

# Cloning and expression of human gamma-interferon cDNA in *E. coli*

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## Abstract

Prior to the production of human gamma interferon using recombinant DNA technology, it had been produced mainly upon mitogenic induction of lymphocytes in very low amounts, which evidently hampered its characterization and its medical applications. The recombinant gamma interferons produced in larger quantities in prokaryotic systems retain their biological activities, and can be used clinically in the treatment of various viral, neoplastic and immunosuppressed conditions or diseases. In this study, a cDNA sequence coding for human gamma interferon was synthesized from mRNA template extracted from induced human T lymphocytes. The cDNA was then amplified by PCR, cloned in an expression vector, and transformed into *Escherichia coli*. The polypeptide produced through the expression of this DNA sequence in *E. coli* showed immunological and chemical properties resembling authentic human IFN- $\gamma$ .

**Keywords:** Gamma interferon, complementary DNA, Polymerase chain reaction, cloning, expression, polyclonal antibodies, Immunoblotting.

## INTRODUCTION

Interferons are secretory proteins, which are produced by leucocytes, fibroblasts and lymphocytes. Their role in anti-tumor and anti-viral responses has been established (Gray and Goeddel, 1982). Human interferons are divided into 3 major groups based

upon characteristics such as antigenicity, biological based and chemical traits. These groups are called alpha beta and gamma interferons. The gene cluster encoding the interferons contains 12 or more genes for alpha and one for beta interferon with 40 to 50% similarities at the DNA level. Gamma interferon (IFN- $\gamma$ ), which is produced by T lymphocytes, encoded by a gene located on chromosome 12. The gene has 3 introns and its final product is a glycoprotein consisting of 146 amino acids containing 30% carbohydrates. It has been shown that the carbohydrate of this molecule is not necessary for its biological activity (Gray and Goeddel, 1982; Bloom, 1980; Zoon *et al.*, 1980; Taniguchi *et al.*, 1980; Yip *et al.*, 1981; Fleishmann, 1981; Langford *et al.*, 1979; Reddy *et al.*, 1997).

IFN- $\gamma$  operates through attachment to specific receptors on the target cells. A broad range of biological activities has been attributed to IFN- $\gamma$  including specific antiviral activities, potentiation of the antiviral activities of IFN- $\alpha$  and - $\beta$ , and preventing the growth of neoplastic cells and macrophages. Also it boosts the production of the major histocompatibility complex (MHC) II molecules on macrophages and other cells (Bass, 1997; Reddy *et al.*, 1997; Gray *et al.*, 1982; Falkner and Maurer-Fogy 1996; Musch *et al.*, 1998; Blackburn *et al.*, 2000; Asao and Fu, 2000).

Earlier, the production of IFN- $\gamma$  for structural studies and clinical use was mainly limited to human cell sources, by exposing T lymphocytes to mitogenic stimuli, or by translating mRNA in oocytes. Besides the low expression, there are also various problems in the purification of this biomolecule. With the development of recombinant DNA technology, human

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IFN- $\gamma$  cDNA sequence has been identified and cloned into a prokaryotic expression vector. It was then proved that the produced recombinant IFN- $\gamma$  has the same immunological and chemical properties as authentic IFN- $\gamma$  (Langford *et al.*, 1979; Reddy *et al.*, 1997; Nathan *et al.*, 1981; Gray *et al.*, 1982; Beyer *et al.*, 1998). Moreover, other procedures for mass production and purification of these products were simplified (Langford *et al.*, 1979; Vassileva-Atanassova *et al.*, 2000; Zlateva *et al.*, 1999; Arora and Khanna 1996; Kendrick *et al.*, 1998; Blackbourn *et al.*, 2000; Xu *et al.*, 1997; Tatsumi and Sata, 1997).

The aim of this study was to clone the human IFN- $\gamma$  encoding cDNA in a suitable prokaryotic expression vector in order to produce the protein in *E. coli*. Upon biochemical characterization of the recombinant protein, methods for large scale expression and purification and finally, studying its biological activities will be the next goals of this research work. There is no doubt that mass production of the recombinant products in the country is of particular importance because of high demand and high economic importance.

## MATERIALS AND METHODS

### Mitogenic stimulation of human lymphocyte:

Human blood lymphocytes were isolated using Ficoll (Sigma, USA) and the viability assay was performed using a standard hemacytometer chamber. About  $10^5$ - $10^6$  cells were added to each well of the 24 wells cell culture plate containing RPMI culture medium and 10% Fetal Calf Serum (FCS) (GIBCO-BRL, Germany). Cell proliferation was observed during 72 hours after the stimulation of the cells with phytohemagglutinin (PHA) (Sigma, USA).

**cDNA synthesis:** Total RNA was extracted from the stimulated T-lymphocytes using the TRIZOL™ solution (GIBCO-BRL, Germany). The quality of the purified RNA was determined by denaturing agarose gel electrophoresis. Then, mRNA extraction was performed using the mRNA extraction kit (Roche, Germany). The cDNA synthesis was performed using Roche first strand cDNA synthesis kit. Briefly, purified poly(A)-mRNA was used in reverse transcription reaction mixture containing oligo-dT primer (10  $\mu$ M), reverse transcriptase (RT) buffer (10x), dNTP (1 mM), RNase inhibitor (50 units) and RT enzyme (20 units) and incubated at 37°C for 1 hour.

**Primer design:** Two gene-specific primers (FA1 and FA2) were designed using the human gamma-interferon DNA sequence available in the GenBank database (Accession number, E00598):

1. FA1:

5'- TTGGGTGAGCTCGGCTGTTACTGCCAGGACCCA-3'  
(with *SacI* site underlined).

2. FA2:

5'-AGGAGCGGCCCGCTCTGGGATGCTCTTCGACCT-3'  
(with *NotI* site underlined).

### PCR amplification of the IFN- $\gamma$ encoding gene:

*SacI* and *NotI* restriction enzyme sites were introduced in the FA1 (upstream) and FA2 (downstream) primers, respectively. A standard PCR protocol using the synthesized cDNA, gene-specific primers (FA1 and FA2), 10x PCR buffer, dNTP (2 mM) and *Taq* DNA polymerase (5 units/ $\mu$ l) (Roche, Applied Science, Germany) was employed. Altogether, 35 cycles, including 94°C for 1 min, 57°C for 1 min and 72°C for 1 min for each cycle was carried out using Perkin-Elmer, 9600 thermal cycler. The PCR products were then analyzed by agarose gel electrophoresis.

**Cloning procedure:** The PCR products were digested in a reaction buffer containing 10x buffer and the two *SacI* and *NotI* restriction enzymes (Roche, Germany), and incubated at 37°C for 5 hours. The vector (pHEN1 plasmid) (a pUC derivative) (BioLabs, England) was also digested with the same enzymes under similar conditions. The digested products were then purified with a DNA purification kit (Roche, Germany). The 3:1 molar ratio of vector-insert was used for the ligation reaction containing 10x ligase buffer and T4 DNA ligase enzyme (Roche, Germany) and incubated at 18°C overnight. The ligated material was used for the transformation of *E. coli* (TG1 strain) (Stratagene, USA) according to the standard protocol (Sambrook *et al.*, 1989).

**Colony-screening by PCR:** The obtained colonies from the transformation experiment were used in a PCR-colony assay using one universal reverse primer (URP) in pHEN1 vector (upstream) and one gene-specific primer (FA2) (downstream). The ampicillin resistant colonies were used as DNA template in a standard PCR protocol (as mentioned above) and cycled 35 times: 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds.

**Restriction enzyme mapping of the PCR products:**

The amplified PCR products were digested with *DraI* restriction enzyme (Roche, Germany). There are two adjacent *DraI* sites in the middle of the IFN- $\gamma$  DNA sequence.

**Plasmid preparation and digestion:** The positive colonies from the PCR-screening experiment were grown in LB-AMP (100  $\mu$ g/ml) glucose (1%) (Sigma, USA) and plasmid preparation was performed according to the standard protocol (Sambrook *et al.*, 1989). The prepared plasmids were then digested with the same enzymes used for cloning and analyzed by agarose gel electrophoresis.

**Sequencing:** One of the recombinant clones, D4, was sequenced using an ABI 373A automated sequencer and the obtained data were compared with GenBank database using the Blast engine search program.

**Production of polyclonal antibody:** Two rabbits (Razi Institute, Iran), aged 12 weeks, were immunized with 50  $\mu$ g of a commercial human IFN- $\gamma$  plus complete Freund's adjuvant (Sigma, USA). After two weeks the reminding injection was given with the same amount of antigen but supplemented with incomplete Freund's adjuvant. The third and fourth injections were performed after every two weeks using 25  $\mu$ g of antigen without adjuvant. Blood was collected from the rabbits before and after each injection. To detect the maximum amount of antibody in the serum, blood was also collected on the fourth, fifth and sixth days after the fourth injection. The specificity of antibody against IFN- $\gamma$  was determined by ELISA method (Friguet *et al.*, 1989) and Western blotting using standard protocol (Sambrook *et al.*, 1989).

**Subcloning of IFN- $\gamma$  encoding gene into pET21a:**

Recombinant plasmid (pHEN1+insert) was digested with *SacI-NotI* restriction enzymes and the IFN- $\gamma$  DNA fragment was recovered from agarose gel using Roche DNA purification kit. Plasmid DNA pET21a (expression vector) was also cut with the same restriction enzymes and purified with DNA purification kit (Roche, Germany). A 3: 1 molar ratio of vector-insert was used for the ligation reaction containing 10x ligase buffer and T4 DNA ligase enzyme (Roche, Germany) and incubated at 18°C overnight. The ligated material was used for the transformation of *E. coli* (B121 strain) (Stratagene, USA) according to the stan-

dard protocol (Sambrook *et al.*, 1989). Recombinant clones were screened by colony-PCR assay and restriction enzyme digestion as described above.

**Expression of IFN- $\gamma$  cDNA in *E. coli*:** For protein expression, from an overnight pre-cultured (1 ml) recombinant clones carrying the IFN- $\gamma$  gene, new cultures (10 ml) of 2x TY-Amp (100  $\mu$ g/ml) glucose (1%) (Sigma, USA) were inoculated and grown to OD<sub>600</sub>=0.7. Following this procedure, the cells were pelleted and washed once with fresh medium. Finally, each pellet was resuspended in 10 ml 2x TY-Amp contained 1 mM Isopropylthio- $\beta$ -D-galactoside (IPTG) (Sigma, USA) and grown for overnight at 28°C. The expressed protein in cytoplasm was extracted and analyzed by the SDS-PAGE. The Western blotting technique was performed according to the standard protocol (Sambrook *et al.*, 1989). As primary antibody rabbit antisera against IFN- $\gamma$ , and as the secondary antibody-conjugate goat-anti-rabbit HRP were used (Sigma, USA).

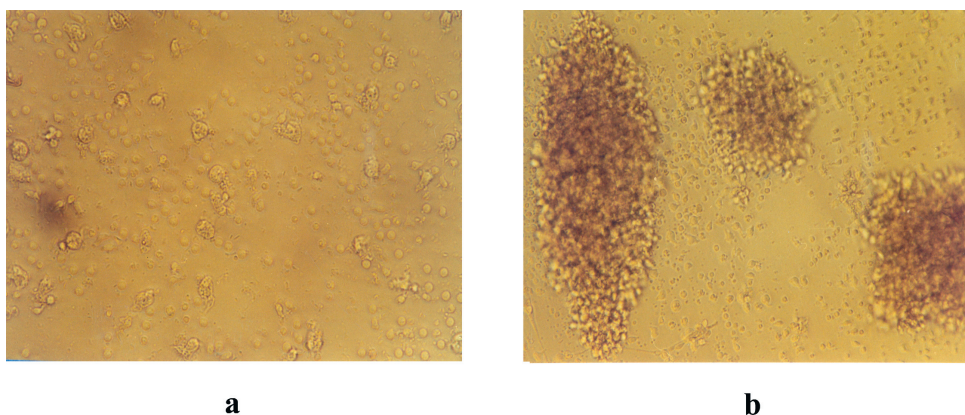
## RESULTS

Cultured human T lymphocytes were stimulated by PHA and the proliferated cells were used for mRNA extraction and cDNA synthesis. The result is shown in figure 1.

For PCR experiments, two gene-specific primers were designed according to the available DNA sequence of IFN- $\gamma$  in GenBank. Figure 2 shows the DNA sequence of the IFN- $\gamma$  and the position of the FA1 (upstream) and FA2 (downstream) primers. As it is obvious in the sequence, the encoding human IFN- $\gamma$  DNA consists of 498 bp, 60 bp of which is leader sequence and the rest of the sequence (438 bp) is related to the interferon protein. The designed primers only amplified the coding region of the IFN- $\gamma$  (146 aa) and the leader segment has been excluded.

PCR experiments were carried out using the gene-specific primers and the synthesized cDNA and these results are shown in figure 3a in which a specific band of 461 bp in length is evident.

In order to primarily confirm that the amplified PCR products are indeed the authentic IFN- $\gamma$  gene, a restriction enzyme mapping was performed on the PCR product. There are two consecutive *DraI* restriction enzyme sites in the middle of IFN- $\gamma$  coding region



**Figure 1. Stimulation of human T lymphocytes by PHA (10 mg/ml).** a) uninduced cells, b) induced cells. In these figures the resting (a) and induced cells (b) are compared and three major clones of lymphocytes are evident upon induction by PHA.

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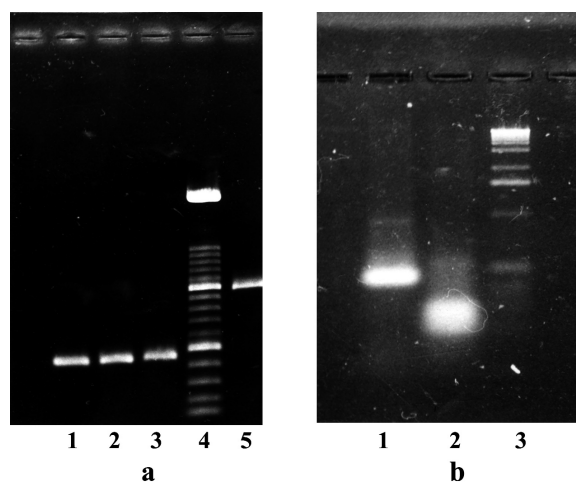
1   gatcagcttg atacaagaac tactgatttc aacttctttg gcttaattct ctcggaaacg
61  atgaaatata caagttatat cttggctttt cagctctgca tcgttttggg ttctcttggc
121 tgttactgcc aggacccata tgtaaaagaa gcagaaaacc ttaagaaata ttttaatgca
181 ggtcattcag atgtagcgga taatggaact cttttcttag gcattttgaa gaattggaaa
241 gaggagagtg acagaaaaat aatgcagagc caaattgtct ccttttactt caaacttttt
301 aaaaacttta aagatgacca gagcatccaa aagagtgtgg agaccatcaa ggaagacatg
361 aatgtcaagt ttttcaatag caacaaaaag aaacgagatg acttcgaaaa gctgactaat
421 tattcggtaa ctgacttgaa tgtccaacgc aaagcaatac atgaactcat ccaagtgatg
481 gctgaactgt cgccagcagc taaaacaggg aagcgaaaaa ggagtcagat gctgtttcga
541 ggtcgaagag catcccagta atggttgtcc tgcttgcaat

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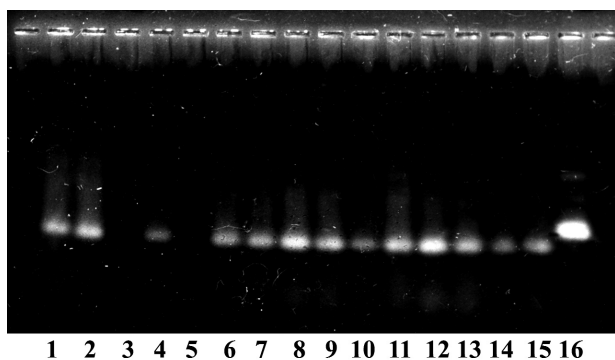
**Figure 2. Coding sequence of the human IFN- $\gamma$  gene region. Only the sense strand is shown.** The positions of the designed upstream primer (FA1) and downstream primer (FA2) are underlined (see Materials and methods). Two *DraI* restriction enzyme sites in the middle of the sequence are shown in Bold (positions 298 and 307, respectively).

(Fig. 2). The results of restriction digestion showed that the PCR products were digested and two overlapping fragments sized 202 bp and 259 bp were generated (Fig. 3b).

The amplified PCR product was cloned into pHEN1 vector and transformed into *E. coli*. The results of the cloning experiment were about 200-300 colonies that were screened by colony-PCR technique. Figure 4 shows the result of the colony-PCR experiment. According to the DNA molecular weight marker, the amplified fragments from the PCR-colony experiment results are about 594 bp in length that is the sum of the distance between the universal reverse primer (URP) to the *SacI* site in pHEN1 (156 bp) and the coding region of IFN- $\gamma$  (438 bp). As a control for PCR reaction, a recombinant colony harboring a known fragment (600 bp in length, cloned in to pHEN1 vector) was used which produce a PCR product of 756 bp in length, using the same URP and a gene-specific primer. The results of plasmid preparation and restric-



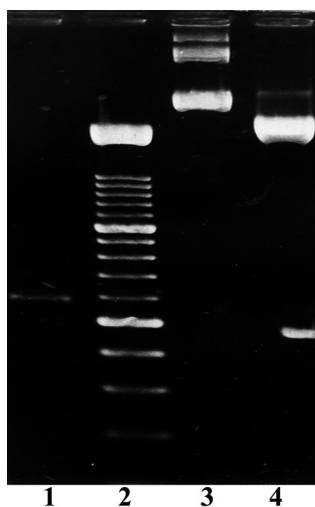
**Figure 3. a) PCR amplification of the IFN- $\gamma$  coding region using FA1 and FA2 primers.** Lanes 1, 2 and 3 are the same PCR products; lane 4 is a DNA ladder of 100 bp, lane 5 an unrelated PCR products of 1 kb. b) **Restriction enzyme digestion of the PCR products by *DraI* enzyme.** Lane1, uncut PCR product; lane 2 digested PCR product; and lane 3 DNA ladder of 100 bp. The size of the digested PCR product is in the range of 202-259 bp.



**Figure 4. Colony-PCR screening.** A universal reverse primer (URP) (in pHEN1) and a gene-specific downstream primer (in IFN- $\gamma$ ) have been used in PCR. Lanes 1-15 are amplified products (about 594 bp in length) of the obtained recombinant clones. As shown, clones no. 3 and 5 are negative. The PCR control (lane 16) is an amplified product of about 756 bp in length.

tion enzyme digestion are shown in figure 5. One of the selected clones (D4) was digested with *SacI* and *NotI* restriction enzymes and a fragment of the correct size (445 bp in length) was resulted.

**Figure 5. *SacI* and *NotI* restriction enzyme digestion of the D4 plasmid DNA.** Lane 1, control fragment of 600 bp (P21); lane 2, DNA ladder of 100 bp; lane 3, uncut D4 recombinant plasmid; lane 4 cut D4 recombinant plasmid. The released fragment has migrated below the 500 bp fragment of the DNA ladder.



5'-(47)

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TAAGAAGGAGATATACCTATGGCTAGCATGACTGGTGGACAGCAAATGGGTGCGGGATCCGAATTCGAGCTC
GCGTGTTACTGCCAGGACCCATATGTAAGAAGCAGAAAACCTTAAGAAATATTTTAATGCAGGGTCAT
TCAGATGTAGCGGATAATGGAACCTTTTCTTAGGCATTTTGAAGAATTGGAAAGAGGAGAGTGACAG
AAAAATAATGCAGAGCCAAATTGTCTCCTTTTACTTCAAACCTTTTAAAAAAGTTTAAAGATGACCAGAG
CATCCAAAAGAGTGTGGAGACCATCAAGGAAGACATGAATGTCAAGTTTTTTTTTTAGCAACAAAACCC
CCCCACATGACTTCGAAAAGCTGACTAATTATTCGGTAACTGACTTGAATGAACAACGCAAAAGCAATAC
ATGAACATCCAAGTGATGGCTGAACTGTGCCAGCAGCTAAAAACAGGGAAGCGAAAAAGGAGTCAG
ATGCTGTTTCGAGGTGCAAGAGCATCCAGAGCGGCCGCACTCGAGCACCACCACCACCACACTGAGA
TCCGGCTGCTAA-3' (612)
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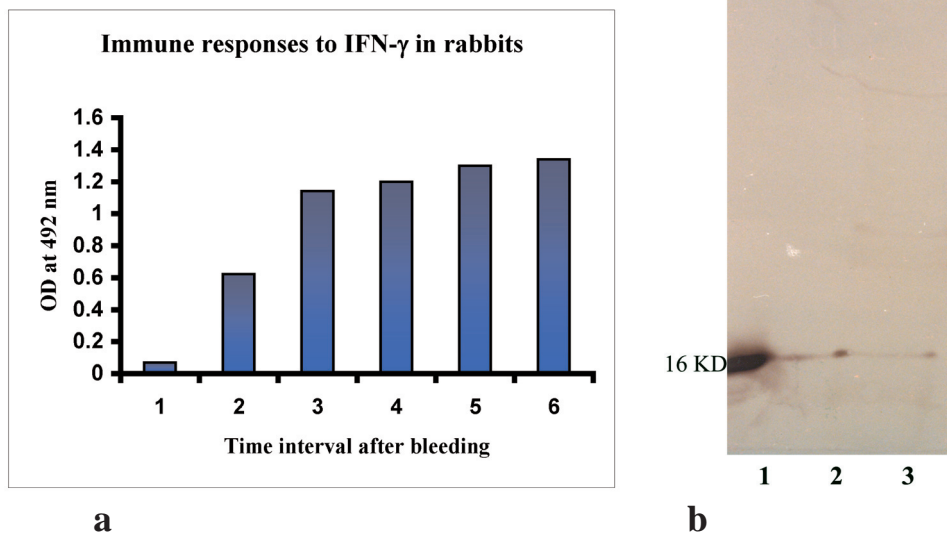
**Figure 6. The sequencing result of the cloned IFN- $\gamma$  coding region (D4 clone).**

In order to finally confirm the results of the cloning experiments, the recombinant plasmid was sequenced. The obtained sequencing data was run on GenBank using the BLAST engine search program, which showed the highest similarity with the human IFN- $\gamma$  (Fig. 6). According to the blasting results, there is a complete match between the cloned sequence and the IFN- $\gamma$  sequence data in GenBank.

Producing polyclonal antibodies against IFN- $\gamma$  was performed to supply the need in different stages of various experiments. The produced antibodies were evaluated in Western blotting and ELISA experiments, and it was shown that these antibodies are able to specifically recognize the commercial IFN- $\gamma$  as well as the IFN- $\gamma$  produced in the stimulated T-lymphocytes in cell culture soup (Fig. 7).

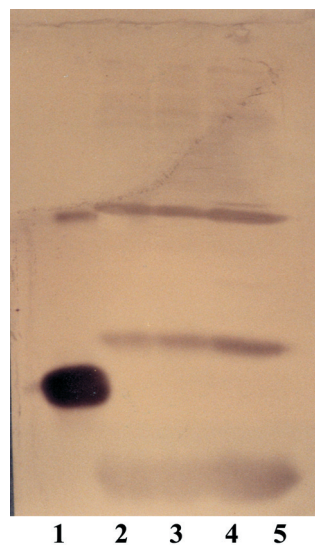
Following the confirmation of the cloned human IFN- $\gamma$  gene by sequencing, it was subcloned into an expression vector (pET21a). The gene segment is under the control of *T7* promoter in the vector and the expressed protein is accumulated in the cytoplasm. There is also a peptide tag (His-Tag) at the end of the cloned gene fragment that is useful for purification and detection purposes. After stimulating of individual colonies, the produced proteins were run onto SDS-PAGE and stained with Coomassie Brilliant blue. In parallel, an identical gel was blotted on nitrocellulose membrane and the expressed proteins were detected using the rabbit antisera followed by anti-rabbit-IgG peroxidase conjugate. The result of this work is shown in figure 8.

As it is clear in the picture, the produced protein by D4 (about 20 kD) and two other recombinant clones (Fig. 4) are recognized by the same polyclonal antibodies prepared against commercial human IFN- $\gamma$  (about 16 kD). The differences in the size are due to



**Figure 7. a) Rabbit immune response to human IFN- $\gamma$  detected by ELISA technique.** The absorbance was read at 492 nm. X-axis shows the time interval after bleeding: 1, Negative control; 2, 14 days after the 1st injection; 3, 14 days after the 2nd injection; 4, Day four after the 4<sup>th</sup> injection; 5, Day 5 after the 4<sup>th</sup> injection; 6, Day 6 after the 4<sup>th</sup> injection. **b) Western blotting of IFN- $\gamma$  as well as the cell culture soup using produced rabbit antisera.** Lane 1, commercial human IFN- $\gamma$ ; Lane 2 and 3, cell culture soup.

**Figure 8. Western blotting of the expressed IFN-g in *E. coli* as well as commercial IFN- $\gamma$ .** Lane 1: commercial human IFN- $\gamma$ ; lanes 2 (D4), 3 (D6) and 4 (D8): positive clones; lane 5: negative control. The protein dimers or aggregates can be seen in commercial IFN- $\gamma$  as well as in the recombinant clones. The low molecular weight proteins (below 5kD), which are probably protein degradations or some nonspecific antibody interactions.



some extra tag sequences in the vector both at the beginning and at the end of the IFN- $\gamma$  DNA sequence. The presence of protein dimers or aggregates is evident in both commercial sample and the expressed recombinant proteins. There are also some low molecular weight proteins (below 5 kD), reacting with the antibodies which are either degradations of the recombinant protein, or nonspecific interactions of polyclonal antisera with bacterial proteins.

## DISCUSSION

The human gamma interferon has not only an antiviral and cytotoxic activity in common to all human interferons, but also shows a potentiating effect on these activities with  $\alpha$ - and  $\beta$ -interferon. Also, the *in vitro* antiproliferative effect of IFN- $\gamma$  on tumor cells is believed to be 10- to 100-fold greater than the other interferon classes. This result, together with its pronounced immunoregulatory role, suggests a much more pronounced antitumor potency for IFN- $\gamma$  and clearly pointing to a potentially promising clinical candidate for cancer treatment (Bloom, 1980; Zoon *et al.*, 1980; Bass, 1997; Fleishmann, 1981; 1979; Reddy *et al.*, 1997; Falkner and Maurer-Fogy, 1996; Musch *et al.*, 1998; Blackbourn *et al.*, 2000).

It was perceived that the application of recombinant technology would be the most effective way of providing the requisite larger quantities of human immune interferon. It was then shown that the materials so produced exhibit bioactivity admitting of their use clinically in the treatment of a wide range of viral, neoplastic, and immunosuppressive conditions or disease (Yip *et al.*, 1981; Langford *et al.*, 1979; Nathan *et al.*, 1981; Gray and Goeddel, 1982; Blackbourn *et al.*, 2000). Gray, Goeddel and co-workers were the first

who reported expression of a recombinant gamma interferon (rIFN- $\gamma$ ) Yip *et al.*, 1981 and Gray *et al.*, 1982), which has proven to exhibit the characteristics of human gamma interferon, i.e., anti-viral and anti-proliferative activity coupled with loss of activity at pH 2. Since then, various expression systems as well as purification protocols have been developed in order to increase the final yield of the recombinant protein and to boost its efficacy (Langford *et al.*, 1979; Vassileva-Atanassova *et al.*, 2000; Zlateva *et al.*, 1999; Arora and Khanna, 1996; Kendrick *et al.*, 1998; Blackbourn *et al.*, 2000; Kontsek *et al.*, 2000).

In the present study, based on the IFN- $\gamma$  DNA sequences obtained from GenBank, two gene-specific primers (FA1 and FA2) were designed and used in a PCR experiment. The PCR conditions for the amplification of IFN- $\gamma$  gene region was optimized and finally, a fragment of expected size (461 bp in length) was obtained. In order to confirm that the amplified DNA corresponds to the IFN- $\gamma$  cDNA sequence, restriction enzyme mapping was performed followed by sequencing. There are two internal *DraI* restriction enzyme sites within IFN- $\gamma$  DNA sequence (Fig. 2), the first one at position 298 and the second one at position 307. To confirm the cloned IFN- $\gamma$  cDNA fragment, restriction enzyme analysis was performed as well as sequencing of each plasmid DNA prepared from individual ampicillin resistant colonies. The results of digestion together with sequencing data showed that the correct fragment has indeed been cloned. Sequencing of the IFN- $\gamma$  gene was problematic due to the presence of stretches of homonucleotides (mainly As and Ts). After several trials on different DNA samples, we obtained a fair sequencing data from D4 clone. Comparing the IFN- $\gamma$  cloned fragment from D4 plasmid DNA sequence with the IFN- $\gamma$  DNA sequence available in the GenBank, there is almost a complete match between the query and subject sequences. There is a short region almost in the middle of the D4 DNA sequence that does not match with the GenBank IFN- $\gamma$  DNA sequences. This is mainly due to the tandem repeat of T and A nucleotides in this region and can only be improved by repeating the sequencing process under specific conditions.

The IFN- $\gamma$  encoding gene was subcloned into pET21a vector was under the control of *T7* promoter that was also linked to a His-tag DNA sequence that is useful for purification and detection purposes. As shown in figure 8, some of the extracted proteins from *E. coli* lysate, as well as the commercial IFN- $\gamma$  protein,

reacted positively with the rabbit IFN- $\gamma$  antisera. The presence of the extra bands (almost twice in size) in the induced samples, as well as in the commercial IFN- $\gamma$  protein sample can possibly be due to misfolding, aggregation or dimerization. There are several reports on the misfolding and aggregation of recombinant IFN- $\gamma$  protein (Zlateva *et al.*, 1999; Arora and Khanna 1996; Vandebroek and Billiau, 1998; Vassileva-Atanassova *et al.*, 1999; Zlateva *et al.*, 1999) in particular due to the presence of CYS-TYR-CYS sequence in the beginning of the IFN- $\gamma$  polypeptide and substitution of CYS residues with other amino acids can alleviate the problem. The difference in size between the commercial IFN- $\gamma$  protein and our recombinant clone is due to the presence of some 37 extra amino acids (on average equivalent to 4.1 kD) in the beginning (T7-Tag) and at the end (His-Tag) of the recombinant proteins. These extra Tag sequences are only for purification or detection purposes and can easily be removed in the final stage of IFN- $\gamma$  production for medical applications.

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