Interferon Preparations as Modifiers of Immune Responses[1] (36868)

WERNER BRAUN AND HILTON B. LEVY
(With the technical assistance of M. J. Rega)

Institute of Microbiology, Rutgers University, New Brunswick, New Jersey 08903; and Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md. 20014

Interferon (IF) preparations are known to contain enhancers and blockers of interferon action (1, 2) as well as modifiers of phagocytic cell functions (3). Interferon preparations also are known to prime relevant cells for subsequent interferon induction by either viruses or synthetic inducers (4–6) and they have been reported to possess antitumor activity (7).

Except for the recent report of interferon's capability to stimulate phagocytosis (3), only minor attention has been paid to the possible influence of interferon preparations on immune responses. One of us previously noted variable effects of IF preparations on enhancing graft-versus-host reactions in mice (8). We have now explored the influence of IF preparations on antibody formation in mice and although the effects have been modest and variable, we have decided to place them on record in order to call further attention to the presence in IF preparations of activities which are not immediately relatable to the antiviral action of IF.

Materials and Methods. Antibody formation to sheep red blood cells (sRBC) was assayed, in the absence or presence of intraperitoneally administered interferon, in 6–8 weeks old CFW mice; interferon was administered at the time of immunization in all of the tests here reported. The number of antibody-forming spleen cells (AFC) was determined by the technique of Jerne *et al.* (9), 48 hours after i.v. immunization with 10^8 sRBC, *i.e.*, at a time when a modification of the rate of activation of antibody-forming cells is detected most easily (10). Each experimental group contained 5 mice and all experiments were performed at least twice.

Three mouse interferon preparations were employed, (1) a mouse serum interferon prepared by i.v. injection of Newcastle Disease virus (11), with a titer of 15,000 International Units (IU)/ml, (2) a tissue culture interferon prepared in L cells through the use of NDV, with a titer of 3000 IU/ml, and (3) a tissue culture interferon, prepared in a line of 3T3 cells transformed by Maloney sarcoma virus (12), and having a titer of 50,000 IU/ml, and referred to as preparation C243.

In several tests, heated (56°, 60°, or 65° for 30 min) preparations of C243 were employed; the antiviral titers of these preparations were, respectively, 3000, 700, and 300.

Poly A, poly U (= poly A:U, Miles Laboratories, Elkhart, Ind.) was administered i.v. at time of immunization. The amount used in all tests reported here was 30γ/mouse, *i.e.*, an amount that causes only a modest enhancement of antibody responses, in contrast to 300γ/mouse which produces strong adjuvant effects (10). COAM (= chlorite-oxidized amylose) was obtained through the kindness of Dr. A. Billiau of the University of Leuven, Belgium, and was administered i.p., 3 hr prior to immunization, at a concentration of 3 mg/mouse, i.p. (13, 14)

Results. Using sheep red blood cells as antigen in mice, it was observed that the administration of either mouse serum IF or tissue culture IF produced an enhancement of the early antibody response (Table I). This enhancement was modest in comparison to potent adjuvants such as poly A:U or chlorite-oxidized amylose (COAM) (Table III) and the response of individual animals tended to vary as reflected in the high standard error of the averages. Furthermore, oc-

[1] Studies by W. B. were supported by National Institute of Health Grant AI 09343 and National Science Foundation Grant GB 20162.

TABLE I. Influence of Two Mouse Interferon Preparations on Antibody Formation to sRBC in Mice.

Treatment of spleen donors	Av no. (± SE) of AFC/10^6 nucleated spleen cells after 48 hr
sRBC (10^8)	159.8 ± 16.6
sRBC + Serum IF[a] (15,000 IU)	486.0 ± 103.0
sRBC + T. C. IF[a] (3,000 IU)	261.9 ± 42.9
Untreated	12.7 ± 3.7

[a] Both preparations were administered i.p., 1.0 ml/mouse.

casional groups of mice (less than 10%) failed to show enhanced activities after immunization in the presence of IF. A rabbit-serum IF preparation was without activity in the mouse system.

Subsequent studies were conducted with a high-potency mouse-tissue culture IF (C243). This preparation reduced early antibody formation when used at relatively high concentrations but produced moderate enhancement at low concentrations (Table II). When a very low concentration of this IF preparation (5×10^{-5} ml = 2.5 IU) was administered in conjunction with a non-stimulatory concentration of poly A:U (which is a potent immunoenhancing agent at higher levels), a marked enhancement of antibody formation was observed.

A potentiation of the effects of the IF preparation in the presence of low levels of poly A:U is also evident in Table III which presents data on the effects of heat treatment of the IF preparation. These tests were performed in an attempt to determine whether the observed effects of IF preparations on antibody formation were attributable to the IF activity in the preparation or to accessory factors. It can be seen from Table III that the immunoenhancing effects became more pronounced as the antiviral activity of the preparation decreased, being highest in the presence of poly A:U when the IF preparation was heated to 60°. It will be noted that in these tests the IF concentration was re-

TABLE II. Influence of Various Concentrations of an Interferon Preparation (IF C243) on Antibody Formation to sRBC in the Absence and Presence of a Low Amount of Poly A:U. Results are from three separate tests.

Treatment and spleen donors			Av no. (± SE) of AFC/10^6 nucleated spleen cells after 48 hr
sRBC (10^8)			299.0 ± 38.4
sRBC + 0.2 ml IF	(1×10^4 IU)		152.2 ± 24.6
sRBC + 0.1 ml IF	(5×10^3 IU)		163.3 ± 26.3
— 0.2 ml IF	(1×10^4 IU)		16.2 ± 7.3
Untreated			52.9 ± 18.1
sRBC (10^8)			267.1 ± 49.1
sRBC + 0.05 ml IF	(2.5×10^3 IU)		404.7 ± 84.8
sRBC + 0.005 ml IF	(2.5×10^2 IU)		436.3 ± 50.3
sRBC + 0.0005 ml IF	(2.5×10^1 IU)		282.2 ± 23.4
Untreated			24.0 ± 9.4
sRBC (10^8)			256.6 ± 28.5
sRBC + 0.005 ml IF	(2.5×10^2 IU)		511.6 ± 119.7
sRBC + 0.0005 ml IF	(2.5×10^1 IU)		388.1 ± 34.6
sRBC —		+ AU (30γ)	312.6 ± 142.2
sRBC + 0.005 ml IF	(2.5×10^2)	+ AU (30γ)	443.4 ± 126.3
sRBC + 0.0005 ml IF	(2.5×10^1)	+ AU (30γ)	391.4 ± 30.2
sRBC + 0.00005 ml IF	(2.5 IU)	+ AU (30γ)	957.2 ± 78.6
Untreated			22.7 ± 8.9

TABLE III. Influence of Heating on the Effect of IF C243 on Antibody Formation to sRBC in the Absence and Presence of a Low Amount of Poly A:U.

Group number	Treatment of spleen donors		Av no. (± SE) of AFC/10^6 nucleated spleen cells after 48 hr
1	sRBC		161.5 ± 12.1
2	sRBC + 0.005 ml IF[a]		256.6 ± 62.1
3	sRBC + 0.005 ml IF 56°		293.9 ± 85.2
4	sRBC + 0.005 ml IF 60°		360.3 ± 56.8
5	sRBC + 0.005 ml IF 65°		356.7 ± 45.3
6	sRBC —	+ AU (30γ)	445.4 ± 86.2
7	sRBC + 0.0005 ml IF[b]		396.7 ± 29.2
8	sRBC + 0.0005 ml IF 56°	+ AU (30γ)	510.0 ± 48.1
9	sRBC + 0.0005 ml IF 60°	+ AU (30γ)	783.7 ± 36.4
10	sRBC + 0.0005 ml IF 65°	+ AU (30γ)	532.0 ± 35.0
11	sRBC —	+ COAM (3 mg)	1580.6 ± 297.8
12	sRBC + 0.005 ml IF	+ COAM (3 mg)	294.6 ± 38.5
13	Untreated		40.7 ± 16.4

[a] (2.5×10^2 IU).
[b] (2.5×10^1 IU).

duced by one log (to 0.0005 ml) in the groups that received a combination of IF plus poly A:U (groups 7–10), compared to the IF concentration used in the absence of poly A:U (groups 2–5). This was done because parallel studies with isoproterenol as a modifier of immune responses (15) had revealed that stimulatory effects of isoproterenol in combination with low amounts of poly A:U occurred only when the isoproterenol concentration was at a very low level, which produced no stimulation by itself, whereas higher concentrations caused a reduced response in the presence of poly A:U (see Discussion). We, therefore, chose for groups receiving IF + poly A:U an IF concentration that, when administered alone (see Table II), was too low to produce significant enhancement.

Table III also contains data on the effects of COAM, with and without interferon. COAM is an oxidized amylose, which has been shown to have antiviral (13) and adjuvant (16) activities. It can be noted that the potent immunoenhancing effect of COAM is nearly completely eliminated in the presence of interferon. This reversal resembles what has been seen previously under conditions where immunoenhancing modifiers of endogenous cAMP levels were used at excessively high concentrations (17–19).

Discussion. All available IF preparations are mixtures of a variety of substances in addition to the protein(s) with antiviral activity. Interpretations of any biological effects must take this fact into consideration. In the present studies we have noted a modest capability of mouse IF preparations of different origins to stimulate the activation of antibody-forming spleen cells in mice, the effectiveness being limited to a relatively narrow concentration range; at higher concentrations inhibitory effects are observed. This type of biphasic response has been noted in most of the recent studies on the influence of modifiers of endogenous cAMP levels on the activation of immune responses (17, 18). Thus when animals are immunized in the presence of adenyl cyclase-enhancing poly A:U in combination with increasing amounts of theophylline, a phosphodiesterase inhibitor and consequently a stabilizer of cAMP, a reduction in immune response occurs at high concentrations of theophylline and a stimulation at low levels (17, 18). Similarly, low concentrations of adenyl cyclase-stimulating isoproterenol enhance antibody formation when administered in conjunction with anti-

gen and poly A:U, whereas high concentrations of isoproterenol, or a combination of high concentrations of poly A:U and low concentrations of isoproterenol, will reduce the magnitude of the immune response (15). The data here reported for the reversal of COAM effects by interferon thus resemble those obtained with appropriate combinations of modifiers of the endogenous cAMP system.

Since the effects of IF preparations resemble those observed with a known modifier of endogenous cAMP levels, studies were initiated recently to explore both adenyl cyclase activity and cAMP levels in mouse spleen cells exposed to IF *in vitro* (C. Shiozawa, unpublished data). The initial results indicate an elevation of both adenyl cyclase activity and cAMP levels in IF-exposed cells from CBA mice (*e.g.*, p moles of cAMP/10^6 cells 60 min after addition of 250 IU IF:7.7; in unexposed control populations: 3.8. Adenyl cyclase activity measured in terms of p moles of ATP converted into cAMP within 40 min at 37° following 20 min exposure of 4×10^7 spleen cells to 250 IU IF:22.2; for unexposed control populations: 15.5).

The results obtained after heating of the IF preparation would suggest that the effects on antibody formation may be distinct from the antiviral activity of the preparation, since a decrease in antiviral titers was associated with an increase in immunoenhancing activity. It would almost appear as if the antiviral factor may be antagonistic to the immunoenhancing factor but other explanations are possible. For example, heating may reduce an excessive amount of material that has both antiviral and cAMP-modifying activity and may thus bring the concentrations of the responsible factor to the narrow range required for immunoenhancing activity. The latter explanation would be consistent with the conclusions reached by Huang *et al.* (3) who have attributed the effect of IF preparations on macrophage activity to IF itself.

Several activities of IF preparations have recently been demonstrated in addition to antiviral activity (1–8). The present report adds an additional capacity, namely, alteration of the magnitude of immune responses. It is now possible to ask whether the reported anti-tumor effects of IF might not be attributable to the type of effect here noted, *i.e.*, either an effect on the immune response or possibly a direct effect on cAMP levels in tumor cells, some of which have been recently found to manifest a susceptibility to agents that are known to modify the cAMP system (19). The possibility that cAMP levels may also play a role in the antiviral activity of IF is an intriguing one but will require a great deal more study.

Summary. Within certain dose ranges, preparations of mouse serum interferon or tissue-culture interferon show a modest capacity to increase the number of antibody-forming spleen cells in mice as measured by the Jerne plaque technique. When low levels of polyadenylic polyuridylic acid (= poly A:U), *i.e.*, an amount that causes only modest stimulation, were used together with amounts of the interferon preparations that also by themselves are ineffective, a good stimulation of early appearance of antibody-forming cells was produced. High concentrations of IF preparations reduced antibody formation, and the potent immunoenhancing effects of chlorite-oxidized amylose (COAM) were almost completely abolished in the presence of low concentrations of interferon preparations. These findings suggest certain similarities between the effects of interferon preparations and those of agents known to modify the cyclic AMP system. Such suspected relationships are supported by preliminary data demonstrating a capacity of IF preparations to elevate adenyl cyclase activity and cAMP levels in mouse spleen cells.

1. Isaacs, A., Rotem, Z., and Fantes, K. H., Virology **29**, 248 (1966).

2. Chany, C., and Brailovsky, C., Proc. Nat. Acad. Sci. U.S.A. **57**, 87 (1967).

3. Huang, K. Y., Donahoe, R. M., Gordon, F. B., and Dressler, H. R., Infection & Immunity **4**, 581 (1971).

4. Levy, H. B., Buckler, C. E., and Baron, S., Science **152**, 1274 (1966).

5. Burke, D. C., and Isaacs, A., Brit. J. Exp. Pathol. **39**, 78 (1958).

6. Margolis, S., Oië, H., and Levy, H. B., J. Gen. Virol. **15**, 119 (1972).

7. Gresser, I., Coppey, J., Fontaine-Brouty-Boye,

D., Falcoff, R., Falcoff, E., and Zajdela, A., Nature (London) **215**, 174 (1967).

8. Levy, H. B., Adamson, R., Carbone, P., DeVita, V., Gazdar, A., Rhim, J., Weinstein, A., and Riley, F., *in* "Biological Effects of Polynucleotides" (R. F. Beers and W. Braun, eds.), p. 55. Springer-Verlag, New York (1971).

9. Jerne, N. K., and Nordin, A. A., Science **140**, 405 (1963).

10. Braun, W., Ishizuka, M., Yajima, Y., Webb, D., and Winchurch, R., *in* "Biological Effects of Polynucleotides" (R. F. Beers and W. Braun, eds.), p. 139. Springer-Verlag, New York (1971).

11. Baron, S., and Buckler, C. E., Science **141**, 1061 (1963).

12. Oië, H., Gazdar, A., Buckler, C. E., and Baron, S., J. Gen. Virol. **17**, 107 (1972).

13. Claes, P., Billiau, A., DeClerq, E., Desmyter, J., Schonne, E., Vanderhaeghe, H., and DeSomer, P., J. Virol. **5**, 313 (1970).

14. Billiau, A., Desmyter, J., and DeSomer, P., J. Virol. **5**, 321 (1970).

15. Braun, W., and Rega, M. J., Fed. Proc. **31**, Abs. (1972).

16. Billiau, A., Eyssem, H., David, G., and DeSomer, P., Proc. Soc. Exp. Biol. Med. **137**, 626 (1971).

17. Braun, W., and Ishizuka, M., J. Immunol. **107**, 1036 (1971).

18. Braun, W., and Ishizuka, M., Proc. Nat. Acad. Sci. **68**, 1114 (1971).

19. Webb, D., Braun, W., and Plescia, O. J., Cancer Res. **32**, 1814 (1972).

Received July 21, 1972. P.S.E.B.M., 1972, Vol. 141.