Attempted Suppression of the Interferon Response in the Mouse (34900)

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The relative roles of various immune defense mechanisms in natural infection can best be clarified through studies in which each part of the host response is selectively suppressed. For example, depression of cellular immunity by antilymphocyte serum has clearly demonstrated the importance of this aspect of the immune response in viral (1), mycobacterial (2), and parasitic (3) infection. Likewise, the limited humoral immune response in children with Bruton's hypogammaglobulinemia leads to an increased incidence and severity of bacterial and *Pneumocystis carinii* infections (4). In previous studies, the role of interferon in microbial infection has only been established in an additive way (5–7). Exogenous interferon and interferon inducers have been found to delay the morbidity and decrease the mortality caused by a number of organisms. Subtractive studies of interferon's role as a host defense mechanism require specific suppression of the interferon response.

Interferon has been suppressed *in vitro* with heparin (8), 7-12-dimethylbenz-anthracene (DMBA) (9), and actinomycin (10, 11). Heparin and DMBA have no known effect on humoral or cellular immunity. But actinomycin has a more general suppressive effect on the immune response; it markedly inhibits the primary humoral response of rabbits to ovalbumin (12).

In pursuance of a model for the specific suppression of interferon, we have expanded the *in vitro* observations to observations in the whole animal. We have also examined the effects of antilymphocyte serum, antimacrophage serum, and splenectomy on the interferon response, since lymphocytes, macrophages, and the spleen have all been implicated as primary sites of interferon synthesis. The effect of phytohemagglutinin was also studied since, in certain situations, it has immunosuppressant properties (13). After the mice were pretreated with the various suppressants, live Newcastle disease virus (NDV) was injected intravenously and the interferon response was quantitated. Newcastle disease virus was chosen as the inducer to stimulate the response to a viral infection. By using the same inducer, animal, and assay throughout, we were able to compare the responses to the various compounds examined.

Materials and Methods. Materials. Actinomycin D was kindly supplied by Merck, Sharp & Dohme Research Laboratories, Rahway, New Jersey. Sodium heparin was obtained from Eli Lilly and Company, Indianapolis, Indiana; DMBA from Sigma Chemical Co., St. Louis, Missouri; and phytohemagglutinin P (Bacto) from Difco Laboratories, Detroit, Michgian.

Interferon induction. ICR strain mice, 5–8 weeks old and weighing 24–28 g, were pretreated with suppressants at doses and times outlined in the "Results" section. All suppressants were injected intraperitoneally unless otherwise noted. Controls received comparable volumes of appropriate diluent. After pretreatment, animals were injected intravenously in the tail vein with 6×10^7 pfu of NDV, Roakin strain. Blood for interferon assay was collected from the axillary vein under ether anaesthesia; it was allowed to clot and serum was then removed. Single samples represent pooled serum from 4 mice. In all experiments interferon was quantitated at 7 hr because its level in serum was maximal at this time (Borden, Murphy and Gary, unpublished observations). In certain

instances interferon was also measured at 2 hr.

Interferon assay. A Mengo virus plaque reduction assay in L cells was used to assay interferon activity. Each interferon sample was treated with a 0.15 vol of 1.5 *M* perchloric acid, centrifuged to remove the precipitate, and dialyzed overnight against 50 vol of Earle's balanced salt solution, pH 7.4. Samples were then tested for complete viral inactivation in primary duck embryo cells. Serial twofold dilutions of interferon samples were prepared in growth medium (Eagle's MEM supplemented with 10% tryptose phosphate and 10% calf serum). Forty-eight hr L cell monolayers, grown in 35-mm dishes under 3% CO_2, were inoculated in triplicate with 0.5 ml of appropriate interferon dilution. After 2 hr at 37°, monolayers were overlayed with 2.0 ml of maintenance medium (M199 supplemented with 2% newborn calf serum) and were held at 37° for another 4 hr. The interferon preparation and medium were then removed. The monolayer was washed with Earle's balanced salt solution, pH 7.4, and was inoculated with 0.2 ml of a Mengo virus suspension containing 40–70 pfu. After a 60-min incubation for viral absorption, the monolayers were overlayed with 2.5 ml of media M199 with 5% newborn calf serum and 1% Difco purified agar containing 0.2 mg/ml of protamine. After 48 hr of incubation, neutral red was overlaid, and the plaques were counted. Interferon units are expressed as the reciprocal of the dilution causing 50% plaque reduction. This assay system was quite reproducible; replicate mouse serum samples, prepared and assayed at the same time, had a mean of 9.32 $\log_2$ units with a standard deviation of 0.15 $\log_2$ units. Thus, three standard deviations were within 0.5 of a $\log_2$ unit. A standard interferon preparation included with each assay showed a variation of no more than 10%.

Viral inhibitory activity met the criteria for interferon including stability at pH 2.0, solubility at 100,000*g*, nondialyzability, inhibition by actinomycin, and species specificity.

Results. None of the experimental treatments was effective in completely suppressing interferon production in mice (Table I). Heparin was injected 6 and 2 hr prior to NDV and again 4 hr after NDV. The first two doses were 200 μg and the final one was 100 μg; larger doses were fatal. There was no suppression of the interferon response (Table I), although the clotting time at 7 hr was prolonged to greater than 30 min. Because it was insoluble in water, alcohol, or other nontoxic solvents, DMBA was dissolved in Freund's adjuvant. Four days prior to NDV the mice received either 100 μg of DMBA or an equal volume of Freund's adjuvant alone. This amount of DMBA, if distributed equally through the animal, was 100-fold greater than that which had been used *in vitro*. No depression of interferon titer was observed (Table I).

Actinomycin was diluted in 0.85% saline, and animals were treated with 40-μg doses, 10-μg doses, or saline on two occasions, 24 and 2 hr prior to virus challenge. A parallel experiment determined that a single 20-μg dose constituted an LD_{50}, and a single 40-μg dose caused 100% mortality at 3 days. At 2 hr serum interferon titers in the group which received 40-μg doses were 50% lower than titers of the control group, but titers of animals receiving all other dosages were normal.

Seven, 4, and 1 day before injection of the viral inducer, rabbit antimouse-thymocyte serum[1] was inoculated. Each animal received 0.5 ml and controls received an equal amount of normal rabbit serum. The interferon response in the experimental group was 50% of that in the control group (Table I). Rabbit antimouse macrophage serum, from a lot previously shown to be effective in reducing serum clearance of collodial carbon (14), was given intraperitoneally on 3 successive days before NDV. The total dose, including an additional 0.3 ml given intravenously 2 hr before NDV, was 1.8 ml. Controls again received an equal dosage of normal rabbit serum. No suppression of the interferon re-

[1] Kindly assayed and provided by Dr. Charles Shepard, National Communicable Disease Center.

TABLE I. Interferon (IF) Production by Mouse After Experimental Pretreatment.

Experimental suppressant	Dosage and time[a]	Time of IF[a]	IF/ml[b]		Percentage reduction
			Control	Exp.	
Heparin	200 μg, —6, —2 hr	2	156	175	0
	200 μg, —6, —2 hr; 100 μg, +4 hr	7	980	960	0
DMBA	—4 days	7	608	494	10
Actinomycin	10 μg, —24, —2 hr		512	512	0
	40 μg, —24, —2 hr	2	256	128	50
	40 μg, —24, —2 hr	7	1024	1024	0
Anti thymocyte serum	0.5 ml, —6, 3, 0 days	7	512	256	50
Anti macrophage serum	1.8 ml over 3 days	2	350	350	0
		7	512	512	0
Splenectomy	—3 weeks	7	512	256	50
Phytohemagglutinin	—24 hr	7	608	256	55

[a] Time before or after induction of interferon with Newcastle disease virus.
[b] Expressed as reciprocal of dilution causing 50% plaque reduction.

sponse was found at either 2 or 7 hr (Table I).

Mice were splenectomized 3 weeks before challenge with interferon inducer. A laparatomy was performed on control animals at the same time. The interferon response in splenectomized animals was 50% of that of control animals (Table I). A single 500-μg dose of phytohemagglutinin in saline was injected 24 hr before virus challenge. This dose has previously been reported to be nontoxic and effective in suppressing the primary and secondary immune response to sheep erythrocytes in mice (13). At 7 hr interferon activity was just slightly less than 50% of control values (Table I).

Discussion. Deficiencies in our understanding of the mechanism of interferon induction hinder the search for a specific interferon suppressant effective *in vivo*. Interferon synthesis presumably occurs when the interferon cistron of the cell is derepressed by the inducer. This message is transcribed and translated, and interferon is released. An interferon suppressant might act at any of these steps of protein synthesis, against the interferon synthesizing cell *per se*, or as an inhibitor of interferon action. We have examined the *in vivo* effect of substances which act on the interferon synthesis mechanism and against the cell. Fournier *et al.* (15) have recently described an inhibitor of interferon action which was isolated from human chorionic and amniotic membranes; it terminated the virus resistant state induced by interferon. Its effect *in vivo* is unknown.

Heparin (8) and DMBA (9) have been reported to be interferon suppressants *in vitro*; their mechanisms of action are unknown but are hypothesized to be suppression of interferon synthesis (8, 9). Their effect *in vivo* is consistent with this hypothesis. Actinomycin also suppressed interferon synthesis *in vitro* (10, 11), presumably by blocking interferon mRNA synthesis. However, actinomycin failed to suppress the interferon response in the mouse; these results confirm the previously reported finding of Younger *et al.* (16). In rabbits, on the other hand, the interferon response was suppressed 95% by actinomycin (17). This anomalous response of mice is unexplained.

Antithymocyte serum, antimacrophage serum, and splenectomy might all be expected to depress interferon titer significantly, since the target cells of these treatments have been implicated as principal sites of interferon production (18). However, in this study, no more than 50% reduction of serum response was found. These findings are in accord with those of others who have tried similar reticulo-lymphatic suppressants. Ne-

onatal ablation of thymus-derived lymphocytes by thymectomy had no effect on the interferon response (19, 20). Antilymphocyte serum only partially suppressed the interferon response in this study, a finding similar to one previously reported (21). Combined thymectomy and antilymphocyte serum treatment had no effect on the interferon response to synthetic pyran polymers (22). In comparison with the potent effect of antithymocyte serum and thymectomy on cellular immunity, the partial interferon suppression observed with antithymocyte serum does not seem biologically significant (23).

The normal interferon response by mice treated with antimacrophage serum suggests that macrophages do not play a primary role in interferon production following intravenous NDV challenge. However, reticuloendothelial blockade by thoratrast has been reported to suppress interferon in mice (24, 25) and, at 3 hr but not 7 hr, in rabbits (26). Splenectomy suppressed, but did not abolish, the interferon response. This finding supports the report of Fruitstone *et al.* (27), who also used NDV, and Subrahmanyan and Mims (25), who used influenza virus, as stimulators of interferon synthesis.

The cell(s) and organ of interferon origin remain uncertain [for review of studies concerning site of interferon origin see Vilcek (18)]. Lymphocytes and macrophages have been proposed as major sources because they have been found to synthesize interferon *in vitro*, but many other cell types, both ectodermal and mesodermal, have also been shown to synthesize interferon *in vitro*. The spleen has been implicated as the organ of maximal interferon production because incubated tissue slices and titration of the whole organ have yielded larger amounts than have been found in any other organ. However, it appears that, in response to infection, many cell types produce interferon, since maximal interferon production occurs most consistently at the site of viral multiplication (18). That no single suppressive agent in this study abolished the response supports this hypothesis. It appears that no unique, specially differentiated system for interferon production exists, and that many cell types are capable of its synthesis.

Summary. The interferon response after pretreatment of the adult mouse with heparin, dimethylbenzanthracene, actinomycin, antilymphocyte serum antimacrophage serum, phytohemagglutinin, or after splenectomy, was investigated. After the mice were pretreated, interferon response was induced by intravenous injection of Newcastle disease virus; serum interferon was then quantitated. None of the treatments completely suppressed interferon production. These findings support the hypothesis that interferon is synthesized *in vivo* at several sites and by many cell types.

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Received Mar. 20, 1970. P.S.E.B.M., 1970, Vol. 134.