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Acta Medica Iranica
2009;47(4) : 7-12

IN VITRO SEMI-QUANTITATIVE DETERMINATAION OF HUMAN GAMMA-INTERFERON EXPRESSION BY RT-PCR

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Abstract:

Secreted cytokines of Th1 (T-helper)/Th2 cells play an important role in the pathogenesis of many diseases. Th1 cells secrete predominantly IFN-γ and IL-2 which regulate cell-mediated immunity against intracellular pathogens and tumors. In this study, expression of IFN-γ was studied using semiquantitative RT-PCR. In brief, lymphocytes of a healthy donor were stimulated with PHA (1µg/106 cell/ml) in cell culture at different incubation times (0, 4, 8, 12, 24, 48 and 72 hours) to express IFN-γ. Total RNA was extracted and cDNA synthesized. A sequence (273 bp) between two oligonucleotide primers (chosen from two different exons of the IFN-γ gene sequences) was amplified using a heat-stable DNA polymerase. In semi-quantitative RT-PCR, we used a serial dilution (1/2, 1/4, ...) for cDNA in order to determine the titer of cDNA which gives visible band in agarose gel (2%) electrophoresis. Results show the highest level of IFN-γ expression was achieved after 4 hours activation with PHA and it was stable at least for 22 hours. Then it fell to baseline level.

Keywords:

semi-quantitative ، IFN-γ ، PHA

TUMS ID: 3436

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