Brief Definitive Report

DNA REARRANGEMENT AND CONSTITUTIVE EXPRESSION OF THE INTERLEUKIN 6 GENE IN A MOUSE PLASMACYTOMA

By THOMAS BLANKENSTEIN, ZHIHAI QIN, WEIQUN LI, and TIBOR DIAMANTSTEIN

From the Institut für Immunologie, Klinikum Steglitz, Freie Universität Berlin, D-1000 Berlin 45, Federal Republic of Germany

The association between the plasmacytoma phenotype and the activation of cellular onocogenes by chromosomal translocation or retroviral insertion is well documented (1, 2). The role of IL-6, a pleiotropic lymphokine (3), during the development of plasmacytomas is not yet clear. IL-6 seems to be involved in the establishment of pristane-induced plasmacytomas in vivo (4), and is required for their growth in vitro (4, 5). A possible autocrine role of IL-6 has been proposed for human multiple myelomas (6). In contrast, paracrine rather than autocrine regulation was suggested to promote myeloma cell growth (7).

Several plasmacytoma lines have been analyzed for alterations in the IL-6 locus. A DNA rearrangement due to the insertion of an intracisternal A particle (IAP) was detected in the cell line MPC11 (8). The constitutive IL-6 expression in MPC11 suggests the involvement of IL-6 in the development of certain myeloma/plasmacytomas according to the "autocrine growth hypothesis" (9).

Materials and Methods

Southern Blot Analysis. High molecular weight DNA extraction and Southern blot analysis have been performed according to standard procedures (10). The nick-translated 1.1-kb Eco RI fragment from plasmid pHP1B5 (11) has been used as IL-6 probe. The cell lines used for Southern blot analysis were obtained from the American Type Culture Collection (Rockville, MD).

Construction and Screening of a Recombinant λ Phage Library. 5–15-kb enriched fragments of Bam HI-digested DNA of MPC11 were ligated into Bam HI/Eco RI pretreated EMBL3 arms and packaged in vitro using Gigapack Gold extracts (Stratagene, La Jolla, CA), resulting in 2×10^6 recombinant phages. Plaque hybridization to the IL-6 probe, λ phage growth, plaque purification, DNA extraction, restriction analysis, and subcloning of restriction fragments into the M13 vector were done according to standard procedures (10).

DNA Sequence Analysis. Subclones containing Eco RI or Eco RI/Bam HI fragments, as outlined in Fig. 2, were constructed into M13mp19. Nucleotide sequence analysis was performed by the dideoxy chain termination method (12).

RNA Dot Blot Analysis. RNA extraction has been done according to standard procedures (10). Twofold serial dilutions of RNA extracted from MPC11, NIH3T3, and P388 cells were blotted onto a nitrocellulose filter by use of a slot blot device and hybridized to the IL-6 and

Address correspondence to Thomas Blankenstein, Institut für Immunologie, Universitätsklinikum Steglitz, Hindenburgdamm 27, D-1000 Berlin 45, FRG.

the β-actin probe (2-kb Pst I fragment of plasmid pA1). The P388 cells had been stimulated with 10 μg/ml LPS for 16 h before RNA extraction.

IL-6 Assay. 5×10^3/0.2 ml of 7TD1 cells (5) were cultured in DMEM supplemented with 10% FCS, 0.24 mM L-asparagine, 0.55 mM L-arginine, 50 μM 2-ME, 0.1 mM hypoxanthine, and 16 μM thymidine for 48 h with serially diluted supernatants derived from plasmacytoma lines. The cultures were pulsed with 0.2 μCi [^{3}H]thymidine for 12 h, and thymidine incorporation was determined. The mean value of triplicate wells of a representative assay is shown. Background incorporation of 1,700 cpm was substracted.

Results and Discussion

Several plasmacytoma lines were analyzed by Southern blots using the IL-6 probe. Upon Eco RI digestion, a new band appears in MPC11 in comparison with BALB/c liver DNA (Fig. 1 *a*). Restriction analysis using different restriction enzymes (Hind III, Xba I, and Bam HI) confirmed a DNA rearrangement in the IL-6 locus of MPC11 (Fig. 1 *b*). Moreover, the reduction of band intensities suggested that the mutation had occurred in the 5′ region of the IL-6 locus and that presumably one out of two alleles was rearranged.

A genomic library was constructed with Bam HI-digested DNA of MPC11 in λ phage EMBL3. Subsequently, recombinant phages were isolated containing the rearranged 13-kb Bam HI fragment and the unaltered 5.2-kb Bam HI fragment. The latter fragment, which contains exons 4 and 5, showed an identical restriction site pattern in comparison with the previously isolated germline IL-6 gene (13). A restriction map of the rearranged 5′ region of the MPC11 IL-6 locus, including the first three exons, in comparison with the germline restriction map is shown in Fig. 2. It reveals the insertion of a 2.5-kb fragment close to the first exon of the IL-6 gene. A strategy for DNA sequence analysis was devised (Fig. 2) in order to detect the integration site of the new sequence relative to the IL-6 exons in MPC11 and

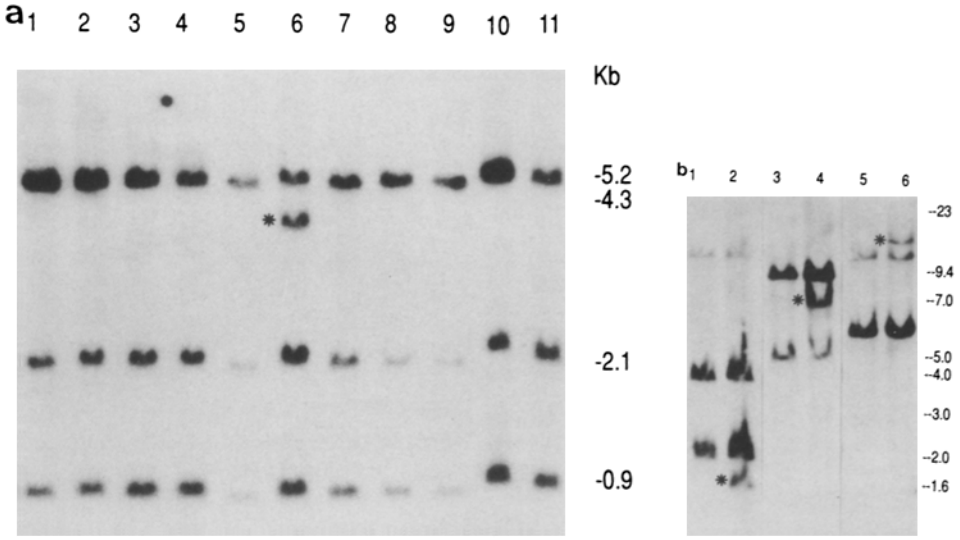

FIGURE 1. (*a*) Southern blot analysis with Eco RI-digested DNA using the IL-6 probe. (*1*) S194; (*2*) MOPC-21; (*3*) X63.Ag8.653; (*4*) P1.17; (*5*) HOPC 1F/12; (*6*) MPC11; (*7*) XS63; (*8*) MOPC 315; (*9*) MOPC 31G; (*10*) J558L; (*11*) BALB/c liver. (*b*) Southern blot analysis with MPC11 and BALB/c liver DNA using different restriction enzymes. (*1*, *3*, and *5*) BALB/c liver DNA; (*2*, *4*, and *6*) MPC11 DNA; (*1* and *2*) Hind III; (*3* and *4*) Xba I; (*5* and *6*) Bam HI.

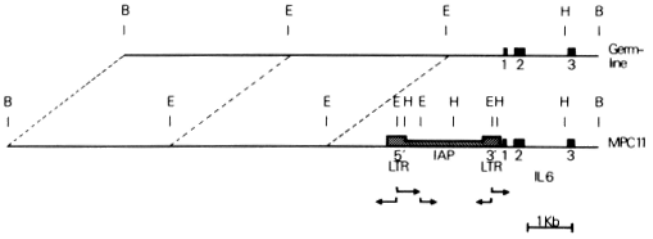

FIGURE 2. Restriction map of the 5′ region of the rearranged IL-6 allele in MPC11 compared with the germline configuration. The first three IL-6 exons are indicated by black boxes. The hatched region denotes the inserted IAP. The arrows indicate the sequencing strategy. B, Bam HI; E, Eco RI; H, Hind III.

to determine the nature of the inserted element. Comparison of nucleotide sequences derived from the rearranged IL-6 locus to the germline IL-6 sequences (14) and to the Genbank Data Base confirmed the insertion of a mobile element belonging to the retrovirus-like family of IAP 18 bp 5′ of the putative transcriptional start site (14), thereby placing the IL-6 control regions 2.5 kb upstream (Fig. 3).

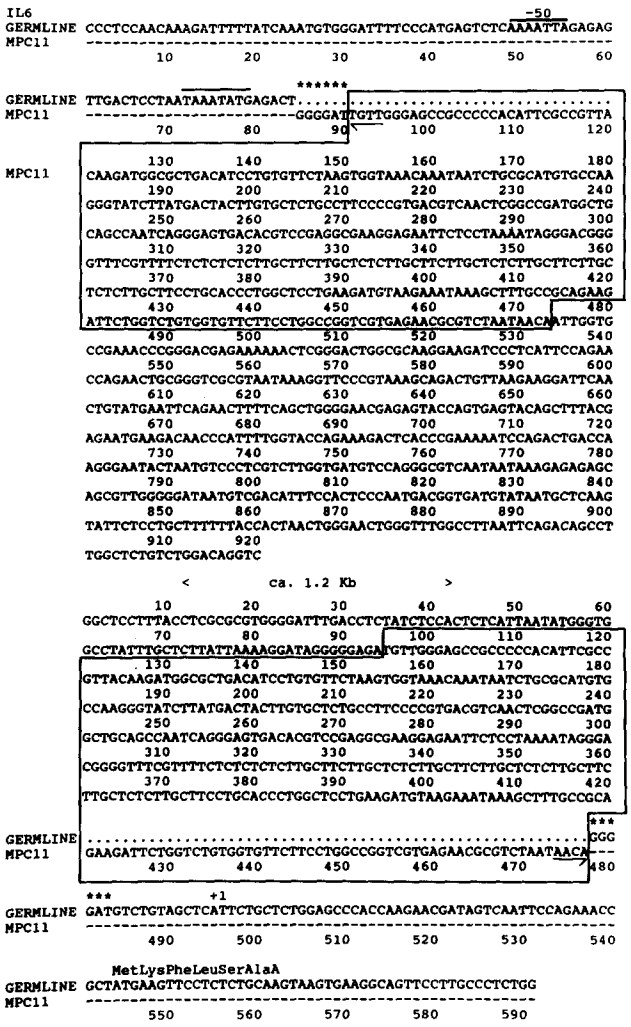

FIGURE 3. Nucleotide sequence analysis of the two junctions of the IAP genome within the rearranged IL-6 allele of MPC11. The IL-6-related sequences from position −103 into the first intron are compared with the germline sequence. Exhibited are the first exon (amino acid translation above the sequence), the transcriptional start site (+1), the potential TATA boxes (at −30 and −50), and a 6-bp duplication at the IAP integration site (indicated by asterisks). Within the two boxes, the LTRs are located with the 4-bp inverted repeat at the ends, indicated by arrows. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession numbers X51457, 8, 9.

IAPs occur ~1,000 times per haploid mouse genome, have similarities to the proviral form of retroviruses, but exist only intracellularly (15), and their association with a malignant phenotype has been documented (2, 16).

The IL-6-derived sequences of the rearranged allele from position −103 extending into the first intron are identical to the germline sequence (14), except for a 6-bp duplication adjacent to the IAP integration site, a common feature of IAP integration, which is thought to occur by retrotransposition. The IL-6–IAP is >95% homologous to the sequence of the IAP that is integrated 5′ to the IL-3 gene in WEHI-3B (17), including a characteristical 4-bp inverted repeat at the termini. Alignment of the two sequences (not shown) shows that the size of the identical LTRs in IL-6–IAP is 383 bp, due to an internal duplication, and that almost the entire potential coding region is deleted, explaining the small size of IL-6–IAP (~2.5 kb). The integration occurred 5′ to 3′ with respect to the IL-6 locus previously thought to result in low or no expression of the adjacent gene (2).

To ascribe a possible functional role to the IL-6–IAP integration, IL-6 activity was assayed in the culture supernatant of MPC11 by using the IL-6 indicator cell line 7TD1 (5) (Fig. 4 *a*). Whereas X63.Ag8-653 and J558L, which contain the IL-6 gene in germline configuration, do not secrete detectable amounts of IL-6, high IL-6 activity was detected in the supernatant of MPC11. The IL-6 expression by MPC11 was confirmed by an RNA dot blot assay (Fig. 4 *b*). Serial dilutions of MPC11, LPS-stimulated P388, and NIH3T3 RNA were hybridized in parallel to the IL-6 and the β-actin probes, showing an estimated 50-fold higher IL-6 expression level in MPC11 in comparison with LPS stimulated P388 cells.

These results document that in addition to the translocation between c-*myc* and the Igh locus (18), a second mutational event has occurred in MPC11. They are con-

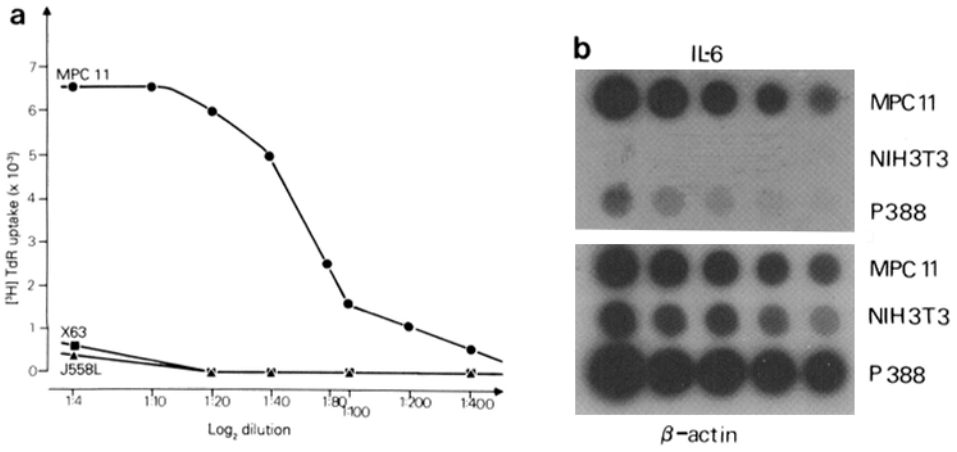

FIGURE 4. (*a*) Cell proliferation assay measured by [^{3}H]thymidine incorporation in cpm with the IL-6 indicator line 7TD1 in the presence of serial dilutions of supernatants collected from plasmacytomas MPC11, X63.Ag8-653, and J558L. (*b*) Dot blot analysis with twofold serial dilutions of RNA extracted from MPC11-, NIH3T3-, and LPS-activated P388 cells. As probes, the IL-6 and the β-actin probes were used.

sistent with a multistage transformation process and suggest that IL-6 acting as autocrine growth factor may contribute to the development of certain plasmacytomas.

Summary

To study the potential involvement of IL-6 in the development of plasmacytomas, a number of plasmacytoma lines were analyzed for alterations in the IL-6 locus. A DNA rearrangement due to the insertion of an intracisternal A particle retrotransposon 18 bp 5′ of the transcriptional start site was detected in the cell line MPC11. The IL-6 gene is constitutively expressed in MPC11, suggesting the involvement of IL-6 in the development of certain myeloma/plasmacytomas according to the "autocrine growth hypothesis".

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References

1. Klein, G. 1983. Specific chromosomal translocations and the genesis of B-cell-derived tumors in mice and men. *Cell.* 32:311.
2. Cohen, J. B., T. Unger, G. Rechavi, E. Canaani, and D. Givol. 1983. Rearrangement of the oncogene c-mos in mouse myeloma NSI and hybridomas. *Nature (Lond.).* 306:797.
3. Kishimoto, T., and T. Hirano. 1988. Molecular regulation of B lymphocyte response. *Annu. Rev. Immunol.* 6:485.
4. Nordan, R. P., and M. Potter. 1986. A marophage-derived factor required by plasmacytomas for survival and proliferation in vitro. *Science (Wash. DC).* 233:566.
5. van Snick, J., A. Vink, S. Cayphas, and C. Uyttenhove. 1987. Interleukin-HP1, a T cell-derived hybridoma growth factor that supports the in vitro growth of murine plasmacytomas. *J. Exp. Med.* 165:641.
6. Kawano, M., T. Hirano, T. Matsuda, T. Taga, Y. Horii, K. Iwato, H. Asaoku, B. Tang, O. Tanabe, H. Tanaka, A. Kuramoto, and T. Kishimoto. 1988. Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. *Nature (Lond.).* 332:83.
7. Klein, B., X.-G. Zhang, M. Jourdan, J. Content, F. Houssiau, L. Aarden, M. Piechaczyk, and R. Bataille. 1989. Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by Interleukin-6. *Blood.* 73:517.
8. Laskov, R., and M. D. Scharff. 1970. Synthesis, assembly, and secretion of gamma globulin by mouse myeloma cells. *J. Exp. Med.* 131:515.
9. Sporn, M. B., and A. B. Roberts. 1985. Autocrine growth factors and cancer. *Nature (Lond.).* 313:745.
10. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY.
11. van Snick, J., S. Cayphas, J.-P. Szikora, J.-C. Renauld, E. van Roost, T. Boon, and R. J. Simpson. 1988. cDNA cloning of murine interleukin-HP1: homology with human interleukin 6. Eur. J. Immunol. 18:193.
12. Sanger, F., A. R. Coulson, B. G. Barrell, A. J. H. Smith, and B. A. Roe. 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. *J. Mol. Biol.* 143:161.
13. Qin, Z., G. Richter, T. Diamantstein, and Th. Blankenstein. 1989. Structure and evolution of the mouse Interleukin 6 gene. *Mol. Immunol.* 26:1021.
14. Tanabe, O., S. Akira, T. Kamiya, G. G. Wong, T. Hirano, and T. Kishimoto. 1988. Genomic structure of the murine IL-6 gene. High degree conservation of potential regula-

tory sequences between mouse and human. *J. Immunol.* 141:3875.
15. Finnegan, D. J. 1985. Transposable elements in eukaryotes. *Int. Rev. Cytol.* 93:281.
16. Ymer, S., W. Q. J. Tucker, C. J. Sanderson, A. J. Hapel, H. D. Campbell, and I. G. Young. 1985. Constitutive synthesis of interleukin-3 by leukaemia cell line WEHI-3B is due to retroviral insertion near the gene. *Nature (Lond.).* 317:255.
17. Ymer, S., W. Q. J. Tucker, H. D. Campbell, and I. G. Young. 1986. Nucleotide sequence of the intracisternal A-particle genome inserted 5′ to the interleukin-3 gene of the leukaemia cell line WEHI-3B. *Nucleic Acids Res.* 14:5901.
18. Stanton, L. W., J.-Q. Yang, L. A. Eckhardt, L. J. Harris, B. K. Birshtein, and K. B. Marcu. 1984. Products of a reciprocal chromosome translocation involving the c-myc gene in a murine plasmacytoma. *Proc. Natl. Acad. Sci. USA.* 81:829.