INTERFERON PRODUCTION BY A PRIMARY CULTURE OF HUMAN ENDOTHELIAL CELLS

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There have been many reports of responses of the endothelial cells of blood vessels to the action of substances involved in immunologic inter-relations. For instance, tumor necrosis factor has been found to have a cytostatic action on endothelial cells (EC) [12]. γ-Interferon (IFN-γ) inhibited proliferation induced by EC growth factor and caused reorganization of the cytoskeleton [5]. Interleukin-1 (IL-1) and tumor growth factor (TGF) increased the adhesiveness of the endothelium for monocytes, polymorphs, and blood lymphocytes [4, 6]. Endothelial cells stimulated helper T cells under the influence of IFN-γ, evidence of their importance in immunologic reactions [7]. Some viruses also affected EC. Type I herpes virus, for instance, depressed collagen and fibronectin synthesis in bovine EC [9]. Cultivation of human EC with HTLV-1 virus led to the formation of a multinuclear syncytium and persistence of the virus antigen in them for 3 months [8]. The presence of giant multinuclear EC is an important distinguishing feature of human blood vessels, including atherosclerotic vessels [3].

Since viruses act on EC, it was interesting to demonstrate the ability of EC to produce interferon (IFN) under their influence.

EXPERIMENTAL METHOD

A primary culture of endothelial cells from the human umbilical vein (UVEC) in the confluent monolayer state was used. UVEC were isolated and cultured as described previously [1, 2]. IFN was induced by Newcastle disease virus (NDV), strain H, and influenza virus (IV), strain WSN (activity of the viruses was expressed as EID_{50} – embryonic infection dose causing death of 50% of embryos), and by polyI:polyC ("Serva") with DEAE-dextran ("Sigma") and by staphylococcal enterotoxin A (SEA), produced by the Mechnikov Ufa Research Institute of Vaccines and Sera, Microbiological Industry of the USSR. During the induction of IFN, the viruses and other preparations were added to wells of 6- and 12-well plates with a UVEC monolayer in fresh culture medium. IFN production was analyzed after 24 h. To inactivate the inducing virus, culture fluid was treated with 1N HCl at pH 2.0 for 7 days and neutralized with 1N NaOH. During the study of acid-labile IFN production, some of the culture fluid was treated at pH 2.0 and the rest with β-propriolactone (final dilution 1:4000) in the cold for 24 h. During IFN induction with polyI:polyC and DEAE-dextran (final concentrations 50 and 200 μg/ml respectively) the mixture was prepared immediately before addition and, after incubation for 3 h, the cell mono-layer was washed 3 times with Dulbecco buffer, after which incubation continued for a further 24 h in fresh culture medium. During induction of IFN with SEA it was added to the culture fluid in concentrations of 5, 1, and 0.1 μg/ml and tested after 1-3 days. During induction of IFN with polyI:polyC and SEA, the culture fluid was not treated. IFN was tested by a micromethod on 96-well panels, with addition of twofold dilutions of the test material in a volume of 0.1 ml to wells containing a 3-day monolayer of human diploid fibroblasts (HDF) or a 1-day transplantable culture of bovine kidney (MDBK). After culture of 24 h at 37°C, 100 TCD_{50} (tissue cytopathic dose causing damage to 50% of

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TABLE 1. IFN Production by UVEC

Inducer	Dose	IFN activity, units/ml
NDV	10^9 EID_{50}	16
	10^8 EID_{50}	16
IV	10^7 EID_{50}	2
	10^9 EID_{50}	16
	10^8 EID_{50}	2
	10^7 EID_{50}	2
PolyI:polyC with DEAE-dextran	50 μg/ml	32
	200 μg/ml	
SEA	5 μg/ml	Under 2
	1 μg/ml	» 2
	0,1 μg/ml	» 2

TABLE 2. Typing of IFN Produced by UVEC

Inducer	Sera		
	nonimmune	anti-IFN-α	anti-IFN-γ
NDV	16	<2	16
IV	32	<2	32
PolyI:polyC with DEAE-dextran	32	<2	32
Control			
IFN-α	256	<2	256
IFN-γ	128	128	<2

Legend. Here and in Table 3: IFN activity given in units/ml.

cells of the monolayer) of the indicator virus (murine encephalomyocarditis virus for each HDF and vesicular stomatitis virus for MDBK) was added to the wells in a volume of 0.1 ml. IFN activity was estimted after 24 h and expressed in units/ml, taking as the titer its last dilution inhibiting development of the cytopathic action of 100 TCD_{50} of indicator virus. During titration of the material, 0.1 ml of liquid containing IFN was treated with 0.1 ml of antiserum to human IFN-α or IFN-γ. Antisera to human IFN were generously provided by Dr. V. I. Iovlev (Leningrad Pasteur Institute). Normal nonimmune serum and human IFN-α and IFN-γ were used as the control. After incubation at 37°C for 1.5 h, the residual IFN was determined by the method described above.

EXPERIMENTAL RESULTS

Table 1 gives the results of primary screening of IFN production with the use of different inducers. They show that NDV induce IFN production in UVEC, depending on the dose of virus. IFN titers were 16 units/ml after addition of 10^9 EID_{50} and 10^8 EID_{50} and 2 units/ml for an inducing dose of 10^7 EID_{50}. By contrast with NDV, the inducing dose for IV was 16 units/ml, whereas doses of IV of 10^8 and 10^7 EID_{50} induced IFN production with a titer of 2 units/ml.

PolyI:polyC with DEAE-dextran is a commonly used inducer of human IFN-β in the culture of HDF. We used optimal ratios and final concentrations of the preparations. This combination induced IFN production in UVEC with activity of 32 units/ml. SEA, used to induce IFN-γ, did not induce IFN production.

The next step in the work was to type INF produced by UVEC (Table 2). Antiserum to IFN-α completely neutralized the antiviral activity of IFN-α, but not of IFN-γ. In turn, antiserum to IFN-γ completely neutralized IFN-γ but not IFN-α. IFN obtained after induction of UVEC with NDV, IV, and polyI:polyC were completely neutralized by antiserum to IFN-α but were not neutralized by antiserum to IFN-γ. Consequently, IFN produced by UVEC are of the α-type. Ability to produce one type of IFN by the use of different inducers is evidently a particular feature of endothelial cells.

TABLE 3. Dynamics of IFN Production after Induction of UVEC by IV

Dose of virus, EID_{50}	Culture	Time after induction, h							
		1		6		24		48	
		ALI	ASI	ALI	ASI	ALI	ASI	ALI	ASI
10^9	HDF	Under 2	Under 2	16	Under 2	64	16	16	16
	MDBK	» 2	» 2	4	» 2	4	4	2	2
10^8	HDF	Under 2	Under 2	4	Under 2	32	16	32	32
	MDBK	» 2	» 2	Under 2	» 2	4	4	4	4

We know that IFN-α may be of two subtypes: acid-labile (ALI-α) and acid-stable (ASI-α). There is abundant evidence [11] of the constant presence of ALI-α in the blood of patients with autoimmune processes. Moreover, the blood cells of such patients did not produce IFN spontaneously [10]. In such cases the producers of ALI-α are probably somatic cells, including endothelial cells. More detailed knowledge of ALI-α production by vascular endothelial cells would be interesting from this point of view.

To determine ALI-α activity in the culture fluid, the inducer virus was inactivated by β-propiolactone. This compound inactivates viruses but not IFN. It was found that NDV, after this treatment, lost only its infectivity but not its interferon properties, and when added to a monolayer of HDF it simulated the presence of IFN. After this treatment, IV lost its infectivity but had no interferon properties, so that it was possible to study the dynamics of ALI-α and ASI-α production by UVEC under the influence of IV (Table 3). The results showed that 6 h after induction of the cells with IV in a dose of 10^9 EID_{50} ALI was present in the culture fluid in a titer of 16 units/ml, but no ASI was present. After 24 h activity of ALI and ASI was 64 and 16 units/ml respectively, and after 48 h, it was 16 units/ml. Typing showed the ALI to be IFN-α

Unlike IFN-γ and IFN-β, human IFN-α exhibit antiviral activity in a heterologous culture of MDBK to the same degree as in a homologous culture of HDF. In our model, titers of IFN were 8-32 times lower on an MDBK than on an HDF culture, but in material in which antiviral activity was manifested on an HDF culture, it was also found on an MDBK culture. This is evidence of the special features of IFN-α produced by UVEC.

These experiments thus demonstrated the ability of UVEC to produce IFN-α under the influence of NVD, IV, and polyI:polyC, IV inducing production of ALI-α also. The results are evidence that EC participate in function of the IFN system in vivo.

LITERATURE CITED

1. A. S. Antonov, A. V. Krushinskii, M. A. Nikolaeva, et al., Tsitologiya, No. 10, 1154 (1981).
2. A. S. Antonov, M. E. Lukashev, Y. A. Romanov, et al., Proc. Natl. Acad. Sci. USA, 83, 9704 (1986).
3. A. S. Antonov, M. A. Nikolaeva, T. S. Klueva, et al., Atherosclerosis, 59, 1 (1986).
4. N. P. Bevilacqua, J. S. Pober, M. S. Wheeler, et al., J. Clin. Invest., 76, 2003 (1985).
5. R. Friesel, A. Komorjasa, and T. Maching, J. Cell Biol., 104, 689 (1987).
6. J. R. Gamble, J. M. Harlan, S. J. Klebanoff, and M. A. Vadas, Proc. Natl. Acad. Sci. USA, 82, 8667 (1985).
7. T. D. Geppert and P. E. Lipsky, J. Immunol., 35, 3750 (1985).
8. J. Hoxie, D. M. Mathews, and D. B. Cines, Proc. Natl. Acad. Sci. USA, 81, 7591 (1984).
9. E. J. Macarac, H. M. Kriedman, and N. A. Kefalides, Lab. Invest., 53, No. 3, 280 (1985).
10. C. T. Preble, K. Rothko, J. H. Klippel, et al., J. Exp. Med., 157, 2140 (1983).
11. S. Skurkovich, B. Skurkovich, and J. A. Bellanti, Clin. Immunol. Immunopathol., 43, 362 (1987).
12. A. N. Stolpen, B. C. Guinan, W. Fiers, and J. Pober, Am. J. Pathol., 123, 16 (1986).