Transgenic Mice Expressing a B Cell Growth and Differentiation Factor Gene (Interleukin 5) Develop Eosinophilia and Autoantibody Production

By Akira Tominaga, Satoshi Takaki, Nobuo Koyama, Shigeki Katoh, Ryoji Matsumoto, Masahiro Migita, Yasumichi Hitoshi, Yukio Hosoya, Shigehito Yamauchi,‡ Yoshiyuki Kanai,‡ Jun-Ichi Miyazaki,* Gentaro Usuku,* Ken-Ichi Yamamura,* and Kiyoshi Takatsu

*From the Institute for Medical Immunology and *Institute for Medical Genetics, Kumamoto University Medical School, Kumamoto 860; and the ‡Institute of Medical Science, University of Tokyo, Tokyo 108, Japan*

Summary

Interleukin 5 (IL-5) has been suggested to be involved in the growth and differentiation of B cells and eosinophils. Especially, Ly-1$^+$ B cells, which have been considered to produce autoantibodies, are selectively developed by this lymphokine in long-term bone marrow culture. To envisage the possible engagement of IL-5 in the development of these cells in vivo, transgenic mice carrying the mouse IL-5 gene ligated with a metallothionein promoter were generated. Transgenic mice carrying the IL-5 gene exhibited elevated levels of IL-5 in the serum and an increase in the levels of serum IgM and IgA. A massive eosinophilia in peripheral blood, bone marrow, and spleen, and an infiltration of muscle and liver with eosinophils, were observed. When cadmium-containing saline was injected intraperitoneally into transgenic mice, IL-5 production was augmented about five times within 24 h, and a distinctive Ly-1$^+$ B cell population became apparent in the spleen after 5 d. IL-5 receptors were detected on those cells by monoclonal antibodies against IL-5 receptors. Another interesting finding in these transgenic mice was an increase in polyreactive anti-DNA antibodies of IgM class. It is suggested, therefore, that aberrant expression of the IL-5 gene may induce accumulation of Ly-1$^+$ B cells and eosinophils. Furthermore, this IL-5 transgenic mouse can be a model mouse for eosinophilia, and we can determine the role of IL-5 in the differentiation of Ly-1$^+$ B cells and eosinophils by using this mouse.

The study of IL-5 originated in the search for one of the B cell growth and differentiation factors, named T cell-replacing factor (TRF),[1] that induces antigen-stimulated B cells to differentiate into plasma cells (1–3) or growth of BCL_1 B cell tumor (BCGFII) (4). On the other hand, eosinophil differentiation factor (5) has been known as a diffusible factor from thoracic duct lymphocytes of parasite-infected rats (6). The cDNA cloning (7–9) and the mAb against IL-5 (10) enabled us to identify this molecule as a cytokine that has been known to induce activated B cells into IgM- and IgA-secreting cells (7–12), to maintain the growth of B cells (13–15), to induce the expression of the IL-2R α chain on the surface of B and T lymphocytes (16–18), to stimulate growth and differentiation of eosinophils (5, 9, 19, 20), and to play a major role in T cell-dependent antibody production (21, 22). IL-5 is an inducible hormone (23) produced mainly by T cells after antigen stimulation such as *Mycobacterium tuberculosis* or *Toxocara canis* (24, 25).

We also observed in an in vitro bone marrow culture that IL-5 could work as growth and differentiation factor for eosinophils and that Ly-1$^+$ B lineage cells were selectively maintained in the presence of IL-5 (26). Ly-1$^+$ B cells are a minor population of B lineage cells that are known to produce a restricted immunoglobulin repertoire including autoantibodies (27, 28). It is not clear, however, whether Ly-1$^+$ B cells are involved in the production of autoantibodies in vivo. It is important, therefore, to evaluate whether or not autoantibodies are produced according to the increased incidence of Ly-1$^+$ B cells by continuous exposure of hematopoietic stem cells to IL-5. We describe here the production of transgenic mice by introducing the mouse IL-5 cDNA ligated to mouse metallothionein promoter (mMT-I). A mas-

[1] *Abbreviations used in this paper:* mMT-I, mouse metallothionein promoter; PE-Av, phycoerythrin-conjugated streptoavidin; TNP, 2,4,6-trinitrophenyl hapten; TRF, T cell-replacing factor.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/91/02/0429/09 $2.00
Volume 173 February 1991 429–437

sive eosinophilia and an expansion of Ly-1$^+$ B cells in the spleen were observed in the resulting transgenic mice. The production of polyreactive IgM antibody against single-stranded (ss) and double-stranded (ds) DNA was seen in IL-5 transgenic mice.

Materials and Methods

Construction of the mMT-I-IL-5 Gene and Production of Transgenic Mice. EcoRI-BglII mMT-I promoter fragment from pdBPV-mMT-neo vector (a generous gift from Dr. P. Howley, NIH, Bethesda, MD) (29) was used to express murine IL-5 (mIL-5) cDNA. The 3′ untranslated region of mIL-5 cDNA that contains an AU-rich sequence was removed. The structure of the mMT-I-IL-5 gene is shown in Fig. 1. The XmnI-NruI fragment of this construct was microinjected into the pronuclei of fertilized eggs of C3H/HeN mice as described (30). For screening of transgenic mice, mouse tail DNA was isolated according to the procedures described previously (31) and digested with BamHI. Digested DNA was analyzed as described by Southern (32). The IL-5 probe used was the 461-bp SacI-AccI fragment of pSP6K-mTRF23 (24).

Assessment of IL-5 Activity by BCGFII Assay. IL-5 activities of rIL-5 were assessed by DNA synthesis with the use of T88-M cells (BCGFII assay) (23, 26), and were expressed in nanograms per milliliter. IL-5-dependent T88-M cells were inoculated in a 96-well microtiter plate at a concentration of 1.5×10^4/0.2 ml/well with serially diluted samples to be tested. Uptake of [^{3}H]thymidine by these cells, which had been pulsed with 0.2 μCi/well during the last 6 h of a 48-h culture, was determined in a liquid scintillation counter. DNA synthesis was determined by means of [^{3}H]thymidine incorporation. In each assay, affinity-purified rIL-5 was also included as a standard (23).

ELISA to Detect IL-5. Anti-IL-5 mAb NC17 (100 μg/ml) was coated onto polyvinyl plate wells (Micro Test III; 3911; Becton Dickinson & Co., Oxnard, CA), and the nonspecific binding sites were blocked with PBS (10 mM phosphate, 140 mM NaCl, pH 7.2) containing 2% BSA. Samples were then applied to the wells and incubated overnight at room temperature. After washing the wells with PBS containing 0.05% Tween 20, polyclonal rabbit anti-murine IL-5 antibodies were added to the wells, followed by a 4-h incubation at room temperature. Then, horseradish peroxidase-coupled goat anti-rabbit Ig (Bio-Rad Laboratories, Richmond, CA) and 2,2′-azino-di (3-ethyl)benzthiazoline sulfonic acid (ABTS) were used as a coloring system according to the manufacturer's recommendation. OD was read at 405 nm. Standard curve was obtained by using purified rIL-5.

Northern Blot Analysis. Total RNAs were isolated by the guanidine isothiocyanate method (33). 20 μg of total RNA from each sample was analyzed by Northern blotting as described (34). ^{32}P-labeled SacI-AccI fragment of IL-5 cDNA (prepared by using Oligolabeling kit from Pharmacia Fine Chemicals, Piscataway, NJ) was used as a probe. Results were shown through Bio Analyzer 100 (Fuji Photo Film, Tokyo, Japan).

Fluorescence-activated Cell Sorter (FACS®) Analysis. Total spleen cells, peritoneal exudate cells, and bone marrow cells from transgenic mice and age-matched control mice were prepared after removing red blood cells. They (10^6) were stained with biotinylated rat mAb against CD5 (Ly-1.53.7.3, rat IgG2a; Becton-Dickinson & Co., Mountain View, CA) plus phycoerythrin-conjugated streptoavidin (PE-Av; Becton Dickinson & Co.) and counterstained with anti-B220 (6B2, a kind gift from Dr. I. Weissman, Stanford University, Palo Alto, CA) followed by FITC-conjugated mAb against rat anti-κ (MAR18.5; kindly provided by Dr. J. Kappler, National Jewish Hospital, Denver, CO), or FITC-coupled IgM (LO.MM9; Serotec, Oxford, England). In the case of H7 (a mAb that recognizes ligand-binding moiety of IL-5R, [35–39]) vs. Ly-1 (CD5), cells were stained with FITC-conjugated anti-Ly-1 at first and then counterstained with biotinylated F(ab)′$_2$ fragments of H7 (rat IgG2a) and PE-Av. Negative controls were cells incubated with PE-Av or FITC MAR18.5. To mask the nonspecific binding sites, cells were preincubated with a 20-fold excess amount of subclass-matched control antibodies (rat anti-Lyt-2, 56-6.72; rat IgG2a) for 15 min at 4°C before staining. Other general procedures were performed as described (26). These cells were then suspended in PBS containing 3% FCS and 0.05% NaN_3 and analyzed on a FACScan (Becton Dickinson & Co.).

Titration of Serum Immunoglobulin Levels and Autoantibodies. Serum immunoglobulin levels were assessed by ELISA by using isotype-specific antibodies as described (12). Results were expressed as micrograms per milliliter serum. Anti-ssDNA and anti-dsDNA antibodies were also measured by ELISA as described previously (40) with a slight modification. Briefly, ssDNA or dsDNA purified from calf thymus DNA, according to the method of Stollar and Papalian (41) was coated on 96-well assay plates (3921; Becton Dickinson & Co.) that had been precoated with poly-L-lysine. These plates were blocked with TBS (25 mM Tris, 140 mM NaCl, pH 7.4) containing 5% FCS and 0.05% Tween 20. Sera were diluted 1:100 with TBS containing 5% FCS. After each incubation, the plates were washed with TBS containing 0.05% Tween 20. Bound antibodies were detected with alkaline phosphatase-conjugated rabbit anti-mouse IgG (Pel-Freez Biologicals, Rogers, AZ) or with alkaline phosphatase-conjugated rabbit anti-mouse IgM (Zymed, South San Francisco, CA). The substrate was *p*-nitrophenylphosphate (Sigma Chemical Co., St. Louis, MO). Antibody activity was expressed as the absorbance at 405 nm in a Titerteck Multiscan ELISA reader (Flow Technologies, Inc., Phoenix, AZ). Anticardiolipin and antitrinitrophenyl hapten (TNP) antibodies were similarly measured by ELISA with minor modifications as described previously (42, 43).

Histology. Organs were fixed in phosphate buffered 10% formalin (35 mM phosphate, pH 7.0) and embedded in paraffin. Sections were stained with hematoxylin and eosin.

Results

Transgene Construct. Because mMT-I promoter is known to be expressed and inducible in various organs such as liver, kidney, intestine, heart, testis, muscle, brain, and spleen, we used this promoter for expressing IL-5 (Fig. 1). Murine IL-5 cDNA that has no 3′ untranslated region was inserted into a part of rabbit β-globin gene derived from pKCR vector (44) and ligated to the mMT-I promoter.

Production of IL-5 Transgenic Mice. Two male mice (Nos. 3 and 6) were transgenic and were shown to carry about 5 or 40 copies of transgene by Southern blot analysis, respectively (Fig. 2 *a*). Both founders transmitted transgenes to half of their offspring without regard to their sex. Although half of the offspring of mouse No. 3 integrated IL-5 transgene, IL-5 expression in terms of eosinophilia in peripheral blood (see below) was very poor and variable among mice (~10% eosinophils in hemizygote and <15% in homozygote even in aged mice). We therefore mainly analyzed IL-5 transgenic mice derived from No. 6 mouse in this study.

The discrimination between hemizygotes and homozygotes

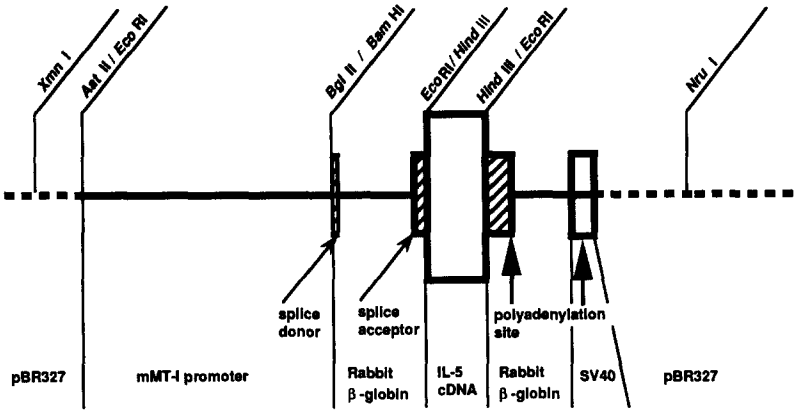

Figure 1. Construct of IL-5 transgene. SV40 promoter of pKCR-mIL-5 was replaced with mMT-I promoter as described in Materials and Methods. The XmnI-NruI fragment was injected into pronuclei of fertilized eggs of C3H/HeN mice.

was possible by measuring the relative density of the 23-kb band (endogenous IL-5 gene) to the 5-kb band (transgene) in the Southern blot described above (Fig. 2 *b*). It was also confirmed by the serum IgM level (Table 1) and the percentage of eosinophils (Fig. 2 *b* and Table 2) in peripheral blood. In No. 6 pedigree, all the individuals with IL-5 transgene have higher level of serum IgM (at least 1.5 times higher than age-matched control) and >20% eosinophils in their peripheral blood.

Expression of the Mouse IL-5 Gene in Transgenic Mice. To determine mRNA expression in IL-5 transgenic mice, Northern blot analysis was performed using total RNA of liver and spleen cells from cadmium-treated normal or IL-5 transgenic mice. Although both endogenous and transgene-derived mRNA were of mouse origin, they were different in their size. IL-5 mRNA from normal IL-5-producing hybridoma B151K12 is 1.7 kb, as described (24), and the transgenic IL-5 mRNA is 1.1 kb, as expected from L cells transfected with pKCR-mMT-I-mIL-5 (the same construct used to make transgenic mice). The latter was probably caused by the removal of the 3′ untranslated region of IL-5 cDNA and the artificial polyadenylation sites. Transcripts with the expected length (1.1 kb) hybridizable with mouse IL-5 cDNA was detected in RNA prepared from liver and spleen cells of the cadmium-treated IL-5 transgenic mice (Fig. 3). Although the 1.1-kb IL-5 mRNA band was also detected in the RNA from spleen and liver of IL-5 transgenic mice without cadmium administration, its expression was low (data not shown). We could not detect either size of IL-5 mRNA in the RNA prepared from spleen and liver cells of cadmium-treated nontransgenic mice under the conditions used in this study. IL-5 transgene expression in liver was very variable, while that in spleen was very constant among four mice of fourth generation.

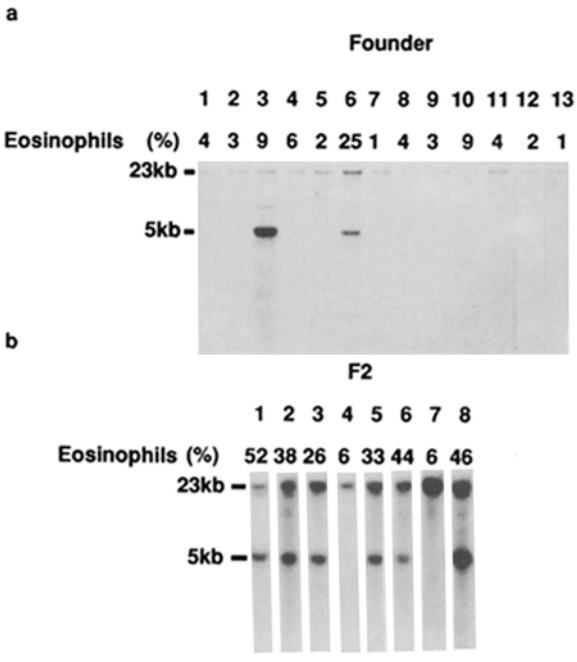

Figure 2. Detection of IL-5 transgene in the founder mouse and its descendant by Southern blot analysis. Percentage of eosinophils in peripheral blood of the individual mouse was also described. Eosinophils were identified by staining the blood smears by May-Gruenwald and Giemsa solution. (*a*) Among 13 offsprings, Nos. 3 and 6 were identified as founder transgenic mice. (*b*) Eight F_2 mice derived from No. 6 were analyzed on the ratio of two IL-5 genes (23 vs. 5 kb) by measuring the intensity of bands in Southern blot.

The serum IL-5 level was determined by both biological assay using the IL-5-dependent T88-M cells and ELISA as described in Materials and Methods. Since we observed similar results in both assay systems, we only described the data by ELISA. The average serum IL-5 level in hemizygotes at 7 wk was 0.11 ng/ml, and that in homozygotes was 2.8 and 16.6 ng/ml at 7 and 25 wk of age, respectively (Table 1). However, we could not found any IL-5 activity in age-matched control mice or in littermate mice (<0.01 ng/ml). Thus, serum IL-5 levels in these homozygous IL-5 transgenic mice were estimated to be at least 1,600-fold higher than normal level in 25-wk-old mice. By injecting cadmium sulfate solution into IL-5 transgenic mice, IL-5 levels in their sera were increased about fivefold within 24 h. These data clearly indicated that the mouse IL-5 transgene, which was inducible upon stimulation with cadmium, was expressed constitutively at least in liver and spleen. IL-5 transgene expression was also observed in bone marrow and kidney (data not shown).

Table 1. *Concentrations of IL-5 and Serum Immunoglobulins of IL-5 Transgenic Mice*

	Control mice (C3H/HeN)		IL-5 transgenic mice derived from No. 6		
			Hemizygotes	Homozygotes	
	7 wk	25 wk	7 wk	7 wk	25 wk
Serum IL-5*	<0.01	<0.01	0.11 ± 0.046	2.8 ± 0.66	16.6 ± 13
Serum Ig‡					
IgM	186 ± 3.3	293 ± 9.0	482 ± 38	1,524 ± 136	1,545 ± 261
IgG1	234 ± 16	736 ± 42	212 ± 12	256 ± 15	876 ± 26
IgG2a	397 ± 19	536 ± 24	375 ± 14	402 ± 23	512 ± 31
IgA	58 ± 8.5	216 ± 20	347 ± 17	229 ± 31	312 ± 11

Results are expressed as the mean ± SE of five female mice in each group at 7 or 25 wk of age.
* Data are measured in nanograms per milliliter.
‡ Data are measured in micrograms per milliliter.

Table 2. *Peripheral Blood Cell Populations in IL-5 Transgenic vs. Control Mice*

Cell type	Control	IL-5 transgenic mice	
		Hemizygotes	Homozygotes
Total white blood cells	8,737 ± 578	17,333 ± 1156	29,800 ± 1682
Eosinophils	183 ± 62	5,175 ± 1087	13,719 ± 1024
Lymphocytes	5,757 ± 574	7,890 ± 520	10,365 ± 845
Monocytes	337 ± 119	468 ± 48	653 ± 60
Neutrophils	2,460 ± 766	3,800 ± 724	5,063 ± 189

Female mice at the age of 25 wk were surveyed for their peripheral blood cell populations. Smears of peripheral blood were stained with May-Gruenwald and Giemsa solution, and their cell types were examined by microscope. The mean values and SE of five mice are shown in cell number per cubic millimeter.

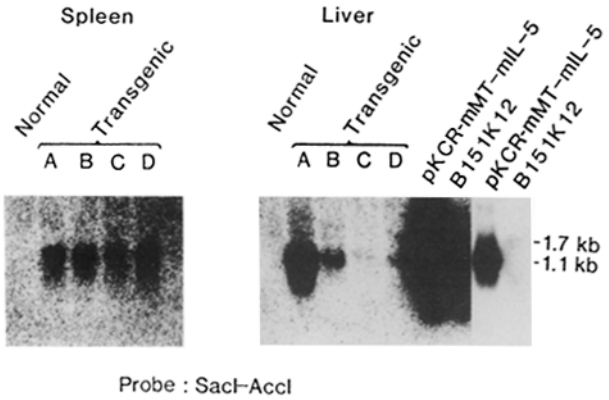

Figure 3. Northern blot analysis of IL-5 transgene expression after cadmium treatment. Individual mice (*A*, *B*, *C*, and *D*) were homozygotes derived from No. 6. 20 μg of total RNA was loaded into each well. B151K12 is a hybrid cell line producing a natural form of IL-5 constitutively. pKCR-mMT-I-mIL-5 is an L cell line transfected with this plasmid that has the same construct with IL-5 transgene. Two lanes at the right hand side are expressed in a decreased sensitivity of Bio-Analyzer 100 to make the bands (1.1 and 1.7 kb) clearer.

Increase in Serum Immunoglobulin Levels and Eosinophils. Serum immunoglobulin levels in these transgenic mice were examined by ELISA. As shown in Table 1, the levels of serum IgM and IgA were approximately eightfold and fourfold higher as compared with those of age-matched control mice that were 7-wk-old, respectively. IgG1 and IgG2a were found to be within control level. In 25-wk-old mice, IgM and IgA levels in the sera of transgenic mice were ~5- and 1.4-fold higher than those of age-matched control mice, respectively. Immunoglobulin levels of the sera of these transgenic mice were not increased by the cadmium treatment.

The most striking feature of transgenic mice was a marked increase in the number of peripheral white blood cells (PBL), of spleen cells, and of peritoneal cells. The total numbers of PBL of hemizygous and homozygous transgenic mice were ~2.0 and ~3.4 times higher than those in littermate control mice, respectively (Table 2). Particularly, the increase in the number of eosinophils was at least 20-fold (hemizygote) to 70-fold (homozygote) compared with that of age-matched control normal mice. Although the number of total lym-

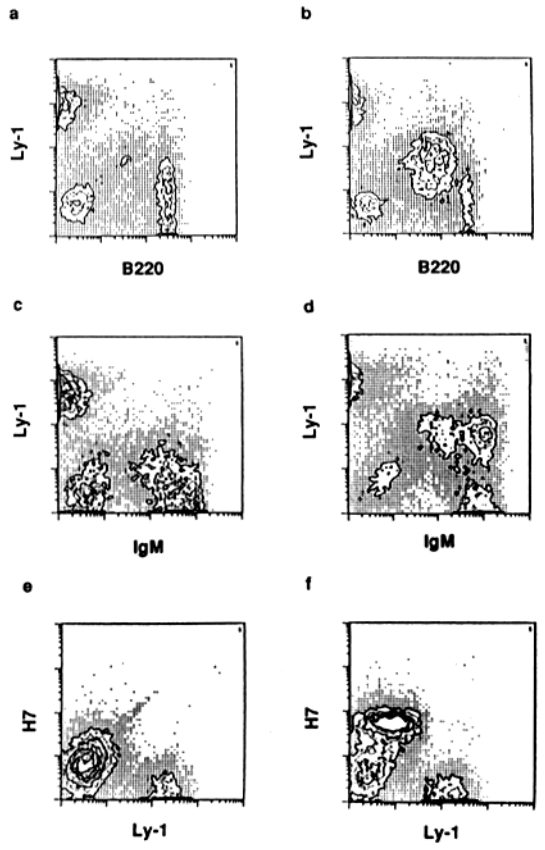

Figure 4. Increase of Ly-1$^+$ B cell population in IL-5 transgenic mice. Scales are expressed in $\log_{10}$. Spleens of 25-wk-old IL-5 transgenic or age-matched control mice were removed 5 d after the cadmium injection and red blood cells were lysed. Total spleen cells were double stained with the antibody combinations described (*a* and *b*) Ly-1 vs. B220; (*c* and *d*) Ly-1 vs. IgM; (*e* and *f*) H7 vs. Ly-1. The cells in the lymphoid gate were analyzed by a flow cytometer. In all experiments, 20-fold class-matched Ig was used to mask the Fc receptors (anti-Ly-2 mAb [56-6.72, rat IgG2a] was used for rat IgG2a). (*a*, *c*, and *e*) Control mice; (*b*, *d*, and *f*) IL-5 transgenic mouse.

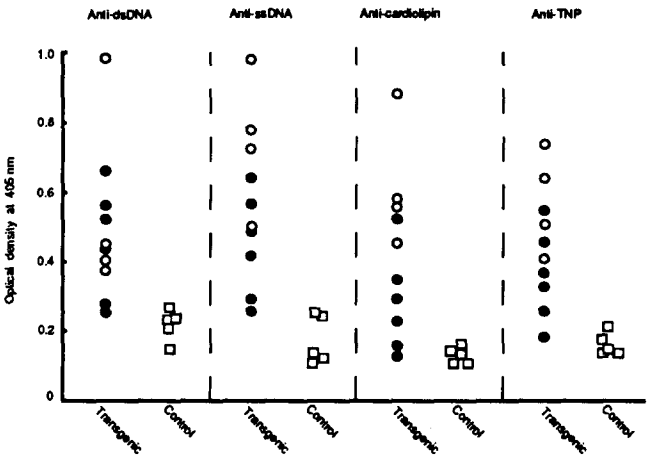

Figure 5. Antigen binding of the IgM antibodies increased in the sera of IL-5 transgenic mice. Open circles indicate the homozygotes for IL-5 transgene. Closed circles are hemizygotes. Open squares are age-matched control mice. All samples were taken from 25-wk-old female mice.

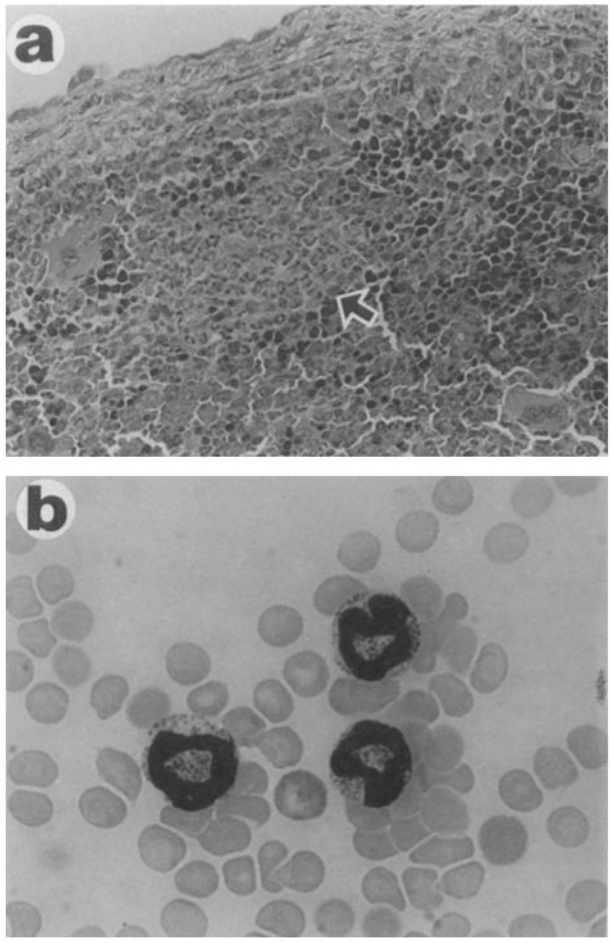

Figure 6. Histology of IL-5 transgenic mice. (*a*) Spleen was fixed in 10% formalin and a section of it was stained with hematoxylin and eosin. An eosinophil colony is indicated by an arrow. (*b*) A smear of peripheral blood was stained with May-Gruenwald and Giemsa solution (*a*, ×300; *b*, ×2000).

phocytes and neutrophils were slightly increased in transgenic mice, the numbers of monocytes were found to remain within normal range. Peritoneal cavity of the littermate control mice contained 2–6 × 10^6 cells. In contrast, there were as many as 2.5 × 10^8 cells in the peritoneal cavity of transgenic mice injected with cadmium. Without cadmium injection, 2–5 × 10^7 cells were found in the peritoneal cavity. About 50% of those cells were eosinophils.

Increase in the Number of Ly-1$^+$ and IL-5R-positive B Cells in the Spleen of IL-5 Transgenic Mice. We stained peritoneal cells, spleen cells, and bone marrow cells from cadmium-injected IL-5 transgenic mice or cadmium-injected control mice with anti-Ly-1 mAb together with various mAbs as described in Materials and Methods. FACS analysis revealed that proportions of IL-5R$^+$ and Ly-1$^+$ B cells of peritoneal cells from IL-5 transgenic mice were unchanged compared with those from littermate control (data not shown). However, a distinctive Ly-1^{dull}- and B220^{dull}-positive population was identified in the spleen cells of IL-5 transgenic mice. Moreover, this weakly Ly-1- and B220-positive population was increased

in its number by the administration of cadmium into the 25-wk-old mice (Fig. 4, *a* vs. *b*). Furthermore, this population has higher density of IgM on its cell surface (Fig. 4, *c* vs. *d*). This Ly-1$^+$ population also expresses IL-5R (H7 antigen) (Fig. 4 *f*). These results suggest that in IL-5 transgenic mice, IL-5R-positive cells are selectively expanded and some of them also coexpress Ly-1 antigens simultaneously.

No difference in the ratio of CD4$^+$ over CD8$^+$ or of α/β^+ over γ/δ^+ was observed among T lymphocytes, and we could not find any increase in allo-killer or MLR activity in IL-5 transgenic mice (data not shown).

Autoantibody Production in IL-5 Transgenic Mice. The increase in the total number of Ly-1$^+$ and IL-5R$^+$ B cells in the peritoneal cavity and the spleen in IL-5 transgenic mice prompted us to examine the production of autoantibodies in the transgenic mice, because Ly-1$^+$ B cells are supposed to produce autoantibodies (45–47). To examine the levels of autoantibodies, we adopted ssDNA, dsDNA, and cardiolipin as test antigens. As a control antigen, TNP was also used. As shown in Fig. 5, we could detect IgM autoantibodies against each antigen in the sera of all IL-5 transgenic mice (six mice are hemizygotes and four mice were homozygote), although the titer in each mouse was variable. These sera reacted also to TNP, and antibody activity to each antigen was absorbed by ssDNA- or dsDNA-coupled Sepharose beads (data not shown), indicating that autoantibodies are polyreactive. No anti-DNA antibodies were found in IgA or IgG fraction (data not shown).

Pathological Findings in Tissues of IL-5 Transgenic Mice. To examine the pathological changes in the IL-5 transgenic mice, two homozygous transgenic mice were killed at 20–25 wk of age. The other two homozygous transgenic mice, which had received four consecutive intraperitoneal injections of cadmium on days 19, 14, 10, and 5 before death, were also subjected to pathological examination. After cadmium treatment, the spleen was enlarged (about five times in cell number) and had white patches consisting of the mass of eosinophils. All the specimens shown in Fig. 6 are from cadmium-injected mice. Both of them showed splenomegaly, and no detectable lymph node enlargement. In the spleen, eosinophils were seen in the marginal area and some of them form colony-like proliferations (indicated by an arrow) all over the spleens (Fig. 6 *a*). Erythroblasts and megakaryocytes were seen more frequently than in normal spleen. Eosinophils were rarely seen in normal spleen cells. Large parts of bone marrow were occupied by mature or immature eosinophils. In the liver, infiltration of eosinophils was mainly observed in Glisson's capsule, and slightly in lobes. Eosinophil infiltration was also seen in the muscle, and derangement of sarcolemma and disappearance of striation were observed. Moreover, we also observed mass of eosinophils along connective or fat tissue sorrounding muscle. In both cases there was infiltration of eosinophils with ring- or U-shaped nuclei and cytoplasm full of red granules. The typical eosinophils seen in peripheral blood of IL-5 transgenic mice were shown in Fig. 6 *b*. The nuclei of eosinophils were found most frequently as a ring-shaped structure, which sometimes showed a folded "figure-eight" structure. The ring form was always an even-sized band (Fig. 6 *b*). Although the mouse neutrophils also had ring-shaped nuclei, they were frequently lobulated. The cytoplasm of eosinophils was filled with small, acidophilic granules which were even in their size. In contrast, the granules of mouse neutrophils were stained poorly and mouse neutrophils appeared to lack cytoplasmic granules.

Discussion

In this study, we describe the generation of a massive eosinophilia and production of polyreactive autoantibodies in IL-5 transgenic mice in which mouse IL-5 cDNA is constitutively expressed. This eosinophilia is characterized as follows: (*a*) the presence of eosinophils with mitosis in spleen, bone marrow, and liver; (*b*) infiltration of many organs with eosinophils, including liver, muscle, heart, lymph node along the bronchus, and Peyer's patches; eosinophils have a tendency to lodge around the connective tissue such as marginal area of spleen, liver, Peyer's patches, and between muscle and fat tissue. Eosinophils probably have some affinity to the certain matrix in connective tissue. In case of muscle, it might be a sign of myositis or fascitis. This feature reminds us of Shulman's disease (48); (*c*) the presence of massive eosinophils in peripheral blood. All these data suggest that IL-5 transgenic mouse can be a model of idiopathic hypereosinophilic syndrome (49). Especially, hypereosinophilic syndrome induced by a graft-vs.-host reaction appears to be caused by IL-5, because IL-5 is known to be produced in a large amount by alloreactive T cells (49, 7).

It is reasonable to expect that in IL-5 transgenic mice, the total number of IL-5R-positive B cells are increased. We also asked if Ly-1$^+$ B cells are increased or not in these transgenic mice, because we observed high incidence of Ly-1$^+$ early B cells in the long-term bone marrow culture on the stromal cells in the presence of IL-5 (26, 50). We used mAb H7 (38, 39) against IL-5R to analyze numbers and distribution of IL-5R-positive cells. In normal mice, most peritoneal surface IgM$^+$ B cells were IL-5R positive, and >70% of IL-5R-positive B cells express Ly-1 antigens on their cell surface (39). On the contrary, only small populations of splenic B cells are H7^{dull} positive, and the H7^{dull} and Ly-1 double-positive cells are marginal (39).

Ly-1$^+$ B cells are a distinct minor subpopulation of B cells and have numerous noteworthy characteristics (for reviews see references 27, 28, 51, 52), such as self-replenishing activity (53–55), particular tissue distribution (abundant in the peritoneal cavity) (46, 53), and production of autoantibodies (45–47). Furthermore, functional studies have suggested that Ly-1$^+$ B cells are selected for the expression of a restricted repertoire of antibody specificities (52, 56). These are the major reasons why Ly-1$^+$ B cells have been considered to be of a separate B cell lineage.

In IL-5 transgenic mice, Ly-1$^+$ B cell population is not relatively increased in peritoneal lymhocytes. In the spleen, however, B220 and Ly-1^{dull}-positive B cells were dramatically increased and many of them were surface IgM and H7 positive.

Ly-1 density on their cell surface seems to be lower than those on peritoneal cells of normal littermate. This distinctive Ly-1$^+$ and B220$^+$ population was also seen in the bone marrow cells, though it was not as clear as in the spleen. This less extensive domination of weakly Ly-1$^+$ and B220$^+$ populations in the bone marrow may partly be explained by the occupation of medullary cavity by eosinophils. This increase in the number of Ly-1$^+$ B cells and eosinophils could be waned by intraperitoneal injection of anti-IL-5 antibody (data not shown).

Mechanism of the preferential increase of Ly-1$^+$ B cell population in the spleen of IL-5 transgenic mice is not fully understood, but direct involvement of IL-5 is highly possible. In vitro growth of Ly-1$^+$ pre-B cells and chronic B cell leukemia cells were shown to depend on IL-5 (4, 7, 14). Recently, we established early B lineage cell lines dependent on stromal cell line ST2 and IL-5 by using Whitlock-Witte type bone marrow culture system. Surprisingly, all 18 clones we established were Ly-1$^+$, while early B lineage cells established in the absence of IL-5 at the same time are rarely Ly-1$^+$ (26, 50). We also presented evidence that bone marrow cells from 3-wk-old mice contains Ly-1$^+$ B cell precursors and these cells could be selectively maintained in the presence of IL-5 and bone marrow–derived stromal cells (50). By culturing bone marrow cells either from 8- or 3-wk-old IL-5 transgenic mice, we found the generation of IL-5-responsive Ly-1$^+$ cells even in the absence of IL-5, suggesting that Ly-1$^+$ B cells may well be maintained in bone marrow of adult IL-5 transgenic mice. However, we could not observe the fully mature Ly-1$^+$ B cells in this in vitro culture system, although they become pre-B cells expressing 2.7-kb membrane type μ mRNA (50). This IL-5 transgenic mouse confirmed the involvement of IL-5 in the development of Ly1$^+$ B cells in vivo.

The findings described in this study indicate the essential role of deregulated production of IL-5 in eosinophilia and preferential growth of Ly-1$^+$ B cells that resulted in the increase in total numbers of Ly-1$^+$ B cells in the spleen. IL-5 is probably involved in the production of polyreactive IgM antibodies in IL-5 transgenic mice, because similar polyreactive IgM autoantibodies containing anti-DNA activity could be elicited by the intraperitoneal injection of 1 μg pure IL-5 (data not shown). However, we do not have direct evidence indicating polyreactive anti-DNA antibodies are produced by Ly-1$^+$ B cells. It is very unlikely that polyreactive anti-DNA antibodies are produced only by Ly-1$^+$ B cells, because there are significant amounts B cells that do not express Ly-1 antigen but express IL-5 receptors.

The fact that those polyreactive anti-DNA antibodies can be recovered from a DNA Sepharose column with 0.5 M NaCl at neutral pH suggests that the antigenic determinant recognized by these autoantibodies is the conformational structure (43). This trait of anti-DNA antibodies is shared with the naturally occurring anti-DNA antibodies in patients with SLE or in lupus model mice (43).

The relation of these polyreactive autoantibodies to autoimmunity is unknown. It is suggested that the class switch of these autoantibodies from IgM to IgG may cause lupus (57). We introduced our IL-5 transgene into mice having genetic backgrounds that facilitate the progression of autoimmunity, such as MRL/lpr or NZW by mating, but so far, no such class switch was observed. Continuous expression of IL-5 might be rather inhibitory for the outbreak of the disease. It might be expressed transiently before the onset of lupus. We are currently trying to induce IL-5 transgenic mice to make a class switch from IgM to IgG.

In general, these polyreactive autoantibodies are known as naturally occurring antibodies in normal serum and are believed to be engaged in the primary protection against infection of microorganisms (27, 28, 53, 58). On the other hand, eosinophils are also believed to be involved in the protection against parasites, and IL-5 is known to be induced when mice are infected with parasites (25, 59). Taken together, IL-5 seems to engage in the primary defense mechanism against the infection of parasites and microorganisms. We are currently investigating if these mice are really resistant against the infection of microorganisms.

We are grateful to Drs. M. Sugimura, T. Nishihara, and T. Honjo for providing pKCR-mTRF plasmid. We are also grateful Drs. K. Onoue, S.-I. Nishikawa, T. Kambara, T. Yamamoto, M. Hirashima, J. Tsuruta, E. Sonoda, and N. Yamaguchi (Kumamoto University Medical School) for the continuous encouragement and valuable suggestions throughout this work. We appreciate Drs. T. Iwanaga, K. Araki, and T. Inomoto for their excellent support for preparing transgenic mice. Ms. Akiko Ohnishi is also acknowledged for her infinite secretarial assistance.

This work was supported in part by a Grant-in-Aid for Cancer Research and for Scientific Research from the Japanese Ministry of Education, Science, and Culture; by the Research Foundation for Pharmaceutical Sciences; by a Grant-in-Aid from the Mochida Memorial Foundation for Medical and Pharmaceutical Research; and by the grant provided by the Ichiro Kanehara Foundation.

Address correspondence to Akira Tominaga, Department of Biology, Institute for Medical Immunology, Kumamoto University Medical School, 2-2-1 Honjo, Kumamoto 860, Japan.

Received for publication 14 August 1990 and in revised form 24 October 1990.

References

1. Schimpl, A., and E. Wecker. 1972. Replacement of T-cell function by a T-cell product. *Nature (New Biol.).* 237:15.
2. Takatsu, K., A. Tominaga, N. Harada, S. Mita, M. Matsumoto, Y. Kikuchi, T. Takahashi, and N. Yamaguchi. 1988. T cell-replacing factor (TRF)/interleukin 5 (IL-5): molecular and functional properties. *Immunol. Rev.* 102:107.
3. Takatsu, K., K. Tanaka, A. Tominaga, Y. Kumahara, and T. Hamaoka. 1980. Antigen-induced T cell-replacing factor (TRF). III. Establishment of T cell hybrid clone continuously producing TRF and functional analysis of released TRF. *J. Immunol.* 125:2646.
4. Swain, S.L. 1985. Role of BCGFII in the differentiation to antibody secretion of normal and tumor B cells. *J. Immunol.* 134:4015.
5. Sanderson, C.J., H.D. Campbell, and I.G. Young. 1988. Molecular and cellular biology of eosinophils differentiation factor (IL-5) and its effect on human and mouse B cells. *Immunol. Rev.* 102:29.
6. Basten, A., and P.B. Beeson. 1970. Mechanism of eosinophilia. II. Role of the lymphocyte. *J. Exp. Med.* 131:1288.
7. Kinashi, T., N. Harada, E. Severinson, T. Tanabe, P. Sideras, M. Konishi, C. Azuma, A. Tominaga, S. Bergstedt-Lindqvist, M. Takahashi, F. Matsuda, Y. Yaoita, K. Takatsu, and T. Honjo. 1986. Cloning of complementary DNA encoding for T cell-replacing factor and identity with B cell-growth factor II. *Nature (Lond.).* 324:70.
8. Azuma, C., T. Tanabe, M. Konishi, T. Kinashi, T. Noma, F. Matsuda, Y. Yaoita, K. Takatsu, L. Hammarstrom, C.I.E. Smith, E. Severinson, and T. Honjo. 1986. Cloning of cDNA for human T-cell-replacing factor (interleukin-5) and comparison with the murine homologue. *Nucleic Acids Res.* 14:9149.
9. Yokota, T., R.L. Coffman, H. Hagiwara, D.M. Rennick, Y. Takebe, K. Yokota, H. Spits, J. de Vries, J. Banchereau, F.D. Lee, N. Arai, and K. Arai. 1987. Isolation and characterization of lymphokine cDNA clones encoding mouse and human IgA enhancing factor and eosinophil colony stimulating factor activities: relation to IL-5. *Proc. Natl. Acad. Sci. USA.* 84:7388.
10. Harada, N., T. Takahashi, M. Matsumoto, T. Kinashi, J. Ohara, Y. Kikuchi, N. Koyama, E. Severinson, Y. Yaoita, T. Honjo, N. Yamaguchi, A. Tominaga, and K. Takatsu. 1987. Production of a monoclonal antibody useful in the molecular characterization of murine T-cell-replacing factor/B cell growth factor II. *Proc. Natl. Acad. Sci. USA.* 84:4581.
11. Matsumoto, M., A. Tominaga, N. Harada, and K. Takatsu. 1987. Role of T cell replacing factor (TRF) in the murine B cell differentiation: induction of increased levels of expression of secreted type IgM mRNA. *J. Immunol.* 138:1826.
12. Sonoda, E., R. Matsumoto, Y. Hitoshi, T. Ishii, M. Sugimoto, S. Araki, A. Tominaga, N. Yamaguchi, and K. Takatsu. 1989. Transforming growth factor induces IgA production and acts additively with interleukin 5 for IgA production. *J. Exp. Med.* 170:1415.
13. Swain, S.L., D.D. McKenzie, R.W. Dutton, S.L. Tonkonogy, and M. English. 1988. The role of IL-4 and IL-5: characterization of a distinct helper T cell subset that makes IL-4 and IL-5 (Th_2) and requires priming before induction of lymphokine secretion. *Immunol. Rev.* 102:77.
14. Harada, N., Y. Kikuchi, A. Tominaga, S. Takaki, and K. Takatsu. 1985. BCGFII activity on activated B cells of a purified murine T cell-replacing factor (TRF) from a T cell hybridoma (B151K12). *J. Immunol.* 134:3944.
15. Karasuyama, H., A. Rolink, and F. Melchers. 1988. Recombinant interleukin 2 or 5, but not 3 or 4, induces maturation of resting mouse B lymphocytes and propagates proliferation of activated B cell blasts. *J. Exp. Med.* 167:1377.
16. Harada, N., M. Matsumoto, N. Koyama, A. Shimizu, T. Honjo, A. Tominaga, and K. Takatsu. 1987. T cell-replacing factor/IL-5 induces not only B-cell growth and differentiation, but also increased expression of IL-2 receptor on activated B cells. *Immunol. Lett.* 15:205.
17. Loughnan, M.S., K. Takatsu, N. Harada, and G.J.V. Nossal. 1987. T cell-replacing factor (IL-5) induces expression of IL-2-R on murine B cells. *Proc. Natl. Acad. Sci. USA.* 84:5399.
18. Nakanishi, K., T. Yoshimoto, Y. Katoh, S. Ono, K. Matsui, K. Hiroishi, T. Noma, T. Honjo, K. Takatsu, K. Higashino, and T. Hamaoka. 1988. Both B151-TRF1 and interleukin 5 regulate immunoglobulin secretion and IL-2 receptor expression on a cloned B lymphoma line. *J. Immunol.* 140:1168.
19. Yamaguchi, Y., T. Suda, M. Eguchi, Y. Miura, N. Harada, A. Tominaga, and K. Takatsu. 1988. Purified interleukin 5 (IL-5) supports the terminal differentiation and of murine eosinophilic precursors. *J. Exp. Med.* 163:46.
20. Yamaguchi, Y., Y. Hayashi, Y. Sugama, Y. Miura, T. Kasahara, S. Kitamura, M. Torisu, S. Mita, A. Tominaga, K. Takatsu, and T. Suda. 1988. Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs in vitro survival: IL-5 as an eosinophil chemotactic factor. *J. Exp. Med.* 167:1737.
21. Rasmussen, R., K. Takatsu, N. Harada, T. Takahashi, and K. Bottomly. 1988. T cell-dependent hapten-specific and polyclonal B cell responses require release of interleukin 5. *J. Immunol.* 140:705.
22. Boom, W.H., D. Liano, and A. Abbas. 1988. Heterogeneity of helper/inducer T lymphocytes. II. Effects of interleukin 4- and interleukin 2-producing T cell clones on resting B lymphocytes. *J. Exp. Med.* 167:1350.
23. Tominaga, A., T. Takahashi, Y. Kikuchi, S. Mita, S. Naomi, N. Harada, N. Yamaguchi, and K. Takatsu. 1990. Role of carbohydrate moiety of IL-5: effect of tunicamycin on the glycosylation of IL-5 and the biologic activity of deglycosylated IL-5. *J. Immunol.* 144:1345.
24. Tominaga, A., M. Matsumoto, N. Harada, T. Takahashi, Y. Kikuchi, and K. Takatsu. 1988. Molecular properties and regulation of mRNA expression for murine T cell-replacing factor (TRF)/IL-5. *J. Immunol.* 140:1175.
25. Yamaguchi, Y., T. Matsui, T. Kasahara, S. Etoh, A. Tominaga, K. Takatsu, Y. Miura, and T. Suda. 1990. *In vitro* changes of hematopoietic progenitors and the expression of interleukin 5 (IL-5) gene in eosinophilic mice infected with *Toxocara canis*. *Exp. Hematol.* In press.
26. Tominaga, A., S.-I. Nishikawa, S. Mita, M. Ogawa, Y. Hitoshi, Y. Kikuchi, and K. Takatsu. 1989. Establishment of IL-5 dependent early B cell lines from bone marrow cells by long-term bone marrow culture. *Growth Factors.* 1:135.
27. Herzenberg, L.A., A.M. Stall, P.L. Lalor, C. Sidman, W.A. Moore, D.R. Parks, and L.A. Herzenberg. 1986. The Ly-1 B cell lineage. *Immunol. Rev.* 93:81.
28. Hayakawa, K., and R. Hardy. 1988. Normal, autoimmune, and malignant $CD5^+$ B cells: the Ly-1 B lineage. *Annu. Rev. Immunol.* 6:197.
29. Law, M.-F., J.C. Byrne, and P.M. Howley. 1983. A stable papillomavirus hybrid plasmid that expresses a dominant selective trait. *Mol. Cell. Biol.* 3:2110.
30. Yamamura, K., H. Kikutani, N. Takahashi, T. Taga, S. Akira,

K. Kawai, K. Fukuchi, Y. Kumahara, T. Honjo, and T. Kishimoto. 1984. Production of transgenic mice integrated human IgG_1 gene. *J. Biochem. (Tokyo).* 96:357.
31. Davis, L.G., M.D. Dibner, and J.F. Battey. 1986. Preparation of DNA from eukaryotic cells. *In* Basic Methods in Molecular Biology. Elsevier, Amsterdam. 42–46.
32. Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503.
33. Chirgwin, J.M., A.E. Pryzbyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5249.
34. Lehrach, H., D. Diamond, J.M. Wozney, and H. Boedtker. 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical re-examination. *Biochemistry.* 16:4743.
35. Mita, S., N. Harada, S. Naomi, Y. Hitoshi, K. Sakamoto, M. Akagi, A. Tominaga, and K. Takatsu. 1988. Receptors for T cell-replacing factor (TRF)/interleukin 5 (IL-5). Quantitation, specificity, and its implications. *J. Exp. Med.* 168:863.
36. Mita, S., A. Tominaga, Y. Hitoshi, K. Sakamoto, T. Honjo, M. Akagi, N. Yamaguchi, and K. Takatsu. 1989. Characterization of high-affinity receptors for interleukin 5 (IL-5) on IL-5 dependent cell lines. *Proc. Natl. Acad. Sci. USA.* 86:2311.
37. Takatsu, K., N. Yamaguchi, Y. Hitoshi, E. Sonoda, S. Mita, and A. Tominaga. 1990. Signal transduction through interleukin 5 receptors. *Cold Spring Harbor Symp. Quant. Biol.* 54:745.
38. Yamaguchi, N., Y. Hitoshi, S. Mita, Y. Hosoya, Y. Murata, Y. Kukuchi, A. Tominaga, and K. Takatsu. 1990. Characterization of the murine interleukin 5 receptor by using a monoclonal antibody. *Int. Immunol.* 2:181.
39. Hitoshi, Y., N. Yamaguchi, S. Mita, E. Sonoda, S. Takaki, A. Tominaga, and K. Takatsu. 1990. Distribution of IL-5 receptor-positive B cells: expression of IL-5 receptor on Ly-1 $(CD5)^+$ B cells. *J. Immunol.* 144:4218.
40. Kanai, Y., and M. Fujiwara. 1985. Naturally occurring antibodies to poly(ADP-ribose) in autoimmune MRL/Mp-lpr/lpr mice. *Clin. Exp. Immunol.* 59:132.
41. Stollar, B.D., and M. Papalian. 1980. Secondary structure of denatured DNA is responsible for its reaction with anti-native DNA antibodies of systemic lupus erythematosus sera. *J. Clin. Invest.* 66:210.
42. Isonishi, S., and Y. Kanai. 1988. Antibody to poly(ADP-ribose) as a predictor of obstetric complications in autoimmune MRL/Mp-lpr/lpr mice: basis for its application to pregnant patients with systemic lupus erythematosus. *Immunol. Lett.* 18:61.
43. Kanai, Y., and T. Kubota. 1989. A novel trait of naturally occurring anti-DNA antibodies: dissociation from immune complexes in neutral 0.3-0.5 M NaCl. *Immunol. Lett.* 22:293.
44. O'Hare, K., C. Benoist, and R. Breathnach. 1981. Transformation of mouse fibroblasts to methotrexate resistance by a recombinant plasmid expressing a prokaryotic dihydrofolate reductase. *Proc. Natl. Acad. Sci. USA.* 78:1527.
45. Hayakawa, K., R.R. Hardy, M. Honda, L.A. Herzenberg, A.D. Steinberg, and L.A. Herzenberg. 1984. Ly-1 B cells: functionally distinct lymphocytes that secrete IgM autoantibodies. *Proc. Natl. Acad. Sci. USA.* 81:2494.
46. Hayakawa, K., R.R. Hardy, and L.R. Herzenberg. 1986. Peritoneal Ly-1 B cells: genetic control, autoantibody, increased lambda light chain expression. *Eur. J. Immunol.* 16:450.
47. Wetzel, G.D. 1989. Interleukin 5 regulation of peritoneal Ly-1 B lymphocyte proliferation, differentiation and autoantibody secretion. *Eur. J. Immunol.* 19:1701.
48. Moutsopoulos, H.M., B.L. Webber, N.A. Pavlidis, G. Fostiropoulos, D. Goules, and L.E. Shulman. 1980. Diffuse fasciitis with eosinophilia; a clinicopathologic study. *Am. J. Med.* 68:701.
49. Spry, C.J.F. 1988. Eosinophils. Oxford University Press, Oxford. 484 pp.
50. Katoh, S., A. Tominaga, M. Migita, A. Kudo, and K. Takatsu. 1990. Conversion of normal Ly-1-positive B lineage cells into Ly-1-positive macrophages in long-term bone marrow cultures. *Dev. Immunol.* In press.
51. Hardy, R.R., and K. Hayakawa. 1986. Development and physiology of Ly-1 B and its human homolog, Leu-1 B. *Immunol. Rev.* 93:53.
52. Rajewsky, K., I. Forster, and A. Cumano. 1987. Evolutionary and somatic selection of the antibody repertoire in the mouse. *Science (Wash. DC).* 238:1088.
53. Forster, I., and K. Rajewsky. 1987. Expansion and functional activity of $Ly\text{-}1^+$ B cells upon transfer of peritoneal cells into allotype-congenic, newborn mice. *Eur. J. Immunol.* 17:521.
54. Hayakawa, K., R.R. Hardy, A. Stall, L.A. Herzenberg, and L.A. Herzenberg. 1986. Immunoglobulin-bearing B cells reconstitute and maintain the murine Ly-1 B cell lineage. *Eur. J. Immunol.* 16:1313.
55. Hayakawa, K., R.R. Hardy, L.A. Herzenberg, and L.A. Herzenberg. 1985. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. *J. Exp. Med.* 161:1554.
56. Pennell, C.A., K.M. Sheehan, P.H. Brodeur, and S.H. Clarke. 1989. Organization and expression of V_H gene families preferentially expressed by $Ly\text{-}1^+$ (CD5) B cells. *Eur. J. Immunol.* 19:2115.
57. Shirai, T., S. Hirose, T. Okada, and H. Nishimura. 1990. $CD5^+$ B cells in autoimmune disease and lymphoid malignancy. *Cli. Immunol. Immunopathol.* In press.
58. Casali, P., and L. Notkins. 1989. Probing the human B-cell repertoire with EBV: polyreactive antibodies and $CD5^+$ B lymphocytes. *Annu. Rev. Immunol.* 7:513.
59. Coffman, R.L., B.W.P. Seymour, S. Hudak, J. Jackson, and D. Rennick. 1989. Antibody to interleukin-5 inhibits Helminth-induced eosinophilia in mice. *Science (Wash. DC).* 245: 308.