PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 159, 94–97 (1978)

Immune Interferon Activates Cells More Slowly Than Does Virus-Induced Interferon[1] (40291)

F. DIANZANI, L. SALTER, W. R. FLEISCHMANN, JR., AND M. ZUCCA

Department of Microbiology, University of Texas Medical Branch, Galveston, Texas 77550

Three antigenically different types of interferon have been found: (a) a 27000–30000 MW protein produced by somatic cells (fibroblast interferon) stimulated by viruses, (b) an interferon produced by leukocytes (leukocyte interferon) also stimulated by viruses (VIF), and (c) immune interferon (IIF), produced by lymphocytes following activation by mitogens or by specific antigenic stimulation (1–3). While the biochemical and biological properties of VIF have been extensively explored and many aspects of its mechanisms of production and action have been clarified, the properties of IIF, especially the mechanisms of activation of the cells, are as yet poorly understood.

Several differences in function between IIF and VIF have been noted. It has been reported that: preparations of IIF exert higher antitumor and immunoregulatory activity as compared with VIF (4, 5), VIF immunosuppressive action but not mitogen induced IIF action is blocked by mercaptoethanol (6), and IIF showed, at least in one system, a decreased ability to inhibit virus yield relative to VIF (7). It seems then reasonable that, since the different types of IF have different mechanisms of induction and show differences of biologic activity, they are likely to manifest important differences in molecular and cellular reactivity (8–10). Since information on this subject could lead to a better understanding of the mechanism of action (antiviral, antitumor, immunoregulatory) of the different types of IF, we have undertaken a comparative study on cellular activation by VIF and IIF.

In previous studies (11, 12) we showed that a very brief reaction (minutes) between VIF and cells at 37° rapidly results in cellular activation which, after removal of IF, is followed 30 min later by the transcription and translation of mRNA for the antiviral protein responsible for the cellular antiviral state. The present study is a comparison of these kinetics of cellular activation using IIF and VIF.

Materials and methods. Human leukocyte interferon (10^6 units/mg protein), induced by Sendai virus, was obtained from the Antiviral Substances Program, NIAID, NIH, and was produced by methods previously described (13). Mouse fibroblast interferon was obtained from the mouse C243 cell line induced with Newcastle Disease Virus as previously described (10^3 units/mg protein; 14). Human immune interferon ($10^{2.4}$ units/mg protein) was obtained from normal lymphocyte cultures stimulated for 4 days with staphylococcal enterotoxin A (SEA). Mouse immune interferon (10^2 units/mg protein) was obtained from mouse splenic cell cultures stimulated with SEA (15). Virus-type interferons were shown to be resistant to 5 days exposure to pH 2 and completely neutralized by specific antibody. Immune interferons were instable at pH 2 and not significantly neutralized by antibody to virus-type interferon (15). Interferon and interferon-induced antiviral activity were measured by the inhibition of the yield of Sindbis virus (human interferon) or GD7 virus (mouse interferon) hemagglutinin in a single cycle yield assay (16) employing tube cultures of human diploid foreskin cells, HF 19, or mouse L cells, strain CCL-1. Interferon titers are expressed as human or mouse reference units. Temperature control at 37° for short periods of time was effected in a waterbath and longer incubations were performed in a 37° incubator containing 4% CO_2 as previously described (11).

Results. Development of antiviral resistance in cells treated with virus-induced or immune interferon. The results of a representative experiment carried out with human leukocyte and immune IFs are shown in Fig. 1. Both

[1] This investigation was supported by DHEW Grant No. 500170 and by CNR Progetto Finalizzato Virus.

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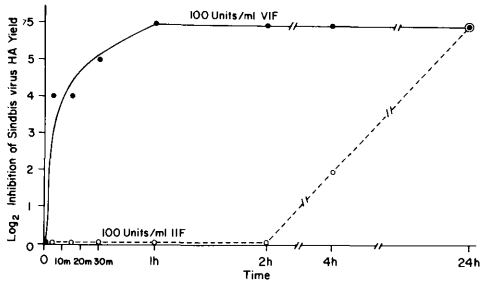

FIG. 1. Development of antiviral activity in human diploid foreskin cells treated for various periods of time with 100 units of either virus-induced or mitogen-induced human interferon.

types of IF were applied at a concentration of 100 reference units per ml as previously described (11). At preestablished times the interferon was removed and the cultures were washed 4 times and challenged with virus (multiplicity of infection, 10). After 1 hr for viral adsorption the cultures were washed 3 times and incubated for 18 hr before titration of viral yield. A control titration of the level of IF activity was included in every experiment. It may be seen that the cell cultures treated with VIF developed substantial resistance after a 5-min treatment and that the degree of resistance to Sindbis virus replication continued to rise thereafter so that 1 hour later it was greater than the measurable level. However, IIF did not induce detectable resistance over 2 hr, and marginal resistance was produced only after 4 hr. The expected degree of antiviral activity was induced after 24 hr treatment.

Similar results were obtained for mouse L cells treated with 300 reference units of either virus induced or immune mouse IF and challenged with GD-7 (Fig. 2). Additionally the same type of kinetics was observed for two more virus-cell systems: vesicular stomatitis virus (human cells, multiplicity of infection, 10) and mengovirus (L cells, multiplicity of infection, 0.1).

Since IIF preparations had a much lower specific activity as compared with VIF preparations, the possibility that some contaminant present in IIF could affect the rate of cellular activation was examined. Specifically cell cultures were treated either with 100 units of VIF, 100 units of IIF or 100 units of VIF plus 100 units of IIF. The cultures were then challenged after 15 min, and 4 hr to determine whether protection in the cultures treated with both types of IF developed according to the kinetics of development of VIF or IIF. The results (Table I) showed that the rapid kinetics of development of the antiviral state induced by VIF was not slowed by the presence of IIF. The same results were obtained either when the two types of interferon were mixed before addition to the cells or when either type was added immediately before the other.

Binding of interferon to cells at 37. It has been shown that cells treated with VIF bind interferon molecules very rapidly (17–21). However data on cellular binding of IIF are not yet available. Since the lack of rapid cellular activation by IIF, as compared with VIF, could be due to different kinetics of cellular binding, experiments were designed to establish the extent of binding of two types of IF under the conditions of rapid activation.

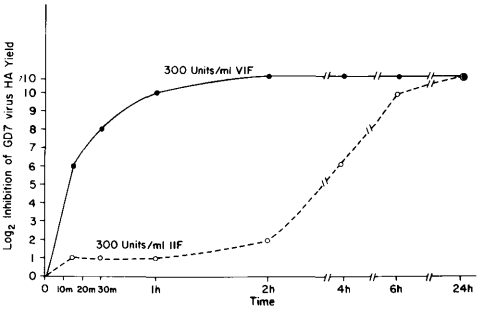

FIG. 2. Development of antiviral activity in mouse L cells treated for various periods of time with 300 units of either virus induced or mitogen induced mouse interferon.

TABLE I. INDUCTION OF THE ANTIVIRAL STATE BY VIRUS-INDUCED INTERFERON, IMMUNE INTERFERON, OR A COMBINATION OF BOTH.

Species of interferon	Type of interferon	Inhibition of virus yield* after treatment for	
		15 min	4 hr
Human	VIF	1.9	2.1
	IIF	0.0	0.8
	VIF + IIF	1.9	2.3
Mouse	VIF	4	>7
	IIF	<1	2
	VIF + IIF	4	>7

* Log_{10} inhibition of Sindbis virus PFU yield (human interferons) or log_2 inhibition of GD-7 virus HA yield (mouse interferons).

Specifically, 0.5 ml of medium containing 1000 reference units per ml of either virus-induced or immune mouse IF were applied to duplicate tube cultures of L cells maintained at 37°. After different periods of time, one group of cultures was washed 6 times with Earle's balanced solution, refed with 0.5 ml of Eagle's medium, and frozen-thawed 3 times to release cell-associated IF. The fluids were then assayed for IF. The results of a representative experiment are shown in Table II.

TABLE II. CELLULAR BINDING OF VIRUS-INDUCED OR IMMUNE MOUSE INTERFERON.

Type of Interferon	Units of interferon associated with cells after (min)				
	5	10	15	30	60
Virus-induced	6	12	10	12	12
Immune	8	10	10	12	12

It may be seen that binding of both VIF and IIF was essentially maximal after 5 minutes incubation at 37° and that the amount of bound IF remained unchanged thereafter. There was no significant difference between the amount of VIF and IIF bound at each time. The IF associated with the cells was approximately 0.6–1.2% of the total IF applied to the cultures. This finding substantially agrees with previous observations on cellular binding of VIF (16–21) and shows that binding of IIF occurs at a similar rate and to a similar degree.

Discussion. It has been previously shown that the development of the antiviral state in cells treated with VIF is triggered immediately by a very brief interaction between IF and cells and continues when the IF is removed from contact with the cells by washing and antibody inactivation (22). This finding has been confirmed for VIF by the data presented in this paper. However IIF, assayed under identical experimental conditions, failed to immediately activate cells after brief contact. Thus in both the human and mouse systems, detectable antiviral resistance was induced by IIF only after several hours of incubation at 37°. The different kinetics of cellular activation by the two types of IF may be due to: (a) Difference of availability of cellular receptors, (b) the presence in the IIF preparation, and not in the VIF preparations, of substances capable of retarding expression of interferon activity under the present experimental conditions, and (c) a different mechanism of activation of the antiviral state.

Studies of cellular binding of the two types of IF did not show any significant difference between their binding activity, suggesting that differences of association by the two IFs with the cell may not play an important role in establishing the different kinetics of activation. However, it should be borne in mind that this relatively durable binding which is usually measured (17–21) may not reflect the transient cell-activating event by which IF induces rapid resistance (22). Specifically it has been shown previously that firm binding to the cell surface is not required for the rapid induction of the antiviral state by VIF and the present finding of different kinetics of activation despite equal kinetics of binding further supports that conclusion.

The hypothesis that a component of the IIF preparation could interfere with the rapid action of the VIF molecule seems less likely since the presence of the slow acting IIF preparation did not inhibit the rapid activation by VIF. However this experiment does not eliminate the possibility of the presence of a substance which only inhibits the rapid action of IIF. Further studies with purified preparations of IIF could test this possibility.

The hypothesis that VIF and IIF may induce the antiviral state through different mechanisms appears likely and deserves further study. If the same biological activity may be evoked through different activation processes, the finding may provide a useful working model for studying several critical cell activities, such as cellular regulation of gene expression, regulation of gene products, and cell membrane receptor functions. Additionally, the different kinetics of activation of the antiviral state by the two types of IF may provide a simple and rapid method to differentiate them.

Summary. The kinetics of activation of the antiviral state by virus induced interferon and by mitogen-induced immune interferon have been studied comparatively. It has been found that both human and murine virus-induced interferons are able to activate the antiviral state after a brief (minutes) contact with the cells. In contrast, several hours were

required for both human and mouse immune interferons to induce a comparable level of antiviral resistance. Experiments measuring the binding of the two interferons to cells showed that there was no significant difference in the rate and degree of binding, suggesting that a different total association of interferon with cells could not account for the slower kinetics of activation by immune interferon. Additionally, the possibility that some contaminants present in the immune interferon preparation could nonspecifically interfere with the rapid induction phenomenon is not supported by the finding that the rapid kinetics of cell activation by virus-induced interferon was not modified by the presence of immune interferon. The interesting possibility which remains is that the two interferons may activate cells by different mechanisms.

The authors are greatly indebted to Dr. G. Georgiades and Mr. M. Langford for the generous gifts of immune interferon and to Dr. S. Baron for helpful suggestions and criticism.

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Received April 24, 1978. P.S.E.B.M. 1978, Vol. 159