Interferon and Cell Division
V. Titration of the Anticellular Action of Interferon Preparations
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ION GRESSER, MARIE-THÉRÈSE THOMAS, DANIÈLE BROUTY-BOYÉ, AND ALVARO MACIEIRA-COELHO

Institut de Recherches sur le Cancer, Institut de Cancérologie et d'Immunogénétique, 94-Villejuif, France

Various murine interferon preparations have been shown to inhibit the multiplication of L 1210 and Ehrlich ascites cells in nonagitated suspension cultures (1–4). In these experiments cells were seeded at a concentration of 2×10^5 cells/ml and cultivated in medium containing relatively large amounts of interferon. The inhibitory effect of interferon was determined by daily cell counts of the different cultures. We report here a more sensitive assay of this anticellular effect, based on the inhibition of L 1210 cell colony formation by interferon preparations incorporated in an agarose nutrient medium base.

Materials and Methods. Cells. L 1210 cells (5) were cultivated in RPMI-1640 (Gibco) nutrient medium supplemented with 20% horse serum in nonagitated suspension cultures in 15-ml pharmacy bottles maintained at 37° (1–3).

Colony formation in agarose. For growth in semisolid medium, 250 to 500 L 1210 cells were seeded in 60-mm petri dishes containing 6 ml of Eagle's basal medium (Gibco), 10% heat-inactivated horse serum, and 0.375% agarose (6, 7). Dilutions of interferon or control preparations were incorporated in the agarose medium [Porterfield (8) had previously shown that interferon diffused sufficiently through agar to exert an antiviral effect on monolayer cell cultures]. Four petri dishes per dilution were utilized. Macroscopic colonies were counted after 14 days of incubation of dishes at 37° in an air–5% CO_2 mixture. The titer of a given interferon preparation was expressed as the reciprocal of the dilution at which a 50% reduction in the number of colonies was observed compared to the number of colonies in control dishes.

Interferon preparations. Mouse interferons were obtained from the nutrient medium of monolayer cultures of MSV-Ia cells (9) inoculated with UV light-inactivated Newcastle disease virus (NDV) or from the brains of Swiss and IC mice inoculated intracerebrally with West Nile virus (WNV) (10). Human interferon (generously provided by Dr. C. Chany) was derived from the nutrient medium of the intact amniotic membrane (11) inoculated with NDV. Rabbit interferon was obtained from the serum of rabbits inoculated intravenously with NDV (12, 13). Control preparations consisted of the medium of uninoculated cell cultures or tissues of uninoculated animals. All interferon and control preparations were treated at pH 2 for 18 hr (preparations containing WNV), or 5 days (preparations containing NDV), and then centrifuged at 60,000*g* for 1 hr in a Martin Christ centrifuge. Some preparations were concentrated 10-fold by forced dialysis. Mouse, human, and rabbit interferon preparations were assayed by the method of inhibition of cytopathic effect (CPE) on monolayer cultures of mouse L cells, monkey BSC cells, and rabbit RK cells inoculated with vesicular stomatitis virus (14, 15). One mouse interferon unit (as expressed in the text) equals 4 NIH International Reference units.

Results and Discussion. The inhibition of L 1210 cell colony formation in agarose containing varying dilutions of mouse MSV-Ia and brain interferon is illustrated in Figs. 1 and 2. The inhibitory titer of these interferon preparations, as determined by the 50% reduction in colony formation, was 1:1120 and 1:960, respectively (Fig. 2). The antiviral

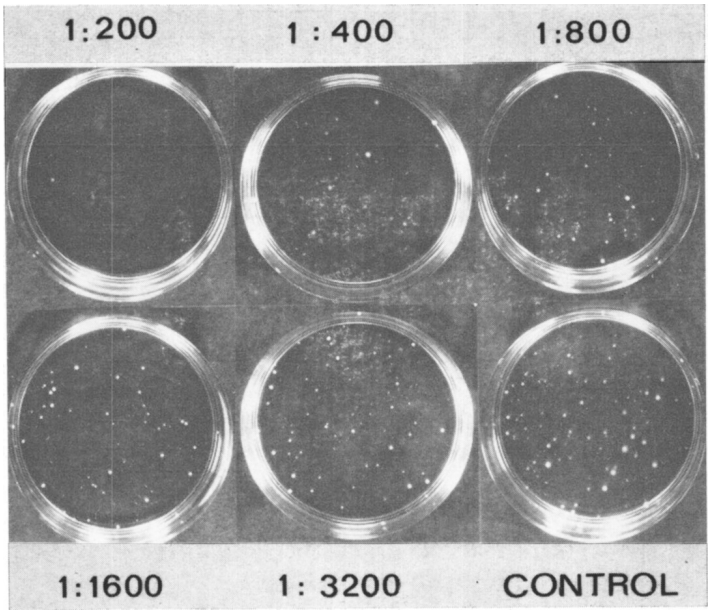

FIG. 1. Dilution of mouse MSV-Ia interferon in agarose: 500 L 1210 cells seeded per petri dish; average number of colonies in 4 control dishes was 142 (range 125–152).

titer of these preparations is given in Table I. This inhibitory effect on colony formation was not observed after pretreatment of an MSV-Ia interferon preparation with 500 μg/ml of crystalline trypsin for 1 hr at 37° or with 0.02 *M* sodium periodate (16) for 2 hr at 37° (Fig. 2). Likewise, no antiviral activity was detected following these treatments (Table I). A slight inhibitory effect on colony formation was observed at a 1:20 dilution of the control brain preparation, whereas the control MSV-Ia preparation was inactive at this dilution (Fig. 2).

Human amniotic membrane and rabbit

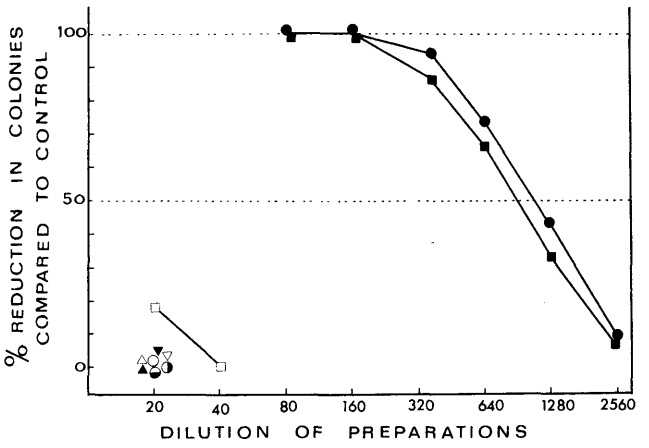

FIG. 2. L 1210 cell colony formation in agarose containing interferon: 250 L 1210 cells seeded per petri dish; average number of colonies in 8 control dishes was 33 (range 30–38 colonies); (●) mouse MSV-Ia interferon; (◒) mouse MSV-Ia interferon pretreated with trypsin; (◑) mouse MSV-Ia interferon pretreated with periodate; (○) control MSV-Ia preparation; (■) mouse brain interferon; (□) control brain preparation; (▼) human amniotic membrane interferon; (▽) control amniotic membrane preparation; (▲) rabbit serum interferon; and (△) control serum preparation.

TABLE I. Antiviral Titer of Interferon Preparations.[a]

Preparations	Cells in assay: Mouse L	Monkey BSC	Rabbit RK 13
Mouse MSV-Ia interferon	1:16,000[b]	—	—
pretreated with trypsin	<1:10	—	—
periodate	<1:10	—	—
MSV-Ia control	<1:10	—	—
Mouse brain interferon	1:16,000[b]	—	—
control	<1:10	—	—
Human amniotic membrane interferon	<1:10	1:512[b]	—
control	<1:10	<1:10	—
Rabbit serum interferon	<1:10	—	>1:6400[b]
control	<1:10	—	<1:10

[a] — = not tested.
[b] Titer per 0.2 ml.

TABLE II. Colony Formation of Interferon Sensitive and Interferon Resistant L 1210 Cells in Agarose Containing Interferon.

Cell type	Dilution of interferon[a] in agarose: None	1:100	1:200	1:400	1:800	1:1600	1:3200
L 1210 S[b]	96[c] (90–100)	0	3 (1–5)	18 (14–23)	46 (43–49)	76 (68–85)	82 (68–94)
L 1210 R[b]	31 (24–41)	29 (24–33)	31 (28–37)	32 (23–36)	39 (35–45)	34 (26–40)	30 (23–34)

[a] Antiviral titer of interferon utilized = 1:8000/0.2 ml.
[b] S = a cloned line of interferon sensitive L 1210 cells; R = a cloned line of interferon resistant L 1210 cells; 500 cells of L 1210 S or L 1210 R seeded/petri dish.
[c] Average number of colonies/petri dish; range observed in 4 petri dishes/dilution is given in parentheses.

serum interferon and their control preparations neither inhibited L 1210 cell colony formation (Fig. 2) nor exerted an antiviral effect on mouse L cells (Table I).

We have previously described the selection of a subline of L 1210 cells resistant to both the anticellular and antiviral effects of different mouse interferon preparations (2, 3). Table II shows that interferon resistant L 1210 cells did form colonies despite the presence of interferon in the agarose medium. Thus, the colony formation inhibition test not only provides a sensitive assay of the anticellular effect of interferon preparations, but also a means of determining the resistance of L 1210 tumor cells to interferon. Utilization of this test may prove of value in determining the sensitivity of other tumor cells to interferon preparations.

Summary. A sensitive assay of the anticellular action of mouse interferon preparations is presented. This technique is based on the inhibition of L 1210 cell colony formation by interferon preparations incorporated in an agarose nutrient medium base.

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Note added in proof: Dr. G. Bodo (Vienna) kindly gave us a purified preparation of mouse cell culture interferon. (Specific activity 2×10^6 units per mg protein). The antiviral titer of this preparation

as assayed in our laboratory was 1 : 256,000. When assayed by the 50% reduction of the number of L 1210 cell colonies in agarose the titer was 1 : 244,000.

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