Volume 11 Number 13 1983 Nucleic Acids Research

Molecular cloning of human interleukin 2 cDNA and its expression in *E. coli*

Rene Devos*, Geert Plaetinck, Hilde Cheroutre, Guus Simons, Wim Degrave, Jan Tavernier, Erik Remaut and Walter Fiers

Laboratory of Molecular Biology, State University of Ghent, Ledeganckstraat 35, B-9000, Ghent, Belgium

Received 6 May 1983; Accepted 14 June 1983

ABSTRACT

A recombinant plasmid containing human interleukin 2 (IL2) cDNA was identified in a cDNA library constructed from mRNA derived from PHA-TPA induced splenocytes. Using this cDNA as a hybridization probe, a DNA fragment containing the IL2 gene was isolated from a collection of hybrid phages derived from human genomic DNA. A unique reading frame was identified from the nucleotide sequence derived from these plasmids coding for a polypeptide of 153 amino acids and containing a putative signal sequence of 20 amino acids. A mature polypeptide starting with either Met-Ala-Pro or Met-Pro was expressed in E. coli under control of the E. coli trp promoter or using a combination of the phage λ P_L promoter and a ribosome binding site derived from phage Mu. The bacterial IL2 polypeptide had a molecular weight of 15,000 daltons and accounted for more than 10% of the total E. coli proteins in fully induced cells; it was biologically active in the T-cell specific DNA synthesis assay, even after recovery from a SDS-containing polyacrylamide gel.

INTRODUCTION

Human interleukin 2 (IL2 or T-cell growth factor) is defined as a protein whose activity allows the long-term proliferation of T-cells following interaction with antigen (1-3). It is an inducible protein synthesized and secreted by activated T-lymphocytes (4) and has been purified from various sources such as human peripheral blood lymphocytes (3,5,6), tonsilar lymphocytes (7), spleen lymphocytes (8), T-cell leukemia (9,10) and T-cell hybridoma cultures (11,12). The molecular weight reported for the SDS-denatured human IL2 ranges from 12000 to 17000 (3,11,13). It is believed that this molecular heterogeneity is dependent on the experimental conditions for the IL2 production (13) and may be explained perhaps by a variable degree of glycosylation (7,14). On the other hand, the finding that IL2 does not bind on a lectin column (3) would seem to indicate that the protein is unglycosylated. Recently, IL2-mRNA isolated from mouse (15), primate (16), human tonsilar (17) and peripheral blood lymphocytes (18) has been translated in Xenopus laevis oocytes, while IL2-mRNA, isolated from a human T-cell line was efficiently translated in a reticulocyte lysate (11). Monoclonal antibodies binding

human IL2 (19,20) and monoclonal antibodies specific for the human IL2 membrane receptor (21) have been prepared. Human IL2 preparations are active on human, rat and mouse IL2-dependent cells (22). IL2 augments NK cell-mediated cytotoxicity in addition to its activation by interferon (23). IL2 has been used extensively for cloning various T-cell subsets (24) and NK cells (25) suggesting a potential use for specific adaptive immunotherapy of neoplasia by systemic transfer of expanded lymphoid cells (26,27). Deficiency in IL2 production has been shown to occur in certain diseases characterized by immunodeficiencies (28,29) supporting its essential role for the full expression of the human immune response.

In this report, we describe the characterization of a human IL2 cDNA gene and its expression in E. coli. The method we have used for cloning the IL2 cDNA was essentially identical to the strategy used for cloning the human immune interferon (IFN-γ) cDNA gene (30). Recently, the human IL2 cDNA gene, derived from a T-cell leukemia was cloned and expressed in eukaryotic cells (31). The availability of an IL2 cDNA-containing plasmid will allow for the large scale production and purification of human IL2 derived from genetically engineered cells. We also report the isolation and characterization of the human genomic IL2 gene.

MATERIALS AND METHODS

1. Biological assay for interleukin 2

Mononuclear cells were isolated from human peripheral blood by means of 'lymphoprep' (Neygaard & Co.). The cells at the interphase were washed twice and cultured at a concentration of 5×10^5 cells/ml in RPMI 1640 medium , supplemented with 1% L-glutamine, antibiotics and 10% fetal calf serum. The cells were stimulated with phytohemagglutinin (PHA) 10 μg/ml (Wellcome) for 20 h at 37°C. The cells were then collected, intensively washed and cultured for another 3 days in medium without PHA. On the fourth day, the cells were supplemented with 5% of a partially purified human IL2 preparation [a 50%-80% $(NH_4)_2SO_4$ precipitate derived from the medium of PHA stimulated splenocytes, redissolved in 1/20 of the original volume, exhaustively dialyzed against phosphate buffered saline (2)]. Every 3 to 4 days, the cells were split at a ratio of 1/2. Living cells from cultures, approximately 2 to 3 weeks old were washed and concentrated in IL2-free medium with 40% fetal calf serum and 10% dimethylsulfoxide to give a final concentration of 2×10^7 cells/ml. Immediately thereafter, cells were frozen in 1 ml glass vials, using a controlled-rate freezer (CRYOSON), applying cooling rates of 1°C/min to -45°C and then quickly to -80°C. The target cells were then stored in liquid nitrogen (33). Immediately before use, the cells were thawed rapidly at 37°C, mixed stepwise with medium and washed. Viability was examined by Trypan blue

exclusion.

The IL2 microassay was performed according to Gillis et al. (4). 100 μl IL2 samples were serially diluted (1/2) in 96-well microtiter plates (Falcon) (in RPMI 1640, 10% fetal calf serum, 1% L-glutamine and antibiotics). The target cells were suspended at 2 x 10^5 cells/ml and 100 μl was supplied to the wells. Microtiter plates were incubated at 37°C for 24 h in a humidified atmosphere of 5% CO_2 in air. Next, 0.5 μCi of ^{3}H-labelled thymidine (Amersham 20-30 Ci/mmole) was added to each microplate well and cultured for an additional 5 h. Cultures were harvested onto glass filter strips (with a cell-harvester; type MASH II). ^{3}H-Thymidine incorporation was determined with a liquid scintillation counter.

The standard (an arbitrarily chosen human IL2 containing supernatant) was defined as containing 100 U/ml and induced maximal proliferation up to dilutions of 1/4 to 1/8 as measured by ^{3}H-thymidine uptake. 30% of the maximal activity of this sample was usually located in the centre of the linear descending part of the standard curve. Each test sample was also tested at serial dilutions and the titre was calculated by a graphical regression analysis. The activity in each test sample was converted into units according to Stadler et al. (20).

2. Construction and screening of cDNA plasmids

Human splenocytes from an individual donor were isolated (37) and cultured for 20 h at 37°C in the presence of PHA (10 μgml^{-1}) and TPA (10 ngml^{-1}). Total RNA was extracted (34) from the washed splenocytes and polyadenylated RNA isolated by oligo dT-cellulose chromatography (Type 3-Collaborative Research). 0.5 mg polyadenylated RNA was fractionated on a 5-20% sucrose gradient in TE-buffer (10mM Tris HCl, pH 7.5, 1mM EDTA) (16 h, 40K, Beckman SW41 rotor, 4°C). Fractions were precipitated with ethanol, dissolved in 30 μl distilled H_2O and assayed for IL2 mRNA activity by microinjection of 30 nl into *X. laevis* oocytes. After 3 days at 23°C, 100 μl of the oocyte bathing medium (containing 0.1% polyethylene glycol 6000 and 0.4% Aprotinin, Sigma) was used for measuring the ^{3}H-thymidine incorporation by PHA stimulated IL2 dependent human PBLs (4,20,33). ds DNA was synthesized (38) using 20 μg polyA$^+$ RNA from fractions for which IL2 activity was observed (3 fractions). The dsDNA between 600 and 750 bp (M3) and between 750 and 1000 bp (M2) was recovered from a 4% polyacrylamide gel, purified on hydroxyapatite and tailed with oligo dG using terminal deoxynucleotidyl transferase (PL Biochemicals). Next, the dsDNA was annealed with pSV529 DNA (39) of which the sticky ends resulting from Bam HI cleavage were filled in with AMV reverse transcriptase and further extended with dC residues. The annealed mixture was used to transform *E. coli* HB101 and resulting colonies derived from each dsDNA

size class (M2 and M3) were picked up and grown individually in wells of microtiter plates. Plasmid DNA was isolated (41) from overnight cultures (0.5 liter Brain Heart Infusion containing 100 μg ml^{-1} carbenicillin) each consisting of a mixture of 50 individual clones, purified by CsCl gradient centrifugation, cleaved with Bam HI followed by sucrose gradient centrifugation. The optical density peak corresponding to "insert-DNA" (30) (5 to 10 μg) was ethanol precipitated, dissolved in TE-buffer and immobilized onto nitrocellulose filters (42) (Schleicher & Schull BA85, 0.45 μm, 9mm^2 squares). A total of 30 filters (18 derived from group M2 and 12 derived from M3) were prehybridized for 2 h at 50°C in 0.5 ml hybridization mixture (40) without RNA and hybridized for 5 h at 50°C in an identical mixture in the presence of 50 μg $polyA^+$RNA isolated from PHA-TPA induced splenocytes, purified on a sucrose gradient and previously assayed for IL2 mRNA activity. RNA eluted from washed filters (40) was assayed for IL2 mRNA activity by microinjection into X. laevis oocytes as described above.

3. Induction of HIL2 in E. coli and SDS-PAG electrophoresis of E. coli extracts

Conditions for induced synthesis of HIL2 by plasmids in strain K-12ΔHIΔtrp were as described by Remaut et al. (46) with minor modifications. Induction of the trp promoter was obtained after tryptophan starvation of E. coli strain K514 growing in M9 medium (47). Cells were collected by centrifugation, dissolved in Laemli sample buffer (48) and electrophoresed in 15% polyacrylamide gels . Alternatively, cells were opened by sonication and after centrifugation, the supernatant and the cell pellet was taken up in Laemli sample buffer and electrophoresed as above.

4. Identification of genomic clones containing HIL2 DNA in human DNA libraries

The methods used in the identification were as described by Tavernier et al. (49).

RESULTS AND DISCUSSION

1. Isolation of IL2 mRNA from PHA-TPA induced human splenocytes

Human splenocytes (30) were stimulated with phytohemaglutinin (PHA) and a phorbol ester (TPA) (32) for 20 h at 37°C after which time the IL2 in the medium was assayed by measuring the proliferation of PHA-stimulated IL2-dependent human peripheral blood lymphocytes (PBLs) (4,20,33), and polyadenylated RNA was isolated from the collected cells (34). Further enrichment of IL2-specific mRNA was obtained by sucrose gradient centrifugation under non-denaturing conditions (35). IL2 mRNA was identified by microinjection of sucrose gradient derived fractions into Xenopus laevis oocytes (36) and the incubation medium was assayed (37) for

IL2 activity. A reproducible activity corresponding to mRNA which sedimented at 10S was observed for most of the individual splenocyte cultures (data not shown). This is in agreement with the sedimentation values reported for IL2 mRNA derived from human PBLs (18) and from human tonsilar lymphocytes (17). Kinetic studies showed that this IL2 activity still accumulated into the medium of microinjected oocytes after 3 days of incubation.

2. Contruction and identification of bacterial clones containing IL2-cDNA sequences

dsDNA was synthesized on polyA$^+$RNA (from a single donor) using standard procedures (38), fractionated by polyacrylamide gel electrophoresis and appropriate size classes, ranging in length from 600 to 750 bp (group M3) and 750 to 1000 bp (group M2) were tailed with oligo dG and inserted into the unique Bam HI site (filled in with AMV reverse transcriptase and tailed with oligo dC) of plasmid pSV529 (39) and used for transformation of E. coli HB101. Since the Bam HI site of the vector pSV529 was restored by the cloning procedure, "insert-DNA" was prepared by Bam HI-digestion of plasmid DNA isolated from mixtures of 50 clones. The "insert-DNA" was then purified by gradient centrifugation and bound onto nitrocellulose. Hybridization of 30 filters (18 filters of group M2 and 12 filters of group M3) with PolyA$^+$RNA derived from PHA-TPA induced splenocytes (and purified on a sucrose gradient) and subsequent elution (40), microinjection into Xenopus laevis oocytes and assay of the oocyte medium for IL2 activity yielded 2 filters, M3-2 and M3-6, which gave a clear positive signal (Table I). Subsequent hybridization of filters containing "insert-DNA" derived from 14 different subgroups (each containing 7 clones) from the group M3-2 with IL2-mRNA, led to the identification of the IL2-cDNA-containing plasmid (M3-2-32, Fig 1A). This was confirmed by hybridizing a filter containing only "insert-DNA" isolated from clone M3-2-32 with IL2-mRNA (Fig 1B). This first positive clone was renamed pSV-HIL2-0. Gel electrophoresis revealed that the Bam HI excised "insert-DNA" was approximately 750 bp in length. Colony hybridization (43,44) of 1300 colonies from group M2 and 1050 from group M3 with a ^{32}P-labelled (45) internal Hinf fragment (500 bp) derived from pSV-HIL2-0 resulted in the identification of one additional clone (M3-6-41, designated pSV-HIL2-1) in the group M3. This second clone is derived from group M3-6 which gave a positive signal in the first hybridization-elution translation screening assay (Table I). The Bam HI excised "insert-DNA", derived from the pSV-HIL2 plasmid preparations was purified by sucrose gradient centrifugation and mapped using restriction enzymes. Further characterization of these insert-DNAs revealed that the insert present in pSV-HIL2-1 was only 250 bp in length and corresponded to an internal sequence within "insert-DNA" derived from pSV-HIL2-0. Such a small insert

TABLE I

Identification of a human IL2 cDNA containing plasmid in a group of 50 individual bacterial clones

Filter No.	Group	cpm*	Filter No.	Group	cpm*
1	M2-1	569	16	M2-16	338
2	2	533	17	17	302
3	3	386	18	18	357
4	4	305	19	M3- 1	248
5	5	293	20	2	7238
6	6	410	21	3	322
7	7	254	22	4	329
8	8	333	23	5	366
9	9	297	24	6	1719
10	10	235	25	7	345
11	11	253	26	8	311
12	12	297	27	9	397
13	13	411	28	10	387
14	14	263	29	11	310
15	15	428	30	12	373
C1		11654			
C2		3861			
blank		314			
standard		20576			

C1 : (Control 1) injection into Xenopus laevis oocytes of polyA$^+$RNA before use in the hybridization-elution assay.
C2 : (Control 2) injection into Xenopus laevis oocytes of polyA$^+$RNA after hybridization with the nitrocellulose filters (non-hybridized RNA).
blank : 100 μl complete medium (RPMI 1640 + 10% FCS) used in the IL2 assay.
standard : partially purified human IL2 preparation from PHA/TPA induced splenocyte cultures.
cpm : ^{3}H-thymidine incorporation of 1/4 dilution of oocyte incubation medium by PHA-stimulated IL2 dependent human peripheral blood lymphocytes.

DNA probably originated through internal dG-tailing at a nick present in the sized ds-cDNA.

3. Nucleotide sequence of the human IL2-cDNA gene and deduced amino acid sequence

a.Characterization of the "insert-DNA" derived from pSV-HIL2-0

Fig. 2 shows a physical map of the IL2 gene together with the sequencing strategy and the restriction map. The nucleotide sequence of the insert derived from plasmid pSV-HIL2-0 and completed with sequence information from the genomic clone is shown in Fig. 3. A nucleotide sequence typical for the 3' end of a eukaryotic mRNA containing the potential polyadenylation signal AAUAAA was identified. Although an ATG start codon

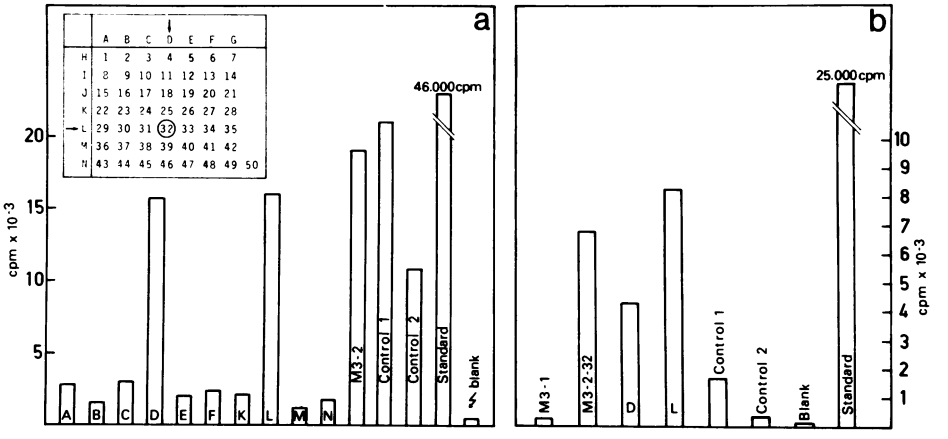


Fig. 1. Screening for a human IL2 cDNA-containing clone in subgroups of M3-2 (A) and identification of an individual clone (B).
A. Plasmid DNA was isolated from 0.5 liter cultures, each containing a combination of 7 individual clones (group A to M, except group N which contained 8 clones, see insert). The DNA was cleaved with Bam HI and the excised "insert-DNA" purified by sucrose gradient centrifugation and immobilized on nitrocellulose filters. Next the filters were hybridized with 80 μg sucrose gradient purified polyA$^+$RNA derived from PHA-TPA induced splenocytes, washed and the eluted RNA injected into X. laevis oocytes. The oocyte bathing medium, C1, C2, standard and blank (see legend to Table I) was used in the IL2 microassay (filters G, H, I and J were not tested).
B. Hybridization-elution-translation assay of filters containing insert DNA isolated from group M3-1 (negative control), subgroups D and L and the individual clone M3-2-32.

and part of its 5'-coding region was missing from the cDNA clone, the nucleotide sequence of pSV-Hil2-0 shows a unique reading frame of 134 amino acids (Fig.3). No potential N-glycosylation sites could be identified within this amino acid sequence. To establish the number of missing nucleotides at the 5'-end of the IL2-cDNA insert, a short (63 bp) restriction endonuclease fragment (Alu I - Hinf I, nucleotide 36-99 in pSV-HIL2-0) (see Fig 2) was isolated, 5'-labelled, strand separated and extended by reverse transcription after hybridization to polyA$^+$RNA derived from induced splenocytes. Analysis of the cDNA product on a denaturing polyacrylamide gel showed a band of around 210 nucleotides (Fig.4). From this result, we can deduce that the insert in pSV-HIL2-0 is approximately 110 nucleotides shorter than the full-length IL2 mRNA (210 minus 99 nucleotides).

b. The nucleotide sequence of the 5'-end of the human IL2 cDNA gene. Isolation of a genomic IL2 gene

To extend further towards the 5'-end the nucleotide sequence of our

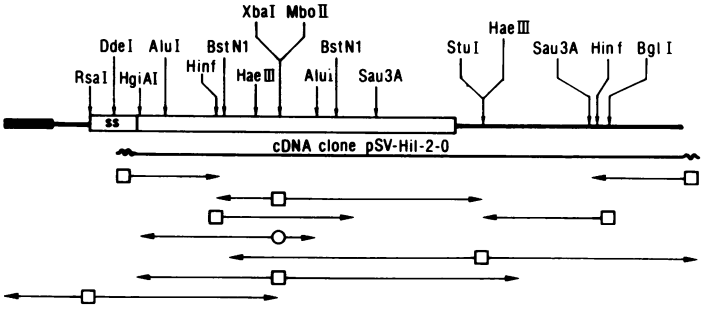


Fig. 2. Restriction map and sequencing strategy for the human IL2 gene. The diagram shows a schematic representation of part of the putative promoter (black box at left), followed by the transcribed region consisting of the 5' untranslated segment (line), the signal sequence (SS bar), the coding region (open bar) and the 3' untranslated sequence (line). The cDNA clone, which started at the second last codon of the signal sequence, is indicated; the wavy line refers to the G/C-tails. The sequence upstream from the cDNA information was derived from a genomic clone. Some restriction sites are indicated on top; all were experimentally verified. Below, the sequencing strategy by the Maxam-Gilbert procedure (55) is given. Open squares correspond to ^{32}P-labeled 5'-ends and open circles indicate 3'-end labeling.

original cDNA clone, we isolated a genomic clone containing the IL2 gene from a human DNA library. Southern hybridization (50) of high molecular weight human DNA, digested with various restriction endonucleases, with ^{32}P-labelled IL2 cDNA suggested that there is only one IL2 gene in the human genome (data not shown). Two human gene banks (51, 52) plated on *E. coli* BHB 2600 were screened in situ (53) using a ^{32}P-labelled IL2-cDNA specific Hinf fragment derived from pAT153-HIL2 (see next section). A total of 5 hybridization-positive phage plaques were isolated from the two banks. One of these (λCH4A-gHil2-1) was shown to contain the total human IL2-gene and was analysed in further detail. The total IL2 gene was then subcloned in pUR250 (54) as two Eco RI restriction endonuclease fragments (pUR-gHil2-1 and pUR-gHil2-2). pUR-gHil2-1 was shown both by restriction analysis and hybridization data to contain the promoter region and the 5'-part of the transcribed human IL2 gene. Detailed restriction analysis of both subclones gave evidence for the presence of at least two introns in the coding region (data to be reported elsewhere). Fig. 3 also shows the nucleotide sequence of the 5'-end of the IL2 gene as deduced from pUR-gHil2-1. The presence of an ATG initiation codon, approximately 60 nucleotides in front of the nucleotide sequence overlapping our original cDNA clone, resulted in a total coding sequence of 153 amino acids. Since the IL2 mRNA is approximately 110 nucleotides longer than the cDNA clone,

Fig. 3. Nucleotide sequence and deduced amino acid sequence of the human interleukin 2 gene. Data derived from the genomic clone (pUR-gHil2-1) is underlined and the sequence, preceding the start of the IL2 mRNA and corresponding to part of the putative promotor, is shown (as DNA) in italics. A TATAAAT-consensus sequence (overriding bar) is present 32 nucleotides upstream from the putative first nucleotide (indicated by an asterisk) of the IL2 mRNA. (This tentative identification of the first nucleotide of the transcript is based on the results of Taniguchi et al. (31) who obtained a full size cDNA clone, and is in agreement with our mRNA mapping data shown in Fig. 4.) The arrow indicates the presumed signal sequence cleavage site (based on the known N-terminal amino acid sequence of natural human IL2). The poly-A signal, AAUAAA, is underlined. The numbers above each line refer to amino acid positions, while those below, to nucleotide positions. Note that the second to last codon, CUG for leucine, was a CUA in the sequence of Taniguchi et al. (31), which indicates a silent substitution at position 503.

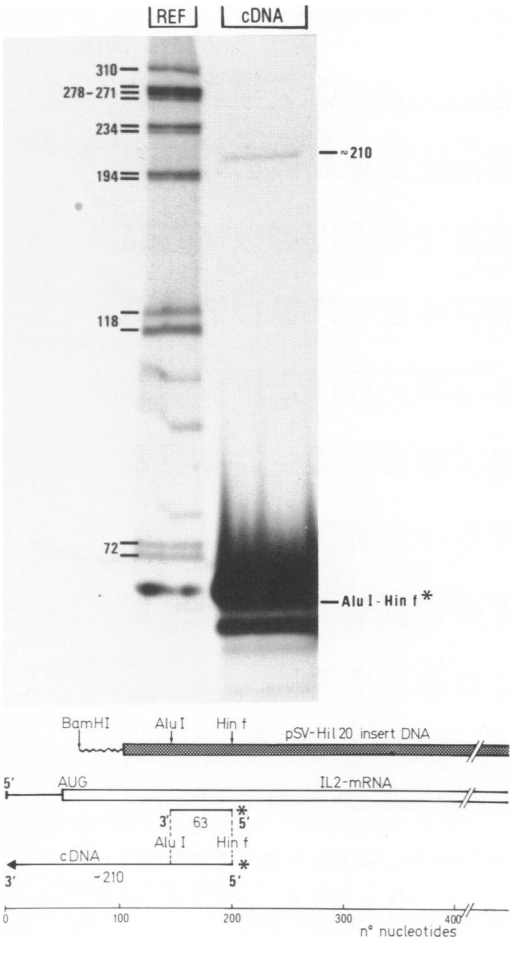


Fig.4. Characterization of the primed extension product from HIL2-mRNA.cDNA. The autoradiogram shows the product obtained after extension of a 63 nucleotide-long restriction fragment hybridized to polyA RNA (derived from mitogen induced splenocytes). Electrophoresis was on a 6% polyacrylamide gel containing 7M urea. Ref: 5'-^{32}P-labeled HaeIII digest of X174-DNA was used as size markers.

pSV-HIL2-0, the presence of this ATG codon results in a 5' non-coding region of approximately 50 nucleotides. We have renumbered our nucleotide sequence on the basis of the results obtained on a presumably complete human IL2 cDNA clone reported recently by Taniguchi et al. (31). Indeed, our sequence data revealed the presence of a TATAAAT-concensus segment, typical for a eukaryotic promoter at position -32 nucleotides as expected. A putative signal peptide sequence of 20 amino acids can be identified (Fig. 3), cleavage of which results in a mature polypeptide of 133 amino acids having a molecular weight of 15,400. The amino acid sequence shows an almost identical number of basic and acidic residues, explaining the neutral pI value observed for the natural human IL2 (3). The polypeptide sequence contains three cysteine residues, two of which

might be involved in an intramolecular disulfide bridge.

The nucleotide sequence we have determined for the IL2 mRNA and amino acid sequence deduced for IL2 is in complete agreement with the data obtained by Taniguchi et al. except for the nucleotide at position 503 which we determined to be G as opposed to A. This substitution does not lead to a change in the amino acid sequence. It should be noted that our clone was derived from normal splenocytes while the one of Taniguchi et al. (31) was derived from the human Jurkat 111 cell line. Also, our assays were done on human PBL while Taniguchi et al. used the mouse cell line CTLL-2. The lack of a potential N-glycosylation site explains why IL2 is not retained on lectin columns (3). The change in heterogeneity of the pI value after treatment with neuraminidase (14) and the observed heterogeneity in molecular weight distribution after induction of IL2 by different stimuli (13) remains to be explained.

4. Expression of human IL2 in E. coli

a. Construction of plasmids

Mature IL2 starts with an alanine residue. From the nucleotide sequence it can be seen that the second amino acid residue (Pro) can be easily exposed after HgiAI cleavage and resection of the 3' protruding end with T_4 DNA polymerase. To add an alanine codon to the 5'-end, the following successive cloning steps were carried out (Fig. 5). The Bam HI insert, derived from pSV-Hil2-0, containing the mature IL2 coding region was inserted into pAT153. The new plasmid, pAT153-Hil2, was cleaved with HgiAI, treated with T_4 polymerase and, after cleavage with Bam HI, the resulting 700 bp fragment was ligated between a Bam HI site and a filled-in Nar I site of pAT153. The reconstructed Nar I site (GGCGCC) is also a Ban I site (isochisomer of HgiCI). The enzyme Ban I cleaves the recognition sequence between the two G residues leaving 5' protruding ends ($G^{\downarrow}GCGCC$). Following filling-in, a GCG codon (Ala) is added to the 5' end of the coding sequence of mature IL2. After Bam HI cleavage, the fragment was cloned between a filled-in Nco I site and a Bam HI site of the expression vectors pPLcMu299 and pTrp321 (obtained form G. Buell, Biogen, S.A.). In these plasmids, the Nco I site is located at the initiator ATG such that the latter can be made accessible for blunt ligation after Nco I cleavage and filling-in with DNA polymerase I (Klenow fragment). Plasmid pPLcMuHIL201, contains the complete coding region for mature IL2 in phase with the initiator ATG of a ribosome binding site derived from phage Mu. The sequence at the initiator ATG then is: TTAGGAGGGTTTTTACC ATG.GCG.CCT. In plasmid pTrpHIL201, the ribosome binding site and initiator ATG derived from the trp attenuator region were used. The sequence at the initiator ATG reads: AAAGGGTATCGATTCC ATG.GCG.CCT. Both plasmids were shown to indeed contain a unique Nco I site as predicted by joining the GCG codon to the blunted Nco I site of the acceptor plasmids.

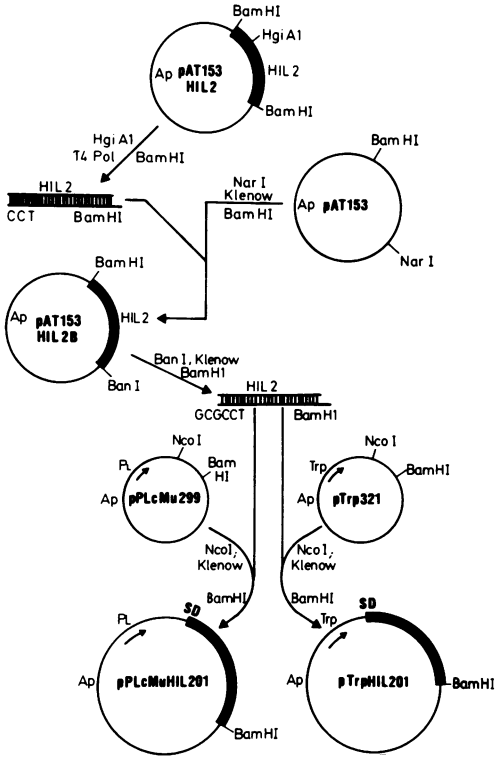


Fig. 5. Plasmid constructions for expression of HIL2 in E. coli (see text for details). Only those restriction sites relevant in the construction scheme have been indicated.

We have also constructed plasmids in which the Pro residue is directly joined to the initiator ATG of both pPLcMu299 and pTrp321. To do this, the blunted HgiA1 site was directly ligated to the filled-in NcoI site of the expression vectors. The resulting plasmids were designated pMuHIL21 and pTrpHIL21. A further derivative, pPLcMuHIL22 is essentially identical to pPLcMuHIL21 except that it contains a smaller vector part derived from pPLc28 (46).

Expression studies with vectors using the P_L promoter were carried out in strain K12ΔH1Δtrp (47). The cells were grown at 28°C and induced at 42°C (46). Induction of the trp promoter was obtained after tryptophan starvation of strain K514λ(48). To test for biological activity of bacterially synthesized human IL2, the cells were opened by sonication; cell debris was spun down and the supernatant was passed through a 0.2μ Millipore filter. These extracts were then assayed for T-cell growth activity. From the results shown in Table 2, it can be concluded that extracts of induced cultures contain IL2 activity, while similar extracts from uninduced cultures scored negative. Both the protein initiating with Met-Ala-Pro, as well as the one initiating with Met-Pro, were biologically

Table II
Expression of HIL 2 in E. coli

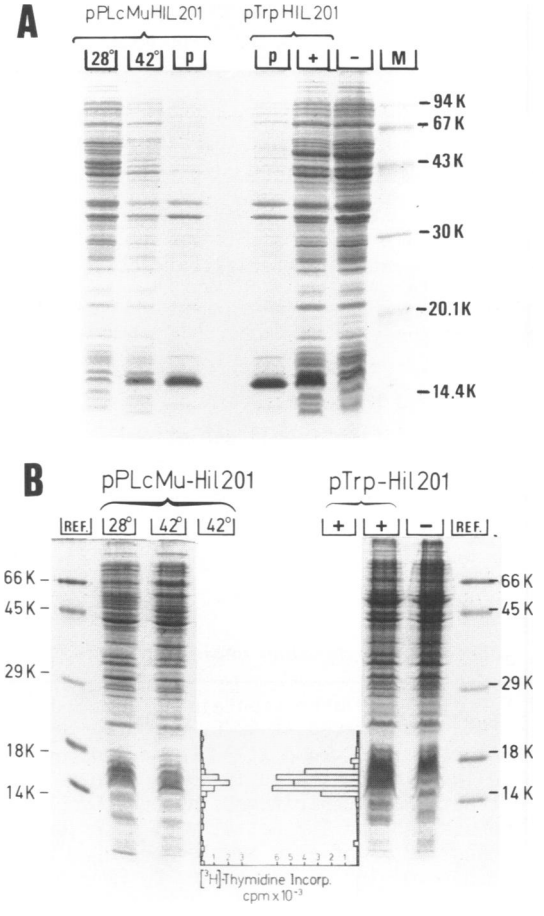
Sample		IL2 Units /ml
A. HIL2 starting with Met-Ala-Pro		
HIL2 standard		100
" "	+ 25% control bacterial extract	70
" "	+ 2.5% " " "	81
pPLcMu-Hil201	28°C	<1
"	42°C	850
pTrp-321		<1
pTrp-Hil201 (induced by tryptophan depletion)		3800
B. HIL2 starting with Met-Pro		
pPLcMu-Hil22	28°C	<1
"	42°C	250
pTrp-Hil21 (induced by tryptophan depletion)		2100

10 ml cultures of E. coli K12ΔH1Δtrp (containing pPLcMu-Hil2 plasmids) grown at 28°C and induced at 42°C, or of E. coli K514λ (containing pTrp-Hil2 plasmids) grown at 37°C and induced by depletion of exogenous tryptophan) were concentrated 10 fold. The cells were lysed by sonication. The extracts were centrifuged, passed through a 0.2μ Millipore filter and assayed for IL2 activity as described in Materials and Methods. 1, 10 and 100 μl of each sample were used at serial dilutions (1/2) and ^{3}H-thymidine incorporation by PHA-stimulated human peripheral blood lymphocytes was measured. IL2 was quantified as described (4,20,33).

active. However, it is not known whether the specific activity of the product starting with Met-Ala-Pro is different from the specific activity of the product beginning with Met-Pro.

b. Physical detection of HIL2 and recovery of biologically active bacterially derived HIL2 from SDS-containing polyacrylamide gels

Total bacterial extracts of E. coli, harboring either plasmid pPLcMu-Hil201 (induced at 42°C) or plasmid pTrp-Hil201 (induced by depletion of exogenous tryptophan) were denatured in SDS and analysed in SDS-containing polyacrylamide gels(48). As shown in Fig 6, a new protein having a molecular weight of about 15K can be detected. This protein is absent in extracts prepared from identical cultures grown at 28°C (for pPLcMu-Hil201) and in extracts prepared from a control culture not



A. Physical detection of bacterially derived HIL2 in a SDS-polyacrylamide gel. 28° and 42°: electrophoresis of total bacterial extracts containing plasmid pPLcMu-Hil201 grown at 28°C and 42°C, respectively. - and +: electrophoresis of total bacterial extracts containing plasmid pTrp321(-) or plasmid pTrp-Hil201(+). p: electrophoresis of bacterial debris obtained by centrifugation of a sonicated bacterial culture containing either plasmid pPLcMu-Hil201 (grown at 42°C) or pTrp-Hil201.
B. Recovery of biologically active bacterially derived HIL2 from SDS-polyacrylamide gel. 28°and 42°: electrophoresis of total bacterial extracts containing plasmid pPLcMu-Hil201 grown at 28°C and 42°C, respectively. - or +: electrophoresis of total bacterial extracts containing plasmid pTrp321 (-) or plasmid pTrp-Hil201(+).

carrying plasmid pTrp-Hil201 and grown at 37°C. From the intensity of the stained band, the protein was estimated to have accumulated to about 5% and 10% of the total cellular protein in the cases of pPLcMu-Hil201 and

pTrp-Hil201, respectively. E. coli harboring plasmids pPLcMu-Hil22 and pTrp-Hil21, designed to express IL2 initiating with Met-Pro, also led to the inducible synthesis of a 15K protein estimated at about 5% and 10% of total cellular protein, respectively (data not shown). SDS denaturation and gel electrophoresis of the bacterial debris obtained after clearing sonicated bacterial extracts by centrifugation showed that most of the synthesized IL2 remains insoluble (Fig 6.A). The biological activity found in the cleared extracts (Table 2.A and 2.B), therefore, is not quantitative and in no relation to the total bacterial synthesis. To determine whether the biologically active HIL2, present in the bacterial cleared extracts, corresponds to the inducible protein having a molecular weight of 15K in SDS-polyacrylamide gels, the total bacterial extracts were denatured with SDS and β-mercaptoethanol and electrophoresed as for Fig 6A. The gel was then cut into 2 mm slices and each slice crushed into 0.2 ml RPMI 1640 + 10% FCS medium. Following overnight incubation at 37°C and centrifugation, the supernatant was removed and the IL2 activity determined as described above. The results show (Fig 6.B) that the HIL2 biological activity, indeed, comigrates with the strong protein band present upon induction of cells containing either plasmid pPLcMuHil201 or plasmid pTrpHil201.

Purification of this bacterially expressed IL2 will provide large amounts of human IL2 needed for studies on cloning and culturing antigen specific T-cell lines. The availability of pure recombinant derived lymphokines such as IFN-δ and IL2 will certainly lead towards a better understanding of the different factors involved in effector cell functions in the immune system.

ACKNOWLEDGEMENTS

We thank Dr. P. Kestens of the UCL-St. Luc Hospital, Brussels; Dr. F. Derom and Dr. J. De Roose of the Academisch Ziekenhuis, Ghent; Dr. P. Kinnaert and Dr. E. Dupont of the Erasmus Hospital, Brussels; Dr. A. Bremer of the St. Pierre Hospital, Brussels; Dr. M. De Broe of the Academisch Ziekenhuis, Antwerp; Dr. J. Nemery of the Algemeen Ziekenhuis, St. Jan, Brugge and Dr. A. Morraye for providing us with human spleens and human tonsils. We also are indebted to Dr. G. Buell, Dr. B. Allet and Dr. E. Kamashima (Biogen S.A., Geneva) for providing us with the expression plasmids pPLcMu299 and pTrp321 as well as valuable information. We thank Chris Opsomer, Rose Wulgaert and Fred Duerinck for excellent help with certain parts of this work and Fred Shapiro and Wim Drijvers for their help in preparing this manuscript. This research was supported by Biogen, S.A.

*Present address: Biogent, Plateaustraat 22, B-9000, Ghent, Belgium

Abbreviations: HIL2, human interleukin 2; IFN, interferon; PBL, peripheral blood lymphocytes; PHA, phytohemagglutinin; FCS, fetal calf serum; TPA, 12-O-tetradecanoyl phorbol-13-acetate

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