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Expression and modeling of human interleukin-2 protein

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Summary: The recombinant plasmid pTargeT.IL-2hu was observed to express IL-2 protein in Vero cells as detected by indirect fluorescent antibody and immunoperoxidase techniques. The modeling of the IL-2 protein using DNASTAR and SWISS-MODEL softwares revealed the structure and epitopes of the protein.

Keywords: Interleukin-2, human, protein modeling

Introduction

Interleukin-2 (IL-2) is a potent cytokine that activates multiple compartments of the immune system including T helper cells, cytotoxic T cells, B cells, macrophages and NK cells (Farrar *et al*., 1981; Kawase *et al*., 1983). The gene is 465 bp in length and encodes a protein of 154 amino acids. Co-administration of plasmids encoding human IL-2 and hepatitis antigen resulted in enhancement of both humoral and cell mediated immune response to hepatitis DNA vaccine (Chow *et al*., 1997). Also IL-2 gene has been used for therapy in various cancers and AIDS to achieve desired immune response (Shah *et al.,* 2006). In this study, we have analyzed the expression of human IL-2 gene in vitro and modeling of the protein.

Materials and Methods

Gene expression

For expression analysis, the recombinant plasmid pTarget.IL-2hu (Saxena et al, 2007) was isolated using Qiagen plasmid mini kit (Qiagen), trasfection was carried out in Vero cells using polyfect transfection reagent (Qiagen) according to manufacturer's instructions and the expression was analysed by indirect fluorescence antibody (IFAT) and immunoperoxidase tests.

Indirect florescence antibody test

Vero cells in 24-well plate with approximately 80% confluent monolayer were transfected with plasmid pTargeT.hu IL-2 and the cells were incubated at 37°C and 5% CO_2. After 48 hours, the cells were fixed with 80% chilled acetone. Mock-transfected vero cells were also fixed as a control. To the

fixed monolayer, 1:50 diluted mouse anti-human IL-2 hyperimmune serum was added and incubated at room temperature for 1 h. After the incubation, the wells were washed twice with PBS and again incubated with goat anti-mouse FITC conjugated secondary antibody and incubated further for 1h at room temperature. Cell monolayer were washed with PBS, mounted in 50% glycerol in PBS and examined under fluorescent microscope.

Immunoperoxidase test

The protocol was similar to IFAT except that, goat anti-mouse HRP as the secondary antibody conjugate was used. The color was developed with DAB (1mg/ml in PBS with 1µl/ml H_2O_2) at room temperature for 5 min.

Protein modeling

The amino acid sequence of human IL-2 was submitted to SWISS-MODEL website online for modeling and the results obtained were visualized using spdbv and Rasmol software. (Kopp and Schwede, 2004; Schwede et al, 2003; Guex Peitsch, 1997; Peitsch, 1995). DNASTAR was also used for modeling.

Results and Discussion

The pTarget.IL-2hu transfected Vero cells showed good immunofluorescence while control healthy cells did not show any fluorescence (Fig.1-2). Similarly the Vero cells transfected with the recombinant plasmid DNA showed enhanced staining reaction in immunoperoxidase test while control cells did not show any staining (Fig.3-4). Modeling of human IL-2 using Protean program of the DNASTAR showed that the IL-2 protein contains alpha, beta, turn and coil regions and has quite distinct antigenic epitopes and has compact helical structure (Fig. 5-6).The SWISS-MODEL software used online produced a very authentic 3-D model which was visualized using spdbv software using pds format files.This recombinant protein is 154 amino acid long with an isoelectric pH 5.55, 117 polar amino acids indicating that the protein is highly hydrophilic. It was then visualized with rasmol software and different models like ribbon, strand, spacefill, ball and stick and back bone were constructed (Fig.7-10). The model in various forms showed the reactive sites, epitopes, groups, secondary structure and various amino acid groups.The protein structure was submitted to database TrEMBL and was assigned the accession # QOGK43.

The expression of the human IL-2 gene in Vero cells showed that the mammalian expression vector pTarget is highly efficient in expressing the gene cloned in it. It further demonstrated that it would express in appropriate host when injected. The modeling of the human IL-2 protein provided through insight into the structural details of the proteins, which included different groups, epitopes, reactive sites and antigenic surfaces.

It has been reported earlier that IL-2 is a potent cytokine that activates multiple compartments of the immune system including T helper cells, cytotoxic T cells, B cells, macrophages and NK cells (Farrar et al., 1981). Wang et al., (1993) reported immune response against HIV after gene inoculation since co-administration of human IL-2 and hepatitis antigen resulted in enhancement of both humoral and cell mediated immune response to hepatitis DNA vaccine (Chow et al., 1997). Several DNA vaccines for animal and human have been developed including rabies (Rai, 2007). In parallel with the earlier attempts to enhance the immune response of vaccines, it can be advantageously used as adjuvant with rabies DNA vaccine for humans. Human IL-2 has also been found beneficial when used in cancer patients and thus it can be used in cancer and related conditions. This study finds that the recombinant human IL2 is expressed in vero cell line is similar to its normal native protein found in body. The topographical studies reveal that the epitopes and hydrophilicity are maintained.

Fig1. Control Vero cells showing no fluorescence.

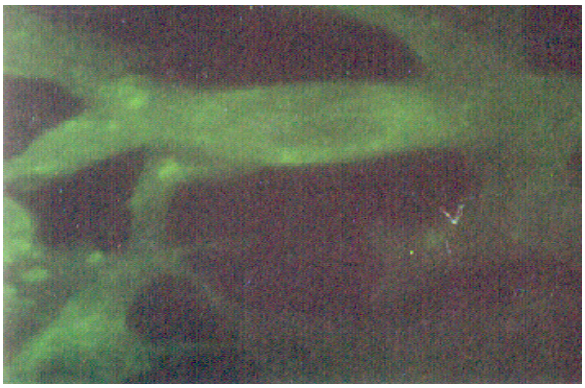

Fig 2. Recombinant plasmid transfected Vero cells showing fluorescence.

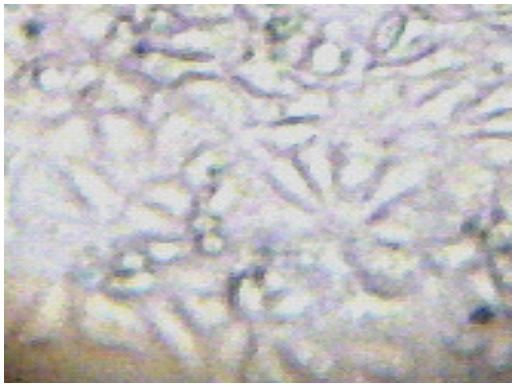

Fig 3. Control Vero cells showing no IPT reaction

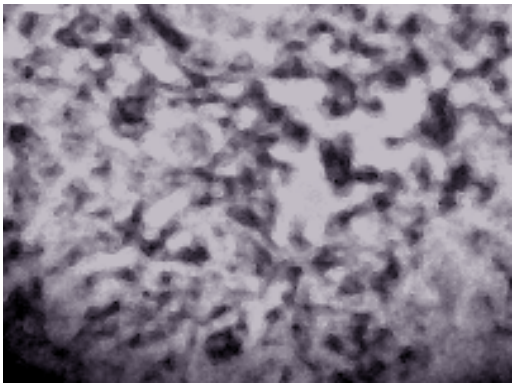

Fig 4 Recombinant plasmid transfected Vero cells showing positive IPT staining.

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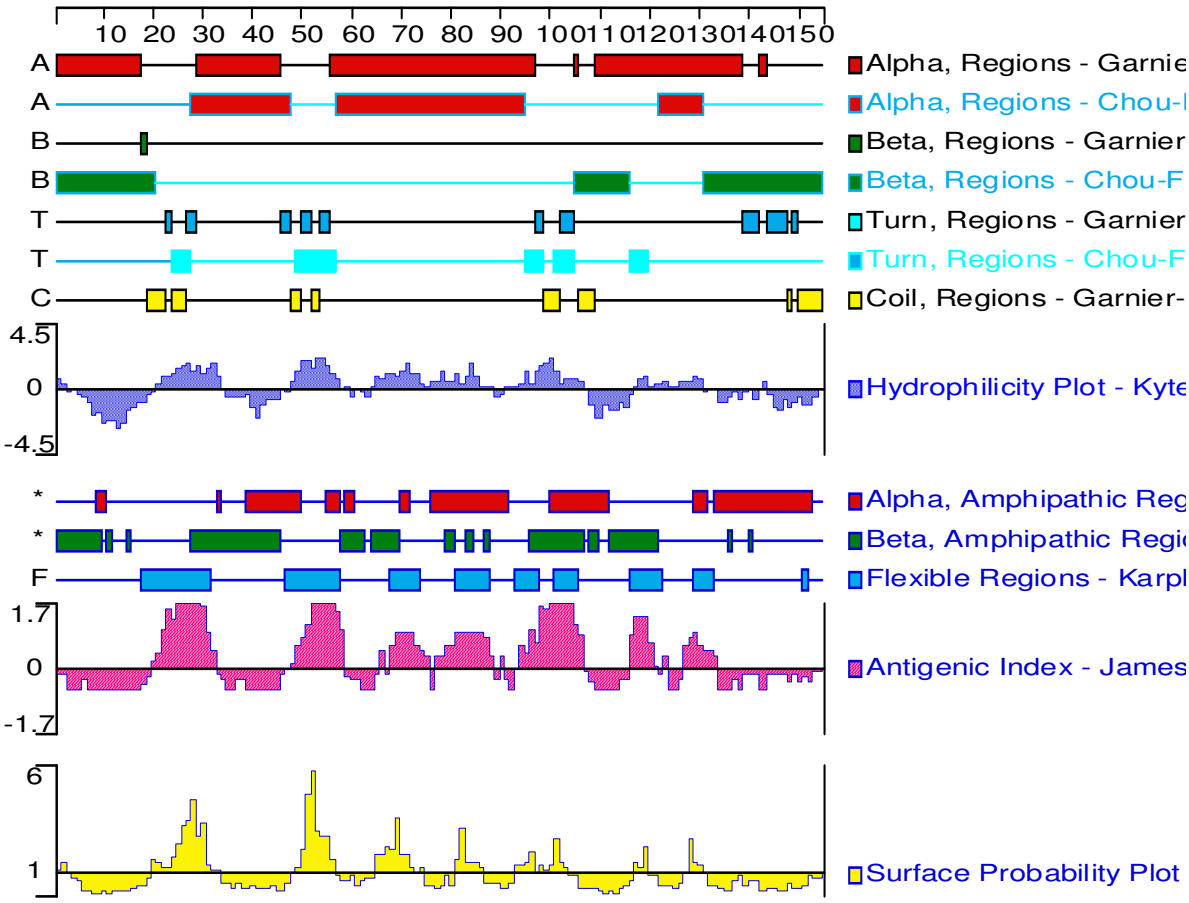

Fig 5. Modeling of human IL2 by protean program of DNASTAR software.

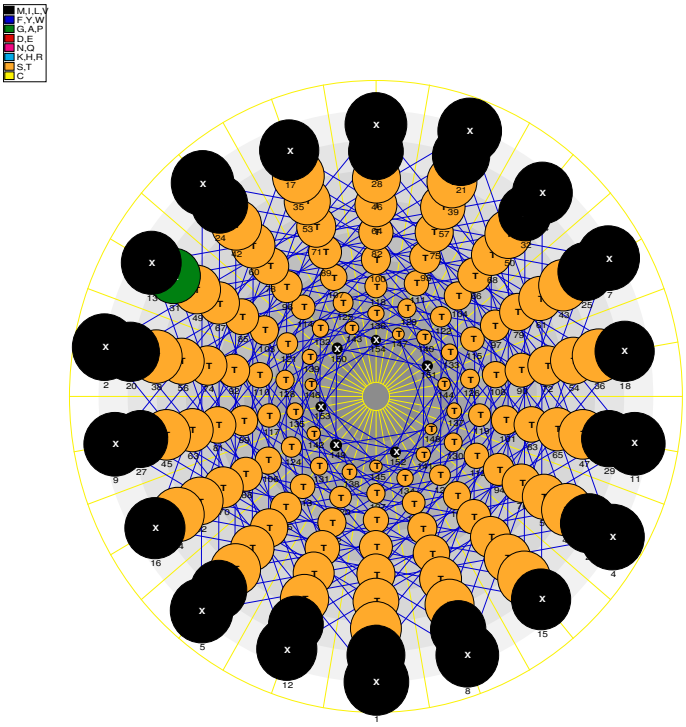

Fig 6. Modelling of human IL2 by protean program of DNASTAR software.showing helical model.

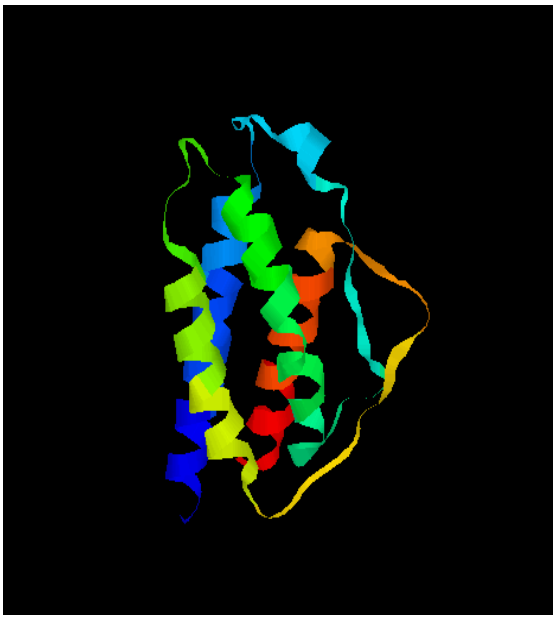

Fig7. Human IL2 model, ribbon, Rasmol

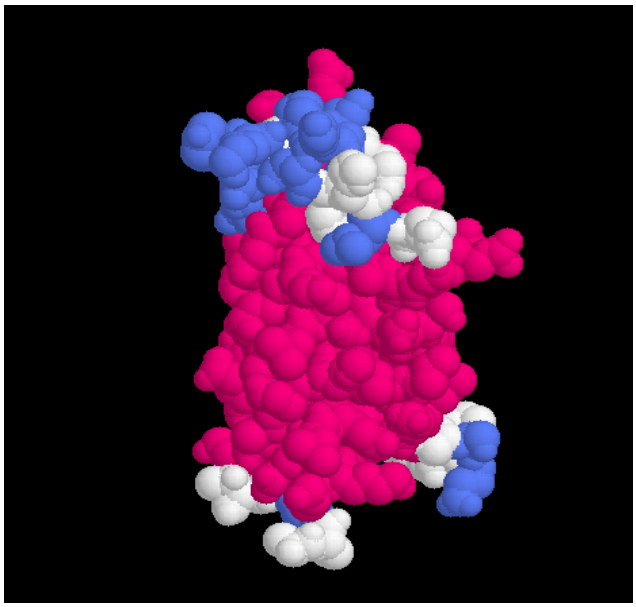

Fig8. Human IL2, strand, Rasmol

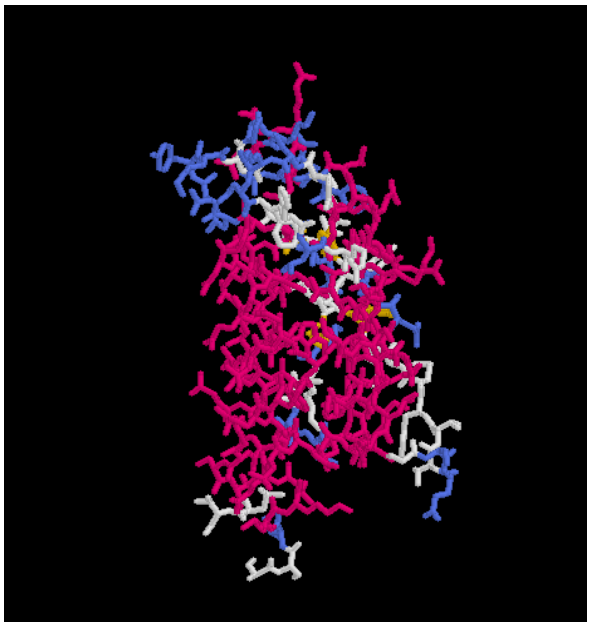

Fig 9. Human IL2,spdbv

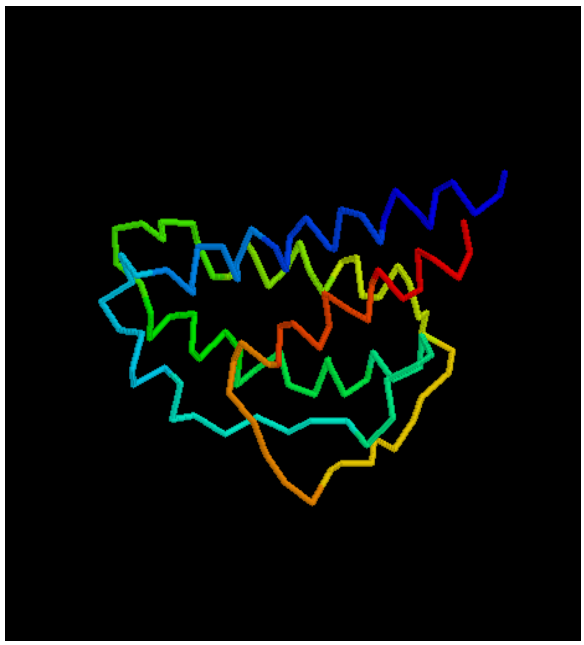

Fig 10. Human IL2, spacefill,Rasmol

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References

Chow YH, Huang WL, Chi WK, Chu YD and Tao MH.(1997). Improvement of Hepatitis B Virus DNA Vaccines by Plasmids Coexpressing Hepatitis B Surface Antigen and Interleukin-2. Journal of Virology 71:169–178.

Farrar WL, Johnson HM, Farrar JJ. 1981. Regulation of the production of immune interferon and cytotoxic T lymphocytes by interleukin 2. J Immunol. 126:1120-1125.

Guex, N. and Peitsch, M. C. (1997) SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modelling. Electrophoresis 18: 2714-2723.

Jürgen Kopp and Torsten Schwede (2004) The SWISS-MODEL Repository of annotated three-dimensional protein structure homology models. Nucleic Acids Research 32, D230-D234.

Kawase I, Brooks CG, Kuribayashi K, Olabuenaga S, Newman W, Gillis S, Henney CS. (1983). Interleukin 2 induces gamma-interferon production: participation of macrophages and NK-like cells. Journal of Immunology. 131:288-292.

Peitsch, M. C. (1995) Protein modeling by E-mail Bio/Technology 13: 658-660.

Rai N, Kaushik P and Rai A. 2006. Expression of cloned rabies glycoprotein gene in mammalian cells. Indian Journal of Virology. 17:18-22.

Saxena A, Rai A, Salunkhe S S, Gupta P K, Tiwari A K, Kumar S, Ahi Y S, Sandey M. (2006). Homo sapiens interleukin-2 mRNA, complete cds, NCBI/EMBL/GenBank accession # DQ861285.

Saxena A, Rai A, Salunkhe S S, Gupta P K, Tiwari A K, Kumar S, Ahi Y S, Sandey M. (2006). interleukin-2 (Homo sapiens) , NCBI/EMBL/GenBank accession # ABI20697.

Saxena A, Rai A, Salunkhe S S, Gupta P K, Tiwari A K, Kumar S, Ahi Y S, Sandey M. (2006). interleukin-2 (Homo sapiens) , UniProtkb-TrEMBL accession # QOGK43

Schwede T, Kopp J, Guex N, and Peitsch MC (2003) SWISS-MODEL: an automated protein homology-modeling server. Nucleic Acids Research 31: 3381-3385.