FUNCTIONAL MURINE INTERLEUKIN 6 RECEPTOR WITH THE INTRACISTERNAL A PARTICLE GENE PRODUCT AT ITS CYTOPLASMIC DOMAIN

Its Possible Role in Plasmacytomagenesis

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IL-6 plays essential roles in immune response, acute phase reaction, and hematopoiesis (1, 2). It is an essential factor for B cell differentiation to antibody-forming cells (3). On the other hand, deregulated expression of the IL-6 gene could be involved in polyclonal plasma cell abnormalities and oncogenesis of plasma cell neoplasias (4–8). IL-6, in fact, functions as a potent growth factor for multiple myelomas (7), as well as plasmacytomas (9, 10). Furthermore, we recently demonstrated the generation of a massive plasmacytosis in IL-6 transgenic mice (11). Therefore, the abnormal expression of either the IL-6 or the IL-6-R genes could play an essential role in plasma cell abnormalities.

The association of chromosomal aberrations with many murine and human tumors suggests that DNA rearrangements may constitute a general mechanism for tumor induction (12). The slowly transforming retroviruses were shown in some cases to activate transcription of a particular cellular *onc* gene (13). The intracisternal A particle (IAP)[1] gene is a member of endogenous proretroviral-like elements present in ~1,000 copies per haploid genome of *Mus musculus* (14). The IAP gene functions as movable elements in the mouse genome (15). The possible role of the IAP gene in oncogenesis was suggested in several studies (16–19).

In this study, we describe the isolation of two species of the cDNAs encoding murine IL-6-R: one is abnormal and the other authentic, from a plasmacytoma cell line and normal spleen cells, respectively. In the cDNA encoding the abnormal IL-6-R, the region corresponding to its intracytoplasmic domain was replaced with a part of long terminal repeat (LTR) of the IAP gene. This replacement could be responsible for the overexpression of IL-6-R on a plasmacytoma cell line. As an intracytoplasmic portion of the IL-6-R is not required for the signal transduction (20),

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[1] *Abbreviations used in this paper:* IAP, intracisternal A particle; LTR, long terminal repeat.

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the integration of the IAP gene into the receptor gene may function as a positive selection element for the development of certain plasmacytomas.

Materials and Methods

Cell Line and Cell Culture. An IL-6 dependent-T cell line, KT-3 (21), was used for transfection experiments. KT-3 cells were maintained in RPMI 1640 medium supplemented with 10% FCS, penicillin (50 U/ml), streptomycin (50 μg/ml), 2 mM glutamine, 50 μM 2-ME, and 2 ng/ml of human rIL-6.

cDNA Library and Screening. cDNA libraries were made utilizing λ phage gt10 from poly(A)$^+$ RNA isolated from plasmacytoma cell line, P3U1, and spleen cells of a BALB/c mouse, as described previously (22). The P3U1 cDNA library was probed with an Fsp I–Ban I fragment of the pBSF2R.236 insert cDNA encoding human IL-6-R (23). Plaques were blotted on Colony/Plaque Screen membranes (New England Nuclear, Boston, MA) and hybridized in 1% SDS, 1 M NaCl, 0.05 M Tris HCl (pH 7.5), 5× Denhardt's solution, and 200 μg/ml salmon sperm DNA at 65°C for 16 h and washed in 2× SSC and 1% SDS at 60°C. To get λ P2 and λ 301, the libraries were probed with an Eco RI–Bam HI fragment (probe 1) or a Fok I–Hind III fragment (probe 2) of λ P1 cDNA, as indicated in Fig. 1 *a*. The filters were hybridized as above and washed in 0.1× SSC and 0.1% SDS at 65°C.

DNA Sequencing. The phage insert cDNAs from positive plaques were cloned into pUC18 and sequenced by utilizing Sequenase (United States Biochemical Corp., Cleveland, OH).

Northern Blot Analysis. Cytoplasmic poly(A)$^+$ RNAs (2 μg/lane of RNA obtained from a variety of tissues of BALB/c mouse and various plasmacytoma cell lines [kindly provided by Dr. S. Ohno, Cancer Research Institute, Kanazawa University, Kanazawa, Japan] except for P3U1; 8 μg/lane RNA from various kinds of cell lines; 1 μg/lane RNA of P3U1) were separated on 1% agarose-formaldehyde gels, transferred to GeneScreen Plus membranes (New England Nuclear), and hybridized with ^{32}P-labeled Eco RI–Bam HI fragment of λ P1 insert cDNA (Fig. 1 *a*, probe 1) in 50% formamide, 1% SDS, 1 M NaCl, and 10% dextran sulfate at 42°C for 16 h. Filters were washed in 2× SSC and SDS at 60°C.

Transfection Experiments and Proliferation Assay. The Eco RI insert cDNA of λ P1 was subcloned at an Eco RI site of pM5G expression vector, kindly provided by Dr. W. Ostertag (24). A Kpn I site of λ 301 was blunted and replaced with an Eco RI site. The 1,410-bp Eco RI fragment, which contains authentic IL-6-R cDNA lacking a 3′ noncoding region, was subcloned at an Eco RI site of pM5G. KT-3 cells were transfected with pM5G containing either λ P1 or λ 301 insert cDNA by electroporation. Selection of transfectants was started on day 3 after transfection with 750 μg/ml of the antibiotic G418 (Sigma Chemical Co., St. Louis, MO).

Stable transfectants (2.5×10^3 cells) were cultured in the presence of various concentrations of murine rIL-6, kindly provided by Dr. Y. Akiyama (Ajinomoto Co., Kawasaki, Japan) or human rIL-6 (25) for 66 h. The cells were pulsed with 0.5 μCi [^{3}H]TdR during the last 6 h and harvested on glass filter paper by an automatic cell harvester (Labo Mash Science Co., Tokyo, Japan). The radioactivity was measured with a scintillation counter (Beckman Instruments, Inc., Fullerton, CA).

Results and Discussion

Murine IL-6-R cDNA clones were isolated from the cDNA library of P3U1 cells that were derived from a BALB/c plasmacytoma cell line, MOPC21. Clones that crosshybridized to the human IL-6-R cDNA, pBSF2R.236 (23), were screened under a nonstringent condition by in situ procedure. 15 positive plaques were identified from 7.3×10^5 plaques. One of them, containing the largest insert cDNA, designated λ P1 (Fig. 1 *a*), was further analyzed. The complete nucleotide sequence of the cDNA insert of λ P1 demonstrated that the cDNA consisted of 1,470 bp and contained a large open reading frame that could encode 440 amino acids (Fig. 1 *b*).

The overall homology with human IL-6-R was 66 and 48% at the DNA and the protein levels, respectively. The hydrophobic regions corresponding to a signal sequence and a transmembrane domain showed much higher homology (74 and 79% at the protein level, respectively). The domain of the Ig superfamily found in the human IL-6-R was also identified in the molecule encoded by the cloned cDNA. All cysteine residues present in the human IL-6-R were found to be conserved in this murine molecule, except for Cys^{211} (human). Furthermore, Trp^{134} (human) residue and Trp^{303}-Ser-Xxx-Trp-Ser^{307} (human) sequence were also found at positions 130 and 300–304, respectively. These residues and four Cys residues (at positions 121, 132, 165, and 176 in human IL-6-R) were found to be conserved among murine erythropoietin R, human IL-2-R β chain, murine IL-4-R, and human granulocyte-macrophage CSF-R, and these receptors have been classified as the IL receptor family (26–30). All data indicated that the λ P1 insert cDNA encoded murine IL-6-R. However, the intracytoplasmic domain of the molecule encoded by the cloned cDNA had no homology with that of the human IL-6-R. It was found that the nucleotide sequence of the cDNA corresponding to the intracytoplasmic domain showed a striking homology (94%) with that of a part of LTR of the IAP gene (Fig. 1 *c*).

Northern blot analysis utilizing the cDNA fragment (Fig. 1 *a*, probe 1) specific to the extracytoplasmic domain detected two species of mRNA (1.8 and 5.5 kb) in P3U1 cells (Fig. 2 *a*). One major mRNA species had a compatible size (1.8 kb) with the cloned λ P1 insert cDNA. But the other mRNA species, the expression of which was much lower than the former one, had a larger size (5.5 kb), compatible with that of human IL-6-R mRNA. Furthermore, the former mRNA species was shown to be hybridized with an IAP-LTR-specific cDNA fragment (Fig. 1 *a*, probe 2), but the latter mRNA was not (data not shown). Moreover, only the second species of the mRNA (5.5 kb) was expressed in the spleen, thymus, and liver of BALB/c mouse, as well as other cell lines, such as the T cell lymphoma cell line, EL-4, and the macrophage cell line, P388D1 (Fig. 2 *a*). The data indicated the presence of IL-6-R mRNA without the IAP-LTR. To isolate this mRNA clone, cDNA clones were obtained that were hybridized with probe 1 but not probe 2. Under these conditions, one clone (λ P2) from the P3U1 cDNA library and 13 clones from the BALB/c spleen cDNA library were obtained. The nucleotide sequences of λ P2 and a representative clone, λ 301 obtained from the BALB/c mouse spleen cDNA library, were determined. In both cDNAs, the nucleotide sequence, as well as deduced amino acid sequence corresponding to both an extracellular and a transmembrane region, were found to be identical with those of the λ P1 cDNA, although the λ P2 insert cDNA lacked a part of 5′ region (Fig. 1 *a*). However, the region corresponding to an intracytoplasmic region, as expected, did not contain the IAP-LTR gene and showed a significant homology with that of human IL-6-R (53% at the protein level) (Fig. 1 *b*). Therefore, these cDNA clones were concluded to encode the authentic murine IL-6-R and the overall homology between murine and human IL-6-R was 69 and 54% at DNA and protein levels, respectively.

To examine whether the IL-6-R with its intracytoplasmic portion encoded by a part of the IAP-LTR gene can transduce the signals, the insert cDNA of λ P1 was subcloned at an Eco RI site of pM5G expression vector (24) and transfected to an IL-6-dependent human T cell line, KT-3 (21). Human IL-6 induced cell growth of KT-3 cells, but murine IL-6 did not. However, KT-3 cells transfected with either

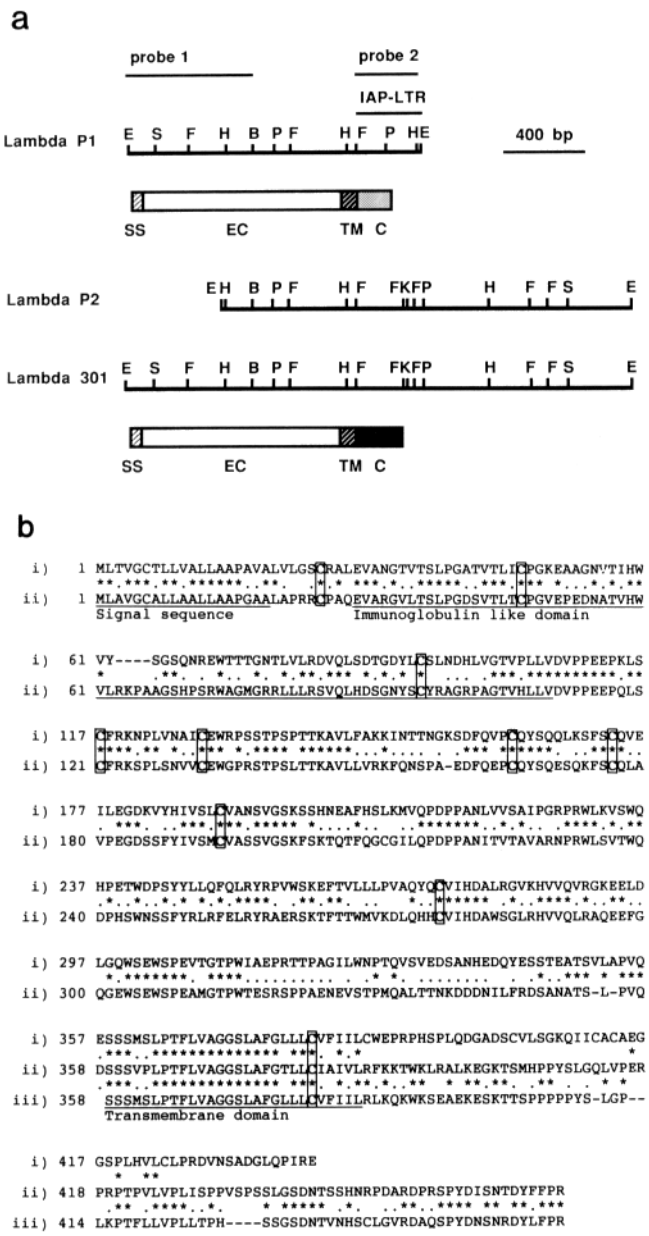

FIGURE 1. (*a*) Restriction endonuclease cleavage maps of the cDNAs encoding abnormal and authentic murine IL-6-R. The open reading frames corresponding to the IL-6-R are also shown by solid boxes: SS, signal sequence; EC, extracellular domain; TM, transmembrane domain; C, intracytoplasmic domain. Abbreviations for restriction enzymes are as follows: B, Bam HI; E, Eco RI; F, Fok I; H, Hind III; K, Kpn I; P, Pst I; S, Sma I. The Eco RI site was newly introduced to the cDNA fragments to make cDNA libraries. (*b*) Comparison of the deduced amino acid sequences of the cloned murine IL-6-R cDNAs with human IL-6-R amino acid sequence. Deduced amino acid sequences of the insert cDNA of λ P1 (*i*) and λ 301 (*iii*) are aligned with that of human IL-6-R cDNA, pBSF2R236 (23) (*ii*). The deduced amino acid sequence between positions of 1 and 357 of the insert cDNA of λ 301 was identical with that of the insert cDNA of λ P1 (data not shown). Gaps are introduced for optimal sequence alignment. The signal sequence, the domain similar to the Ig superfamily, and a transmembrane domain are underlined. Identical amino acids are indicated by asterisks. Conserved cysteine residues are boxed. Some of abnormal or authentic murine IL-6-R cDNA clones were found to be defective in three base pairs, resulting in the lack of Ala^{314} (data not shown). (*c*) Comparison of the nucleotide sequences of the 3′ region of the insert cDNA of λ P1 (*top lines*) and that of the IAP-LTR se-

(continued)

the λ P1 or λ 301 cDNA responded to murine IL-6 to proliferate (Fig. 3). The data indicated that the IL-6-R, in which an intracytoplasmic domain was replaced with the molecule encoded by a part of the IAP-LTR gene, could function and transduce the signals. This was in complete agreement with our previous observation with human IL-6-R that an intracytoplasmic domain was not required for the signal transduction (20).

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1159 TGTTGGGAGCCGCGCCCACATTCGCCGTTACAAGATGGCGCTGACA-GCTGTGTTCTAAG
     ************ ********************************  ************
2993 TGTTGGGAGCCGCCCCCACATTCGCCGTTACAAGATGGCGCTGACATCCTGTGTTCTAAG
     └─→U3                                              GRE

1218 TGGTAAACAAATAATCTGCGCATGTGCCGAGGGTGGTTCTCCACTCCATGTGCTCTGCCT
     ***************************** *****   *    *** * ************
3053 TGGTAAACAAATAATCTGCGCATGTGCCAAGGGTATCTTATGACTACTTGTGCTCTGCCT
     enhancer

1278 TCCCCGTGACGTCAACTCGGCCGATGGGCTGCAGCCAATCAGGGAGTGACACGTCCTAGG
     ****************************************** ******* ****** ***
3113 TCCCCGTGACGTCAACTCGGCCGATGGGCTGCAGCCAATCAAGGAGTGATACGTCCGAGG
                                    CAT box

1338 CGAAGGATAATTCTCCTTAATAGGGACGGGG-TTTCGTTTTCTCTCTCTCTTGCTTC--T
     ******* *** ******** ********** ************************
3173 CGAAGGAGAATGCTCCTTAAGAGGGACGGGGTTTTCGTTTTCTCTCTCTCTTGCTTCTTG
       TATA box                    U3←┘└→R

1395 CTCTCTTGCTTCTTGCTCTCTTGCTTCCTGCACCCTGGCTCCTGAAGATGTAAGAAATAA
     ************************************************************
3233 CTCTCTTGCTTCTTGCTCTCTTGCTTCCTGCACCCTGGCTCCTGAAGATGTAAGAAATAA
                                                        poly A
                                                        signal

1455 AGCTTTGCCGCAGAAG
     ************
3293 AGCTTTGCCGCAG
          R←┘
```

quence of the cDNA encoding IgE binding factor (*bottom lines*). Glucocorticoid responsive element (GRE), enhancer sequence, CAT box, TATA box, and poly A addition signal are underlined. The whole nucleotide sequences of the cDNA encoding murine IL-6-R (λ P1 and λ 301 cDNA) have been submitted to the EMBL/GenBank Data Libraries under the accession numbers X51976 and X51975, respectively.

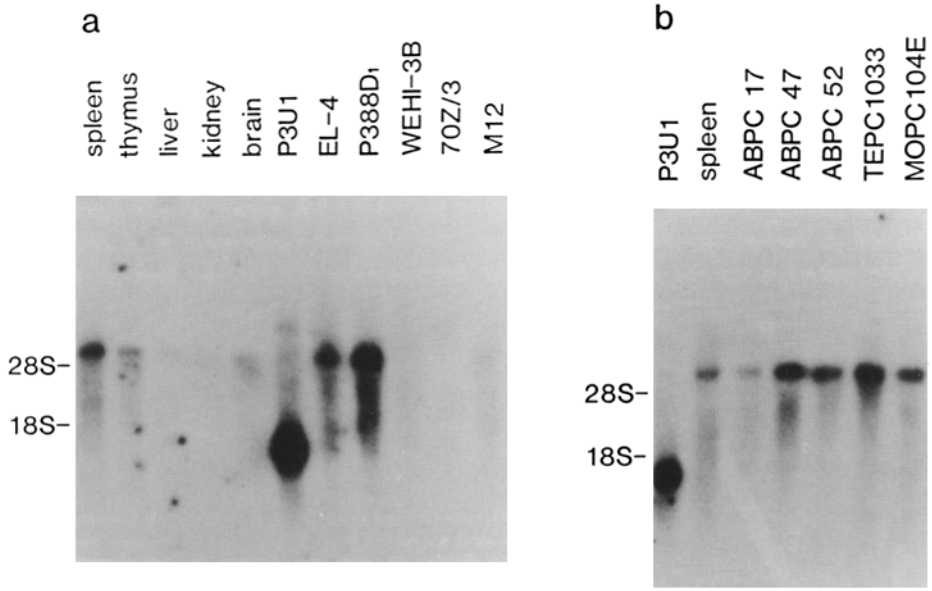

FIGURE 2. Expression of IL-6-R mRNA in a variety of cells and cell lines (*a*) and plasmacytomas (*b*). Cytoplasmic poly(A)$^+$ RNAs (2 μg/lane of RNA obtained from the spleen, thymus, liver, kidney, and brain of BALB/c mouse and various plasmacytoma cell lines, except for P3U1; 8 μg/lane of RNA from various kinds of cell lines; 1 μg/lane RNA of P3U1) were separated on 1% agarose-formaldehyde gels, transferred to nylon filters, and hybridized with ^{32}P-labeled Eco RI-Bam HI fragment of λ P1 insert cDNA. P3U1, plasmacytoma cell line; EL-4, T cell lymphoma cell line; P388D1, macrophage-like cell line; WEHI-3B, myelomonocytic leukemia cell line, 70Z/3, pre-B cell line; M12, B lymphoma cell line; ABPC17, ABPC47, ABPC52, TEPC1033 and MOPC104E, plasmacytoma cell lines.

IAP has been demonstrated to act as an insertional element that upregulates (17, 19) or downregulates (31, 32) the relevant gene expression. IAP-LTR consists of three regions, U3, R, and U5, and is classified into two groups on the basis of the size of the R region (33). IAP-LTR found in the IL-6-R gene showed a striking homology with that found in murine IgE binding factor (34) and contained U3 and R regions in which the enhancer sequence was included (Fig. 1 *c*). The study of the organization of the human IL-6-R gene showed that a transmembrane domain and an intracytoplasmic domain of human IL-6-R were encoded by different exons (unpublished data). Therefore, the overexpression of the IL-6-R mRNA with the IAP-LTR sequence in P3Ul cells could be due to the enhancer activity of the IAP-LTR gene that was supposed to be inserted between the exons encoding a transmembrane and an intracytoplasmic domain of the murine IL-6-R gene. Stabilization of mRNA may be another possible mechanism for the enhanced expression of the rearranged IL-6-R gene with IAP-LTR.

The IAP gene is expressed in early mouse embryos and many murine plasmacytomas (35, 36). The possible role of the IAP gene in oncogenesis was suggested in several studies: the insertion of the IAP gene in the c-*mos* or the IL-3 gene in plasmacytoma and a myeloid leukemic cell line resulted in the activation of the c-*mos* or the IL-3 gene, respectively (16–19). Overexpression of IL-6-R may make cells more sensitive to IL-6 (20). Since IL-6 is a potent growth factor for plasmacytomas and deregulated expression of the IL-6 gene has been suggested to be involved in the oncogenesis of plasma cell neoplasias (8), the deregulated expression of the IL-6-R gene could contribute to accelerate the IL-6-induced promotion of the malignant transformation of plasma cells. A unique feature of an IL-6-R system consisting of two molecules, a ligand binding chain and a signal transducer, allowed the IL-6-R containing the molecule encoded by a part of the IAP-LTR gene instead of the normal intracytoplasmic domain to function for the transduction of the growth signals in plasmacytomas. Although, the insertion of the IAP-LTR gene was not commonly observed in various plasmacytomas (Fig. 2 *b*), this rearrangement may provide a positive pressure for the selection in the development of certain plasmacytomas.

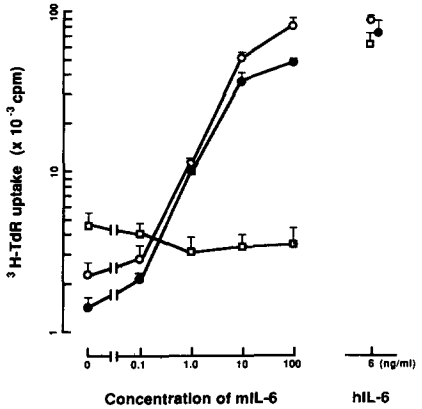

FIGURE 3. Murine IL-6-R containing a molecule encoded by the IAP-LTR gene can transduce the signal. (○) KT-3 cells expressing murine IL-6-R containing the molecule encoded by the IAP-LTR gene instead of normal intracytoplasmic domain. (●) KT-3 cells expressing authentic murine IL-6-R. (□) Normal KT-3 cells. Results are shown as mean ± of quadruplicate cultures.

Summary

Two species of the cDNAs encoding murine IL-6-R (one is abnormal and the other authentic) have been cloned from a plasmacytoma cell line, P3U1, and BALB/c mouse spleen cDNA libraries. In the cDNA encoding the abnormal IL-6-R, the region corresponding to an intracytoplasmic domain was replaced with a part of the long terminal repeat of the intracisternal A particle gene (IAP-LTR). The authentic IL-6-R consists of 460 amino acids with the domain of the Ig superfamily. The overall homology between murine and human IL-6-R was 69 and 54% at DNA and protein levels, respectively. The extracellular domain after the Ig-like domain of murine IL-6-R was found to have an homology with those of murine erythropoietin R, human IL-2-R β chain, murine IL-4-R, and human granulocyte-macrophage CSF-R, as in the case of human IL-6-R, and these receptors have been classified as members of the IL receptor family.

In P3U1 cells, the expression of the mRNA encoding abnormal IL-6-R was much higher than that of the mRNA encoding authentic IL-6-R. An IL-6-dependent human T cell line, KT-3, which did not respond to murine IL-6, acquired the responsiveness to murine IL-6 when transfected with the cDNA encoding abnormal IL-6-R, indicating that abnormal IL-6-R lacking a normal cytoplasmic domain can function. Since IL-6 functions as a potent growth factor for murine plasmacytomas, overexpression of abnormal IL-6-R may function as a positive selection element for the development of certain plasmacytomas.

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