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The Continued Presence of Interferon is not Required for Activation of Cells by Interferon (39851)[1]

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A very brief (minutes) reaction between interferon and cells at 37° rapidly results in induced cellular resistance to virus (1) which is believed to be mediated by induced antiviral protein (AVP) (2). It has been determined that there is a 30-min time period between the brief exposure to interferon and the beginning of transcription of the mRNA for the proposed antiviral protein (3, 7). This 30-min period may be necessary for (a) a continuing process of activation of cells that requires the presence of interferon, or (b) a more complicated process which is initiated by the brief contact of the cell with interferon and proceeds in its absence. The present study was undertaken to help determine whether or not the rapid induction of the AVP which is initiated by reaction of interferon with cells continues to occur after its complete removal. Acquisition of this type of information is important for understanding the regulation of genetic expression of the interferon system.

Materials and methods. Human leukocyte interferon was obtained from the Antiviral Substances Program, NIAID and was produced by methods previously described (4). Titrations of interferon and of the interferon-induced antiviral state were performed by the inhibition of the yield of Sindbis virus hemagglutinin in a single-cycle yield assay employing tube cultures of human foreskin cells, HR203 (5). Interferon units are expressed as human reference units. Temperature control at 37° for short periods of time was obtained with a water bath, and longer incubations were performed in an incubator containing 4% CO_2. Additional control of temperature at 37° was obtained with the use of prewarmed media and wash fluids. Using the water bath, and an electronic thermometer, temperature shifts to 37° were shown to occur within 2 min. Sheep gamma globulin containing antibody to human leukocyte interferon was kindly supplied by Dr. C. Anfinsen, NIH, Bethesda, Maryland and was used at a concentration capable of inhibiting the activity of 1000 units/ml of human leukocyte interferon. At this concentration it was shown that the antibody did not bind to cells. A normal sheep gamma globulin control was included in each experiment employing the antibody.

Results. Binding of interferon to cells and development of resistance after brief exposure to interferon at 0 to 37°. It has been shown that cells treated with interferon bind interferon molecules very rapidly (6-9). However, it has not been determined whether the continued presence of bound interferon is required for the completion of the induction process or whether induction may be triggered by a more fleeting type of temperature-dependent interaction. To clarify this point preliminary experiments were designed to establish the extent of binding of interferon under conditions which may or may not permit the development of antiviral resistance after brief contact with interferon. Specifically, 0.5 ml of medium containing 300 units/ml of interferon was applied to two groups of duplicate cultures at 0 or 37°. After 15 min the interferon was removed, and the cultures were washed four times with Earle's balanced salt solution (2 ml per wash) and fed with 0.5 ml of interferon-free Eagle's medium containing 2% fetal bovine serum. One group of the cultures was then subjected to three cycles of freezing and thawing to release cell-associated interferon which was then assayed. Representative results are shown in Table I, columns 1-3. There was no signifi-

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TABLE I. EFFECT OF TEMPERATURE ON DEVELOPMENT OF RESISTANCE AFTER BRIEF EXPOSURE TO INTERFERON AT 0 OR 37°

Interferon pretreatment (300 units/ml for 15 min) at:	Interferon (units/ml)			Incubation before virus challenge		
	Cell-Bound	Subsequently released after 2 hr at 37°		None	2 hr at 0°	2 hr at 37°
0°	5	15	Units of HA yield[a]	32	32	2
			Percentage of inhibition	0	0	94[2]
37°	9	7	Units of HA yield[a]	<2	<2	<2
			Percentage of inhibition	94[b]	>94[b]	>94[b]

[a] Significance at the $p = 0.05$ level of inhibition of virus HA yield relative to controls occurs at threefold decrease.

[b] $p < 0.05$.

cant difference between the amounts of interferon bound to the cells at 0 or 37° or the amounts subsequently released from cultures after continued incubation for 2 hr at 37°. The cell-associated interferon was not newly produced since the interferon preparation does not show interferon-inducing activity. The somewhat different rates of binding at 0 and 37° observed in different systems (8, 9) were not detectable under our experimental conditions.

Comparisons of the cell binding of interferon at 0 and 37° with the subsequent development of resistance could help determine whether or not the cell-bound interferon was the cause of rapid resistance. Therefore, in addition to the above experiment, duplicate cultures, after a 15-min reaction with 300 units/ml of interferon at 0 or 37°, were washed and challenged immediately or incubated at either 0 or 37° for an additional 2 hr before virus challenge. Virus resistance after the additional incubation is shown in Table I, columns 5–6. It may be seen that resistance was manifested after pretreatment for 15 min at 37° (column 4). However, at 0° no resistance was detected after the initial 15-min reaction with interferon or after the additional 2-hr incubation at 0°. Instead, resistance was shown after pretreatment for 15 min at 0° and a subsequent 2-hr incubation at 37°. As reported previously (8) this resistance is probably induced by the small amount of interferon which is residual in the culture after washing. Taken together these results imply that the interferon–cell interaction at 37° initiates a process more complex than simple binding. This comes from the finding that cells at 0° bind the same amounts of interferon as those at 37°, but subsequent warming from 0 to 37° does not result in rapid development of resistance.

To better define the development of resistance after exposure of cells to 300 units/ml of interferon for 15 min at 0 or 37°, the kinetics were studied. The cultures were washed four times at the respective temperatures, refed with interferon-free medium, and incubated at 37° for varying periods before virus challenge. As shown in Fig. 1, the rate of development of resistance after brief exposure of cells to interferon at 0° was much slower than the rate at 37°. Thus, the cell-bound interferon alone could not account for the rapid developing resistance at 37°, since an equal amount of interferon was bound at 0°, but the rate of development of resistance after further incubation at 37° was much slower than in the cultures initially exposed to interferon at 37°.

Role of interferon which has eluted from cells in the development of resistance under the present conditions. The slow development of resistance after brief reaction of interferon with cells at 0° and subsequent incubation at 37° has been reported to result from elution of cell-associated interferon which then reacts with the cells as reported previously (8). To assess the role of cell-bound and subsequently eluted interferon in the development of resistance at 37°, elution of cell-bound interferon at 37° was allowed to occur into large volumes of medium (5 ml rather than 0.2 or 0.5 ml) as had been employed previously (2). Under these conditions the concentration of eluted interferon would be 1/10th to 1/25th the concen-

tration of interferon eluted into 1 volume of medium. Since antiviral activity is proportional to the concentration of interferon (11), elution into a larger volume would decrease the development of resistance. Thus, cultures were exposed in the standard manner to 300 units/ml of interferon at 0 or 37° for 15 min and washed four times at the respective temperature. Groups of cultures were challenged (a) immediately with virus suspension in 0.2 ml of medium; (b) immediately with the same concentration of virus in 5 ml of medium; (c) with virus after a 2-hr incubation at 37° in 0.5 ml of medium; and (d) after a 2-hr incubation at 37° in 5 ml of medium. To disperse the eluting interferon, cultures were shaken gently every 5 min during the 2-hr incubation period. Subsequently, unadsorbed virus was removed by rinsing, and virus yields were determined after the usual incubation time.

The results, shown in Table II, demonstrate the expected inhibition of virus production when the cultures were preincubated at 0° for 15 min with interferon and further incubated for 2 hr at 37° in the small volume of medium. Also as anticipated a similar 2 hr incubation of cultures with large volumes of medium or virus inoculum did not result in the development of significant resistance. In comparison preincubation at 37° for 15 min with 300 units/ml of interferon induced highly significant resistance regardless of the volumes of subsequent incubating medium or virus inoculum.

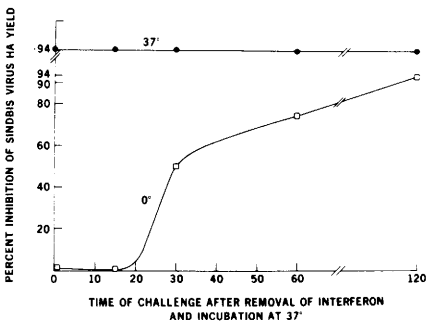

FIG. 1. Development of antiviral activity in cells treated with interferon at 0 (●——●) and 37° (□——□) for 15 min and then incubated at 37° for the times indicated before challenge with Sindbis virus.

Since resistance did not develop in cells pretreated with interferon at 0° and then incubated in large volumes of medium at 37°, it was confirmed that the slow development of resistance under the 0° conditions of Fig. 1 was due to elution of cell-associated interferon which then slowly induced resistance in the cells. More importantly, it may be concluded that eluting interferon did not play a role in the development of resistance at 37°, since eluting volume did not effect the development of this resistance although elution does occur (see Table I).

Effect of removal of cell-bound interferon by antibody on the development of resistance. Another way to test the relationship of cell-associated interferon to the activation of the resistant state is to determine the effect of inactivation of cell-bound interferon by antibody on the development of resistance. If the development of resistance requires the presence of cell-bound interferon during the entire 30-min period between the initial interferon cell reaction and transcription, then brief treatment of these cells with antibody to interferon at 37° should prevent the rapid development of

TABLE II. EFFECT OF VOLUME OF ELUTING MEDIUM AND ANTIBODY TO INTERFERON ON DEVELOPMENT OF RESISTANCE AFTER BRIEF EXPOSURE TO INTERFERON AT 0 OR 37°

Interferon pretreatment (300 units/ml for 15 min) at:		Incubation before challenge				
		None	2 hr at 37°	2 hr at 37° with 10 vol of medium	2 hr at 37° with antibody to interferon	Challenge with 25 vol of medium
0°	Units of HA yield[a]	32	2	16	32	32
	Percentage of inhibition	0	94[2]	50	0	0
37°	Units of HA yield[a]	<2	<2	<2	<2	<2
	Percentage of inhibition	>94[b]	>94[b]	>94[b]	>94[b]	>94[b]

[a] Significance at the $p = 0.05$ level of inhibition of virus HA yield relative to controls occurs at a threefold decrease.

[b] $p < 0.05$.

resistance. For this purpose cultures which had been pretreated at 0 or 37° for 15 min with 300 units/ml of interferon were washed and incubated for an additional 2 hr at 37° in the presence of high-titered antibody to interferon using standard volumes of medium. The antibody did not prevent the development of resistance in cells briefly reacted with interferon at 37° (Table II). Controls showed that the concentration of antibody used: (a) inhibited 1000 units of interferon in medium; (b) inactivated interferon bound to cells during a 15-min reaction at 37° (but did not abolish resistance); and (c) prevented the development of resistance in cells with interferon bound at 0°. The failure of anti-interferon antibody to prevent development of resistance after brief adsorption of interferon at 37° supports the conclusion that the process which leads to genetic derepression of the AVP can continue to function after removal of the inducing interferon.

Discussion. The present findings help define the characteristics of the interferon–cell interaction which lead to the transcriptional and translational events for production of the resistance factor (AVP). Basically, the present findings indicate that the initial and brief interaction of interferon with cells at 37° initiates a process which, in the absence of continued interferon, subsequently leads to derepression of the genetic site(s) for antiviral activity. The evidence is considered below.

Prior evidence indicates that the initial reaction of interferon with cells occurs at the cell surface (12) and the interferon is not consumed in the process (13). However, simple binding of interferon to the cell surface is not sufficient to initiate the process, since binding at 0° does not lead to activation of the cellular process which leads to development of resistance. The activation of the cell by interferon requires an incubation temperature of 37°, thereby indicating a temperature-dependent event. The ineffectiveness of the interferon–cell reaction at 0° could occur because interferon binds to cells differently at 0 and 37°, or that the cell can respond appropriately to interferon only at 37°. After the cell has been activated by brief contact with interferon at 37°, the continued presence of interferon is no longer required for the subsequent transcription (30 min later) which leads to resistance (3), since all detectable interferon may be removed by washing and applying antibody without impairing the later development of resistance. Thus, it seems reasonable to conclude that the brief reaction of interferon with human cells at 37° initiates a temperature-dependent cellular process which no longer requires the presence of interferon and leads 30 min later to the derepression of the cistron for the AVP. Although previous reports indicated that this activation might occur after simple binding at 0° (7, 8, 9), it did not occur under the present experimental conditions.

It is not known whether or not the mechanisms of rapid development of resistance are qualitatively different from those of slower development following use of low concentrations of interferon. A simple explanation would seem to be a common mechanism for both with different kinetics depending on the concentration of interferon and the temperature of incubation.

Summary. This study was undertaken to help determine whether or not the reaction of interferon with cells which leads to the rapid induction of the antiviral state (a) is initiated by the first binding of interferon to cells and (b) continues to occur after removal of the interferon. Experiments measuring the binding of interferon to cells over 15 min at 0 and 37° and the development of resistance showed that there was no significant difference between the amount of interferon bound to the cells at the two temperatures, but the rate of development of resistance was slowed if the initial binding occurred at 0°. This indicated that cell-bound interferon alone could not account for the rapidly developing resistance since equal amounts of interferon were bound at 0° and 37° but the rates of development of resistance were different. Experiments employing different volumes of medium for elution of interferon bound to cells at 0 or 37° showed that the eluting volume influenced the development of resistance in cells initially exposed to interferon at 0° but not in cells initially exposed at 37°. This indicated that the eluting interferon did not play

a role in the rapid development after initial reaction at 37° as it does after initial reaction at 0°. The failure of antibody to interferon to prevent the development of resistance after brief reaction of interferon with cells at 37° indicates that the continued presence of cell membrane-bound interferon is not required for development of this resistance. The present findings when taken together with previous findings indicate that a brief temperature-dependent interaction between interferon and human cells initiates one or more cellular processes which no longer requires the presence of interferon and leads 30 min later to the derepression of the cistron which controls the development of the antiviral state.

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