Successful Induction of Locally Produced (Lung) Interferon During the Hyporesponsive State (37440)

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As discussed by Paucker and Boxaca (1) and Ho, Postic and Ke (2), successive administration of an interferon inducer *in vivo* does not lead to progressively increased levels of circulating interferon. Rather, there is a circumscribed period during which each subsequent response to the inducer beyond the initial one is considerably less than found originally. This has been called the hyporesponsive state. This phenomenon has been of concern to those contemplating the use of interferon inducers clinically since it is difficult to induce sustained high levels of circulating interferon over a prolonged time. Of concern also, have been the nature and efficacy of host defenses to virus infection during this hyporesponsive period. Using a convenient model consisting of mice infected with the PR8 strain of influenza virus, an attempt was made to further explore details of the hyporesponsive state. A period of approximately 72 hr after intranasal inoculation of PR8 is required in order to induce maximal lung interferon. Therefore, there is ample time to make animals hyporesponsive to interferon induction using a nonviral inducer prior to the time that maximal stimulation of interferon by the PR8 influenza virus would normally occur. The effects of the hyporesponsive state on interferon induction by the influenza virus can then be evaluated.

Animals made hyporesponsive to repeated intraperitoneal or intravenous injections of a synthetic polynucleotide, polyinosinic–polycytidilic acid (poly I:poly C), did not exhibit hyporesponsiveness to induction of interferon by the virus locally in the lung. Therefore, it appears that the definition of the hyporesponsive state, as based on measurement of serum interferon, must be tempered by a consideration that local sites may not be hyporesponsive and may be capable of giving a primary interferon response.

Materials and Methods. Animals. Female, general purpose Swiss mice weighing 15–20 g were used in these experiments (obtained from Veterinary Resources Branch, NIH). All animal experiments involved at least 10 animals/test group and serums and lungs from animals were pooled as indicated. All lungs were rinsed thoroughly at time of harvest in an attempt to eliminate surface blood. Ten percent suspensions by weight of the lungs were made in Eagle's minimal essential medium (MEM) with 2% calf serum and used for the interferon assays as detailed below.

Interferon inducers. The PR8 strain of influenza virus was prepared in the allantoic fluid of 10–11 day old embryonated eggs. Assay for virus was performed using a standard HI test with chicken RBC. The PR8 pool titered 1:128 in this test and has previously been shown to contain $10^{6.3}$ EID_{50}. It was diluted 1:10 before use in these experiments.

The synthetic polynucleotide, poly I:poly C was obtained from P. L. Biochemicals in the lyophilized state and was reconstituted with pyrogen-free water as needed.

Interferon assay. Twofold serial dilutions of 1 ml of the material to be tested were placed on primary mouse embryo cells and assayed as previously described (3). Briefly, following overnight incubation and removal of the test materials, the cells were challenged with vesicular stomatitis virus, Indiana

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strain (VSV) at a multiplicity of infection of approximately 2–10. About 16–18 hr later, fluids were harvested and assayed for VSV. The reciprocal of the last dilution causing at least a 0.5 $\log_{10}$ decrease in virus yield was taken as the titer of interferon. Lung suspensions to be assayed for interferon content were first acidified to pH 2 for 72 hr. This eliminated the presence of infectious PR8 influenza virus as well as any residual poly I:poly C. Interferon was characterized by its species specificity, resistance to acidification (pH 2) and its broad antiviral action.

Experimental design. Each set of studies involved four groups of animals and lasted for 3 days. Each mouse in the first group received only one inoculation of 100 μg of poly I:poly C either intraperitoneally or intravenously on Day 3 of the experiment, 6 hr before its termination. A second group of animals were injected with poly I:poly C on Days 0 through 3 in order to contrast reactivity to repeated injections to that to a single one and thus, evaluate the ability of the mice to develop hyporesponsiveness. A third group of mice were inoculated intranasally (IN) with influenza virus on Day 0 and simultaneously received the first injection of poly I:poly C which was repeated on Days, 1, 2 and 3. Finally, mice in the fourth group were only inoculated IN with influenza virus on Day 0. Serums and organs for interferon assay were collected on Day 3, 6 hr after the last poly I:poly C injection or 72 hr after inoculation of influenza virus.

Results. Early experiments confirmed the report by Isaacs and Hitchcock (4) that, following IN inoculation of influenza virus, peak interferon titers were obtained 72 hr postinfection. This occurred prior to any appreciable mortality from the influenza virus infection.

Table I illustrates typical experiments. Six hours following a single ip injection of poly I:poly C, serum interferon measured 1:1024 and interferon was also present in the lung suspension at a titer of 1:16. Whether this represented actual interferon induction in lung tissue, or that present in the serum contained in the lungs is not certain.

Following multiple ip injections of poly I:poly C only (on Days 0, 1 and 2) a "hyporesponsive" state was reached since, in animals in this group, on Day 3, poly I:poly C injected 6 hr prior to the termination of the experiment was unable to stimulate measurable serum interferon. Interferon also was not found in the lungs. Thus, a regimen of poly I:poly C given ip on Days 0, 1 and 2 makes the animals "hyporesponsive" to further ip stimulation by poly I:poly C.

The lungs of animals in the group receiving influenza virus IN only had interferon in a titer of 1:1024. In this particular experiment, there was little interferon found in the serum (titer of 1:8) although in other experiments, titers of up to 1:16 have been found.

TABLE I. Serum and Lung Interferon Induction During "Hyporesponsive Period."[a]

Treatment	Interferon titer			
	Poly I:poly C intraperitoneally		Poly I:poly C intravenously	
	Serum	Lung	Serum	Lung
Poly I:poly C × 1 6 hr before termination of experiment	1024[b]	16	1024	16
Poly I:poly C × 3	8	8	64	32
Influenza virus IN × 1; poly I:poly C × 3	8	512	8	128
Influenza virus IN × 1; no poly I:poly C	8	1024	8	128

[a] 72 hr after start of experiment.

[b] Reciprocal of highest dilution showing interferon-like activity.

There was little or no hyporesponsiveness to the induction of lung interferon by the influenza virus in animals in the group which received multiple injections of poly I:poly C as well as influenza virus IN since titers of 1:512 were found in the lungs of these animals. Thus, the production of lung interferon was unaltered by the presence of the "hyporesponsive" state in the serum.

It was possible that following ip injection of the poly I:poly C the inducer was not able to reach lung tissue and therefore the lung was not made hyporesponsive. In order to further explore this, similar experiments were performed except that the poly I:poly C was given intravenously. As shown in Table I, the pattern of results were similar to those noted with ip injection of the poly I:poly C.

In these experiments, the PR8 strain of influenza virus was found to be relatively insensitive to interferon. No differences in the influenza virus titer of lung tissues were found in animals receiving only influenza, or influenza and multiple doses of poly I:poly C. The interferon found in the serum and lung suspensions after exposure to systemic poly I:poly C had no effect on the amount of virus recovered from the lung which titered approximately 1:8 HA units.

An additional possibility, as suggested by the experiments of Buckler *et al.* (personal communication) and which could explain our results was that the response to a viral stimulus of interferon differs from the response to a nonviral interferon stimulus. This was explored by repeating the above experiments except that poly I:poly C was used exclusively for both the ip and the IN inoculations. The results showed the same pattern. Thus, multiple ip doses of poly I:poly C, which made the animals hyporesponsive to further interferon induction in serum after ip inoculation of this compound, had no effect on the level of interferon in the lung achieved after multiple IN inoculations of poly I:poly C (titer of 1:64 which did not differ significantly from the control titer of 1:32 in animals receiving only a single poly I:poly C IN).

The converse of the above experiments was also performed. Animals which were inoculated with influenza virus 72 hr previously and which were actively producing lung interferon were then stimulated with a single iv injection of poly I:poly C 6 hr before sacrifice. As shown in Table II the fact that interferon was being produced in the lungs in response to the influenza virus appeared to have no effect on the serum response of these animals to the poly I:poly C. The serum interferon level of 1:1024 was the same whether or not the animals had received influenza virus and were making lung interferon.

TABLE II. Serum and Lung Interferon Induction by Poly I:Poly C.

Treatment	Interferon titer in Lung	Interferon titer in Serum
Poly I:poly C × 1 ip	16[a]	1024
Influenza × 1, IN[b]; poly I:poly C, × 1, ip 72 hr later[c]	128	1024

[a] Reciprocal of highest dilution showing interferon-like activity.
[b] 72 hr prior to termination of experiment.
[c] 6 hr prior to termination of experiment.

Discussion. The results indicate that, in animals which might be considered to be hyporesponsive based on serum interferon response, high levels of interferon may be produced in lung tissue. However, it is possible that there exists a type of blood–lung barrier similar to the blood–brain barrier. In 1936, Fox showed that the lungs were poorly permeable to serum antibody (5) and it is possible that, in the mouse, this is also true for serum interferon. If so, this would be in contrast to the rabbit, in which active local induction of interferon in lung slices taken 5 min after iv inoculation of poly I:poly C was demonstrated by Ho and Ke (6).

The interferon levels in the lungs of mice even after only one injection of poly I:poly C were considerably lower than those in serum. These findings substantiate those of Kleinschmidt and his colleagues (7, 8) who showed that systemically induced interferon does not reach lung tissue and thus cannot afford protection against viral infections of the lower respiratory tract.

Another possibility is that lung tissue is less prone to become hyporesponsive to repeated interferon inducers than other organ systems. This hypothesis was tested by doing repeated (four) IN inoculations of poly I:poly C and then comparing lung titers of interferon to those produced after one IN exposure to poly I:poly C. The results occasionally, but not consistently, indicated that lung hyporesponsiveness did develop (unpublished data). While this possibility cannot be excluded because the results are not consistent, it is also possible that following each IN inoculation, the inducer was placed into a different area of the lung which was still able to respond. However, recently, Blach-Olszewska and Skurska (personal communication) have shown a lack of hyporeactivity in brain tissue after repeated intracerebral inoculations of virus. Thus, hyporesponsiveness may be a phenomenon limited to the production of circulating interferon.

It is also possible that there is more than one type of cell population capable of responding to an interferon inducer and that iv or ip inoculations stimulated one type whereas IN inoculation involves another. The concept that the immune response of the lung is somehow separate from the systemic immune response has already been discussed in work of Waldman, Spencer and Johnson (9) utilizing influenza virus vaccine. Our results would support the concept that this might also be the case for interferon production.

It seems clear that our results and those of others indicate that hyporesponsiveness is a relative term and that individual organs may be capable of unimpaired interferon response during the so-called hyporesponsive period. Recovery from a virus infection during the "hyporesponsive" state may not be due solely to the residual protection of the initially induced interferon, but may be due to the production of high levels of interferon by specific target organs.

Summary. High levels of interferon can be induced locally in the lungs of mice shown to be hyporesponsive to induction of serum interferon. However, induction of local interferon in the lungs of mice did not similarly inhibit the induction of serum interferon.

1. Pauker, K., and Boxaca, M., Bacteriol. Rev. 31, 145 (1967).

2. Ho, M., Postic, B., and Ke, Y. H., *in* "Interferon Ciba Foundation Symposium," p. 19. Churchill, London (1968).

3. Singer, S. H., Barile, M. F., and Kirschstein, R. L., Proc. Soc. Exp. Biol. Med. 131, 1129 (1969).

4. Isaacs, A., and Hitchcock, G., Lancet 2, 69 (1960).

5. Fox, J. P., J. Immunol. 31, 7 (1936).

6. Ho, M., and Ke, Y. H., Virology 40, 693 (1970).

7. Kleinschmidt, W. J., Ann. N.Y. Acad. Sci. 173, 547 (1970).

8. Kleinschmidt, W. J., and Streightoff, F., Infec. and Immunity 4, 738 (1971).

9. Waldman, R. H., Spencer, C. S., and Johnson, J. E. III, Cell. Immunol. 3, 294 (1972).

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