M. Chused, J. P. Reeves, and A. D. Steinberg. 1984. Studies of lymphoproliferation in MRL-lpr/lpr mice. *J. Immunol.* 133:1955.
21. Murphy, E. D. 1981. Lymphoproliferation (lpr) and other single locus models for murine lupus. *In* Immunologic Defects in Laboratory Animals. Vol. 2. Plenum Publishing Corp., New York. 143–173.
22. Fujiwara, M., and A. Kariyone. 1984. One-way occurrence of graft-versus-host disease in bone marrow chimeras between congenic MRL mice. *Immunology.* 53:251.
23. Theofilopoulos, A. N., R. S. Balderas, Y. Gozes, M. T. Aguado, L. H. Hang, P. R. Morrow, and F. J. Dixon. 1985. Association of lpr gene with graft-vs.-host disease–like syndrome. *J. Exp. Med.* 162:1.
24. 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 function. *Immunol. Rev.* 54:225.
25. 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.
26. Sarmiento, M., A. L. Glasebrook, and F. W. Fitch. 1980. IgG or IgM monoclonal antibodies reactive with different determinants on the molecular complex bearing Lyt-2 antigen block T cell-mediated cytolysis in the absence of complement. *J. Immunol.* 125:2665.
27. Klein, J., V. Hauptfeld, and M. Hauptfeld. 1975. Evidence for a fifth (G) region in the H-2 complex of the mouse. *Immunogenetics.* 2:141.
28. Fisher, C. L., R. A. Eisenberg, and P. L. Cohen. 1988. Quantitation and IgG subclass distribution of anti-chromatin autoantibodies in SLE mice. *Clin. Immunol. Immunopathol.* In press.
29. Budd, R. C., M. Schreyer, G. G. Miescher, and H. R. MacDonald. 1987. T cell lineages in the thymus of *lpr/lpr* mice. Evidence for parallel pathways of normal and abnormal T cell development. *J. Immunol.* 139:2200.
30. Perkins, D. L., J. Michaelson, and A. Marshak-Rothstein. 1987. The lpr gene is associated with resistance to engraftment by lymphoid but not erythroid stem cells from normal mice. *J. Immunol.* 138:466.
31. Cronin, P. S., A. P. Sing, L. H. Glimcher, V. E. Kelley, and C. L. Reinisch. 1984. The isolation and functional characterization of autoimmune clones expressing inappropriate Ia. *J. Immunol.* 133:822.
32. Kelley, V. E., N. M. Farber, J. M. Williams, and T. B. Strom. 1986. Inability of autoimmune mice with the lpr gene to spontaneously produce interleukin 3. *Eur. J. Immunol.* 16:464.
33. Chandy, K. G., T. E. DeCoursey, M. Fischbach, N. Talal, M. D. Cahalan, and S.

Gupta. 1986. Altered K^+ channel expression in abnormal T lymphocytes from mice with the lpr gene mutation. *Science* (*Wash. DC*). 233:1197.

34. Katagiri, T., T. Mori, T. Nakano, K. Ueno, and K. Kano. 1984. Aberrant expression of Forssman and Paul-Bunnell antigens on lymph node cells of MRL/Mp-lpr/lpr mice. *J. Immunol.* 133:3143.
35. Samelson, L. E., W. F. Davidson, H. C. Morse, III, and R. D. Klausner. 1986. Abnormal tyrosine phosphorylation on T cell receptor in lymphoproliferative disorders. *Nature* (*Lond.*). 324:674.