VIROLOGY

Regulation of Human Fibroblast Interferon Gene Expression by Human Leukocytic Interferon and Cytopathic α-Virus

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Natural human α-interferon and Sindbis α-virus induce production of β_1-interferon mRNA in human fibroblasts. Accumulation and regulation of β_1-interferon mRNA splicing after induction with α-interferon and virus were different. Expression of the β_1-interferon gene was assessed by conjugated reverse transcription and polymerase chain reaction (semi-quantitative variant).

Key Words: *β-interferon; α-interferon and α-virus induction; regulation of β-interferon mRNA transcription; conjugated reverse transcription and polymerase chain reaction*

Interferons (IF) are a group of inducible proteins providing for the resistance to RNA and DNA viruses by reacting with cells of vertebrates [1,7]. Three IF are known: leukocytic (α), fibroblast (β), and immune (γ): they are coded by different genes. Different inductor-cell systems are characterized by predominant production of one IF type: α-IF in virus-infected leukocytes, β-IF under the effect of double-stranded RNA (dsRNA) and viruses in fibroblasts, and γ-IF in response to mitogens and antigens in blood lymphocytes and splenocytes. Analysis of IFs induced in an organism shows 3 IF types in the blood in different amounts. "Priming" effect (boosting of production and effect of one IF by pretreatment of cells with low doses of another IF type) indicates mutual regulation of different IF types. Elucidation of the mechanism of such regulation is important for medical application of IF preparations. At present this problem is almost not studied.

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We tested the capacity of natural human α-IF (Egis) to induce expression of β_1-IF gene in human fibroblasts (HF). Participation of the universal transcription factors NFkb, ATP, HMG proteins and similar regulatory factors IRF1, IRF2 in the production and action of α- and β-IF [2,13] is a sufficient basis for mutual induction.

Double-stranded RNAs formed during virus replication are believed to be active structures inducing IF production in an α-virus infection. We investigated the effect of viral infection on the spectrum of mRNA β_1-IF produced in HF. The apoptosis system is activated in cells infected with α-viruses triggering a cytopathic effect [8]. We tried to reveal the differences in the regulation of β_1-IF gene expression in normal and α-virus-infected HF. A Sindbis-like Karelian fever virus was used.

Regulation of β-IF production at the level of mRNA transcription was studied mainly in HF treated with a synthetic poly (I)-poly(C) complex (dsRNA analog). At least 5 different β-IF mRNA from 0.65 to 1.8 t.n.p. long are formed [10]. Their translation products are neutralized by anti-β_1-IF serum and are

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therefore considered related. The gene coding for β_1-IF is located in the 9th chromosome, and the β_2-IF gene is in the 5th chromosome. The β_1-IF [9] and β_2-IF genes are cloned and sequenced. Nucleotide sequences of other β-IF genes are unknown. The β_2-IF mRNA, unlike the β_1-IF mRNA, is induced in HF by cytokines (growth factors and tumor necrosis factor). β_1-IF complementary DNA (cDNA) in the coding site is about 200 nucleotides shorter than β_2-IF cDNA. Nucleotide sequences of β_1- and β_2-IF are characterized by a low level of homology. The greatest homology (45%) is observed between β_1-IF and α_1-IF genes, but their genes give no cross hybridization. Moreover, there are no antigen crossings between the proteins they code for.

We investigated for the first time the transcription of human β_1-IF mRNA using conjugated reverse transcription and polymerase chain reaction (RTR-PCR) [3]. A semiquantitative variant of this method is used for assessing the levels of cytokines genes expression in various biological systems [5].

MATERIALS AND METHODS

HF (M19 strain) were cultured in 2-liter rollers in Eagle's medium with glutamine and 10% fetal calf serum. After a monolayer was formed (in 48 h at 37°C), the cells were treated with 1000 IU/ml α-IF (purified natural human α-IF, Egys; 2×10^6 U/ml) in the presence of the protein synthesis inhibitor cycloheximide (50 μg/ml) for 4 h at 37°C (conditions of mRNA superinduction) [2]. HF were infected with Karelian fever virus (strain LEIV 9298, infective titer 9 lg PFU/ml) with multiplicity of infection 5 PFU/cell. After 1-h virus adsorption at 37°C, the cells were washed and incubated in fresh growth medium for 4 h at 37°C. Control HF (no α-IF and no virus infection) were incubated in the medium without cycloheximide for 4 h at 37°C.

RNA was isolated from HF by the guanidine-thiocyanate-phenol-chloroform method [4]. For removing DNA, RNA was reprecipitated with 3 M ammonium acetate. Oligonucleotide primers (direct: 5'-TGCAGCAGTTCCAGAAGGAGG-3' and reverse: 5'-TCCAGTCCCAGAGGCACAGGC-3') for PCR and hybridization probe: 5'-CCTGGAAGAAAAA CTGGAGAAAGA-3' detecting specific β_1-IF mRNA were calculated using Oligo 3.4 software and a published nucleotide sequence [9]. Interactions between selected oligonucleotides with heterologous templates β_2-IF [15], α_{1B}- [14], α_2- [12], and γ-IF [6] in PCR and hybridization were considered impossible, because the number of errors at sites of probable "precipitation" was at least 8 and the heating temperature (55°C) allows for no more than one error. No sites homologous to primers were found in the structure of Sindbis virus genome RNA [11].

PCR was carried out in 2 stages: 1) RTR: cDNA synthesis on summary cellular RNA templates by reverse transcriptase from avian myeloblastosis virus (20 U/μl, Omutninsk Chemical Plant) with universal oligo (dT) 16 primer in a standard reaction mixture for reverse transcription (40 μl) at 42°C with 1-10 μg total cellular RNA and 40 U reverse transcriptase [5]; and 2) PCR amplification on cDNA template (5 μl RTR mixture, intact and 1/10 diluted) with Taq-polymerase (5 U/μl, Biopol) of 40-50 cycles in a standard mixture [3] in a Bioexcellence DNA incubator (Techne). The size of resultant PCR products was determined by mobility in 1.4% agarose gel with ethidium bromide in relation to the marker position. Blot- and dot-hybridization recommended by Promega on Hybond-N membranes (Amersham) in 50% formamide were used. PCR products (intact and 1/10 diluted) were applied onto membranes, denatured with 0.5 M NaOH, neutralized with 0.5 M Tris-HCl, pH 7.0, heated at 80°C for 2 h, and incubated first in prehybridization and then in hybridization buffers (50% formamide, 6×SSC — Saline Sodium Citrate, and 1% SDS), 1% Tween-20, 100 μg/ml salmon seminal DNA) for 19 h at 46°C with ^{32}P-probe (100×10^6 degrad./mg) to the internal site of β_1-IF cDNA sequence. Membranes were washed from radioactivity 4 times in 1×SSC and twice in 0.1×SSC at 45°C. Dried membrane was exposed onto an x-ray film for 18 h and developed.

RESULTS

Semiquantitative variant of RTR-PCR detects changes in the level of transcription of specific cytokine mRNA, because the amount of PCR products depends on dilution of RNA and dilution of complementary DNA (cDNA) obtained in RTR on poly(A)RNA with a universal oligo (dT) primer [5]. Previously, we studied changes in the induction of mRNA of the IF system enzymes.

Figure 1 (*a-c*) shows the results of detecting β_1-IF mRNA in HF by RTR-PCR and subsequent analysis of the products by electrophoresis in agarose gel with ethidium bromide and blot- and dot-hybridization with ^{32}P-probe. Four discrete types of PCR products (400-700 bp) were obtained on the total RNA template from normal (intact) HF in RTR-PCR with primers to β_1-IF cDNA; only 2 of them reacted with internal ^{32}P-probe. Treatment with purified leukocytic IF (1000 IU/ml, Egis) increased the β-IF gene expression more than 10-fold (according to dot-hybridization). Only 1 DNA type predominated among PCR products (according to agarose electrophoresis and

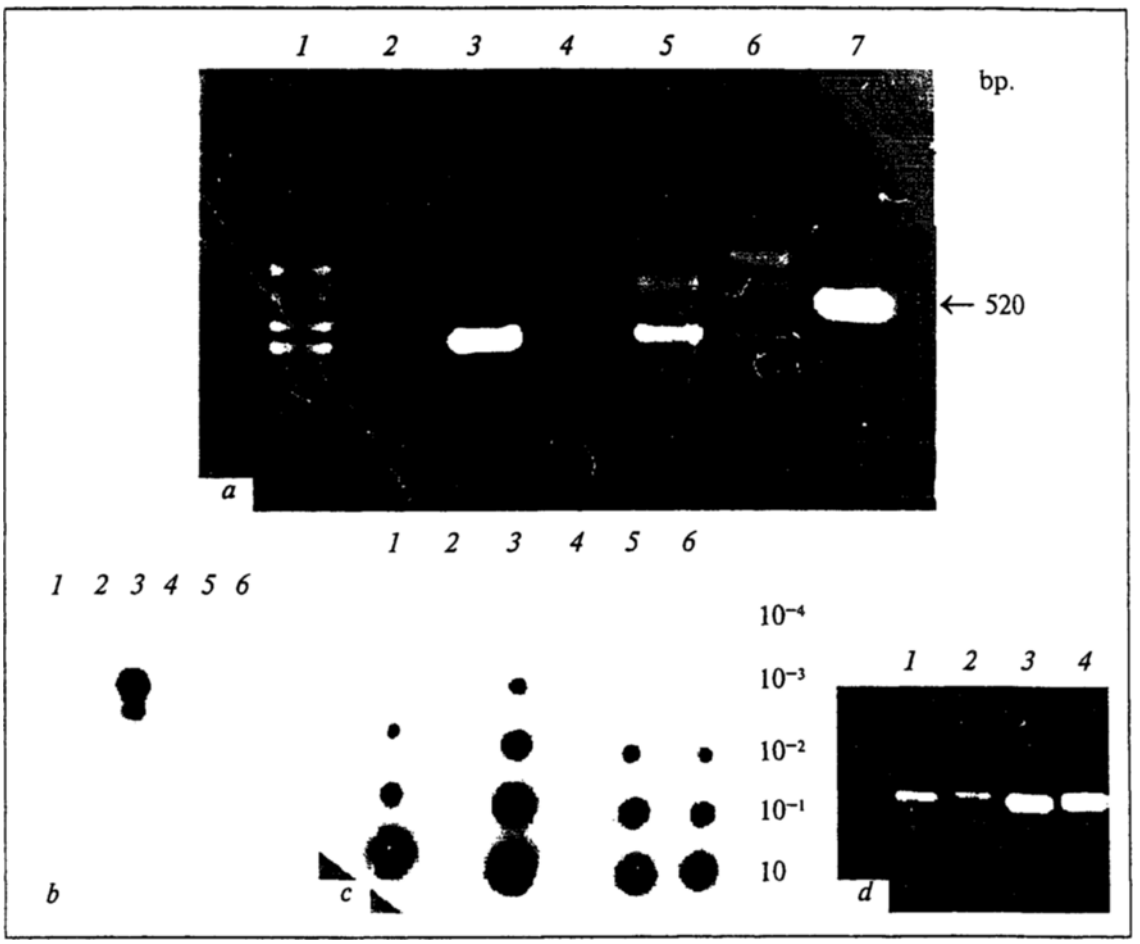

Fig. 1. Detection of β_1-interferon mRNA in human fibroblasts by reverse transcription (RTR) and polymerase chain reactions (PCR) with subsequent analysis of DNA products. *a*) electrophoresis in 1.4% agarose gel with ethidium bromide (50 amplification cycles); *b*) blot-hybridization; *c*) dot-hybridization on membranes with ^{32}P-probe. Control cells (*1*, *2*); induction of α-interferon (*3*, *4*); α-virus infection (5, 6). PCR with intact cDNA RTR (1, 3, 5) and that diluted 1:10 (2, 4, 6). Dot-hybridization with 10^{-1}-10^{-4} dilutions. PCR product 520 bp in size is the marker; *d*) agarose gel (1.4%) electrophoresis of PCR products obtained on α-virus (Karelian fever virus) RNA from infected human fibroblasts with primers to NSP1 (methyltransferase) and E1 (envelope protein) gene sites. PCR: 15 cycles (*1*, *2*) and 20 cycles (*3*, *4*).

blot-hybridization), whose size was similar to that expected for β_1-IF cloned cDNA (393 bp).

Viral RNAs were produced (Fig. 1, *d*) and β_1-IF mRNA were accumulated in cultures infected with Karelian fever virus after 4-h incubation, as shown by RTR-PCR (Fig. 1, *a-c*). Viral RNAs in cells were detected with paired primers to NSP1 (522 bp product) and E1 (506 bp) gene sites [3]. The content of viral RNA in infected cells is much higher than the content of β_1-IF mRNA. Virus-specific PCR products can be detected after 15-20 amplification cycles, whereas interferon-specific DNA only after 45-50 cycles (as shown by agarose gel electrophoresis with ethidium bromide). The β_1-IF mRNA in virus-infected cells is represented by several types, two of which predominate. A 393-bp long PCR product (expected for β_1-IF mRNA) prevails. The total level of β_1-IF gene expression in viral infection is higher than in control cells, i.e., specific DNA products are detected when intact and 1/10 diluted cDNA is used in PCR. The result of viral induction points to the presence of heterogeneous β_1-IF mRNA types in the total RNA preparation from control and infected cells. RTR-PCR analysis demonstrated for the first time the degree of similarity of primary structure of different types of β_1-IF mRNA. Detection of β_1-IF mRNAs varying in size with the same pair of primers permits regarding them as stages in genesis. The differences in the spectrum of β_1-IF mRNAs induced by α-IF and α-virus may result from impairment of their slicing mechanism in viral infection. Our results on β_1-IF mRNA transcription are in line with previous data on heterogeneous sizes of β_1-IF mRNAs induced by poly-(I)-poly-(C).

Stimulation of the β_1-IF mRNA transcription and slicing with natural α-IF demonstrates mutual

regulation of type I IF gene expression. We believe that mutual stimulation of biological activities of IF is due to activation of their mRNA transcription. During exposure of HF to α-IF, activation of β-IF and 2,5-oligoadenylate synthetase are correlated. A cause-and-effect relationship between these processes is probable. Small size of 2,5-oligoadenylate synthetase (40-46 kD) can contribute to maturation of mRNA precursors [7]. Thus, as the level of this enzyme increases, the production of final β_1-IF mRNA in HF also increases. By contrast, Sindbis virus caused fragmentation of cellular DNA and did not increase the low expression of the 2,5-oligoadenylate synthetase gene in HF. This may account for accumulation of intermediate β_1-IF mRNA types in control HF and in HF infected with α-virus.

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