SOME PATTERNS OBSERVED IN THE INTERFERON EFFECT

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SOME PATTERNS OBSERVED IN THE INTERFERON EFFECT

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(The authors found that the level of cell resistance resulting from the effects of interferon depends upon the concentration of the drug and the period consumed in administration, and also upon the multiplicty of the infection which is present. Resistance was first noted 1-2 hours following administration of interferon, following which it reached a maximum by the 5th-6th hours, remaining at a constant level infefinitely as long as interferon continued present in the culture fluid. For maximum effect, however, the interferon should be in contact with the cells previously. When the latter precaution was observed, the culture cells showed complete protection from the after-effects of the virus. On the other hand, the administration of even large amounts of interferon 2-3 hours following inoculation resulted in gradual reduction of resistance. The time between removal and the start of such reduction was 6-9 hours; and the time was independent of both interferon concentration and infection multiplicity. This period, evidently, corresponds to the period of functioning of so-called antiviral protein. The paper includes 2 tables, 4 illustrations, and a bibliography of 13 items).

The outcome of the infection of a virus-cell-interferon system depends both on the special features of each of those components, and on the quantitative relations existing between them. The most reliable index of the effectiveness of interferon action is the level of resistance of the cells to the virus infection.

[redacted]

In their study of the phenomenological aspects of interferon action, the authors demonstrated the substantial importance of the periods during which the drug was added, and of the interferon concentration and the multiplicity of the infection, as well as of the sensitivity of the chosen system [1-6].

In this connection, Group A arboviruses are distinguished by heightened sensitivity to interferon action; they were chosen by the authors as a model for the study of the basic patterns exhibited in the rise and development of cell resistance.

Material and Methods

Viruses. The virus of Venezuelan equine encephalomyelits (VEE) was used; this was obtained from the virus museum of the D. I. Ivanovskiy Institute of Virology, USSR Academy of Medical Sciences. The virus was passivated on chicken embryo fibroblasts. Plaques under agar [7] were used to determine infectivity of the virus.

Cells. A monolayer of trypsinized, 48-hour culture of the fibroblasts, prepared by the usual method, was used for a cell supply.

Interferons. Homologous interferons, obtained by the method of double infection, or by the method of suspended culturing [8-10]. Interferon titer was expressed in inhibiting units produced by the suppression of 50% plaques (IU_{50}/ml). In all of the tests the dynamics of the development of resistance produced by the interform, and its level, were determined by degree of suppression of virus reproduction, this being expressed as the difference in logarithms (of test animals and control animals).

13

[redacted]

Reagents. A lyophilized preparation of actinomycin D was dissolved in a phosphate buffer (pH 7.2) directly before use.

Results of Research

Dynamics of the development of interferon-induced resistance. The cells were treated with interferon in a concentration of 300 IU/ml. Next, at every hour, the interferon was carefully washed off, following which the cells were again infected with various doses of VEE virus—0.01 and 10 biol. poison units per cell. After 18 hours following the infection, the culture liquid was collected and the virus titer of the liquid determined.

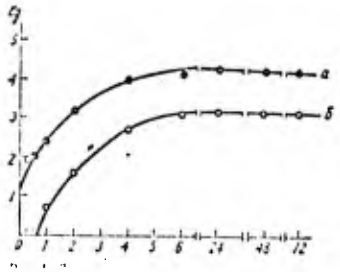

Figure 1. Dynamics of the development of interferon-induced cell resistance, and its dependence on the multiplicity of infection.

On the x-axis is plotted the time following administration of the interferon (in hours); on the y-axis, the level of resistance. The upper curve represents VEE virus in a dose of 0.01 biol. poison units; the lower curve represents a a dose of 10 units per cell.

The maximum resistance for the two doses in question was noted after 4-6 hours. The curves shown above run parallel—an indication of the identical rate of acquisition of resistance by the cells, with no dependence upon infection dose. As is clear from Fig. 1, given less multiplicity of infection, and observance of simple, equivalent conditions, the degree of resistance was higher. The resistance level achieved by the 5th-6th hours did not vary indeterminately for a long time if the condition of constant presence of interferon in the culture liquid was observed.

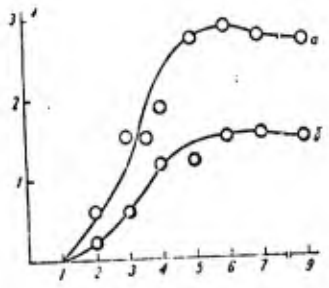

Figure 2. Relationship between resistance level and interferon concentration.

The upper curve corresponds to an interferon concentration of 100 IU/ml; the lower, to a concentration of 25 IU/ml. Other designations are the same as in Figure 1.

Relationship between resistance level and interferon concentration.

Interferon in concentrations of 100 and 25 IU/ml was added to the culture

[redacted]

liquid; then after 1 hour it was removed, and the cells, following careful washing, were infected with VEE virus (10 biol. poison units/cell). The titer of the virus in the culture liquid was determined 18 hours following infection.

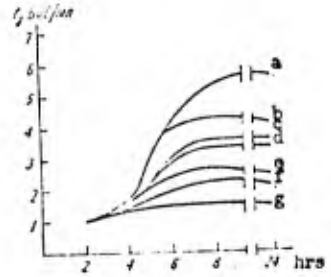

Figure 3. Suppression of VEE virus reproduction, as related to schedule of administration of interferon.

Curves: a - control; b - simultaneous administration and infection; c - administration 15 minutes before infection; d - 30 minutes before infection; e - 1 hour before infection; f - 2 hours before infection; g - 24 hours before infection. Poison units are plotted on the y-axis (in logarithms of units per ml).

The cells achieved maximum resistance 5-6 hours following administration of the interferon as viewed independently of its concentration (Figure 2). Degree of cell resistance with use of the 25 IU/ml dose of interferon was only about half as great as with use of the 100 IU/ml dose. Consequently, the resistance of the cells stands in direct proportion to the interferon concentration. In other words, by increasing or decreasing the interferon dose, it is possible to regulate the intensity of cell

immunity.

Effect of interferon as a function of the time of its application. Interferon in a concentration of 320 IU/ml was introduced into the culture liquid 24, 2 and 1 hrs, and also 30 and 15 min, before infection; and also immediately following infection of the cells with 1-5 biol. poison units per cell of VEE virus. The titer of the infection virus was determined from samples taken every 2 hrs.

In every instance, including the application of interferon (Fig. 3 above) simultaneously with the infection, there was a distinct suppression of the reproduction of the virus, and this was more marked the later the time of application. The most prominent suppression of virus reproduction was noted in the case of pre-infection application of interferon (from 2 to 24 hours before infection). When the pre-infection period was 24 hours, suppression of virus reproduction was virtually complete.

The authors determined the basic patterns involved in the rise and development of cell resistance in connection with times of application of interferon to a liquid of infiltrated cultures (with respect to the moment of infection). Interferon in a concentration of 160 IU/ml was applied to the culture liquid immediately following infection, and also 2, 4 and 6 hours after infiltration of the chicken fibroblasts by VEE virus. The infection does varied between the limits of 0.01 and 10 biol. poison units per cell. Samples were taken every 2 hours to determine the content of infection virus.

TABLE 1

Level of Cell Resistance as It Depends on Time of Application of Interferon and Multiplicity of Infection

Time of application of interferon[1]	Multiplicity of infection (in biol. poison units per cell)			
	0.02	0.1	1.0	10
Immediately following infection	3.4[2]	2.4	2.0	0
2 hrs following infection	2.0	1.3	0	0
4 hrs following infection	0.7	0.4	0	0
6 hrs following infection	0.2	0.4	0	0

[1]The interferon was applied in all tests in quantities of 160 IU/ml.

[2]Degree of suppression of VEE virus reproduction in comparison with the control.

It was established (Table 1) that the smaller the virus dose used to infect, the more pronounced the action of the interferon. One possible explanation of this was that with low multiplicity of infection, the interferon is able to protect the surrounding cells not originally infiltrated by the virus, and is therefore able to prevent subsequent cycles of reproduction.

Patterns observed as a result of interferon action when the agent is removed from the culture liquid. To determine the time of activity of a hypothetical antivirus protein (AVP), cells were given a preliminary treatment with interferon of concentration 160 IU/ml for a period of 4-24 hrs. Following this, the interferon was washed off, and the cells were infected

with VEE virus (dose 1-10 biol. poison units per cell). The rise and dynamics of virus reproduction under these conditions was studied. It was observed that virus reproduction in cells already processed with interferon set it 7-8 hours following removal of the interferon (Figure 4).

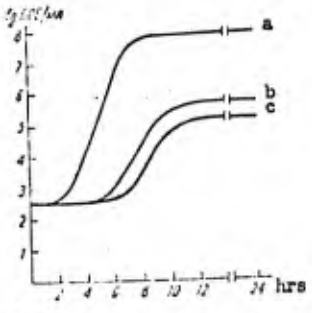

Figure 4. Length of the latent period of development of VEE virus, as it depends on the time of preliminary processing of the cells with interferon.

a - control; b - preliminary treatment of cells with interferon for 4 hrs; c - preliminary treatment for 6 hrs.

Since the latent period of VEE virus development amounts to 3 hrs, reproduction of the virus in the test with interferon began 4-5 hours that observed in the control. Along with this, the intensity of virus reproduction was found to be significantly lower. The final titer in the test was only one-half to one-quarter that of the control.

Delay of the start of VEE virus reproduction by 4-5 hours beyond that in the control was found not to depend upon the pre-incubation of

cells with interferon. This fact enabled the authors to assume that an AVP is accumulated in the use of one and the same concentration of interferon up to a certain level. After 7-8 hours following removal of the interferon, one observes a gradual fall in AVP activity, accompanied by loss of resistance and by onset of virus reproduction with destruction of cells.

Nor was the period of activity of the AVP found to depend upon the dose taken (Table 2). Multiplicity of infection, from 0.01 to 100 biol. poison units per cell, had no effect on the extent of the latent period following removal of interferon applied 4 and 24 hrs before infection.

TABLE 2

Relationship between Length of Latent Reproduction Period of VEE Virus and Infection Multiplicity

Multiplicity of infection (biol. poison units per cell)	Latent period (in hrs) following removal of interferon	
	interferon applied 4 hrs before infection	interferon applied 24 hrs before infection
0.01	7-8	7-8
1	7-8	7-8
100	7-8	7-8

To demonstrate that loss of resistance by cells following removal of interferon is the result of deterioration of AVP resulting from cessation of biosynthesis of m-RNA for the AVP, the authors carried out the following experiments. Interferon was introduced into the culture liquid in a concentra-

tion of 25 IU/ml, then carefully washed out at the end of 1, 2, 3, 4, 5, 6 and 7 hours, following which to half the samples was added actinomycin D in a concentration of 2 μg/ml. The cells were infected with VEE virus (10-20 biol. poison units per cell). Next, 18 hours following infection, the infection titer of the virus was determined.

After carefully washing out the interferon, just as with addition of actinomycin D, no further build-up of resistance took place. Here resistance in both cases was equivalent, being fixed, as it were, at the level achieved at the moment of addition of the interferon. It should be stressed that the interval of time after which resistance began to fall off as a result of simple washing out of the interferon or addition of actinomycin D, amounted to 7-8 hours, just as in the preceding experiment.

The data obtained support the conclusion that there is no long-term action on the part of the interferon adsorbed by the cells. In other words, any interferon which might be preserved following washing, deteriorates rapidly, and plays no sort of substantial role in maintaining cell resistance which had reached a certain level.

Discussion of Results

The data cited above made it possible to confirm a direct interrelationship between the effectiveness of interferon action, as it inhibits virus reproduction, and the reaction of cells to virus infection. To bring out maximum this inhibitor is capable of, some preliminary contact between interferon and cells must be achieved for several hours, at a temperature of 37°C, and with a fairly high concentration of the interferon. This conclusion is,

in fact, quite in agreement with that of other writers on the subject [6, 11, 12].

The development of inferon-induced cell resistance is tied in, as we suppose, with the formation of m-RNA for the AVP, and is possible only following a latent period of 1-2 hrs. In addition, only a sufficiently high multiplicity of infection can insure such a result: with low multiplicity, the inhibiting action of the interferon appears at once following its introduction into the culture liquid. All this probably serves to explain the "medicinal action" of interferon--in other words, its ability to inhibit virus reproduction when it is applied 2 and 4 hours following infection, by which time the virus dose is relatively small.

Removal of the interferon from the culture solution means prevention of further formation of the AVP, rapid (7-8 hrs.) breakdown of AVP so far synthesized, and, of course, loss of the achieved level of cell resistance.

The period between removal of interferon and the start of reduction in cell resistance is quite independent of interferon concentration and multiplicity of infection. Possibly, however, it reflects the length of the functional life of the AVP.

Another result of the removal of interferon from the virus-cell-interferon system is the development of the typical process of virus infection following a certain latency period [13]. As was established in the authors' experiments, the duration of this period depends upon the particular conditions of the test (interferon concentration, infection multiplicity, and time of incubation of the infected cells with interferon).

1. S. R
2. N. B
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10. M. M
11. Yu. V p.
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SOME REGULARITIES OF INTERFERON LITER

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