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Generation of plasmacytomas with the chromosomal translocation t(12;15) in interleukin 6 transgenic mice

(BALB/c/genetic background/plasmacytosis)

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ABSTRACT **The mechanisms through which pristane or mineral oil can induce plasmacytomas in BALB/c or NZB mice are not fully understood, but involvement of interleukin 6 (IL-6), a growth factor for plasmacytomas and myelomas, has been strongly suggested. To clarify the role of IL-6 in plasmacytomagenesis, a human IL-6 cDNA was introduced into mouse germ lines under the transcriptional control of the murine major histocompatibility complex class I (H-2L^d) promoter. IL-6 transgenic mice of C57BL/6 origin developed a massive plasmacytosis but not plasmacytomas. However, introduction of BALB/c genetic background into IL-6 transgenic mice could generate monoclonal transplantable plasmacytomas with the chromosomal translocation t(12;15). These results provide firm evidence of the critical role of IL-6 in the plasmacytoma development.**

It has been suggested that deregulated expression of a variety of peptide growth factors could play a critical role in neoplastic transformation in an autocrine or paracrine fashion (1, 2). Overproduction of several growth factors, such as granulocyte–macrophage colony-stimulating factor (3), interleukin 6 (IL-6) (4), and transforming growth factor α (5–7), in transgenic mice was reported to induce a massive hyperplasia of their relevant target cells, but abnormal production of such growth factors *per se* could not generate true malignant tumors, although transforming growth factor α-induced hyperplasia was strongly suggested to result in neoplastic transformation with the additional genetic changes hypothesized (5–7).

Almost 30 years ago, Potter and Boyce (8) generated plasmacytomas in BALB/c mice by intraperitoneal injection of mineral oil. Plasmacytoma growth factor, which is now known to be identical to IL-6 (9–11), was suggested to be responsible for plasmacytoma generation (12, 13), but details of the mechanism were not known. The generation of mineral oil- or pristane-induced plasmacytomas is restricted to a few selected strains of mice, such as BALB/c and NZB (14), suggesting the involvement of certain genetic factors for the IL-6-dependent plasmacytomagenesis. Indeed, IL-6 transgenic mice of C57BL/6 origin showed massive plasmacytosis but did not develop transplantable tumors (4). In this study, we report the generation of transplantable monoclonal plasmacytomas with the chromosomal translocation t(12;15) in IL-6 transgenic mice with a BALB/c genetic background.

MATERIALS AND METHODS

Animals. C57BL/6J, BALB/cA, and BALB/cA-*nu/nu* mice were obtained from specific-pathogen-free (SPF) stocks of Nippon Clea, Osaka. Fertilized C57BL/6J eggs were microinjected with DNA. Transgenic mouse lines were maintained in a conventional mouse facility. BALB/cA-*nu/nu* mice were intraperitoneally injected with 0.2 ml of pristane (2,6,10,14-tetramethylpentadecane; Aldrich) 1 week before the transplantation of cells from transgenic mice. BALB/cA-*nu/nu* mice were maintained under specific-pathogen-free conditions.

Construction of the L^d–IL-6 Transgene. The simian virus 40 early promoter region in the vector pKCR3 (15) was replaced by the 1.4-kilobase-pair *Sph* I–*Bam*HI fragment of the H-2L^d promoter from pL^{d}4 (16), resulting in plasmid pLG-1. A 340-base-pair 3′ untranslated region was deleted from the human IL-6 cDNA (17) with BAL-31 exonuclease to increase the stability of the IL-6 mRNA. After *Eco*RI linker ligation, the 770-base-pair *Eco*RI fragment was inserted into the *Eco*RI site of the rabbit β-globin sequence in pLG-1. The 3.3-kilobase-pair *Sph* I–*Xho* I fragment (L^d–IL-6) was isolated and microinjected into fertilized eggs from C57BL/6 mice as described (18).

Human IL-6-Specific ELISA. The concentration of human IL-6 in transgenic mouse serum was estimated by the human IL-6-specific ELISA as described (19).

Isotype-Specific ELISA. The ELISA used goat anti-mouse immunoglobulins (Cappel Laboratories) and alkaline phosphatase-conjugated affinity-purified rabbit anti-mouse immunoglobulin antibodies specific for each isotype (Zymed Laboratories) as described (19). Myeloma proteins of appropriate subclass were used as standards [IgG1, αBSF2-166; IgG2b, αBSF2-77; IgM, αBSF2-60 (19); IgA, IgG2a, and IgG3 were obtained from Organon Teknika–Cappel].

SDS/PAGE of Transgenic Mouse Serum. Transgenic mouse serum (0.5 μl) was electrophoresed through a SDS/4–20% gradient 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).

Transplantation of Plasma Cells into BALB/cA-*nu/nu* Mice. Approximately 1–5 × 10^6 cells of the spleen, lymph node, and ascites cells were transferred into the peritoneal cavity of pristane-pretreated BALB/cA-*nu/nu* mice.

Southern Blot Analysis. High molecular weight DNAs were extracted. DNAs were digested with either *Eco*RI or *Xba* I and subjected to Southern blot analysis using the random-primer-labeled *Eco*RI–*Hin*dIII fragment that contained the mouse immunoglobulin heavy-chain joining region segment 4 (J_H4) as described (20).

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Abbreviations: IL-6, interleukin 6; J_H4, heavy-chain joining region segment 4.

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Chromosome Analysis. Metaphase spreads were prepared from ascites tumor cells without Colcemid treatment. G-banding analysis was performed as described (21). Well-spread metaphase plates were photographed for karyotype analysis, and their locations were recorded. Chromosomes were identified, and the translocation breakpoints were located as described (21).

RESULTS

Generation of C57BL/6 L^d–IL-6 Transgenic Mice. The L^d–IL-6 DNA was microinjected into fertilized eggs from C57BL/6 (B6) mice. Four founder B6 L^d–IL-6 transgenic mice were obtained, and three founders were found to transmit the transgene to their progeny. Human IL-6 (0.1–5 ng/ml) was detected in the sera of B6 L^d–IL-6 transgenic mice by using an ELISA that specifically detects human IL-6 (19). Human IL-6 mRNA was detected in the spleen, lymph nodes, thymus, heart, lung, liver, kidney, and intestine, but not in the brain (data not shown). The following pathological features of B6 L^d–IL-6 transgenic mice were essentially the same as those of B6 E_μ–IL-6 transgenic mice as reported (4): (*i*) fatal plasmacytosis with the polyclonal increase in serum immunoglobulins, mainly IgG1 (100- to 400-fold), (*ii*) mesangial proliferative glomerulonephritis, and (*iii*) an increase in mature megakaryocytes in the bone marrow. However, these plasma cells were not transplantable to syngeneic B6 mice, indicating that IL-6 alone is not sufficient for the generation of plasmacytomas.

Introduction of BALB/c Genetic Backgrounds into B6 L^d–IL-6 Transgenic Mice. There is a remarkable strain dependence of murine plasmacytomagenesis. Most inbred strains other than BALB/c and NZB are resistant to the induction of plasmacytomas (14). To elucidate the genetic influence on the plasmacytomagenesis, one of the B6 L^d–IL-6 transgenic mouse lines (B6 L^d46) was backcrossed to BALB/c mice (Fig. 1). An increase in the level of serum immunoglobulins was accelerated in (BALB/c × B6 L^d46)F_1 transgenic mice compared to that of B6 L^d46 transgenic mice (Fig. 2). In the BALB/c × (BALB/c × B6 L^d46)F_1 backcross transgenic progeny, three out of nine mice (L^d46-7-5, L^d46-7-8, and L^d46-7-2) showed an increase in serum IgA level (50- to 200-fold) at 30 weeks of age, although they initially showed a preferential increase in IgG1. The other backcross transgenic progeny and all of B6 L^d46 transgenic mice showed an increase in IgG1 (100- to 400-fold) but not in IgA. The three mice with increased serum IgA levels developed ascites at ≈40 weeks of age. At this time, mesenteric lymph nodes were enlarged, but swelling of submandibular, axillary, and inguinal lymph nodes was not severe. These findings were in contrast to those observed in B6 L^d–IL-6 transgenic mice in which enlargement of axillary and inguinal lymph nodes was prominent. Lymph nodes were composed of plasma cells and immunoblasts. Infiltration of plasma cells was also observed in the spleen and the bone marrow.

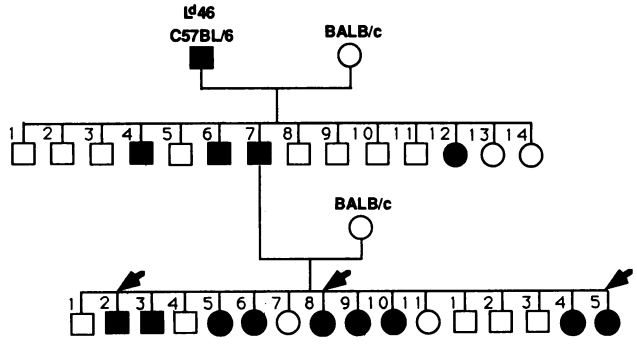

FIG. 1. Pedigree of L^d46 transgenic mice. Mouse B6 L^d46 was backcrossed to BALB/c mice. Male mice are indicated by squares, and female are indicated by circles. Mice bearing the transgene are indicated by solid symbols; mice without it are indicated by open symbols. L^d46-7-5, L^d46-7-8, and L^d46-7-2 (indicated by arrows) developed ascites at 37, 45, and 45 weeks old, respectively.

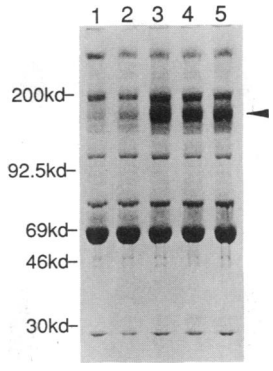

FIG. 2. Acceleration of an increase in the level of serum immunoglobulins in a BALB/c genetic background. Transgenic mouse serum (0.5 μl) was electrophoresed through an SDS/4–20% gradient polyacrylamide slab gel under nonreducing conditions and the gel was stained with Coomassie brilliant blue R250. Lanes: 1 and 2, sera from two B6 L^d46 F_1 mice (20 weeks old); 3–5, sera from three (BALB/c × B6 L^d46)F_1 mice (17 weeks old). The arrowhead indicates the band that contains the immunoglobulins.

Transplantability of Plasma Cells Proliferated in Transgenic Mice. We examined whether these plasma cells were transplantable to pristane-pretreated BALB/cA-*nu*/*nu* mice. The recipient nude mice developed ascites 4–8 weeks after the transplantation and many tumor masses were observed in the peritoneal connective tissues. There were many plasma cells in the ascites and some of them were multinucleated. These plasma cells were found to contain large amounts of cytoplasmic IgA by immunofluorescent staining. The ascites tumors were found to contain human IL-6 at 1–3 ng/ml. Thus transplantable plasmacytomas were generated in IL-6 transgenic mice with a BALB/c genetic background.

Immunoglobulin Heavy Chain Gene Rearrangements and G-Banded Karyotypes of Plasmacytomas. To examine the clonality of these plasma cells, Southern blot analysis of the DNAs derived from L^d46-7-2 and L^d46-7-5 was performed using the J_H4 probe (20). As shown in Fig. 3, a rearranged

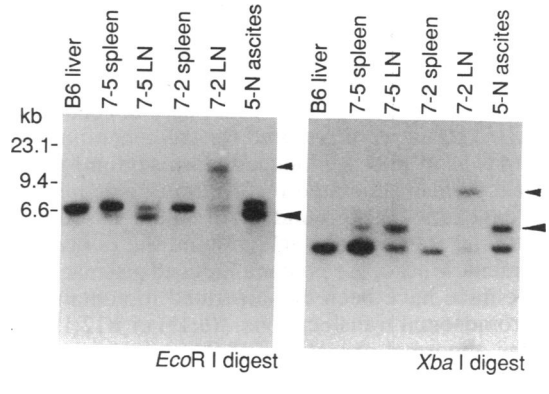

FIG. 3. Southern blot analysis of the immunoglobulin gene. DNAs (5 μg per lane) obtained from indicated organs were digested with either *Eco*RI or *Xba* I and subjected to Southern blot analysis utilizing the random-primer-labeled *Eco*RI–*Hin*dIII fragment containing J_H4 (20). The arrowheads indicate rearranged monoclonal J_H4-bearing fragments. Lanes: 7-5 spleen, DNA obtained from L^d46-7-5 spleen; 7-5 LN, DNA from L^d46-7-5 lymph node; 7-2 spleen, DNA from L^d46-7-2 spleen; 7-2 LN, DNA from L^d46-7-2 lymph node; 5-N ascites, DNA from ascites cells of nude mouse transplanted with plasmacytoma cells from L^d46-7-5. kb, kilobase pairs.

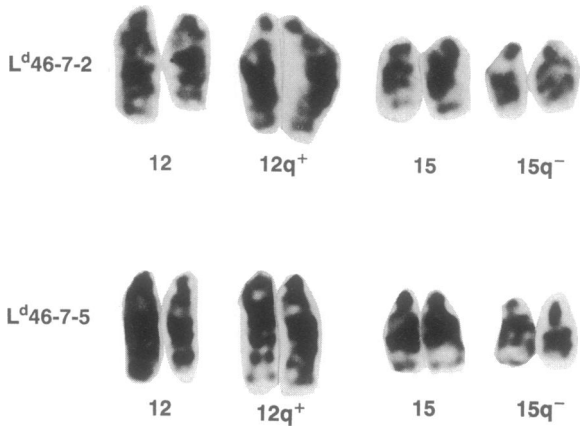

FIG. 4. Chromosomal translocation t(12;15) in the plasmacytoma. Plasmacytomas derived from L^{d}46-7-2 and L^{d}46-7-5 mice were intraperitoneally transplanted into BALB/cA-*nu/nu* mice. Metaphase spreads were prepared from ascites tumor cells in these BALB/cA-*nu/nu* mice. G-banding was performed as described (21). Well-spread metaphase plates were photographed for karyotype analysis, and their locations were recorded. Identification of chromosomes and translocation breakpoints was as described (21).

monoclonal J_H4-bearing fragment was detected in both DNAs, indicating the monoclonality of these transplantable plasmacytomas.

Most of the pristane-induced plasmacytomas in BALB/c mice have nonrandom chromosomal translocations, t(6;15) or t(12;15) (14). Therefore, analysis of G-banded karyotypes was performed. The plasmacytomas generated in both L^{d}46-7-2 and L^{d}46-7-5 were near-tetraploid and contained the t(12;15) translocation (Fig. 4). Furthermore, the c-*myc* gene was found to be rearranged (data not shown).

DISCUSSION

In this study, we demonstrated that overexpression of the human IL-6 gene in IL-6 transgenic mice could induce plasma-cell neoplasia through a massive polyclonal plasmacytosis. Overexpression of IL-6 was found to be enough for the generation of polyclonal plasmacytosis in IL-6 transgenic mice of B6 origin. These plasma cells were not found to be transplantable into syngeneic mice, although the histological findings were compatible with those of malignant plasmacytomas (4). In contrast, IL-6 transgenic mice that were backcrossed to BALB/c mice developed monoclonal transplantable plasmacytomas. The data indicated that in addition to the overexpression of the IL-6 gene, certain genetic factors of BALB/c strain were required for the generation of plasmacytomas as in pristane-induced plasmacytomagenesis.

Plasmacytomas generated in IL-6 transgenic mice with a BALB/c genetic background were found to contain the chromosomal translocation t(12;15) and the c-*myc* gene rearrangement. Most of the pristane-induced plasmacytomas in BALB/c mice have been demonstrated to contain nonrandom chromosomal translocations, t(6;15) or t(12;15), resulting in the abnormal expression of the c-*myc* gene (21, 22). Plasmacytomas generated in v-*abl* transgenic mice also contain the c-*myc* gene rearrangement (23). A retrovirus bearing v-*myc* can induce plasmacytomas in pristane-treated mice and these plasmacytomas do not contain the c-*myc* gene rearrangement, suggesting that v-*myc* expression can make up for the c-*myc* expression (24). All data indicate that deregulated expression of the c-*myc* gene may play a role in murine plasmacytomagenesis.

More than 60% of pristane-induced plasmacytomas in BALB/c mice produce IgA. IgG producers are also induced, but IgM producers are rare (14). However, the high levels of v-*myc* expression can induce IgM producers in pristane-treated mice without a chromosomal translocation, suggesting that immunoglobulin class switch event may influence chromosomal translocation (24). The IL-6 transgenic mice with a BALB/c genetic background showed the acceleration of an increase in serum immunoglobulins in comparison with B6 transgenic mice. This may not be directly linked to the development of IgA plasmacytomas because this increase was limited to the IgG1 subclass. The interesting question is whether this acceleration of the increase in serum immunoglobulins and the susceptibility to pristane and/or IL-6-induced plasmacytomagenesis in BALB/c mice are related. Genetic analysis of IL-6 transgenic mice between B6 and BALB/c may provide useful informations.

Intraperitoneal injection of mineral oil or pristane induces the formation of granulomatous tissue where generation of plasmacytomas is observed (8). The growth of these plasmacytomas was found to be dependent on a factor produced by the cells in the granulomatous tissue, and this factor has been shown to be identical to IL-6 (9, 11, 14). These previous observations and present results support the hypothesis that the induction of plasmacytomas by mineral oil or pristane is at least in part due to continuous production of IL-6 in the granulomatous tissue. It is conceivable that certain genetic changes occur during the course of IL-6-induced expansion of polyclonal plasma cells, resulting in the generation of monoclonal malignant plasmacytomas. Alternatively, the compartment of plasma-cell precursors may contain the so-called plasmacytoma precursors that have certain abnormal protooncogenes. IL-6 could induce plasma-cell differentiation of these precursors and, during this period, one of these precursors could transform into a plasma-cell neoplasia with the additional genetic changes in protooncogenes. In either case, aberration of oncogene expression may be influenced by a BALB/c genetic background.

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