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Interferon Cell Species Specificity: Role of Cell Membrane Receptors (38198)

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(Introduced by C. Chany)

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In somatic monkey-mouse hybrid cells, the receptors for monkey and for mouse interferon are distinct. When small amounts of interferon of both types are added to the cells, the antiviral effect is cooperative. In addition, both interferons can be potentiated by actinomycin D, 4–6 hr after the administration of interferon. These data, together with other observations previously published, seem to indicate that receptors are solely responsible for the cell species specificity, while there is probably only one mechanism needed for the establishment of the antiviral state in the hybrid cells. In the hybrids here studied, this machinery could be of murine origin.

The biological effect of interferon is restricted to the homologous cell species (1). Interspecies activity was occasionally reported (2), mostly limited to phylogenetically closely related animals, sometimes to more distant species (3, 4). The aim of the present study is to determine: (a) whether this cell species specifically is related only to interferon receptors located in the cell membrane; or (b) if the whole cellular machinery responsible for the antiviral state is specific of the cell.

Material and Methods. Cell lines. A previously described somatic monkey-mouse hybrid cell system (MKCV III) was used for this study (5). The hybrid cells are sensitive to both types of interferon that protect the parental cells. The genetic sites for interferon production and action are asyntenic (5, 6). From the original hybrid cell population, 2 clones were isolated and selected: clone 4, which contains 19.84 ± 1.2 biarmed monkey chromosomes, and about 68 telocentric mouse chromosomes; and clone 2, which contains 6.36 ± 0.66 monkey chromosomes and 62 mouse chromosomes.

The quantity of monkey-specific antigens in the hybrid cells was studied using antibodies labeled with peroxidase and specific to the parental simian cells. These studies showed that in clone 4, about 21% of the parental monkey antigens were present; while in clone 2, less than 8.6% were found (C. Grunewald-Krembel and R. Wicker, unpublished experiments).

Interferons. Two types of interferon were employed. Murine interferon was prepared using LM or MSV-IF cells, induced by the Hertfordshire strain of Newcastle disease virus (NDV), irradiated with UV (5000 erg/mm^2). Primate interferon was prepared using human white blood cells (WBC), induced by NDV. The techniques of the production of interferon were previously described (7, 8). Murine interferon was titered with regard to a reference prepared by N.I.H., primate interferon with regard to an INSERM reference.

Viruses. In all the experiments, the cells were infected with vesicular stomatitis virus (VSV), Indiana strain, at a multiplicity of infection of 0.1 PFU/cell.

Results. Two effects of interferon on these hybrid cells were investigated: (i) the establishment of the antiviral state, and

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(ii) the enhancement of the establishment antiviral state by the addition of actinomycin D 4–6 hr after induction with interferon (9).

The cells (MKCV III) were treated for 24 hr with increasing concentrations of mouse interferon and simultaneously with a constant amount of primate interferon (50 INSERM ref. units), which induces only a 2–4-fold decrease of the viral yield in the hybrids. The results are shown in Fig. 1. Mouse interferon, at concentrations of 15–30 NIH ref. units, had practically no antiviral effect. However, when mouse and primate interferon were combined, the antiviral state increased 50–100-fold. When high concentrations of mouse interferon were used, primate interferon had only a slight additive effect, or none at all.

On the other hand, the cells were treated with increasing concentrations of primate interferon, and simultaneously with a constant amount of murine interferon (10 ref. units). The results are shown in Fig. 2: when primate interferon had only a slight effect, the addition of a small amount of murine interferon was sufficient enough to activate the 2 interferons. The same results were obtained in MKCV III clone 4. When parental cells were treated with homologous interferon, the addition of heterologous interferon had no effect.

In further experiments, the potentiating effect of actinomycin D was assayed sep-

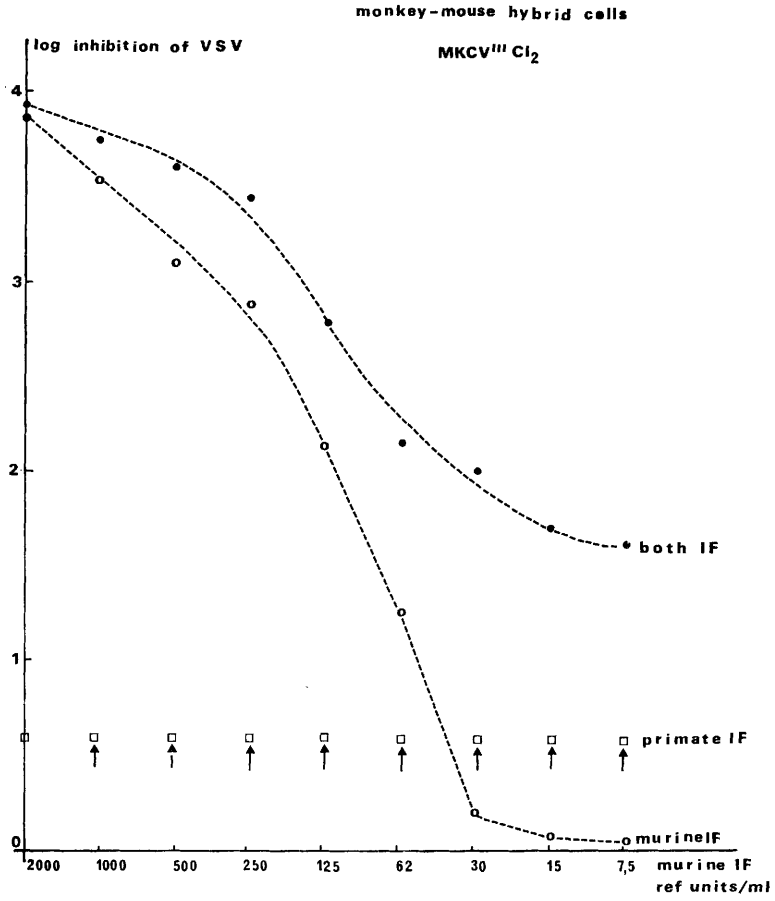

FIG. 1. Dose-response relationship between the amount of interferon is expressed as the log of the viral yield in control/ the log of the viral yield in interferon-treated cells. ●- - -● Cells treated with both murine and primate interferons. ○- - -○ Cells treated with murine interferon. □ □ Cells treated with a constant amount of primate interferon.

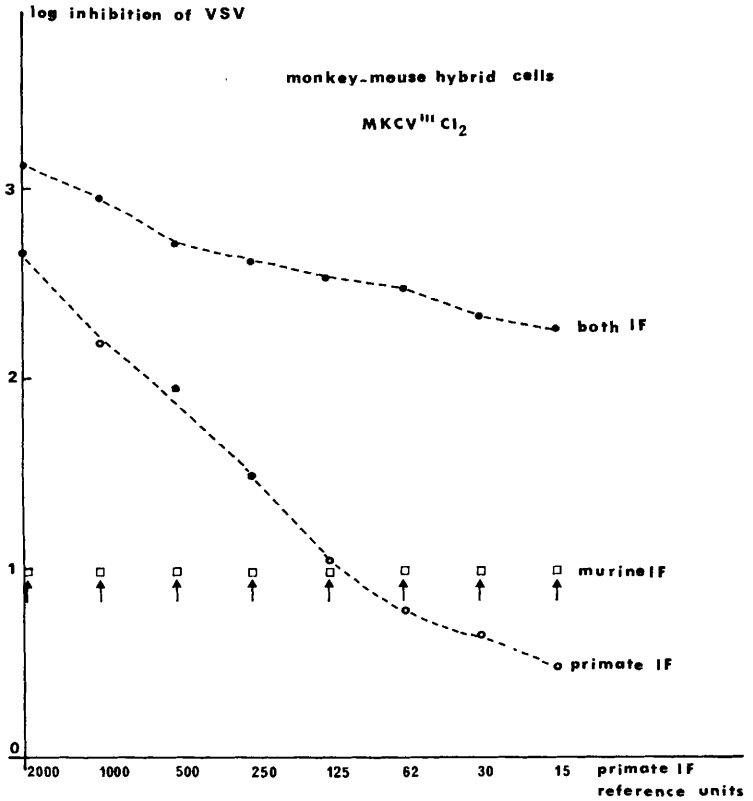

FIG. 2. Dose-response relationship between the amount of interferon is expressed as the log of the viral yield in control/ the log of the viral yield in interferon-treated cells. ●---● Cells treated with both murine and primate interferons. ○---○ Cells treated with primate interferon. □ □ Cells treated with a constant amount of murine interferon.

arately for primate or mouse interferon in hybrid clones. In the parental cells, actinomycin D increased the antiviral effect of mouse and primate interferons respectively. The hybrid cells were incubated with either murine (320 ref. units) or primate (800 ref. units) interferon for 5 hr. Actinomycin D was then added at different concentrations ranging from 0.05 to 0.2 μg/ml. After 1 hr, actinomycin D was removed and the cells incubated with fresh medium for 20 hr. The cells were then challenged with VSV, and the viral yield was assayed in L cells. The results are summarized in Table I. Murine interferon was potentiated in both clone 2 and clone 4, while primate interferon was significantly potentiated only in clone 4. In this clone, the potentiating effect of increasing concentrations of actinomycin D was comparable for both murine and primate interferon in parallel sets of experiments.

Discussion. The results of the experiments here presented are in favor of the hypothesis that only the cell receptors, located on the membrane, govern the cell species specificity of the different biological effects of interferon. Firstly, we have previously shown that monkey and mouse cell receptors can be distinguished by their sensitivity to trypsin. In the hybrid cell and in parental cells, trypsin destroys selectively the receptors for primate interferon, but not the receptors for murine interferon (10). Since in the hybrid cells, the receptors for the 2 interferons are different, it is likely that no competition for receptors between the 2 species of interferon occurs when simultaneously added to the cells. Secondly, since the dose-response effect is sigmoidal

TABLE I.[a]

Actinomycin D (μg/ml)	Transcription inhibition	MKCV^{III} Cl$_2$ Interferon		MKCV^{III} Cl$_4$ Interferon	
		Murine	Primate	Murine	Primate
0	0%	2.08	1.65	1	0.84
0.05	20%	2.43	1.80	1.6	1
0.10	40%	2.45	1.75	1.83	1.54
0.15	55%	2.6	1.65	2.08	1.65
0.20	65%	2.85	1.47	2.3	1.78

[a] Potentiating effect of actinomycin D on the antiviral state induced by murine or primate interferon in monkey-mouse hybrid cells. The results are expressed as the log of the viral yield in control virus + actinomycin D/ the log of the viral yield in interferon + actinomycin D-treated cells.

for interferon, a critical concentration of this substance is necessary to trigger the cell receptors and unleash the synthesis of the antiviral machinery in the cells. When the concentration of mouse interferon is low, the small amount of primate interferon added is enough to render both interferons effective, while a comparable increase of the amount of each interferon separately has no effect. This is only conceivable if, in the hybrids, the receptors for the 2 interferons are different, but the antiviral machinery the same, and if the dose-response relationship between interferon and the antiviral protection is sigmoidal. Thirdly, in clone 4, a potentiating effect of actinomycin D is observed for both primate and mouse interferon separately; while in clone 2, only the effect of mouse interferon is enhanced. The lack of potentiating effect of primate interferon in clone 2 supports our previous hypothesis that a critical level of the antiviral state is necessary in order to demonstrate the potentiating effect of interferon by antimetabolites (9) (11). Since the quantity of monkey antigens in the cell membrane of clone 2 is low (as shown by peroxydase-labeled antibodies to monkey antigens), it is likely that the number of receptors for primate interferon is insufficient to reach this critical point.

The responsibility of cell membrane receptors for the species specificity of interferon is also substantiated by previous observations of Duc-Goiran *et al.* (4). They show that human amniotic (HAM) interferon protects embryonic rat cells, while human WBC interferon does not. The dose-response curves of HAM interferon were sigmoidal in primate cells, but linear in rat cells. On the other hand, rat interferon had no effect in primate cells.

The present data are consistent with the idea that each of the species of interferon acts separately on the membrane to initiate the first steps in the development of cellular resistance to virus and that both triggering events activate a common intracellular mechanism.

The localization of the cell species specific properties of interferon in the membrane receptor sites is of theoretical importance for the study of the mechanism of interferon-receptor interactions, and for the mechanism of induction of the antiviral state.

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