INHIBITION OF THE MULTIPLICATION OF MYXOVIRUS AND ARBOVIRUS BY CHEMICALLY MODIFIED RIBONUCLEIC ACIDS FROM THE HOST CELLS.

Jean-Pierre Ebel, Jacques-Henry Weil, Gisèle Beck and Claudine Bollack.

Laboratoire de Chimie Biologique, Faculté des Sciences, Strasbourg (France).

and

Louis Colobert[*] and Pierre Louisot[*]

Division de Microbiologie du Centre de Recherches du Service de Santé des Armées, Lyon (France).

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When animals or cultures of animal cells are infected by a virus, they produce substances, such as interferons, which are able to inhibit viral multiplication (Isaacs and Lindenmann, 1957). The viral nucleic acid alone is able to cause in the host cell an identical production (Henle and Paucker, 1958; Paucker and Henle, 1958). Non-viral nucleic acids, extracted from cells of animal, plant or bacterial species different from the host cell (heterologous nucleic acids) can also induce the synthesis of interferons or related substances (Isaacs, 1961; Rotem et al., 1963). In this communication we show that ribonucleic acids homologous to the ones of the host cells, and which are normally unable to produce substances that inhibit viral multiplication, become able, when they are chemically modified, to induce in the host cells a complete or almost complete resistance to an infection by Myxoviruses and Arboviruses.

We have used two different systems : the system "Myxovirus parainfluenzae I (virus Sendai) - cells of calf renal cortex" and the system "Arbovirus A (virus Sindbis) - fibroblasts of chicken embryos".

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[*] Present address : Laboratoire de Chimie Biologique, Faculté de Médecine 8, Avenue Rockefeller, Lyon (France).

In the first system we have studied the influence of a homologous ribonucleic acid, either intact or partially degraded (total cytoplasmic RNA extracted from cells of calf renal cortex), modified by methylation

Table 1

Inhibition of the multiplication of Myxovirus parainfluenzae I, strain 52 (Kuroya et al., 1953) on cells of calf kidney in the presence of the same initial concentration (100 µg/ml) of homologous or heterologous, modified or unmodified RNA.

Experimental conditions of viral multiplication	Inhibition*
In the presence of native homologous RNA	0 to 4
In the presence of degraded homologous RNA	0 to 3
In the presence of brominated homologous RNA(1)	13
In the presence of brominated homologous RNA(2)	23
In the presence of methylated homologous RNA(3)	33
In the presence of methylated homologous RNA(4)	87 to 100
In the presence of native heterologous RNA (yeast total RNA)	36
In the presence of degraded heterologous RNA (yeast total RNA)	35
In the presence of methylated heterologous RNA (yeast total RNA(5))	36

* expressed as a percentage of normal viral multiplication on untreated cells.

(1) Brominated homologous RNA : 0.5 mole of bromine was added per RNA nucleotide in the reaction mixture. Average percentages of modified bases : 8-bromoguanine : 10 %; 5-bromouracile and 5-bromocytosine : 3 %.

(2) Brominated homologous RNA : 1.5 mole of bromine was added per RNA nucleotide in the reaction mixture. Average percentages of modified bases : 8-bromoguanine : 30 %; 5-bromouracile : 25 %; 5-bromo - cytosine : 8 %.

(3) Methylated homologous RNA : 2 moles of dimethylsulfate were added per RNA nucleotide in the reaction mixture. Average percentages of modified bases : 7-methylguanine : 10 %; 1-methyladenine : traces.

(4) Methylated homologous RNA : 4 moles of dimethylsulfate were added per RNA nucleotide in the reaction mixture. Average percentages of modified bases : 7-methylguanine : 25 %; 1-methyladenine : 8 %; 3-methylcytosine : traces.

(5) Methylated heterologous RNA (yeast total RNA) : 6 moles of dimethylsulfate were added per RNA nucleotide in the reaction mixture. Average percentages of modified bases : 7-methylguanine : 30 %; 1-methyl - adenine : 12 %; 3-methylcytosine : traces.

(Bollack et al., 1965) or by bromination (Duval and Ebel, 1965). We have compared its efficiency to that of a heterologous ribonucleic acid (yeast RNA) either chemically modified or unmodified. The results obtained are summarized in table 1. They show the remarkable capacity of the methylated homologous RNA to induce in the host cells a complete resistance to a subsequent viral infection. A heterologous RNA, either chemically modified or unmodified, has a much smaller inhibitory effect on a later infection. No difference was observed between native and partially degraded RNA's.

In the second system we have also studied the influence of a homologous RNA, namely a total RNA from 9-day- old chicken embryos, modified by methylation (Bollack et al., 1965), allylation (Bollack and Ebel, in preparation), acetylation (Keith and Ebel, in preparation) or treatment with nitrous acid (Carbon, 1964). We have also compared the efficiency of these RNA's to that of heterologous RNA's (RNA from calf renal cortex and yeast RNA). The percentages of inhibition measured in this system were determined by the technique of plaque formation (Porterfield et al., 1960). The results obtained are summarized in table 2. They show the remarkable efficiency of the chemically modified homologous RNA's which, at a concentration of 100 µg/ml, cause an almost complete inhibition of viral multiplication. With much smaller concentrations of modified homologous RNA's (10 to 20 µg/ml), the inhibition of viral multiplication is already very significant, as the multiplication is only 50 % of that observed with the untreated cells.

Furthermore we have observed that the total RNA, extracted from chicken embryos of a strain different from the one which was used for the culture of host cells ("semi-homologous" RNA), has some inhibitory effect on viral multiplication (see table 2).

We have checked that chemically modified homologous RNA's do not exert their inhibitory effect through a depression of the cell metabolism : the cell respiration, measured in the first system in the presence of methylated RNA, is even superior to the one of untreated cells. Furthermore, microscopic examination of the cell cultures showed that the doses of chemically modified homologous RNA's which were used have an obvious eutrophic effect on the development of the cells.

In the two systems where cells were treated with modified (for instance methylated) homologous RNA's, we have observed the presence of a

Table 2

Inhibition of the multiplication of Sindbis virus (strain AR 339) on fibrobasts of chicken embryos, observed in the presence of the same initial concentration (100 µg/ml or 200 µg/ml) of chemically modified homologous RNA or of heterologous RNA.

Experimental conditions of viral multiplication	Inhibition*	
	100 µg/ml	200 µg/ml
In the presence of homologous RNA	0	0
In the presence of "semi-homologous" RNA	45	55
In the presence of heterologous RNA (yeast RNA)	25	40
In the presence of heterologous RNA (RNA from calf renal cortex)	32	50
In the presence of methylated homologous RNA(1)	86	92
In the presence of allylated homologous RNA(2)	89	94
In the presence of acetylated homologous RNA(3)	78	85
In the presence of homologous RNA treated with nitrous acid (4)	84	94

* expressed as a percentage of normal viral multiplication on untreated cells and observed with two initial concentrations of RNA.

(1) Methylated homologous RNA : 4 moles of dimethylsulfate were added per RNA nucleotide in the reaction mixture. Average percentages of modified bases : 7-methylguanine : 25 %; 1-methyladenine : 8 %; 3-methylcytosine : traces.

(2) Allylated homologous RNA : 50 moles of allylbromide were added per RNA nucleotide in the reaction mixture. Average percentage of modified base : 7-allylguanine : 8 %.

(3) Acetylated homologous RNA : 4 moles of acetic anhydride were added per RNA nucleotide in the reaction mixture. Average percentage of modified base : N_4-acetylcytosine : 30 %.

(4) Homologous RNA treated with nitrous acid : 0.5 volume of $NaNO_2$ 3 M were added to 1 volume of a solution of 5 mg per ml RNA in acetate buffer 0.25 M pH 4.3 . Average percentages of desamination : cytosine : 5 %; adenine and guanine : 4 %.

substance which has anti-virus activity, is soluble, cannot be sedimented by centrifugation at 145,000 g for 4 hours, precipitates upon addition of ammonium sulfate, does not dialyse out, is stable at pH 2 for 16 hours at 0°C, is not sensitive to ribonuclease (1 to 4 µg/ml, for 30 to 60 minutes at

22°C), but is sensitive to trypsin (100 µg/ml for 30 minutes at 37°C) and to papain (6.5 units/ml for 1 hour at 22°C), and is deeply altered by successive freezing and thawing. All these properties remind of those of the substances called interferons (Isaacs and Lindenmann, 1957; Lampson et al., 1963). But the characterization of such substances does not exclude the existence of different protecting mechanisms in our systems.

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