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Molecular cloning and expression of human gamma interferon (IFN- γ) full cDNA in Chinese Hamster Ovary (CHO) cellsZamani, A.^a, Afshari, J.T.^b, Alikhani, M.Y.^c^a Department of Immunology, Medicine School, Hamedan University of Medical Sciences, Hamedan, Iran^b Department of Microbiology, School of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran^c Department of Immunology and Allergy, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract

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Background: IFN- γ is mostly secreted by activated CD4⁺, CD8⁺ T cells and NK cells. This cytokine has immunomodulatory, anti-cancer and anti-microbial effects and is important for prophylaxis, diagnosis and treatment of chronic infections and cancers. Objective: The purpose of this study was to clone the full cDNA of human IFN- γ and express it in CHO cell line. Methods: Lymphocytes from a healthy individual were isolated and activated by phytohaemagglutinin (PHA) in vitro. After 4 hours, total RNA extracted and first cDNA strand was synthesized. cDNA was amplified with primers containing EcoRI and NotI sites. The amplified fragment and the pCDNA3.1 vector were cut by EcoRI and NotI and ligated. The construct (pCDNA3.1-IFN- γ) was transferred into E.coli (DH5 α strain) using CaCl₂ method and selected by plating on a medium containing ampicillin. The construct sequence was confirmed by PCR and sequence analysis. Construct expression was achieved by performing a calcium phosphate-mediated transfection into CHO cells and followed by selection of stable drug (G418) resistant clones by limiting dilution assay (LDA). The IFN- γ production by transfected CHO cells was measured using ELISA technique. Results and Conclusion: Out of 33 grown transformed bacterial colonies, only 6 had the entire sequences of the inserted fragment and one of them was used for the transfection experiment. Out of 768 wells, 5 clones produced more than 100 ng/ml/10⁶ cells of IFN- γ . Among the 5 clones, one with the maximum production of INF- γ (143 ng/ml/10⁶ cells) was selected and used for propagation.

Reaxys Database Information

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Author keywords

cDNA; CHO; IFN-

Indexed keywords

EMTREE drug terms: ampicillin; antibiotic g 418; calcium chloride; calcium phosphate; complementary DNA; gamma interferon; phytohemagglutinin; RNA

EMTREE medical terms: article; bacterium colony; cell isolation; Chinese hamster; controlled study; dilution; DNA strand; DNA vector; enzyme linked immunosorbent assay; Escherichia coli; gene amplification; genetic transfection; human; human cell; in vitro study; interferon production; lymphocyte; molecular cloning; polymerase chain reaction; protein expression; RNA extraction; sequence analysis

Chemicals and CAS Registry Numbers: ampicillin, 69-52-3, 69-53-4, 7177-48-2, 74083-13-9, 94586-58-0; antibiotic g 418, 49863-47-0, 83855-92-9; calcium chloride, 10043-52-4; calcium phosphate, 10103-46-5, 13767-12-9, 14358-97-5, 7758-87-4; gamma interferon, 82115-62-6; phytohemagglutinin, 9008-97-3; RNA, 63231-63-0

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