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ISOLATION AND CHARACTERIZATION OF A HUMAN INTERLEUKIN 2 GENE

Shuji Mita, Shuichiro Maeda, Kenshi Obaru, Naoki Nishino, Kazunori Shimada, Toshio Hirano*, Kaoru Onoue*, Tomoko Ogawa** and Hideyuki Ogawa**

Department of Biochemistry, Kumamoto University School of Medicine, *Department of Biochemistry, Institute for Medical Immunology, Kumamoto University School of Medicine, Honjo, Kumamoto 860 and **Department of Biology, Faculty of Science Osaka University, Toyonaka, Osaka 560, Japan

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SUMMARY: An interleukin 2 (IL-2) gene was isolated from a Charon 4A human gene library. Electron microscopic examination of 15 heteroduplexes formed between the genomic DNAs and the IL-2 cDNAs demonstrated that the size of the IL-2 gene is about 5.1 ± 0.5 kb and that there are at least two introns in this gene. Nucleotide sequence of the 5' flanking region of the IL-2 gene showed a homology with that of the corresponding region of the human immune interferon gene.

Interleukin 2 (IL-2), a lymphokine possessing potent effects on various immune responses (1, 2), is produced by T cells after stimulation with antigen or mitogen in the presence of macrophage-derived interleukin 1 (3, 4). To initiate a study on mechanisms of IL-2 induction, we have cloned human IL-2 mRNAs from a cDNA library prepared from phytohemagglutinin (PHA)-stimulated tonsillar lymphocytes (5), and have demonstrated by Southern blot analysis of human placental DNA that there is only one IL-2 gene in the human genome (5).

We now report the isolation and characterization of a human IL-2 gene. Efrat et al. (6) recently found that cultivation of human tonsillar lymphocytes in the presence of PHA leads to the induction of IL-2 and the kinetics of immune interferon (IFN-γ)

Abbreviations: cDNA, complementary DNA; mRNA, messenger RNA; IL-2, interleukin 2; T-cell, thymus-derived cell; PHA, phytohemagglutinin; IFN-γ, immune interferon; IFN-α, leukocyte interferon; kb, kilobase pairs; bp, base pairs.

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induction by PHA in the same culture are remarkably similar to those of IL-2 (6). As a first step toward elucidating the regulatory mechanisms of transcription of IL-2 and IFN-γ genes, we sequenced the 5' flanking region of the genomic IL-2 gene and compared our findings with those from the corresponding region of the human IFN-γ gene (7). We found that the homology extends from the "TATA" box (8) upstream for about 60 bp.

MATERIALS AND METHODS

Enzymes and chemicals: Restriction enzymes and T4 polynucleotide kinase were purchased from Takara Shuzo Co., E. coli DNA ligase from NEB, bacterial alkaline phosphatase and E. coli DNA polymerase I from BRL, DNase I from Sigma and calf intestinal alkaline phosphatase from Boehringer-Mannheim. All these enzymes were used according to the supplier's recommendations. (α-^{32}P)dCTP (3,000 Ci/mmol) was obtained from Amersham and (γ-^{32}P)ATP (3,000 Ci/mmol) from NEN. Formamide was obtained from Nakarai Chemicals Ltd..

DNAs used as probes: An IL-2 cDNA fragment carried in pHIG5-3 has already been characterized (5) and corresponds to a complete human tonsillar IL-2 mRNA. A 5' part of the cDNA fragment was excised from this plasmid as described (5) and was used as a 5' end probe.

Cloning procedures: A Charon 4A human gene library consisting approximately of $1X10^6$ independently derived clones was prepared as described (9, 10). These phage clones carry about 15 to 20 kb-size DNA fragments obtained from the partially EcoRI digested human placental DNAs. The general procedures for screening of the human genomic DNA library, DNA isolation, nick translation and DNA blot hybridization were performed as described previously (9). All of the cloning procedures were carried out in accordance with the guidelines of the Ministry of Education, Science and Culture, Japan for recombinant DNA. DNA sequences were determined according to Maxam and Gilbert (11).

Heteroduplex analysis by electron microscopy: The DNAs derived from a Charon 4A clone (named Lm HIG1) carrying a whole human IL-2 gene, Charon 4A and the HindIII-cleaved pHIG5-3 were mixed, denatured for 5 minutes at 25°C in 1mM EDTA/0.1M NaOH, neutralized and hybridized for 1 hr at 25°C in 70% formamide/10mM EDTA/0.1M Tris-HCl, pH 8.0/0.25M NaCl. The molecules were photographed as described elsewhere (12), using a JOEL model JEM-100S electron microscope.

RESULTS AND DISCUSSION

Isolation of a human IL-2 genomic DNA clone: A Charon 4A human gene library constructed as described in the Materials and Methods was screened by in situ plaque hybridization, using the ^{32}P-labeled IL-2 cDNA (5) as a probe. One out of $6X10^5$ plaques gave a positive hybridization signal and was named Lm HIG1.

To analyze the structural features of the IL-2 gene carried by the Lm HIG1 DNA, Southern blot analysis of the Lm HIG1 DNA was carried out (9, 13). At first, Lm HIG1 DNA was digested with EcoRI, HindIII and EcoRI plus HindIII. The digests were then electrophoresed on 0.6% agarose gels and blotted onto nitrocellulose filters. The DNA blots were hybridized with the nick translated IL-2 cDNA probe.

From the results shown in Fig. 1 A a, the EcoRI and HindIII cleavage sites present on the insert of Lm HIG1 were inferred as shown in Fig. 1 B. To further confirm this restriction map, we subcloned the 3.5 kb, 3.6 kb and 3.7 kb EcoRI fragments separately into the EcoRI site of pBR322. The ^{32}P-labeled IL-2 cDNA probe hybridized with both the 3.5 kb and 3.6 kb EcoRI fragments containing subclones, but not with the 3.7 kb EcoRI fragment containing subclone. Moreover, the 5' end probe prepared from the pHIG5-3 hybridized only with the 3.5 kb EcoRI fragment and not with the 3.6 kb EcoRI fragment (data not shown). These results support the idea that the arrangement of the three EcoRI fragments is in the order shown in Fig. 1 B and the 3.5 kb EcoRI fragment contains the 5' end region of the IL-2 gene.

The EcoRI digest of the Lm HIG1 DNA showed a single hybridization band of 3.5 kb and the HindIII digest, two bands of 3.3 kb and 2.1 kb on the autoradiogram (Fig. 1 A b). These hybridization patterns were identical with those obtained previously with the human placental DNA (5). The EcoRI and HindIII double-digest of the Lm HIG1 DNA showed three hybridization bands of 2.8 kb, 2.1 kb and 0.8 kb, respectively (Fig. 1 A b). The 2.1 kb band apparently corresponds to the 2.1 kb HindIII band (Fig. 1 A b). The appearance of the 2.8 kb and 0.8 kb hybridization bands in this digest is consistent with the restriction map shown in Fig. 1 B. All the genomic DNA regions hybridizable with the

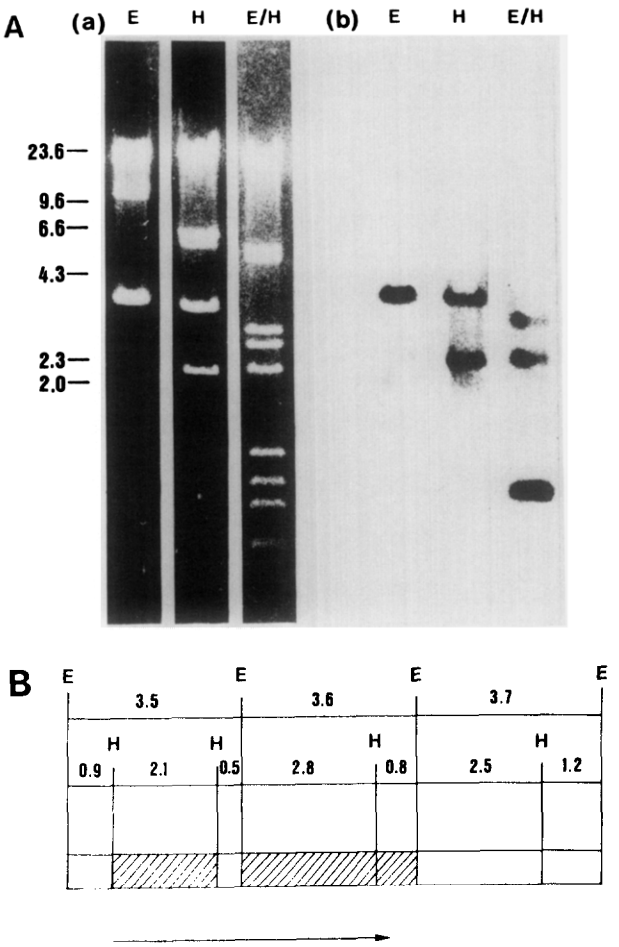

Fig. 1. Restriction mapping of the human IL-2 gene region. (A), Restriction and hybridization analyses of Lm HIG1 DNA. Lm HIG1 DNA was digested with restriction endonucleases EcoRI (E), HindIII (H), or EcoRI plus HindIII (E/H). The fragments were separated by electrophoresis, transferred to nitrocellulose filters and hybridized with the ^{32}P-labeled IL-2 cDNA probe (9, 13). (a), A photograph of the ethidium bromide-stained gel in UV light. (b), An autoradiogram of the hybridized filter. Lambda DNAs cleaved with HindIII were used as size markers, and the sizes of DNA fragments are indicated in kb on the left. (B), Restriction map of the human IL-2 gene region. Hatched blocks indicate the fragments hybridizing with the ^{32}P-labeled IL-2 cDNA probe. The horizontal arrow indicates the direction of transcription. Numbers above the horizontal lines indicate the size of the fragments in kb.

IL-2 cDNA probe are given in Fig. 1 B, and there are two HindIII cleavage sites within these regions. We have already shown that the IL-2 cDNA contains no HindIII sites (5). Accordingly, these results suggest that the genomic IL-2 gene contains at least two

introns. All the results summarized in Fig. 1 indicate that the 11 kb genomic DNA insert carried by Lm HIG1 contains a whole human IL-2 gene.

Organization of the human IL-2 gene: To examine the structural organization and the approximate size of the IL-2 gene, we performed heteroduplex analyses. The Lm HIG1 DNA was hybridized with the HindIII-cleaved pHIG5-3 DNA in the presence of Charon 4A DNA and the resulting heteroduplexes were examined by electron microscopy. The Charon 4A DNA was added to the hybridization mixture in order to convert the Charon 4A arms of the Lm HIG1 DNA into double-stranded DNAs and to contrast the IL-2 gene flanking DNA regions as single-stranded DNAs. We examined 15 heteroduplexes and Fig. 2 A shows a typical one. The IL-2 gene obviously contains two introns in the coding region. This result is consistent with the results shown in Fig. 1. A larger single-stranded tail derived from the HindIII-cleaved pHIG5-3 DNA serves to determine the 3' side of the IL-2 gene. One intron, located on the 5' side of the gene, is estimated to be 2.4 ± 0.2 kb and another is 1.9 ± 0.2 kb. The sizes of three exons 1 through 3, are 250 ± 30 bp, 150 ± 20 bp, and 380 ± 30 bp, respectively (Fig. 2). These results indicate that the size of the human IL-2 gene is about 5.1 ± 0.5 kb. It should be noted that detection by electron microscope of small single-stranded loops is difficult, and the exact number of introns is now being determined by nucleotide sequence analyses.

Nucleotide sequence of the 5' region of the IL-2 gene: To compare the sequence of the 5' flanking region of the human IL-2 gene with that of the corresponding region of the IFN-γ gene, the 2.1 kb HindIII fragment was extracted from the 3.5 kb EcoRI fragment and was confirmed to hybridize with the 5' end probe prepared from the pHIG5-3 (Fig. 1 B and Fig. 3 A). This HindIII

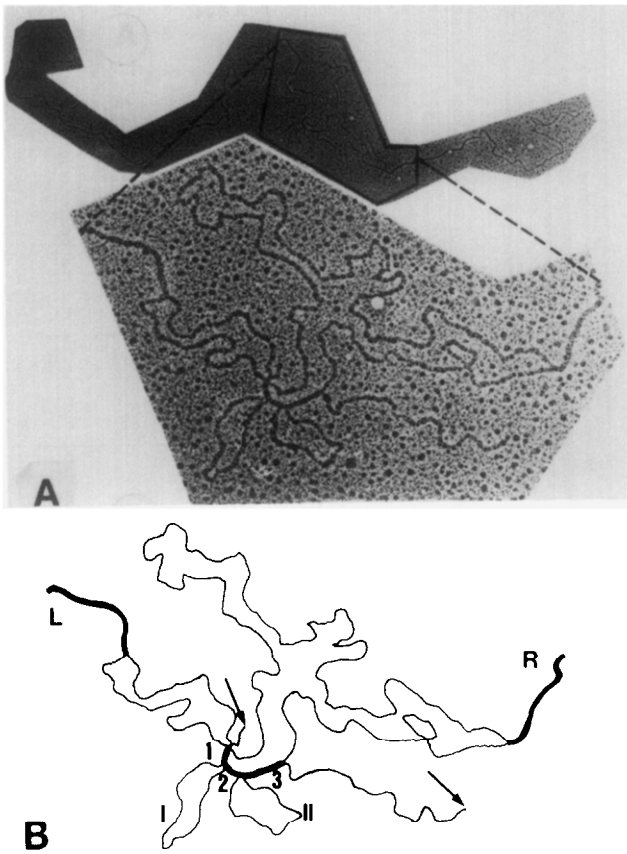

Fig. 2. Electron microscopic analysis of the human IL-2 gene. Electron micrographs of a heteroduplex molecule consisting of the Lm HIG1, pHIG5-3 and Charon 4A DNAs (A) and its diagram (B) are presented. In the diagram, heavy lines indicate double-stranded DNAs and thin lines, single-stranded DNAs. The Charon 4A arms are marked with R and L. The numbers 1 through 3 correspond to exons, beginning at the 5' end of the gene, and the numbers I and II indicate introns. The total length of the IL-2 cDNA is 788 bp (5) and is used as an internal reference to estimate the length of double-stranded DNAs. The two single-stranded DNA tails formed by the heteroduplex between the HindIII-cleaved pHIG5-3 and the IL-2 genomic DNAs are indicated by arrows and are calculated from our previous data (5) to be 280 bp and 2.5 kb, respectively. They serve as internal references to estimate the length of single-stranded DNAs.

fragment was digested with RsaI and the two RsaI fragments hybridizing with the 5' end probe were excised from the agarose gel. We found that only the larger of the two RsaI fragments contained the 5' flanking region of the IL-2 gene (Fig. 3 A), and was further digested with HinfI. Both strands of the resulting 197 bp HinfI/RsaI fragment have been sequenced (Fig. 3 A). The 196 nucleotides preceding the initiation codon (ATG)

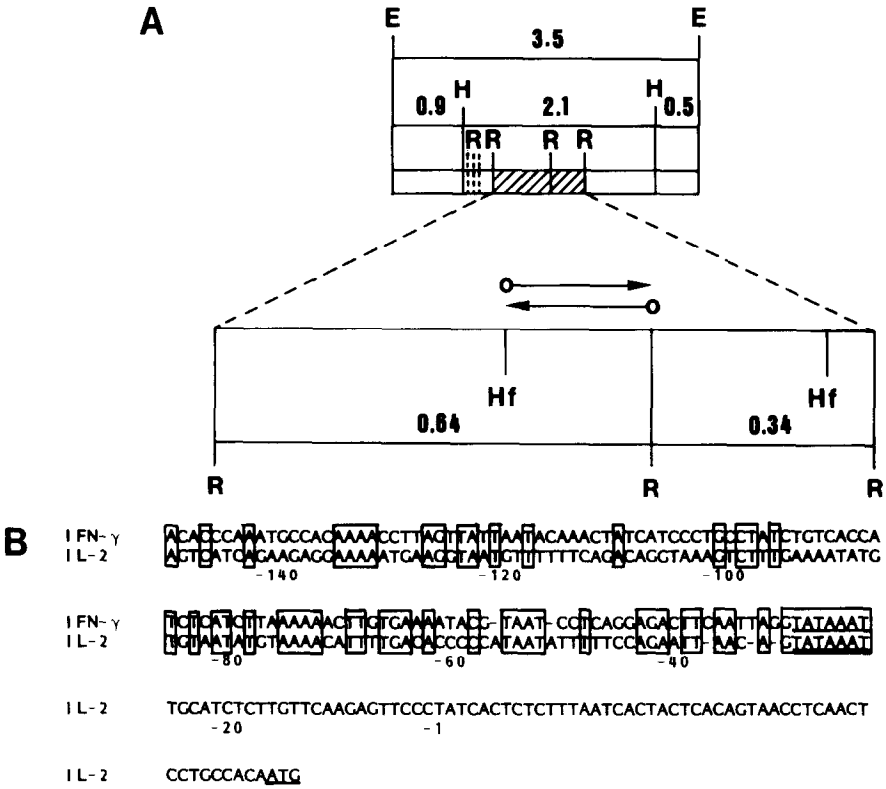

Fig. 3. Sequencing of the 5' region of the human IL-2 gene. (A), The sequencing strategy and physical map of the 5' region of the IL-2 gene. A hatched block indicates the RsaI fragments hybridizing with the 5' end probe. Numbers above the horizontal lines indicate the size of the fragments in kb. The end labeled sites are marked with open circles. The direction and extent of sequencing are shown by arrows. Symbols are EcoRI (E), HindIII (H), RsaI (R) and HinfI (Hf). Only those RsaI sites on the 2.1 kb HindIII fragment are shown. The vertical broken lines indicate RsaI sites, the exact positions of which have yet to be determined. (B), Nucleotide sequence of the 5' region of the human IL-2 gene and comparison of the sequence with that of the human IFN-γ gene. Nucleotides are numbered according to the positions from the putative initiation site for transcription upstream (-). The IFN-γ sequence is from Gray & Goeddel (7). A few gaps are introduced to increase alignment and (-) indicates introduction of one nucleotide gap. Identical nucleotides are enclosed. The initiation triplet ATG and the "TATA" box are underlined.

of the IL-2 gene are presented in Fig. 3 B. The 47 bp of the 5' untranslated region is in complete agreement with that of the previously sequenced IL-2 cDNA (5). The TATAAAT sequence is 32 nucleotides upstream from the putative transcriptional initiation site, and probably corresponds to the "TATA" box (8). A comparison of the sequences of the 5' flanking regions of IL-2 and

IFN-γ genes (7) revealed a 62% homology (Fig. 3). This homology extends from the "TATA" box upstream to nucleotide number -85 (Fig. 3). Another short segment of homology was noted from nucleotide number -117 to -134, as shown in Fig. 3.

We found no significant homology among the 5' flanking regions of the human IL-2, IFN-α (14) and β-globin genes (15) (data not shown). The presence of a homology in the 5' flanking regions of the IL-2 and IFN-γ genes suggests that these regions play an important role in the regulation of gene functions. We are now examining the role of the 5' flanking sequences.

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