Maturational Arrest from $CD4^+8^+$ to $CD4^+8^-$ Thymocytes in a Mutant Strain (LEC) of Rat

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Summary

A mutant strain (LEC) of rats was found to have a novel defect in T cell maturation, that is, arrest of differentiation from $CD4^+8^+$ to $CD4^+8^-$ but not to $CD4^-8^+$ thymocytes. FACS analyses demonstrated a deficiency in the $CD4^+8^-$ T cell subset in the thymus and a marked decrease in $CD4^+$ T cells in peripheral lymphoid organs. Expression of the T cell receptor (TCR)/CD3 complex in $CD4^+8^+$ and $CD4^-8^+$ thymocytes of LEC rats was normal. Expression of class II major histocompatibility complex (MHC) in the thymus of LEC rats was also the same as that of normal rats. These results indicate that maturational arrest occurs only in the transition pathway from $CD4^+8^+$ to $CD4^+8^-$ thymocytes, and that this mutation can not be attributed to the default of expression of either TCR/CD3, CD4, or class II MHC antigen. Consequently, dysfunction of helper T cells was observed in LEC rats, while killer T cells and B cells functioned normally. Although the complete identification of the origin of this mutation requires further studies, it is hoped that such investigations will throw light on the mechanism of positive selection.

Pre-T cells differentiate into mature T cells in the thymus. During this process, potentially autoreactive thymocytes are clonally eliminated (negative selection) (1–4), while other thymocytes receive differentiative signals by interacting with thymic stromal cells (positive selection) (5, 6). The positive selection hypothesis is supported by the alteration of MHC restriction from the donor type to be recipient type in bone marrow chimera mice, and also by the alteration of surface marker antigens (CD4 and CD8) expressed on thymocytes from double-positive ($CD4^+8^+$) to single-positive (either $CD4^+8^-$ or $CD4^-8^+$) phenotypes. The interaction between MHC and either TCR, CD4, or CD8 antigen molecules has been shown to be a prerequisite for the differentiation of $CD4^+8^+$ into either $CD4^+8^-$ or $CD4^-8^+$ cells (7–17). Thus, administration of anti-TCR antibody to neonatal mice blocks the appearance of matured T cells (12), and administration of either anti-class II MHC or CD4 antibody to neonatal mice or thymus in organ culture blocks the differentiation of $CD4^+8^+$ cells to $CD4^+8^-$ cells (10, 11, 16). Moreover, administration of either anti-class I MHC or CD8 antibody to neonatal mice blocks differentiation to $CD4^-8^+$ cells (7–9, 17). However, the molecular mechanism of positive selection is unknown.

We report here a novel mutant rat (LEC) in which differentiation from $CD4^+8^+$ to $CD4^+8^-$ cells in the thymus is blocked genetically but differentiation to $CD4^-8^+$ cells is normal. This is the first report of an animal model with a genetic block at the specific stage in T cell differentiation. Although the origin of this mutation is still unknown, this mutant should be useful in studies on the mechanism of positive selection in the thymus.

Materials and Methods

Rats. LEC/Tj, LEA/Tj, and ACI/Tj rats were maintained in our laboratory in specific pathogen-free (SPF)[1] conditions. LEC rats were originally established at the Center for Experimental Plants and Animals (Hokkaido University, Japan) (18). SPF LEC rats were provided by Otsuka Pharmaceutical Co. Ltd. (Tokushima, Tokushima, Japan). WKAH/Slc rats were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Shizuoka, Japan).

Antibodies and Reagents. Anti-rat class II MHC mAb, OX6 (19), ascites was a gift from Dr. H. Kimura, University of Pennsylvania (Philadelphia, PA). The anti-rat TCR-α/β mAb, R73 (20), hybridoma cell line was a gift from Dr. T. Hünig, Universität Würzburg (Würzburg, FRG). Anti-rat CD4 mAb, W3/25 (21), and anti-rat CD8 mAb, OX8 (22), hybridoma cell lines were gifts from

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

Dr. M. Miyasaka, Tokyo Metropolitan Institute for Medical Sciences (Tokyo, Japan). Anti-rat CD3 mAb, 1F4 (23), ascites was a gift from Dr. Y. Hashimoto, Tohoku University (Sendai, Japan). Purified antibodies were labeled with FITC or PE by standard procedures. A Vectastain ABC kit and biotinylated anti-mouse IgG and IgM were purchased from Vector Laboratories, Inc. (Burlingame, CA). A cDNA clone encoding murine IL-4, 2A-E3 (24), was a gift from DNAX Research Institute of Molecular and Cellular Biology (Palo Alto, CA). Other reagents were obtained from the following sources: RPMI 1640, FCS, penicillin, streptomycin, and gentamicin (Gibco Laboratories, Grand Island, NY); PE, mitomycin C, methyl α-D-mannopyranoside, MEM nonessential amino acid, Con A, and PWM (Sigma Chemical Co., St. Louis, MO); streptavidin DuoCHROM and FACS Lysing Solution (Becton Dickinson & Co., Mountain View, CA); murine rIL-2 and rIL-4 (Genzyme, Boston, MA); Nu-Serum (Collaborative Research Inc., Lexington, MA); Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ); DMEM (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan); SRBC (Nippon Bio-Test Labs. Inc., Kokubunji, Tokyo, Japan); DNP^{28}-aminoethylcarboxymethyl-Ficoll (Biosearch, San Rafael, CA); paraformaldehyde (Nacalai Tesque Inc., Kyoto, Japan); [^{32}P]deoxyCTP (Amersham International, Amersham, UK); [^{51}Cr] sodium chromate (New England Nuclear, Boston, MA).

FACS Analyses. Thymocytes were prepared from rats (4–6 wk old) and stained with both FITC-conjugated anti-CD4 and PE-conjugated anti-CD8 mAbs for two-color staining. For three-color staining, thymocytes were first incubated with anti-TCR or anti-CD3 mAb, and then with biotinylated anti-mouse IgG for anti-TCR or biotinylated anti-mouse IgM for anti-CD3. The free arms of biotinylated anti-mouse Ig antibodies were blocked with mouse Ig, and then streptavidin DuoCHROM, FITC-conjugated anti-CD4, and PE-conjugated anti-CD8 mAbs were added. Lymph node cells were prepared from mesenteric lymph nodes and stained by either the one-color or three-color staining procedure described above. Spleen cells were stained with FITC-conjugated anti-CD4 or FITC-conjugated anti-CD8 mAb. Stained cells were fixed in 0.1% paraformaldehyde/saline. Blood cells were treated with FACS lysing solution after staining with either FITC-conjugated anti-CD4 or anti-CD8 mAb. Analyses were performed with a FACScan and either a Consort 30 or FACScan software program (Becton Dickinson & Co.). Dead cells were gated out by forward and side light scatters.

Immunohistochemistry. Frozen thymuses from 5-wk-old rats were sectioned in 6-μm thickness, and sections were stained with hematoxylin and eosin. Adjacent sections were incubated with anti-rat class II MHC mAb, and then with biotinylated anti-mouse IgG and the complex of avidin and biotinylated peroxidase (Vectastain ABC kit). After incubating with 0.02% 3-3′-diaminobenzidine in 0.1 M Tris-HCl (pH 7.6) at room temperature for 5 min, the sections were counter-stained with methylgreen.

CTL Assay. Mesenteric lymph node cells (responder cells) were prepared from LEC ($RT\text{-}1^u$) or LEA ($RT\text{-}1^u$) rats, and 5×10^6/ml cells were cocultured for 5 d with 2.5×10^6/ml ACI spleen cells ($RT\text{-}1^a$) that had been treated with 30 μg/ml mitomycin C for 40 min at 37°C. The culture medium (5 ml) was DMEM supplemented with 5% FCS, 10% Nu-Serum, 10 mM Hepes, 5.5 mM glutamine, 0.16 mM L-asparagine, 0.55 mM L-arginine-HCl, 1× MEM nonessential amino acid, 50 μM 2-ME, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.5 mg/ml gentamicin. After culture, the cells were harvested and suspended in 1 ml of the same medium.

Con A blast cells were prepared by culturing ACI rat spleen cells with 2.5 μg/ml Con A for 2 d. These Con A blast cells were enriched by Ficoll-Paque density gradient centrifugation (1,500 *g*, for 15 min), washed with 2% methyl α-D-mannopyranoside, and labeled with ^{51}Cr by incubating 10^7 cells with 200 μCi [^{51}Cr]sodium chromate for 2 h at 37°C.

An aliquot of the effector cell suspension (100 μl) was incubated with [^{51}Cr]-labeled target cells (10^5 cells) for 15 h at 37°C in a total volume of 200 μl of DMEM. The mixture was then centrifuged at 1,500 *g* for 10 min, and 150 μl of the supernatant was removed for measurement of radioactivity. The cytolytic activity was expressed as specific killing calculated as: $100\times (a-b)/(t-b)$; where a is the ^{51}Cr release in the presence of effector cells, b is spontaneous release from labeled target cells in the absence of effector cells, and t is the total radioactivity releasable by incubating with 0.1% NP-40.

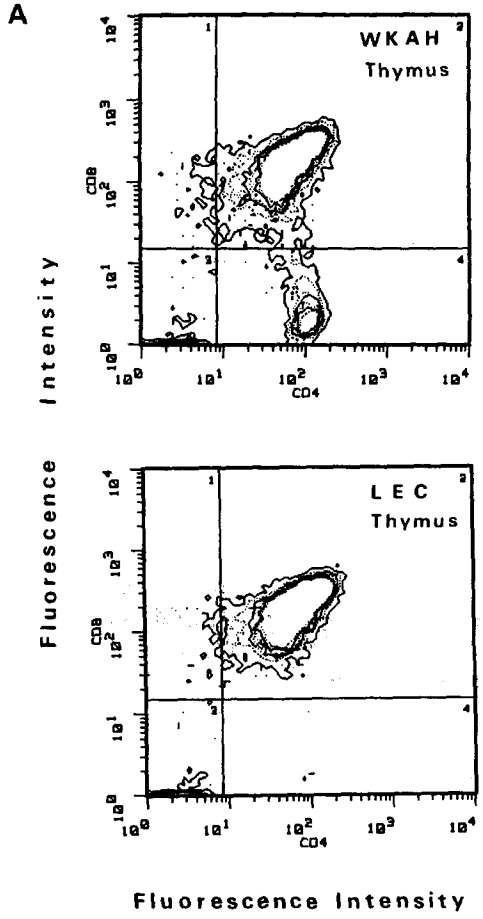
Cytokine Assay. Lymphocytes (5×10^6 cells/ml) prepared from spleen and mesenteric lymph nodes of rats of 4–6 wk old were cultured in the presence of 2.5 μg/ml Con A or 5 μg/ml PWM for 2 d. The culture medium was RPMI 1640 supplemented with 10% FCS, 1 mM sodium pyruvate, 50 μM 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin. After stimulation by mitogens, the culture supernatant was collected, diluted serially with fresh culture medium, and added to cultures of CTLL-2 cells (50% [vol/vol]). Murine rIL-2 and rIL-4 were also serially diluted, from an initial concentration of 1,000 U/ml, and added to the culture system (50% [vol/vol]). CTLL-2 cells (5×10^3/well) were cultured for 2 d. The proliferation of CTLL-2 cells was assayed colorimetrically as described previously (25).

Detection of mRNA for IL-4. Mitogen-stimulated spleen cells were collected, and the RNA fraction was prepared from the guanidine thiocyanate (5.5 M) extracts by cesium chloride (5 M) density gradient centrifugation, as described previously (26), with slight modifications. A sample of 10 μg of RNA fraction was treated with formaldehyde, subjected to 1% agarose gel electrophoresis, and then transferred to a nitrocellulose membrane. A Pst-I fragment (360 bp) for murine IL-4 cDNA was labeled with [^{32}P]deoxyCTP, as described previously (27) (specific radioactivity, 2×10^8 dpm/μg), and used as a probe for hybridization. Hybridization was performed overnight at 42°C in a solution of 0.1 M Pipes-NaOH (pH 6.8), 50% formamide, 0.65 M NaCl, 5× Denhardt's solution (0.1% BSA, 0.1% polyvinylpyrrolidone, and 0.1% Ficoll), 0.1% SDS, 5 mM EDTA, and 100 μg/ml sonicated salmon sperm DNA. The membrane was washed four times at 50°C with a solution of 2× SSC containing 0.2% sodium pyrophosphate and 0.1% SDS, and was exposed to XAR-5 X-ray film (Eastman Kodak Co., Rochester, NY) overnight.

Plaque-forming Assay. Rats were immunized intraperitoneally with SRBC (2×10^8) or DNP-conjugated Ficoll (50 μg). After 5 d, plaque-forming spleen cells were estimated by the method of Jerne and Nordin (28). SRBC and TNP-conjugated SRBC were used as target cells for rats that had been immunized with SRBC and DNP-conjugated Ficoll, respectively.

Results and Discussion

Deficiency in $CD4^+$ T Cells. An LEC (Long-Evans with a cinnamon-like coat color) rat was obtained by a spontaneous mutation at the 24th generation during inbreeding and was originally reported to develop spontaneous hepatitis and hepatocarcinoma (18, 29). However, we found several abnormalities in the lymphoid system of this rat, such as a reduced level of serum IgG (30), hypoplasia of the thymus and the spleen, and hypoplasia of the medulla in the thymus. Thus, we examined the distribution of T cell subsets in the thymus



by FACS analyses using anti-CD4 and -CD8 mAbs (Fig. 1 *A* and Table 1). In the cell population from normal rat (WKAH) thymus, most of the thymocytes (76%) express both CD4 and CD8 antigens ($CD4^+8^+$ cells), with smaller numbers of CD4 single-positive cells ($CD4^+8^-$, 15%), CD8 single-positive cells ($CD4^-8^+$, 5.5%), and double-negative cells ($CD4^-8^-$, 3.5%). In contrast, the thymus of LEC rats contains scarcely any $CD4^+8^-$ cells (<1%). However, no significant differences were observed in the percentages of $CD4^-8^+$ and $CD4^-8^-$ cells in WKAH and LEC rats. The percentage of double-positive cells was 12% higher in LEC rats than in WKAH rats; this increase seemed to correspond to the percentage of disappearance of $CD4^+8^-$ cells. These results indicate that LEC rats are defective in the transition pathway from $CD4^+8^+$ to $CD4^+8^-$ cells.

We also examined the distribution of T cell subsets in peripheral lymphoid organs of the two strains. As shown in Fig. 1 *B* and Table 1, the most striking finding was that the percentages of $CD4^+$ cells in three kinds of peripheral lymphoid organs were much lower in LEC rats than in WKAH rats. CD4 antigen was expressed by one quarter of the spleen cells in WKAH rats but by only 5% of those in LEC rats. Since the anti-rat CD4 mAb (W3/25) crossreacts with macrophages (31), the actual percentage of CD4-bearing T cells in LEC rats seems to be marginal. Although the percentage of $CD8^+$ spleen cells was slightly lower in LEC rats than that in WKAH rats, no significant difference was observed. The lymph nodes of LEC rats contained a relatively large number of $CD4^+$ cells (20%), but the percentage of $CD4^+$ cells was much lower than that in WKAH rats. On the other

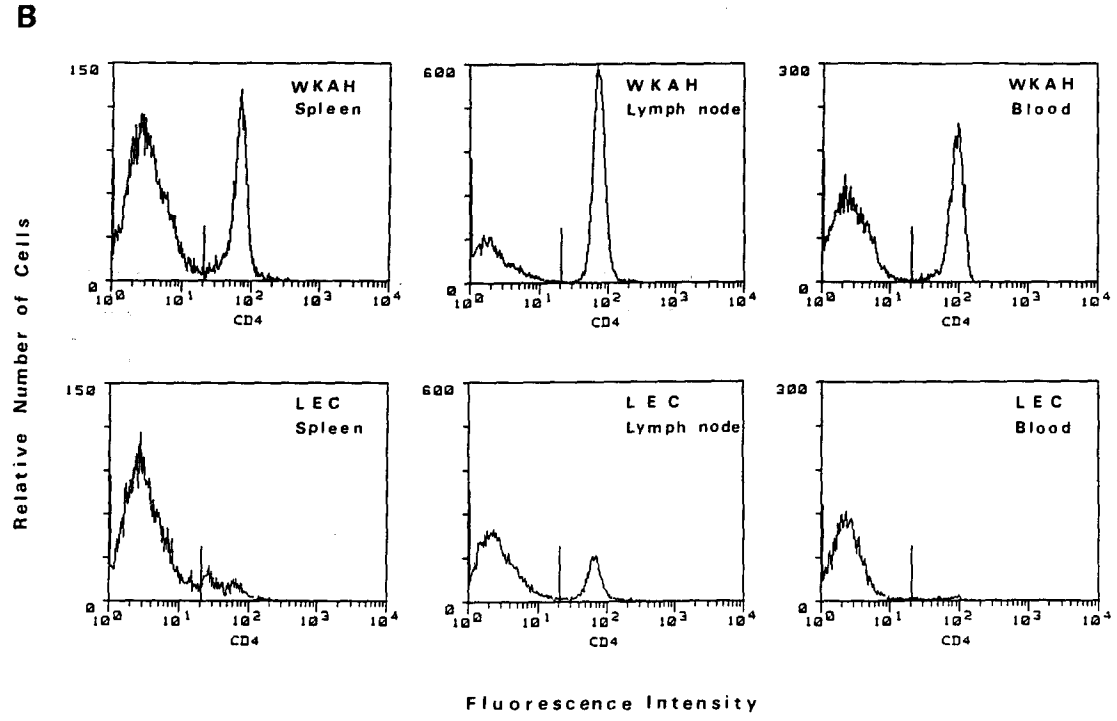


Figure 1. FACS analyses of CD4/CD8 antigens on thymocytes (*A*) and CD4 antigen on spleen, lymph node, and PBL (*B*).

hand, the percentage of $CD8^+$ cells in LEC rats was higher than that in WKAH rats, but this seemed to indicate an apparent increase with a decrease in the number of $CD4^+$ cells on FACS analysis, and did not mean an increase in the absolute number of $CD8^+$ cells. Results for peripheral blood cells were similar to those for spleen cells. These results are consistent with the observation of deficiency in mature CD4-bearing T cells in the thymus of LEC rats.

The CD4/CD8 phenotype of thymocytes and peripheral lymphocytes of F_1 offsprings from LEC and WKAH rats were analyzed (Table 1). The data for F_1 rats were similar to those for WKAH rats. Furthermore, backcrossed progeny of (WKAH × LEC)F_1 and LEC showed a 1:1-segregated phenotype in terms of deficiency in $CD4^+$ T cells in the peripheral blood (T. Yamada, T. Natori, K. Izumi, T. Sakai, T. Agui, and K. Matsumoto, manuscript submitted for publication). These data suggest that this mutation is caused by a single recessive gene.

TCR/CD3 Expression. Interaction between TCR and class II MHC has been shown to be necessary for maturation from $CD4^+8^+$ to $CD4^+8^-$ cells (12–15). Therefore, we examined the expression of TCR/CD3 antigens in each subset of thymocytes (Fig. 2, *A* and *B*). The most important finding was that the TCR/CD3 antigen complex was expressed on $CD4^+8^+$ cells in LEC rat thymus. In LEC thymus, 49.1 ± 11.1% (mean ± SEM; three to four rats) of the $CD4^+8^+$ cells were TCR-α/β^-, and 50.9 ± 11.1% were dull-positive; these values were similar to those in WKAH rat thymus (50.3 ± 4.4% for negative and 49.7 ± 4.4% for dull-positive). A second notable finding was that both TCR-α/β and CD3 antigens were brightly positive in ~40% of the $CD4^-8^+$ cells in LEC rat thymus (31.2 ± 2.8% for TCR-α/β and 41.5 ± 1.1% for CD3), as well as in WKAH rat thymus (40.8 ± 2.8% for TCR-α/β and 45.0 ± 5.8% for CD3). This indicates that differentiation from $CD4^+8^+$ to $CD4^-8^+$ cells is normal in LEC rat thymus. A third finding was that the ratio of TCR/$CD3^+$ cells in the $CD4^-8^-$ subset was less in LEC rat thymus than that in WKAH rat thymus (5.9 ± 0.3% vs. 23.3 ± 4.3% for TCR-α/β and 12.7 ± 0.4% vs. 33.9 ± 3.6% for CD3). The difference between the percentages of $CD3^+$ cells and TCR-α/β^+ cells can be regarded as that of TCR-γ/δ^+ cells, despite the absence of direct evidence for this using anti-rat TCR-γ/δ antibody. The results, therefore, indicate that the ratio of matured $CD4^-8^-$ cells (both TCR-α/β^+ and -γ/δ^+) is less in LEC rats than in WKAH rats. However, it is unclear whether this is related to the defect in maturation from $CD4^+8^+$ to $CD4^+8^-$ cells.

Class II MHC Expression. Since CD4 and TCR/CD3 antigens are expressed on $CD4^+8^+$ cells in LEC rats, it might be supposed that class II MHC antigen is not expressed in the thymus. However, as shown in Fig. 3, class II MHC antigen is expressed in the thymus (both the cortex and medulla) of LEC rats as in WKAH rats, in spite of hypoplasia of the medulla. Furthermore, the data for backcrosses between either (WKAH × LEC)F_1 or (BN × LEC)F_1 and LEC rats showed no hereditary linkage between deficiency in $CD4^+$ T cells and the MHC haplotype (T. Yamada et al., manuscript submitted for publication). These data indicate that this mutation is not a mutation of the class II MHC gene.

Normal Maturation of $CD8^+$ T Cells. The percentage of $CD8^+$ cells was normal in the peripheral lymphoid organs of LEC rats (Fig. 1 and Table 1). Expression of TCR/CD3 antigens by $CD4^-8^+$ subset cells in the thymus was also normal (Fig. 2). We further examined both TCR/CD3 expression and the function of peripheral $CD8^+$ T cells to confirm that the one side of maturational pathway of T cells (maturation of $CD8^+$ T cells) is normal in LEC rats. As shown in Fig. 4, most of the $CD8^+$ lymph node cells were $CD3^+$ (93.7 ± 3.7%, mean ± SEM; three to four rats); further, 66.6 ± 5.8% of the $CD8^+$ cells were also TCR-α/β^+ in LEC rats. In WKAH rats, 95.4 ± 3.1% of the $CD8^+$ lymph node cells were $CD3^+$, and 78.0 ± 9.0% were TCR-α/β^+. This may indicate that the proportion of

Table 1. *The Percent of T Cell Subsets in Various Organs in WKAH, LEC, and (WKAH × LEC) F_1 Rats*

	Thymus				Spleen		Lymph node		Blood	
	$CD4^-8^-$	$CD4^+8^-$	$CD4^-8^+$	$CD4^+8^+$	$CD4^+$	$CD8^+$	$CD4^+$	$CD8^+$	$CD4^+$	$CD8^+$
WKAH	3.6 ± 0.4	14.8 ± 2.3	5.6 ± 0.6	76.0 ± 2.5	24.4 ± 3.1	10.5 ± 2.1	49.4 ± 3.4	17.7 ± 2.1	39.3 ± 1.2	10.6 ± 0.6
LEC	5.1 ± 0.6	0.9 ± 0.1*	6.1 ± 0.6	88.0 ± 0.7*	4.5 ± 0.5*	9.0 ± 1.9	19.4 ± 0.9*	31.4 ± 2.0‡	3.5 ± 0.2*	7.4 ± 1.1§
F_1	4.9 ± 0.4	15.1 ± 0.4	4.5 ± 0.4	75.6 ± 0.7	21.1 ± 2.3	14.2 ± 3.2	53.0 ± 1.2	21.6 ± 1.7	34.0 ± 1.9	12.6 ± 1.4

Thymocytes were stained by the two-color staining method as described in Materials and Methods. Spleen, lymph node, and peripheral blood cells were stained separately with FITC-labeled anti-CD4 or anti-CD8 mAb as described in Materials and Methods. Analyses were performed with FACScan, and the percents of positive cells were estimated using a Consort 30 software program. Data represent the mean ± SEM of observations obtained from 4–20 rats.

* $p < 0.001$ vs. WKAH.

‡ $0.001 < P < 0.005$ vs WKAH.

§ $0.025 < P < 0.05$ vs. WKAH.

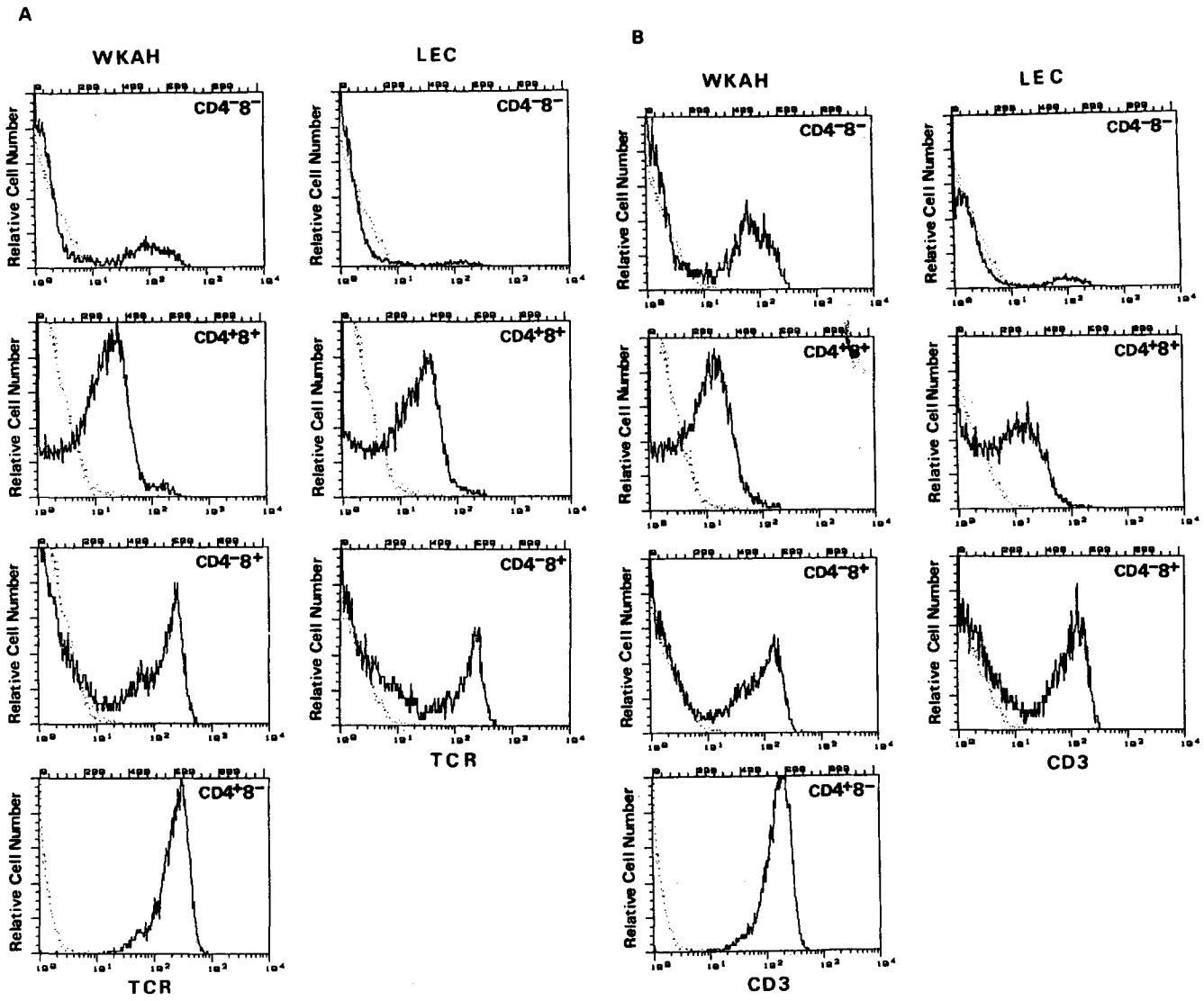


Figure 2. Expression of TCR (*A*) and CD3 (*B*) antigens on each subset of thymocytes in WKAH and LEC rats. Thymocytes stained with three colors were first analyzed with a FACScan using two parameters, FL1 (anti-CD4) and FL2 (anti-CD8), and then 13,000 cells of each subset were collected using a live gate. Collected cells were analyzed by a parameter of FL3 (either anti-TCR or anti-CD3). The dotted line represents the profile of cells stained without either anti-TCR or anti-CD3 mAb. Results are representative of those obtained for three to four rats.

TCR-γ/δ^+ cells in the $CD8^+$ lymph node cells is greater in LEC rats than that in WKAH rats, but it is unknown whether this is related to deficiency in $CD4^+$ T cells in LEC rats. However, the important observation is that most of the $CD8^+$ cells express TCR/CD3 antigens. This was strengthened by the CTL assays of LEC lymph node cells (Fig. 5). When LEC lymph node cells were incubated with spleen cells from ACI rats ($RT\text{-}1^a$) in vitro in the presence of exogenous IL-2, their cytolytic activities showed the same level as those of LEA rat lymph node cells, which have the same MHC haplotype as LEC rats ($RT\text{-}1^u$). This result indicates that $CD8^+$ cells in LEC lymph nodes have a normally functional potential, in spite of the impaired $CD4^+$ T cells.

Dysfunction of Th Cells. Deficiencies in the productions of both IL-2 and IL-4 were consequently observed in LEC rats (Fig. 6, *A* and *B*). When spleen cells from WKAH rats were stimulated by either Con A or PWM, the supernatant from the spleen cells supported the proliferation of CTLL-2 cells, whereas, the supernatant from the spleen cells of LEC rats had a much lower ability to support their proliferation. These data indicate that the supernatant from the spleen cells of WKAH rats contained appreciable IL-2, whereas that of LEC rats did not. Stimulation of the lymph node cells with Con A and PWM revealed the difference between LEC and WKAH rats more clearly (Fig. 6 *A*, *right panel*); the supernatant from control lymph node cells supported the prolifer-

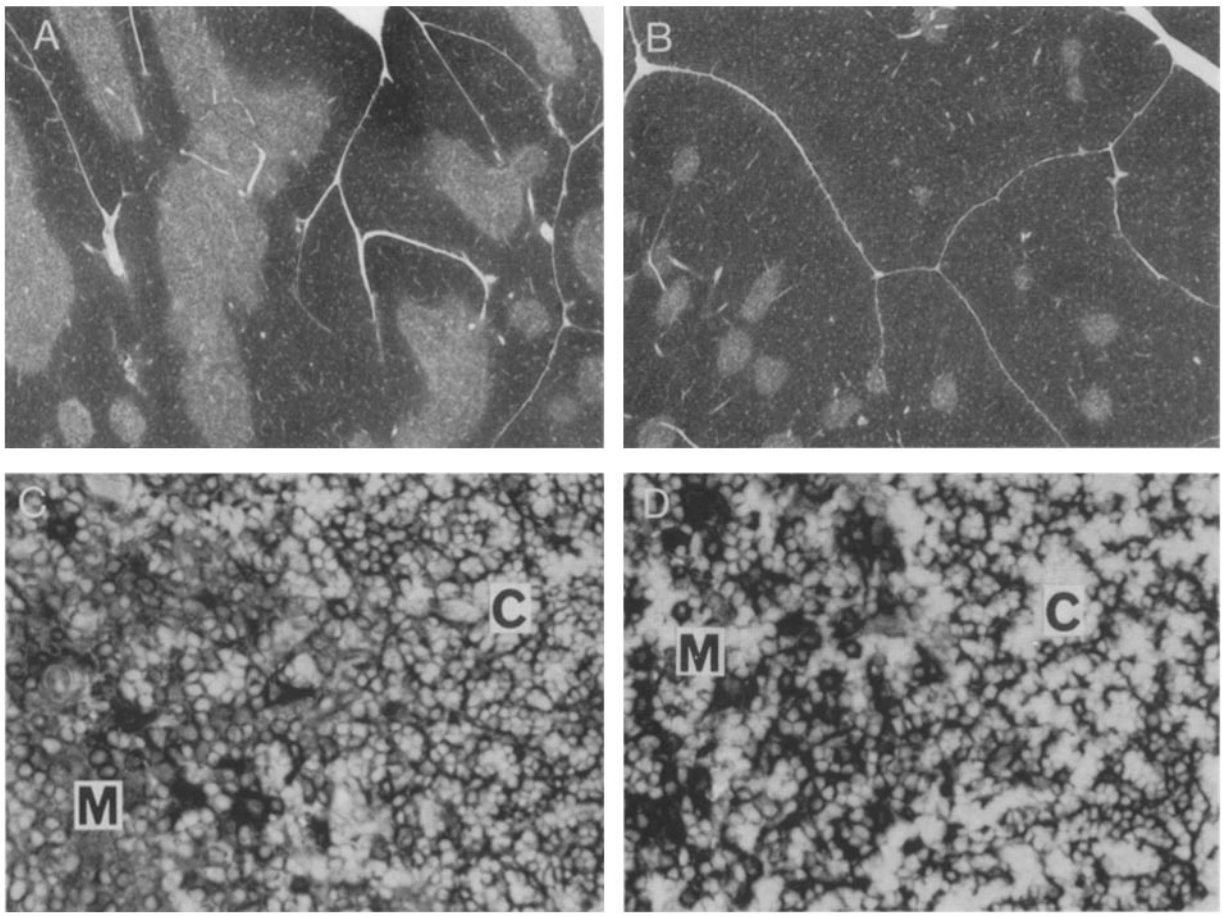


Figure 3. Expression of class II MHC antigen in the thymus. Sections were stained with hematoxylin and eosin (*A* and *B*, ×19.8). Adjacent sections were stained for class II MHC immunohistochemically (*C* and *D*, ×300). (*A* and *C*) Normal (WKAH) rat; (*B* and *D*) LEC rat. *C* and *M* indicate the cortex and medulla, respectively. Note that class II antigens are expressed well in both the cortex and medulla of both strains.

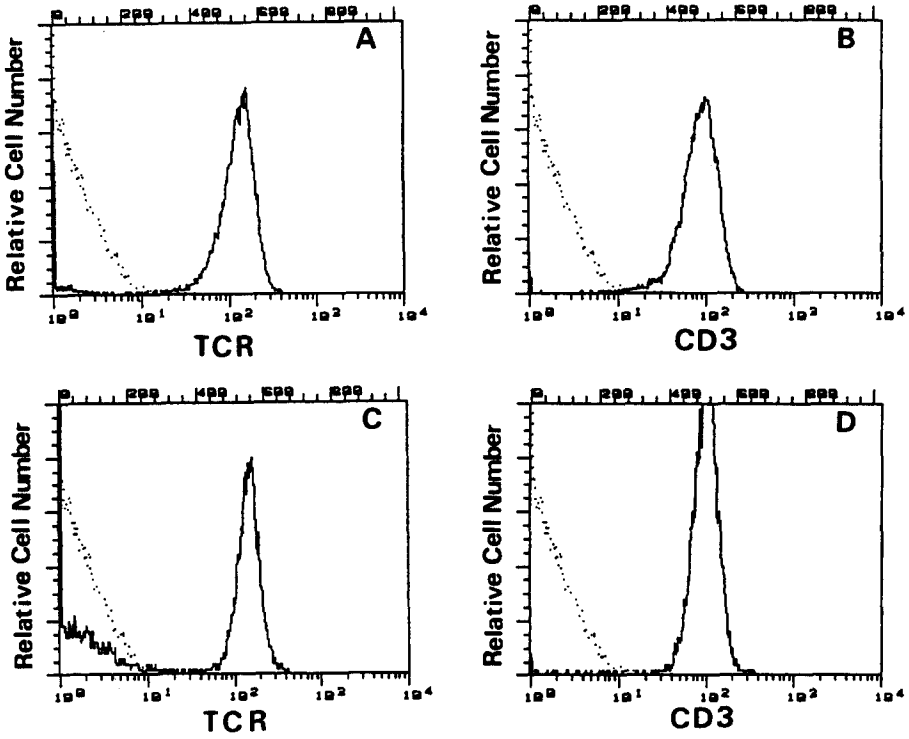


Figure 4. Expression of TCR/CD3 antigens on CD8$^+$ lymph node cells. Lymph node cells stained with three colors were first analyzed with a FACScan using two parameters, FL1 (anti-CD4) and FL2 (anti-CD8), and then 13,000 cells of CD8 single-positive subset were collected using a live gate. Collected cells were analyzed by a parameter of FL3 (either anti-TCR or anti-CD3). (*A* and *B*) WKAH rat; (*C* and *D*) LEC rat. The dotted line represents the profile of cells stained without either anti-TCR or anti-CD3 mAb. Results are representative of those obtained for three to four rats.

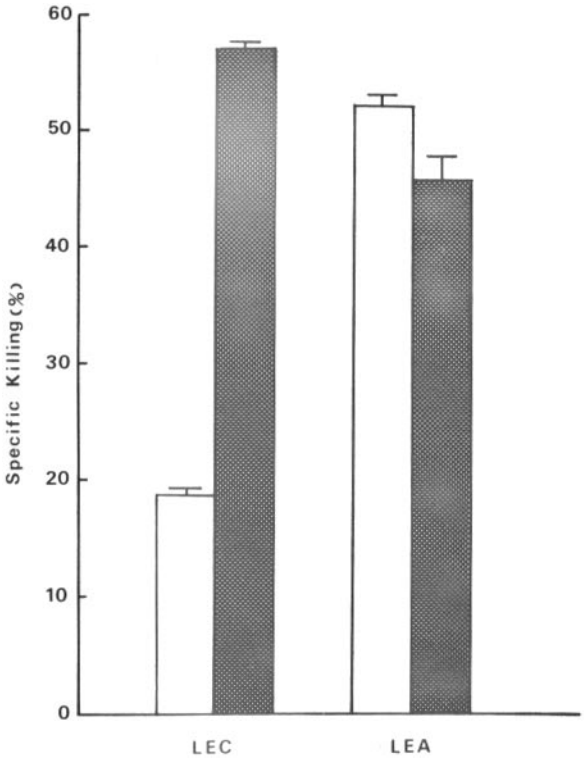


Figure 5. CTL assays in lymph node cells of LEC and LEA rats. Lymph node cells from LEC or LEA rats as responder cells were cocultured with mitomycin C–treated ACI spleen cells as stimulator cells for 5 d in the absence (*open columns*) or presence (10% [vol/vol]) (*filled columns*) of the supernatant of rat Con A blast spleen cells. Cells were harvested and incubated with ^{51}Cr-labeled target cells (ACI Con A blast spleen cells) for 15 h at 37°C. Released ^{51}Cr was counted, and values are expressed as specific killing calculated by the equation given in Materials and Methods. Columns and bars represent means and SEMs of triplicated CTL assays.

ation of CTLL-2 cells, whereas that from LEC lymph node cells did not at all. With respect to IL-4, to which CTLL-2 cells responded weakly, we directly compared the mRNA levels for IL-4 in spleen cells of WKAH and LEC rats (Fig. 6 *B*). Spleen cells from WKAH rats expressed mRNA for IL-4 when stimulated by either mitogen, whereas spleen cells from LEC rats scarcely expressed this mRNA. The deficiencies in the productions of IL-2 and IL-4 support the conclusion that LEC rats have virtually no CD4$^+$ cells, rather than indicating alteration of the antigenic epitopes in their CD4 molecules.

It is of interest to compare the results of IL assays and FACS analyses of the lymph node cells from LEC rats. The CD4$^+$ cells (~20%) in the lymph nodes can be considered to have developed extrathymically, since CD4 single-positive cells do not exist in the thymus of LEC rats. The results suggest that CD4-bearing cells that developed extrathymically do not function as Th cells. The organ that induces expression of CD4 antigen on lymph node cells to supplement for the insufficiency of the thymus is unknown.

From both physical and functional deficiencies in Th cells, LEC rats were expected to be unresponsive to T cell–dependent antigens. In fact, LEC rats did not produce antibodies against SRBC, a T cell–dependent antigen (32), but produced antibodies against DNP-conjugated Ficoll, a T cell–independent antigen (33), as well as WKAH rats (Fig. 6 *C*). Spleen cells from LEC rats produced antibodies against DNP-conjugated Ficoll without immunization more efficiently than spleen cells from WKAH rats. However, the ratio of the plaque-forming cells in immunized and nonimmunized rats were almost consistent between LEC and WKAH rats. These results indicate that Th cells are defective in helping the antibody production, but B cells produce antibodies normally in LEC rats.

The origin of the mutation in LEC rats is unknown. CD4 antigen molecules, in spite of normal expression of antigenic epitopes, may be defective and so can not interact with class II MHC molecules or can not adequately transfer the signals into intracellular elements. Another possibility is that there is a defect in the TCR, such as the lack of the interaction with class II MHC molecule or of the signal transduction. A third possibility is a defect in production or release of putative thymic factors by stromal cells. To obtain further immunological information on this novel mutation, we have started to make a congenic strain of WKAH rats bearing only the recessive gene, *thid* (Th immunodeficiency). Through studies of this rat at a molecular level, we hope that the molecular mechanism of positive selection may be elucidated.

Recently, an interesting human case of immunodeficiency was reported (34). Although this patient showed maturational arrest in the transition pathway from CD4$^+$8$^+$ to CD4$^-$8$^+$ cells, the similarity is very interesting between this patient and LEC rats. As further investigations in humans develop, the findings of such immunodeficient patients may increase. LEC rats may become useful as an animal model for such a genetic immunodeficiency in humans.

We thank Drs. T. Natori, H. Kimura, K. Okumura, and Y. Katsura for helpful discussions; Drs. A. M. Kruisbeek, N. Shinohara, and J. Meligeni for critical review of the manuscript; and Ms. K. Morita for typing the manuscript. We also thank the Science Information Division, the Ministry of Education, Science and Culture of Japan, for aid in buying some of the equipment used in this work.

This work was supported by a research grant from the Ministry of Education, Science and Culture of Japan.

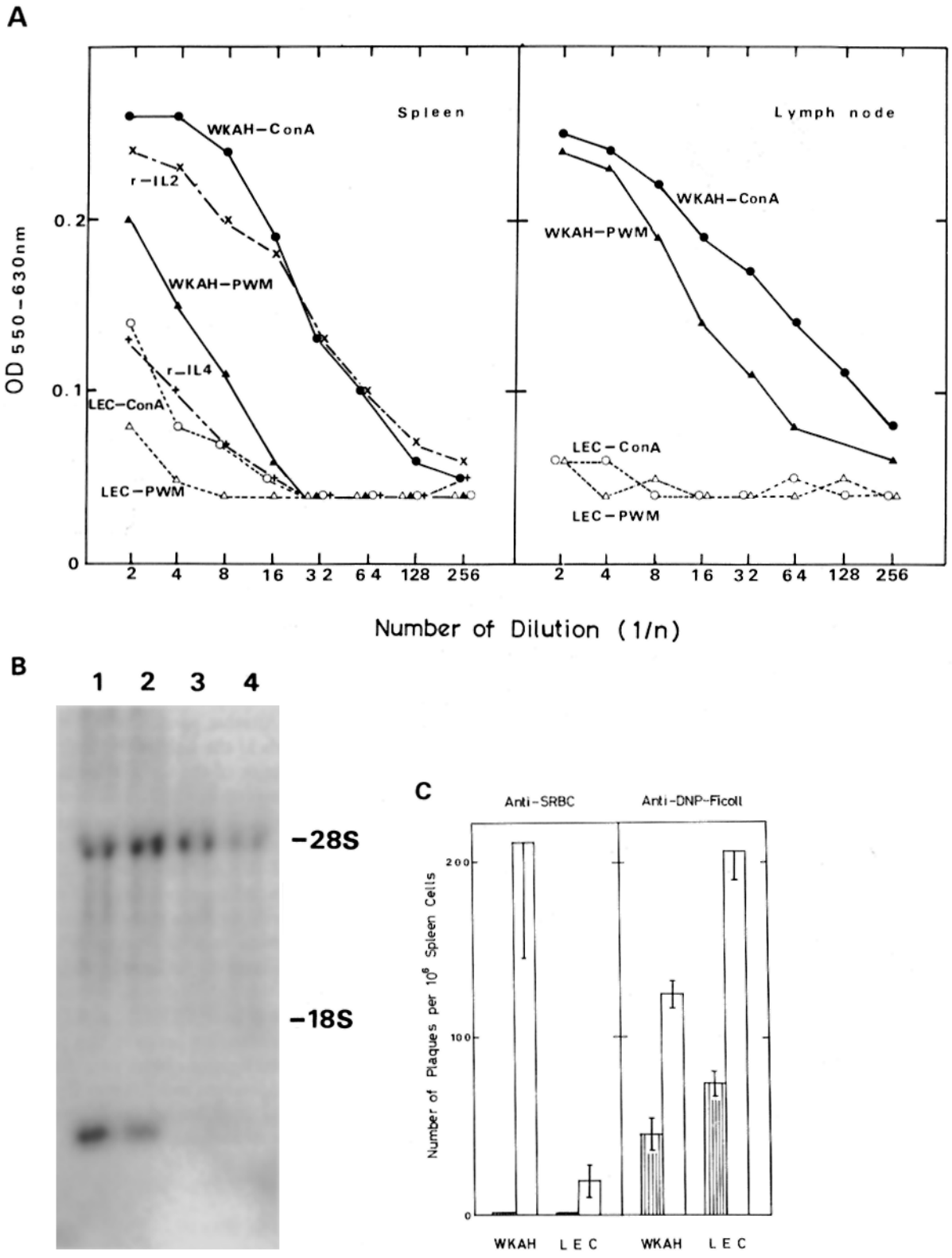


Figure 6. Deficiency in Th cell function in LEC rats. The supernatant from spleen and lymph node cells stimulated by either Con A (*circles*) or PWM (*triangles*) was tested for ability to support growth of CTLL-2 cells (*A*). Filled and open symbols represent data from WKAH and LEC rats, respectively. Murine rIL-2 (x) and rIL-4 (+) was used as a positive control. Results are representative of those obtained in three independent experiments on spleen cells and two independent experiments on lymph node cells. The expression of mRNA for IL-4 was examined simulataneously in the extracts of the spleen cells (*B*). (Lanes *1* and *2*) WKAH rat; (lanes *3* and *4*) LEC rat; (lanes *1* and *3*) stimulation by Con A; (lanes *2* and *4*) stimulation by PWM. (*C*) Plaque-forming assay in spleen cells from normal (WKAH) and LEC rats immunized with either SRBC or DNP-conjugated Ficoll. The basal levels of plaque formation by spleen cells from nonimmunized rats are shown by vertically striped columns. Columns and bars represent means ± SEMs of values for three rats. The results are representative of those in two experiments.

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Received for publication 30 April 1990 and in revised form 27 August 1990.

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