INFECTION AND IMMUNITY, Apr. 1978, p. 55-57
0019-9567/78/0020-0055$02.00/0

Vol. 20, No. 1
Printed in U.S.A.

Rapid Activation of the Interferon System In Vivo

FERDINANDO DIANZANI,[1]* PIETRO GULLINO,[2] AND SAMUEL BARON[1]

Department of Microbiology, University of Texas Medical Branch, Galveston, Texas 77550,[1] and Laboratory of Pathophysiology, National Cancer Institute, Bethesda, Maryland 20014[2]

Received for publication 6 October 1977

Experiments were carried out to study the kinetics of local interferon production in the subcutaneous tissues of rats stimulated with Newcastle disease virus. Specifically, the interferon produced and released in the extracellular fluids was collected at various intervals of time in micropore chambers implanted into the subcutaneous tissue of rats. Interferon was detected at moderate titers 1 h after induction, and it was present at high titer at 2 h. The interferon levels remained remarkably high in the samples collected after 3, 5, and 24 h, and in some rats it was still detectable after 48 and 72 h. Since control experiments showed that it requires 2 to 3 h for interferon to penetrate the chambers, it may be concluded that high concentrations of interferon are present in the extracellular fluid within 1 h of induction. The evaluation of the kinetics of production and of the concentrations attained in the extracellular fluid suggests that in a solid tissue a cell infected by a potent interferon inducer may produce interferon early enough and in sufficient quantity to protect neighboring cells before the production of progeny virions.

Previous findings in vitro have shown that (i) moderate to high concentrations of interferon (IF) can induce a strong antiviral state after a brief (minutes) contact with the cells (3), and (ii) L cells induced with Newcastle disease virus (NDV) and then resuspended at cell concentrations approaching those existing in the body develop a strong antiviral state in less than 1 h (5). These data raise the possibility that in vivo the IF produced by an induced cell and secreted into the small extracellular space may reach concentrations high enough to induce rapidly the antiviral state.

These concentrations have been theoretically estimated by assuming that: (i) a single cell releases its IF over a period of 6 h; (ii) the half-life of IF in the extracellular fluid is similar to the rapid turnover phase in the serum (10 min); (iii) the volume of extracellular space surrounding a cell in a solid tissue varies from 12 to 120 μm^3; and (iv) IF production in vivo may cover the same range as in cell cultures (0.005 to 0.05 unit/cell). The resulting estimates are reported in Table 1. It can be seen that an estimated concentration of 3,000 to 30,000,000 units/ml may occur in the extracellular space of an F-producing cell (3).

To verify this estimation, experiments were carried out using micropore chambers incorporated into the subcutaneous tissues of rats to collect the IF released in the extracellular fluid at various intervals of time after induction by virus.

(A preliminary report of this work was given at the 9th International Immunobiological Symposium, Zagreb, Yugoslavia, October 1975.)

MATERIALS AND METHODS

Seven to 10 days before induction of IF, micropore chambers (diameter, 13 mm; capacity, 150 to 200 μl; porosity, 45 μm) were implanted in the subcutaneous tissues of female Sprague-Dawley rats (200-g average weight) as previously described (6). During this period of time the host's fibroblasts layer the external face of the membranes. The cells, however, are unable to pass through the membranes, but the fluid bathing the cells can diffuse through and be collected by aspiration through a polyethylene catheter attached to the chamber. Immediately before induction the fluid in the chambers was collected (zero time), and then 0.1 ml of suspension of NDV (B1 strain) containing 10^9 egg infectious doses/ml was placed in the chambers. At pre-established times thereafter the fluid contained in the chambers was collected, diluted 1:100, and titrated for IF activity after inactivation of residual NDV by pH 2 treatment for 5 days and addition of antibody to NDV. At least three rats were used for each point. Fluids collected from each animal before administration of NDV or collected after application of uninfected allantoic fluid were used as controls.

IF titrations were performed in rat embryo fibroblast cultures as a single-cycle hemagglutination yield reduction assay, using Sindbis virus as the challenge virus as previously described (4). A threefold reduction with respect to the control virus yield was considered significant. The antiviral activity found in the samples was shown to be IF mediated in accordance with the current criteria (pH 2 stability, activity against heter-

ologous viruses, inactivity on heterologous cells, blocking by actinomycin D).

RESULTS

The results of a representative experiment are reported in Table 2. It may be seen that 100 units of IF per ml was detected in two out of three rats tested 1 h after induction, and it was present, in titers up to 200,000 units/ml, in all the rats tested 2 h after induction. The IF levels remained remarkably high in the 3-, 5-, and 24-h samples and in some rats were still detectable after 48 and 72 h.

Previous studies of diffusion rates of fluid into chambers under analogous conditions (6, 9) established that the rate is low. A preliminary confirmation under the present experimental conditions was carried out using equilibrating molecule. ^{24}Na-labeled saline was injected intravenously, and at various intervals of time the amount of radioactivity in the plasma and in the chamber fluid was determined. An equilibrium was reached only 2 to 3 h after the injection. It may then be inferred that in the studies in rats the IF detected in the chambers at each of the early times represents only a fraction of the amount actually produced. To test this hypothesis the diffusion rate of IF was measured in vitro in chambers filled with Eagle medium supplemented with fetal bovine serum and immersed in the same medium containing 3,000 reference units of IF per ml. After the indicated times of incubation at 37°C (with frequent, gentle shaking), the IF titer was determined in duplicate chambers and the surrounding fluid. The results are reported in Fig. 1. It can be seen that after 2 h the IF concentration within the chambers was only 1% of the external concentration and an equilibrium was established only between 2 and 3 h of incubation. Since it requires 2 to 3 h for IF to penetrate the chambers fully, the detection of high concentrations of IF in the chambers implanted into the subcutaneous tissue of rats 2 to 3 h after induction (Table 2) indicates that the rats stimulated with NDV actually had produced these very large amounts of IF within 1 h after the induction.

TABLE 1. *Estimated range of concentrations of IF in the intercellular space surrounding a producing cell*[a]

IF (units) produced by 2×10^5 cells	IF yield/cell (units)	Vol of intercellular space/cell (μm^3)	Estimated concn of IF (units/ml) in the intercellular space/cell
10^4	0.05	12	$10^{7.5}$
		120	$10^{6.5}$
10^2	0.0005	12	$10^{5.5}$
		120	$10^{4.5}$
10	0.00005	12	$10^{4.5}$
		120	$10^{3.5}$

[a] Values have been calculated with the following formula and rounded off to the nearest half-$\log_{10}$: (IF yield per culture ÷ number of cells) ÷ intercellular volume per cell × fraction of 24-h production time represented by 10-min IF yield.

DISCUSSION

This study shows that the IF system is activated extremely early in the virus-induced subcutaneous tissues of rats. Earlier studies showed (2, 10) that rats injected intravenously with NDV produce IF, detectable in the plasma and in spleen extracts, as early as 1 to 2 h after the induction, but titers were, respectively, 50 and <10 units/ml after 1 h and did not exceed 100 units after 2 h. Maximum levels in plasma and spleen extracts were not observed until 6 h post-induction and did not exceed 100 and 10,000 units/ml, respectively. Similar kinetics also occur in mice and rabbits induced intravenously with the same inducer (1, 7). Also, rat cells stimulated in culture with Chikungunya virus do not produce detectable IF until 5 h after the induction (4). In the present experiments 100 and 10,000 to 100,000 reference units of IF were detected in the fluid collected from the chambers 1 and 2 h, respectively, after application of NDV. However, the data on the rate of diffusion of IF

TABLE 2. *IF appearance in micropore chambers implanted subcutaneously in rats stimulated locally with NDV*[a]

Rat no.	IF titer ($\log_{10}$ units/ml) at time (h) after stimulation with NDV							
	0	1	2	3	5	24	48	72
1	<2.0	ND	5.4	ND	4.8	4.9	ND	<2.0
2	<2.0	ND	ND	5.3	ND	4.8	2.2	<2.0
3	<2.0	ND	ND	4.2	ND	4.0	<2.0	<2.0
4	<2.0	2.0	3.0	4.0	4.0	4.0	3.0	2.7
5	<2.0	2.0	3.5	ND	4.0	3.5	3.0	2.5
6	<2.0	2.0	4.0	ND	3.0	4.5	3.5	2.0

[a] Each value indicates the IF titer in individual rats. ND, Not determined.

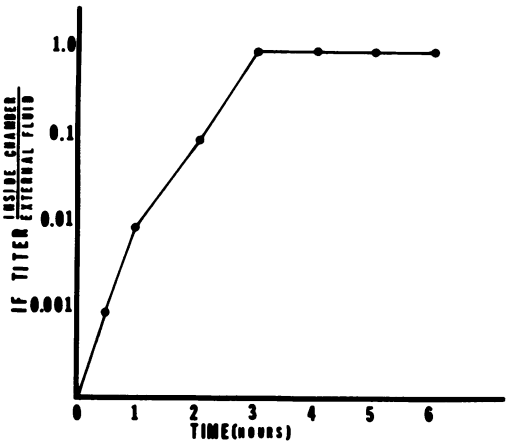

FIG. 1. *Rate of diffusion of IF into micropore chambers from surrounding fluid.*

shown in Fig. 1 indicate that after 1 and 2 h of incubation only 0.1 and 1.0% of the external IF had penetrated the chambers. It is, therefore, reasonable to conclude that the actual concentration of IF in the subcutaneous extracellular fluid was at least 10,000 and 100,000 to 1,000,000 units/ml after 1 and 2 h, respectively, following induction. These values approach those estimated in Table 1 and are much higher than those detected in plasma and in spleen extracts during other studies. It remains to be determined, however, whether this difference is due to more efficient production of IF by subcutaneous cells, induction of more cells by the chamber system, or, more likely, a more efficient collection of IF by the chambers. Whichever interpretation is valid, the data show that in vivo local production of IF can occur extremely early and to high titers.

The persistence of high levels of IF in the chamber from 24 to 72 h may reflect slower decay of IF in the chambers, nonsynchronous production of IF (due to different cell types or continuing induction), or a combination of these.

The biological significance of the presence of high concentration of IF as early as 1 h after the viral infection is clear. In fact, the data provide reasonable evidence that under certain conditions in a solid tissue, a cell infected by a potent IF inducer may produce sufficient IF sufficiently early to protect neighboring cells (and perhaps itself) before the production and release of progeny virions. Although less potent viral inducers require study, the findings reported here may provide an explanation for the occurrence of at least some of the abortive or self-limiting viral infections in nature.

ACKNOWLEDGMENTS

This work was supported by contract 760066384 from Consiglio Nazionale delle Ricerche. Progetto Finalizzato Virus; Public Health Service grant RR 05427 from the Division of Research Resources; and Department of Health, Education and Welfare grant S00170.

We thank Carol Uhlendorf, Lynette Salter, and Flora Grantham for their excellent technical assistance.

LITERATURE CITED

1. **Baron, S., and C. E. Buckler.** 1963. Circulating interferon in mice after intravenous injection of virus. Science **141**:1061–1064.
2. **Billiau, A., and C. E. Buckler.** 1970. Production and assay of rat interferon. Symp. Ser. Immunobiol. Stand. **14**:37–44.
3. **Dianzani, F., and S. Baron.** 1975. Unexpectedly rapid action of human interferon in physiological conditions. Nature (London) **257**:682–684.
4. **Dianzani, F., A. Pugliese, and S. Baron.** 1974. Induction of interferon by a nonreplicating single-stranded RNA virus. Proc. Soc. Exp. Biol. Med. **145**:428–433.
5. **Dianzani, F., I. Viano, M. Santiano, M. Zucca, and S. Baron.** 1977. Effect of cell density on development of the antiviral state: interferon-producing cells: a possible model of *in vivo* conditions. Proc. Soc. Exp. Biol. Med. **155**:445–448.
6. **Gullino, P., S. H. Clark, and F. H. Grantham.** 1964. The interstital fluid of solid tumors. Cancer Res. **24**:780–797.
7. **Ho, M., M. K. Breinig, B. Postic, and J. A. Armstrong.** 1970. The effect of preinjections on the stimulation of interferon by a complexed polynucleotide, endotoxin, and virus. Ann. N.Y. Acad. Sci. **173**:680–693.
8. **Lockart, R. Z., Jr.** 1973. Criteria for acceptance of a viral inhibitor as an interferon and a general description of the biological properties of known interferons, p. 11–28. *In* N. Finter (ed.), Interferon and interferon inducers. North-Holland Publishing Co., Amsterdam.
9. **Swabb, E. A., J. Wei, and P. Gullino.** 1974. Diffusion and convection in normal and neoplastic tissues. Cancer Res. **34**:2814–2822.
10. **Van Rossum, W., and P. DeSomer.** 1966. Some aspects of interferon production *in vivo*. Life Sci. **5**:105–109.