Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 7547–7551, October 1989
Immunology

IgG1 plasmacytosis in interleukin 6 transgenic mice

(B-cell stimulatory factor 2/plasmacytoma growth factor/mesangio-proliferative glomerulonephritis/megakaryocytopoiesis)

SACHIKO SUEMATSU*, TADASHI MATSUDA*, KATSUYUKI AOZASA†, SHIZUO AKIRA*, NAOKO NAKANO*, SHINSUKE OHNO‡, JUN-ICHI MIYAZAKI§, KEN-ICHI YAMAMURA§, TOSHIO HIRANO*, AND TADAMITSU KISHIMOTO*¶

*Division of Immunology, Institute for Molecular and Cellular Biology, Osaka University, 1-3, Yamada-oka, Suita, Osaka 565; †Department of Pathology, Nara Medical University, Kashiwara, Nara 634; ‡Department of Molecular Immunology, Cancer Research Institute, Kanazawa University, Kanazawa 920; and §Institute for Medical Genetics, Kumamoto University Medical School, Kumamoto 862, Japan

Communicated by M. Potter, June 23, 1989 (received for review April 14, 1989)

ABSTRACT **Interleukin 6 (IL-6) has been suggested to be involved in the pathogenesis of polyclonal and monoclonal plasma cell abnormalities. To address this possibility, transgenic mice carrying the human IL-6 genomic gene fused with a human immunoglobulin heavy chain enhancer were generated. High concentrations of human IL-6 and polyclonal increase in IgG1 (120- to 400-fold) in sera of all transgenic mice were observed. A massive plasmacytosis in thymus, lymph node, and spleen and an infiltration of plasma cells in lung, liver, and kidney were observed. However, the plasma cells were not transplantable to syngeneic mice and were found not to contain chromosomal aberrations including c-*myc* gene rearrangements. The evidence indicates that deregulated gene expression of IL-6 can trigger polyclonal plasmacytosis but cannot induce plasmacytoma. It is suggested that additional genetic changes may be required for the generation of plasma cell neoplasia. Other interesting findings in these transgenic mice were the development of mesangio-proliferative glomerulonephritis and an increase in megakaryocytes in bone marrow.**

Interleukin 6 (IL-6) is a cytokine that induces the terminal differentiation of B cells to plasma cells. Furthermore, it acts on a variety of cells including T cells, hepatocytes, hematopoietic stem cells, nerve cells, and mesangial cells (1, 2). Moreover, it has been suggested that deregulated IL-6 production may cause hypergammaglobulinemia in patients with cardiac myxoma (3). Additional evidence has accumulated suggesting that deregulated gene expression of IL-6 is involved in the pathogenesis of polyclonal and/or monoclonal plasma cell abnormalities (1, 2). Deregulated IL-6 production was observed in rheumatoid arthritis (4) and Castleman's disease (5) in which polyclonal plasmacytosis is observed. Furthermore, IL-6 was found to be an autocrine growth factor for human myeloma cells (6).

Potter and Boyce (7) demonstrated that intraperitoneal injection of paraffin oils could induce the generation of plasmacytomas in BALB/cAnPt mice. Plasmacytomas were demonstrated to develop exclusively from cells in oil-induced granulomatous tissues, which were found to produce elevated concentrations of plasmacytoma growth factor(s), suggesting the importance of such growth factor(s) for the generation of plasmacytomas (8). The mouse plasmacytoma growth factor was purified (9, 10) and its cDNA was cloned (11). The results showed that mouse plasmacytoma growth factor is the murine homologue of human IL-6 (2, 12).

The above evidence suggests that deregulated gene expression of IL-6 may be involved in polyclonal plasmacytosis and generation of plasma cell neoplasia. To test this possibility, transgenic mice were produced by introducing the human IL-6 genomic gene fused with the human immunoglobulin heavy chain enhancer (Eμ). A massive plasmacytosis was observed in the resulting transgenic mice.

MATERIALS AND METHODS

Construction of the Eμ–IL-6 Gene and Production of Transgenic Mice. The 6.6-kilobase (kb) *Bam*HI–*Pvu* II fragment encompassing the human IL-6 gene (13) was recloned into *Bam*HI–*Pvu* II sites of pGEM4 vector (pGEM-IL6). The 2.2-kb *Xba* I fragment containing Eμ (14) was inserted into the *Sma* I site (1.3 kb upstream from the most 3′ transcription initiation site of IL-6 gene) of pGEM-IL6 by blunt-end ligation (pGEM-Eμ-IL6) and the *Pvu* II site was changed to a *Sac* I site. The insert containing the Eμ–IL-6 gene was recovered from pGEM-Eμ-IL6 by a single *Sac* I digestion. The schematic construction of the Eμ–IL-6 gene is shown in Fig. 1. Transgenic mice were produced as described (15) utilizing C57BL/6 mice (Nippon Clea, Osaka).

SDS/PAGE and Immunodiffusion Analysis of Serum Immunoglobulin. Murine serum (0.5 μl) was electrophoresed through a 4–20% gradient SDS/polyacrylamide slab gel (Daiichi Pure Chemical, Tokyo) under nonreducing conditions and the gel was stained with Coomassie brilliant blue R250. Molecular mass marker standards used were lysozyme (14.3 kDa), ovalbumin (43 kDa), bovine serum albumin (68 kDa), phosphorylase *b* (97.4 kDa), and myosin heavy chain (200 kDa). Agar gel immunodiffusion against isotype-specific immune sera was performed utilizing mouse monoclonal typing kits (Serotec, Oxford).

Isotype-Specific ELISA. ELISA was performed utilizing goat anti-mouse immunoglobulin (Cappel Laboratories) and alkaline phosphatase-conjugated affinity-purified rabbit anti-mouse immunoglobulin antibodies specific for each isotype (Zymed Laboratories) as described (16). Myeloma proteins of the appropriate subclasses were used as standards (IgG1, α BSF2-166; IgG2b, α BSF2-77; IgM, α BSF2-60 (16); IgA, IgG2a, IgG3 were obtained from Organon Teknika, Durham, NC).

Determination of IL-6 Activity. IL-6 activity was measured utilizing the IL-6-dependent murine hybridoma cell line MH60.BSF2 as described (16).

Northern Blot Analysis. Total RNA was isolated by the guanidine isothiocyanate method and subjected to Northern blot analysis utilizing the ^{32}P-labeled *Taq* I–*Ban* II fragment of pBSF2.38 cDNA as described (12).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IL-6, interleukin 6; Eμ, immunoglobulin heavy chain enhancer; FACS, fluorescence-activated cell sorter.

¶To whom reprint requests should be addressed.

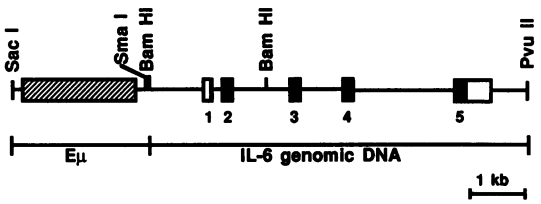

FIG. 1. Construct of the human Eμ–IL-6 gene.

Southern Blot Analysis. High molecular weight DNA was prepared and subjected to Southern blot analysis by using as probe a ^{32}P-labeled 3.8-kb *Xba* I fragment including sequences for heavy chain joining regions 1–4 (J_H1 to J_H4) (17) as described (14).

Fluorescence-Activated Cell Sorter (FACS) Analysis. One million lymph node cells were stained with fluorescein-conjugated rat monoclonal antibody RA3-6B2 (anti-B220) and phycocyanin-conjugated rat monoclonal antibody 30H12 (anti-Thy1.2) or phycocyanin-conjugated anti-B220 plus fluorescein-conjugated rat monoclonal antibody 331.12 (anti-IgM) and analyzed by a dual-laser FACS 440 (Becton Dickinson) equipped with an argon ion laser and a dye laser (18). Dead cells were excluded from the analysis by adding propidium iodide.

RESULTS

Production of IL-6 Transgenic Mice. C57BL/6 transgenic mice carrying the human IL-6 gene were produced by introducing the human genomic IL-6 gene fused with Eμ. Seven founders carrying this transgene were produced. Among these, three founder mice (mice 13, 16, and 24) are described in this report. As judged from hybridizing intensity of bands, one (mouse 13), ≈5 (mouse 16), and ≈20 (mouse 24) copies of the transgene were present in these mice.

Expression of Human IL-6 Gene in Transgenic Mice. To examine whether the human IL-6 gene was expressed in these transgenic mice, the serum IL-6 activity was determined utilizing the IL-6-dependent mouse hybridoma cell line MH60.BSF2 (16). The serum IL-6 activities at 18 weeks (mouse 13), 7 weeks (mouse 16), 7 weeks (mouse 24), and 10 weeks old (mouse 24) were found to be equivalent to the activities exerted by human recombinant IL-6 at 0.8 ng/ml, 20 ng/ml, 1.3 ng/ml, and 1.9 ng/ml, respectively. The serum IL-6 activity in normal mice was less than 0.1 ng/ml. The serum IL-6 activity in these transgenic mice was neutralized by goat anti-human IL-6 antibodies but not by rabbit anti-mouse IL-6 antibodies. Furthermore, mRNA with the expected length (≈1.4 kb) hybridizable with human IL-6 cDNA (12) was detected in RNA preparations isolated from either spleen or lymph node cells of mice 13 and 16 (Fig. 2). The data indicated that the human IL-6 gene was expressed in lymphoid tissues and biologically active human IL-6 was secreted into sera of transgenic mice.

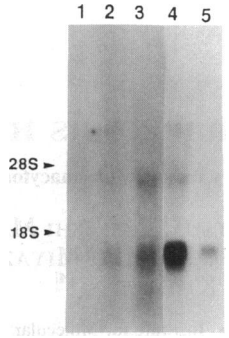

FIG. 2. Expression of human IL-6 mRNA in transgenic mice. The total RNAs (5 μg per lane) obtained from mouse 13 spleen (lane 4), mouse 13 lymph node (lane 5), mouse 24 spleen (lane 2), mouse 24 lymph node (lane 3), and normal C57BL/6 mouse spleen (lane 1) were subjected to Northern blot analysis utilizing the *Taq* I–*Ban* II fragment of pBSF2.38 cDNA as a probe as described (12).

Increase in Serum Immunoglobulin of IL-6 Transgenic Mice. The effects of deregulated gene expression of IL-6 on the serum immunoglobulin levels were investigated. First, the profile of serum proteins in these transgenic mice was examined by SDS/PAGE. As shown in Fig. 3*a*, mouse 24 showed an increase in the immunoglobulin fraction (corresponding to a molecular mass of ≈150 kDa) and a reciprocal decrease in the albumin fraction as it aged. A similar increase

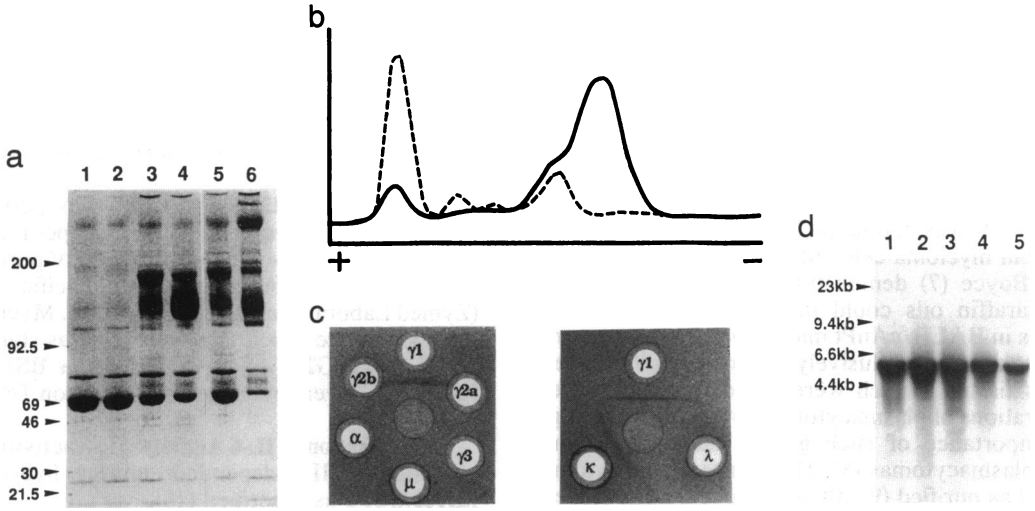

FIG. 3. (*a*) SDS/PAGE analysis of sera obtained from IL-6 transgenic mice. Sera were obtained from IL-6 transgenic mice 13, 16, and 24 or a control littermate, mouse 22, which does not contain IL-6 transgene, and subjected to SDS/PAGE under nonreducing conditions. Lanes: 1, mouse 22 at 7 weeks old; 2, mouse 24 at 4 weeks old; 3, mouse 24 at 7 weeks old; 4, mouse 24 at 10 weeks old; 5, mouse 13 at 7 weeks old; 6, mouse 16 at 7 weeks old. Molecular masses in kDa are indicated. (*b*) Densitometric profiles of cellulose acetate electrophoresis of serum obtained from either mouse 24 at 10 weeks old (solid line) or an age-matched normal control mouse (dashed line). (*c*) Sera diluted 1:1000, obtained from transgenic mice, were subjected to Ouchterlony gel diffusion analysis for immunoglobulin typing. Representative data on mouse 24 are shown. (*d*) DNA (5 μg) obtained from mouse 24 spleen (lane 2), mouse 24 lymph node (lane 3), mouse 13 spleen (lane 4), mouse 13 lymph node (lane 5), and normal C57BL/6 mouse spleen (lane 1) was digested with *Eco*RI and subjected to Southern blot analysis utilizing mouse J_H4 probe (17).

Table 1. Concentrations of serum immunoglobulins of transgenic mice

Mouse	Immunoglobulin, mg/ml					
	IgM	IgA	IgG1	IgG2a	IgG2b	IgG3
Normal C57BL/6 (18 weeks old)	0.76	1.2	0.61	0.24	1.7	0.22
Nontransgenic 22 (10 weeks old)	0.24	0.72	0.60	0.11	0.50	0.17
Transgenic						
13 (18 weeks old)	0.45	0.49	170.0	0.12	2.2	0.42
16 (7 weeks old)	0.56	1.1	75.0	0.40	0.42	0.32
24 (10 weeks old)	2.0	1.3	240.0	0.38	2.9	0.24

Mouse 22 is the nontransgenic mouse that was from the same litter as transgenic mouse 24.

in immunoglobulin fraction was also observed in the transgenic mice 13 and 16. An increase of immunoglobulin fraction without a monoclonal spike was demonstrated by cellulose acetate electrophoresis in all transgenic mice (Fig. 3*b*, representative data on mouse 24). The increased immunoglobulin was limited mainly to IgG1 (γ1, κ) (Fig. 3*c* shows the data on mouse 24). The concentrations of immunoglobulin classes or subclasses were determined by an ELISA. As shown in Table 1, the increase in IgG1 was 120- to 400-fold as compared to those of age-matched control normal mice. However, the other classes of immunoglobulins (IgM, IgG2a, IgG2b, IgG3, and IgA) were found to remain within three times of the immunoglobulin concentrations in normal mice. Essentially the same increase in IgG1 was observed in the other four transgenic mice (data not shown). No apparent oligoclonal or monoclonal rearrangements of immunoglobulin bands were observed by Southern blot analysis utilizing a heavy chain joining region (J_H) probe in the *Eco*RI-digested DNAs obtained from either lymph node or spleen of mice 13 and 24. As shown in Fig. 3*d*, only the germ-line-sized 6.2-kb band was observed. These findings indicated that IgG1 increased polyclonally in these transgenic mice.

Pathological Findings in Tissues of IL-6 Transgenic Mice. To examine the pathological changes in the IL-6 transgenic mice, six transgenic mice were sacrificed at 7–18 weeks of age. The other transgenic mouse (mouse 16), which was dead at 7 weeks old, was also subjected to pathological examination. All mice were found to have splenomegaly and lymph node enlargement. The size of some lymph nodes obtained from the peritoneal cavity was ≈1 cm in diameter. The histological findings were essentially the same among all seven transgenic mice and representative data on mouse 24 are shown in Fig. 4. Lymph nodes were completely replaced by compactly arranged plasma cells characterized by an eccentrically situated nucleus with prominent speckles of chromatin and cytoplasm with a marked peripheral basophilia (Fig. 4*a*). The proliferating plasma cells comprised mature and rather immature forms with mitoses. In the thymus, a massive proliferation of plasma cells was observed (data not shown). In the spleen, a massive proliferation of plasma cells was found frequently preserving the white pulp (data not shown). Fig. 4*b* shows immunofluorescent staining of cytoplasmic immunoglobulin of spleen cells, demonstrating the presence of plasma cells producing immunoglobulin. Furthermore, infiltration of plasma cells was found in the alveolar septa of the lung, the portal region of the liver (data not shown), and the

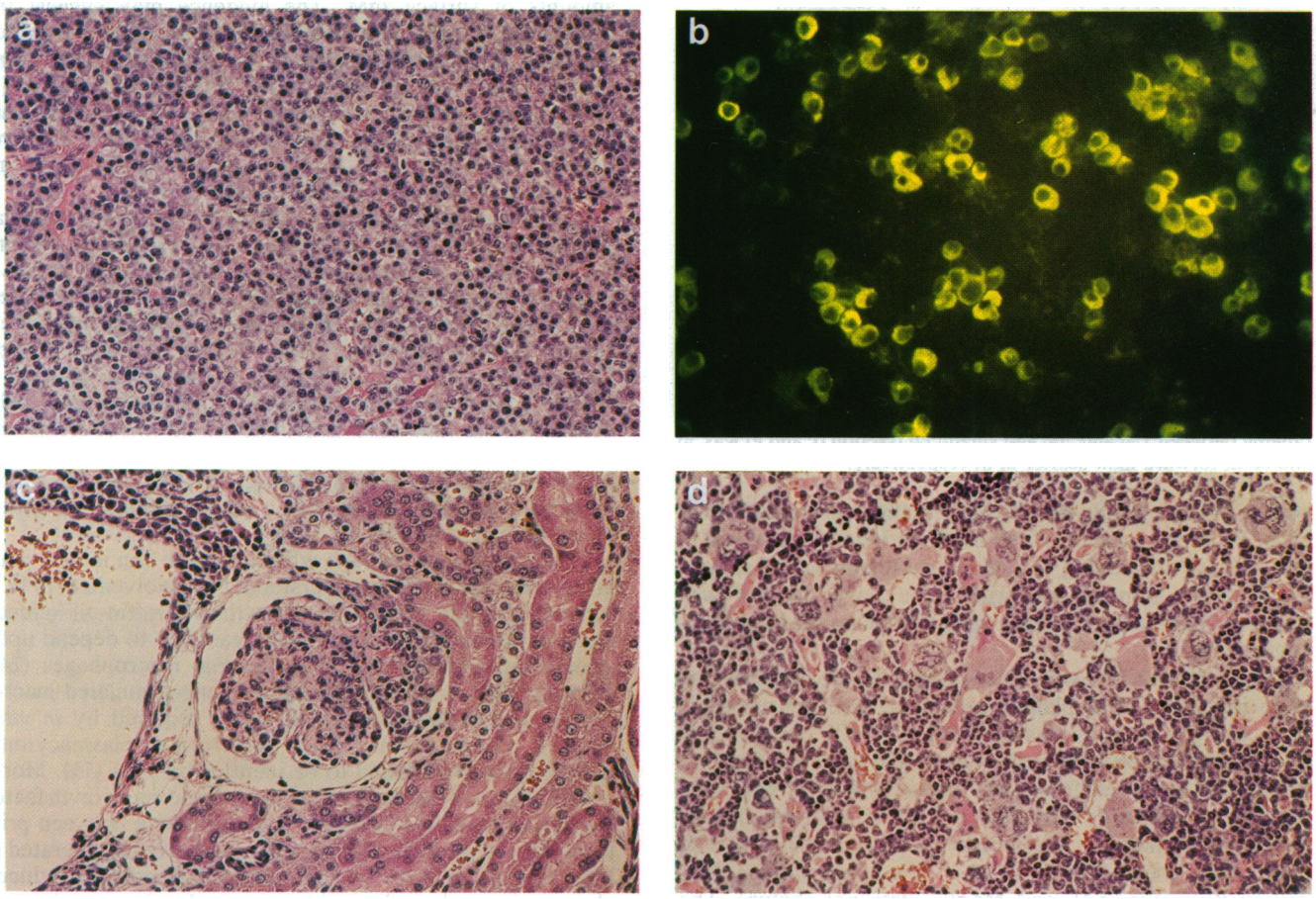

FIG. 4. Pathological findings of transgenic mouse 24. Hematoxylin and eosin staining of lymph node (×310) (*a*), kidney (×310) (*c*), and bone marrow (×310) (*d*). (*b*) Cytoplasmic immunoglobulin of spleen cells was stained with fluorescein-conjugated $F(ab')_2$ fragment of rabbit anti-mouse immunoglobulin (×1000).

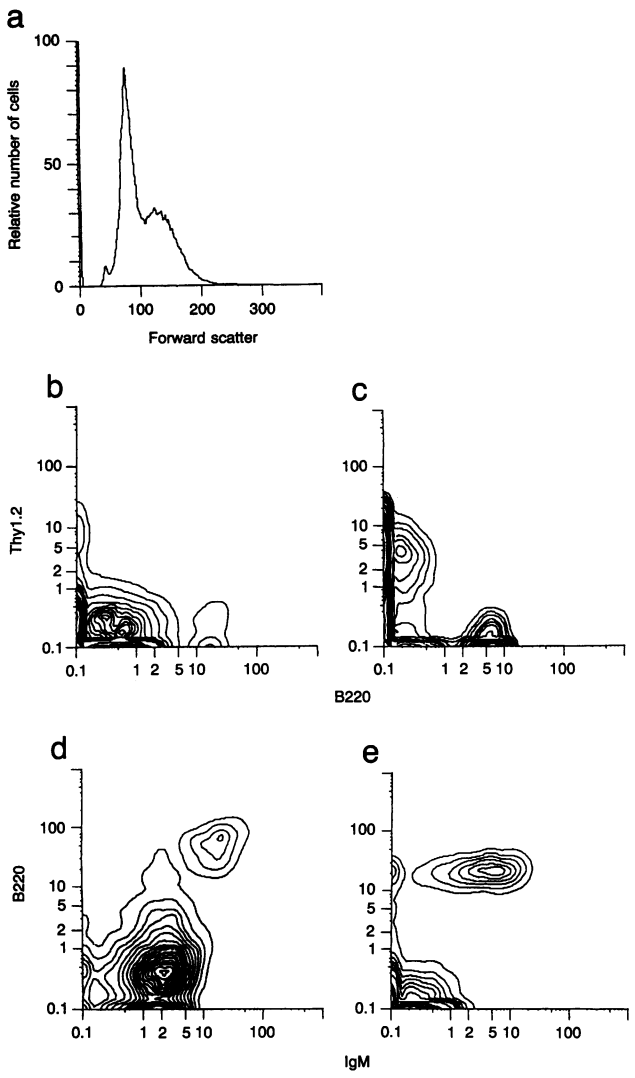

FIG. 5. FACS analysis of lymph node cells. Lymph node cells of transgenic mouse 24 were stained with fluorescein-conjugated anti-B220 and phycocyanin-conjugated anti-Thy1.2 (*b* and *c*) or phycocyananin-conjugated anti-B220 and fluorescein-conjugated anti-IgM (*d* and *e*) and analyzed by a dual-laser FACS 440. The *x* and *y* axes represent forward light scatter and relative number of cells, respectively (*a*), and logarithmic intensities of green and red fluorescences, respectively (*b–e*). The large-cell fraction (*b* and *d*) was gated on cells ranging between 130 and 200 and small-cell fraction (*c* and *e*) was 50 and 80 in forward light scatter in *a*, respectively.

stroma of kidney (Fig. 4*c*). No abnormality was found in liver cells. Profound mesangial cell proliferation was observed in the kidney, displaying a typical pathology of mesangio-proliferative glomerulonephritis. The bone marrow was found to contain some plasma cells and increased megakaryocytes (Fig. 4*d*).

FACS Analysis of Lymph Node Cells. As the lymph nodes were infiltrated with plasma cells (Fig. 4*a*), the antigenic surface of plasma cells was analyzed by FACS. As shown in Fig. 5*a*, the lymph node cells were found to be divided into small- and large-cell fractions. About 40% of lymph node cells were large cells that were not found in normal lymph nodes, suggesting that these cells are plasma cells. Most of these cells were Thy1$^-$, B220$^-$, (Fig. 5*b*), surface IgM dull-positive (Fig. 5*d*), and Mac1$^-$ (data not shown). The staining of the large cells with anti-immunoglobulin showed a similar pattern to that obtained with anti-IgM, indicating that immunoglobulin expressed on the large cells was mainly IgM. The small-cell fraction was found to contain two types of cell populations, as observed in normal lymph nodes; Thy1$^+$, B220$^-$ cells and Thy1$^-$, B220$^+$ cells (Fig. 5*c*). The B220$^+$ cells comprise surface IgM$^+$ and IgM$^-$ cells (Fig. 5*e*).

DISCUSSION

The present study demonstrated the generation of a massive plasmacytosis in IL-6 transgenic mice in which the human IL-6 gene is constitutively expressed. This plasmacytosis is characterized as follows: (*i*) the presence of compactly arranged plasma cells with mitoses in spleen, lymph nodes, and thymus; (*ii*) infiltration of plasma cells in many organs including lung and kidney; and (*iii*) the presence of abnormal large cells that do not express B220, Thy1, and Mac1 in lymph nodes. The plasma cells generated were neither monoclonal nor transplantable to syngeneic mice. Therefore, the plasmacytosis generated in the IL-6 transgenic mice may be the consequence of a massive proliferation of normal plasma cells. Additionally, these plasma cells might represent a preneoplastic stage.

It has been shown that activated B cells expressing IL-6 receptor can respond to IL-6 *in vitro* (1, 2, 19). Therefore, it is very likely that IL-6 directly induced plasmacytosis in these transgenic mice by stimulating cell division and maturation of these B cells. It is possible that other cytokines induced by IL-6 could play a role in expanding the plasma cell population.

Although IgG1 was found to be the major polyclonally increased immunoglobulin heavy chain class, FACS analysis demonstrated the presence of an abnormally large number of cells considered to be plasma cells that expressed small amounts of surface IgM. The evidence may suggest the presence of plasma cells that are under a rapid class switch from μ to γ1. At present there has been no evidence indicating that IL-6 can directly induce immunoglobulin class switch (1, 2). Therefore, the mechanism(s) inducing such a possible class switch in IL-6 transgenic mice is unknown. IgG1 paraproteinemia was reported in SJL/J mice (20). It may be interesting to examine the IL-6 production in SJL/J mice.

Another interesting finding in the IL-6 transgenic mice was the development of mesangio-proliferative glomerulonephritis. This finding confirms the previous observations that IL-6 is a growth factor for mesangial cells and mesangio-proliferative glomerulonephritis may be caused by abnormal expression of IL-6 by mesangial cells (35). An increase in megakaryocytes in bone marrow of IL-6 transgenic mice is in complete agreement with the report by Ishibashi *et al.* (21), demonstrating that IL-6 functions as thrombopoietin and induces the maturation of megakaryocytes. Furthermore, serum albumin was decreased (Fig. 3*a*), in complete agreement with the fact that IL-6 is a major regulator of acute-phase protein synthesis (22, 23).

The mechanisms through which pristane can induce plasmacytomas are not fully understood, but involvement of IL-6 is highly possible. The *in vitro* growth of paraffin-oil-induced plasmacytomas was shown many years ago to depend upon growth factors provided by peritoneal macrophages (24). Subsequently, it was found that pristane-stimulated macrophages also produce a growth factor required by *in vitro* adapted plasmacytomas (8). Furthermore, plasmacytoma growth factor was found to be identical to IL-6 (11). Moreover, IL-6 was demonstrated to be an autocrine growth factor for human myeloma cells (6). One difference between pristane-induced plasmacytomas and plasmacytosis generated in IL-6 transgenic mice is that almost all pristane-induced plasmacytomas have been demonstrated to carry a chromosome translocation generated by recombination of the c-*myc* locus with the immunoglobulin heavy or light chain loci (25, 26), resulting in the deregulated expression of the c-*myc* gene.

However, plasma cells analyzed in IL-6 transgenic mice in this study were found not to contain apparent chromosomal translocations involving the c-*myc* gene (data not shown). Since pristane-induced plasmacytomas are generated in BALB/c or NZB but not in C57BL/6 mice, the results may simply reflect the difference in the genetic background (27).

Infection with Abelson murine leukemia virus or a retrovirus containing either avian v-*myc* or c-*myc*/v-Ha-*ras* was reported to rapidly induce plasmacytomas in pristane-primed but not normal BALB/c mice (28–30), indicating that pristane treatment is one of the essential factors for the development of plasmacytomas. Furthermore, deregulated expression of the c-*myc* gene may play a crucial role in plasmacytomagenesis: v-*myc* expression can accelerate the generation of plasmacytomas as plasmacytomas thus generated lacked the c-*myc* gene rearrangement. Thus v-*myc* expression can replace the requirement for the c-*myc* gene rearrangement in plasmacytomagenesis (29).

It has been demonstrated that there is a significant association in the occurrence of plasma cell neoplasias and preexisting chronic inflammations including rheumatoid arthritis (31). We have demonstrated (4) the deregulated IL-6 gene expression in rheumatoid arthritis. Furthermore, it was shown that there was a correlation between deregulated IL-6 production and polyclonal plasmacytosis in patients with cardiac myxoma (3) and Castleman's disease (5). Moreover, several cases of rheumatoid arthritis, cardiac myxoma, and Castleman's disease were reported to result in the generation of myeloma and/or plasmacytoma (32–34).

The findings in this study indicate the essential role of deregulated production of IL-6 in polyclonal plasmacytosis and suggest that pristane-induced generation of plasmacytoma might be directly associated with the continuous production of IL-6. However, direct evidence indicating this relationship is not yet available. If transfer of the c-*myc* gene and/or other oncogenes into IL-6 transgenic mice could induce transplantable plasmacytomas, then these transgenic mice will provide a useful model for dissecting the process of the oncogenesis of plasma cell neoplasias.

We thank Dr. Edward Barsoumian for a critical review of the manuscript. We also thank Ms. M. Harayama and Ms. K. Kubota for their excellent secretarial assistance. This work was supported in part by Grants-in-Aid for Specially Promoted Research and for Cancer Research from the Japanese Ministry of Education, Science and Culture and by Special Coordination Funds of the Science and Technology Agency of the Japanese Government.

1. Kishimoto, T. & Hirano, T. (1988) *Annu. Rev. Immunol.* **6,** 485–512.
2. Hirano, T. & Kishimoto, T. (1989) in *Handbook of Experimental Pharmacology*, eds. Sporn, M. B. & Roberts, A. B. (Springer, Berlin), Vol. 95, in press.
3. Hirano, T., Taga, T., Yasukawa, K., Nakajima, K., Nakano, N., Takatsuki, F., Shimizu, M., Murashima, A., Tsunasawa, S., Sakiyama, F. & Kishimoto, T. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 228–231.
4. Hirano, T., Matsuda, T., Turner, M., Miyasaka, N., Buchan, G., Tang, B., Sato, K., Shimizu, M., Maini, R., Feldman, M. & Kishimoto, T. (1988) *Eur. J. Immunol.* **18,** 1797–1801.
5. Yoshizaki, K., Matsuda, T., Nishimoto, N., Kuritani, T., Taeho, L., Aozasa, K., Nakahata, T., Kawai, H., Tagoh, H., Komori, T., Kishimoto, S., Hirano, T. & Kishimoto, T. (1989) *Blood*, in press.
6. Kawano, M., Hirano, T., Matsuda, T., Taga, T., Horii, Y., Iwato, K., Asaoku, H., Tang, B., Tanabe, O., Tanaka, H., Kuramoto, A. & Kishimoto, T. (1988) *Nature (London)* **332,** 83–85.
7. Potter, M. & Boyce, C. (1962) *Nature (London)* **193,** 1086–1087.
8. Nordan, R. P. & Potter, M. (1986) *Science* **233,** 566–569.
9. Van Snick, J., Cayphas, S., Vink, A., Uyttenhove, C., Coulie, P. G., Rubira, M. R. & Simpson, R. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9679–9683.
10. Nordan, R. P., Pumphrey, J. G. & Rudikoff, S. (1987) *J. Immunol.* **139,** 813–817.
11. Van Snick, J., Cayphas, S., Szikora, J.-P., Renauld, J.-C., Van Roost, E., Boon, T. & Simpson, R. J. (1988) *Eur. J. Immunol.* **18,** 193–197.
12. Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashiwamura, S., Nakajima, K., Koyama, K., Iwamatu, A., Tsunasawa, S., Sakiyama, F., Matsui, H., Takahara, Y., Taniguchi, T. & Kishimoto, T. (1986) *Nature (London)* **324,** 73–76.
13. Yasukawa, K., Hirano, T., Watanabe, Y., Muratani, K., Matsuda, T. & Kishimoto, T. (1987) *EMBO J.* **6,** 2939–2945.
14. Yamamura, K., Kikutani, H., Folsom, V., Clayton, L. K., Kimoto, M., Akira, V., Kashiwamura, S., Tonegawa, S. & Kishimoto, T. (1985) *Nature (London)* **316,** 67–69.
15. Yamamura, K., Kikutani, H., Takahashi, N., Taga, T., Akira, S., Kawai, K., Fukuchi, K., Kumahara, Y., Honjo, T. & Kishimoto, T. (1984) *J. Biochem. (Tokyo)* **96,** 357–363.
16. Matsuda, T., Hirano, T. & Kishimoto, T. (1988) *Eur. J. Immunol.* **18,** 951–956.
17. Hayday, A. C., Gillies, S. D., Saito, H., Wood, C., Wiman, K., Hayward, W. S. & Tonegawa, S. (1984) *Nature (London)* **307,** 334–340.
18. Hardy, R. R., Kishimoto, T. & Hayakawa, K. (1987) *Eur. J. Immunol.* **17,** 1769–1774.
19. Taga, T., Kawanishi, K., Hardy, R. R., Hirano, T. & Kishimoto, T. (1987) *J. Exp. Med.* **166,** 967–981.
20. Wanebo, H. J., Gallmeier, W. M., Boyse, E. A. & Old, L. J. (1966) *Science* **154,** 901–903.
21. Ishibashi, T., Kimura, H., Uchida, T., Kariyone, S., Friese, P. & Burstein, S. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5953–5957.
22. Andus, T., Geiger, T., Hirano, T., Northoff, H., Ganter, U., Bauer, J., Kishimoto, T. & Heinrich, P. C. (1987) *FEBS Lett.* **221,** 18–22.
23. Gauldie, J., Richards, C., Harnish, D., Lansdorp, P. & Baumann, H. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7251–7255.
24. Namba, Y. & Hanaoka, M. (1972) *J. Immunol.* **109,** 1193–1200.
25. Ohno, S., Babonits, M., Wiener, F., Spira, J., Klein, G. & Potter, M. (1979) *Cell* **18,** 1001–1007.
26. Mushinski, J. F., Bauer, S. R., Potter, M. & Reddy, E. P. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1073–1077.
27. Potter, M. (1984) *Cancer Surveys* **3,** 247–264.
28. Potter, M., Sklar, M. D. & Rowe, W. P. (1973) *Science* **182,** 592–594.
29. Potter, M., Mushinski, J. F., Mushinski, E. B., Brust, S., Wax, J. S., Wiener, F., Babonits, M., Rapp, U. R. & Morse, H. C., III (1987) *Science* **235,** 787–789.
30. Clynes, R., Wax, J., Stanton, L. W., Smith-Gill, S., Potter, M. & Marcu, K. B. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6067–6071.
31. Isobe, T. & Osserman, E. F. (1971) *Ann. N.Y. Acad. Sci.* **190,** 507–517.
32. Wegelius, O., Skrifvara, B. & Andersson, L. (1970) *Acta Med. Scand.* **187,** 133–138.
33. Graham, S. L. & Sellers, A. L. (1979) *Arch. Intern. Med.* **139,** 116–117.
34. Keller, A. R., Hochholzer, L. & Castleman, B. (1972) *Cancer* **29,** 670–683.
35. Horii, Y., Muraguchi, A., Iwano, M., Matsuda, T., Hirayama, T., Yamada, H., Fujii, Y., Dohi, K., Ishikawa, H., Ohmoto, Y., Yoshizaki, K., Hirano, T. & Kishimoto, T. (1989) *J. Immunol.*, in press.