Induction of 2′,5′ oligo(A) synthetase in tumor-bearing mice with encephalomyocarditis (EMC) virus of poly(I)poly(C)

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Infection of 13 month-old C_3H mice with EMC virus or inoculation with the interferon inducer poly(I)poly(C) results in elevated levels of the enzyme 2′,5′ oligo(A) synthetase only in animals with spontaneous tumors (breast cancer or hepatomas). High enzymatic activities are detected in homogenates from liver, spleen, plasma and neoplastic cells of the animals with breast carcinomas and only in the neoplastic liver cells of the animals with hepatomas.

C_3H mice *Encephalomyocarditis virus* *Poly(I)poly(C)* *2′,5′ Oligo(A) synthetase* *Induction of 2′,5′ oligo(A) synthetase* *Spontaneous tumor*

1. INTRODUCTION

Recent studies on the mechanism of action of interferon have revealed the induction, in interferon-treated cells, of a number of proteins which are generally accepted as the enzymes responsible for the multiple effects of interferon [1,2]. One of these enzymes is the 2′,5′ oligo(A) synthetase catalysing the synthesis of an · oligonucleotide, pppA(2′p5′A)n [3]. This oligonucleotide, with its unusual 2′–5′ phosphodiester bonds, activates a latent endoribonuclease and therefore enhances mRNA degradation. There are a few reports which show that the same enzymatic system might operate in experimental mice treated with interferon or interferon inducers since one of the enzymes involved in the system, i.e. the 2′,5′ oligo(A) synthetase is induced in different organs of these animals [4–6].

The C_3H mouse is a laboratory strain which is known to produce spontaneous tumors. The percentage of these animals producing tumors varies according to the breeding status and environmental stress [7]. It has been reported that by the age of 7.2 months, 99% of the female mice produce breast cancer and by 14 months 85% of the males produce hepatomas [8]. The inbred C_3H mice kept in our Institute since 1961 produce spontaneous tumors to a lesser extent (by the age of 10 months 50% of the females and 60% of the males produce breast cancer and hepatomas, respectively).

We estimate here the levels of the 2′,5′ oligo(A) synthetase in various tissues of healthy and tumor-bearing C_3H mice.

2. MATERIALS AND METHODS

2.1. *Interferon inducers*

Encephalomyocarditis (EMC) virus was grown in BHK/C_{13} cells. Its titration was carried out using a plaque assay in the same type of cells. Poly(I)-poly(C) was purchased from Serva, Heidelberg.

2.2. *Animals*

Thirteen-month-old C_3H mice were used for this investigation. Essentially 4 groups of animals were used: female healthy or with breast tumor and male healthy or with hepatomas. EMC virus (5×10^6 p.f.u./mouse) was administered intraperitoneally and poly(I)poly(C) (10 mg/kg body wt) was administered intravenously.

2.3. *Plasma preparation and tissue extracts*

After ether anesthesia, mice were bled from the auxillary vessels, the blood was collected in

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polystyrene tubes containing 50 μl sodium citrate (3 mg/ml), and left for 15–30 min at 4°C. Plasma was collected after centrifugation (2000 × g, 15 min) and stored at −80°C.

Tissues frozen in liquid nitrogen were homogenized mechanically (Dounce homogenizer) in buffer containing 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM DDT and 20 mM Hepes (pH 7.4) [4]. The cell homogenate was then centrifuged at 10 000 × g for 10 min and the supernatant representing the S_{10} cell fraction was stored in liquid nitrogen.

2.4. *Assay of 2′,5′ oligo(A) synthetase*

The activity of 2′,5′ oligo(A) synthetase is estimated by following the conversion of ^{3}H-labelled ATP (Amersham) to adenosine oligomers as in [9]. Incubations were for 24 h at 30°C followed by precipitation of the mixture with 6 vols acetone. The proportion of ATP which is polymerized is calculated by separating the synthesized oligomers from monomers with small DEAE–cellulose columns [4]. The activity of the 2′,5′ oligo(A) synthetase is expressed as the percentage of the substrate (^{3}H-labelled ATP) which is converted to a mixture of oligonucleotides.

3. RESULTS

Apparently healthy animals or animals with spontaneous tumors (C_3H females with breast cancer or males with hepatomas) were injected intraperitoneally with EMC virus (5 × 10^6 p.f.u./mouse). At the times after injection shown in fig.1, the animals were killed and the levels of 2′,5′ oligo(A) synthetase were estimated in various tissues of the neoplastic or healthy animals.

The data presented in fig.1,2 show that:

(i) In healthy animals the activity of the 2′,5′ oligo(A) synthetase is very low at the time of virus infection (points at zero time of the graphs) and virus infected animals (fig.1a–c, fig.2a–c). Only liver cells from either healthy females or males show low, but detectable levels, of synthetase after inoculation with EMC virus (fig.1a,2a).

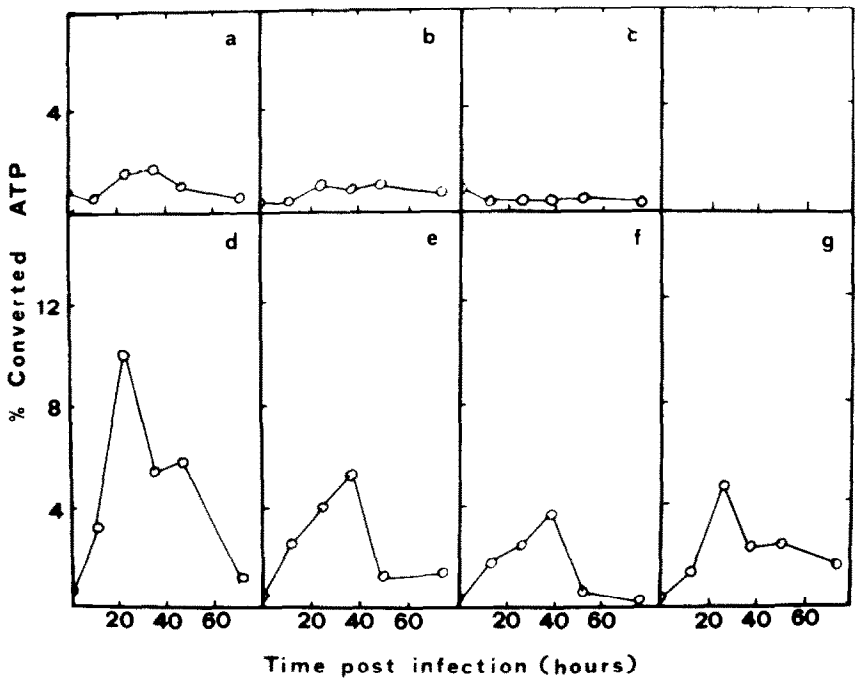


Fig.1. 2′,5′ Oligo(A) synthetase activity in C_3H mice at different times after EMC virus infection. Top row, healthy animals; bottom row, animals with breast cancer. Homogenates prepared from: (a,d), spleen (b,e), plasma (c,f) and neoplastic tissue (g).

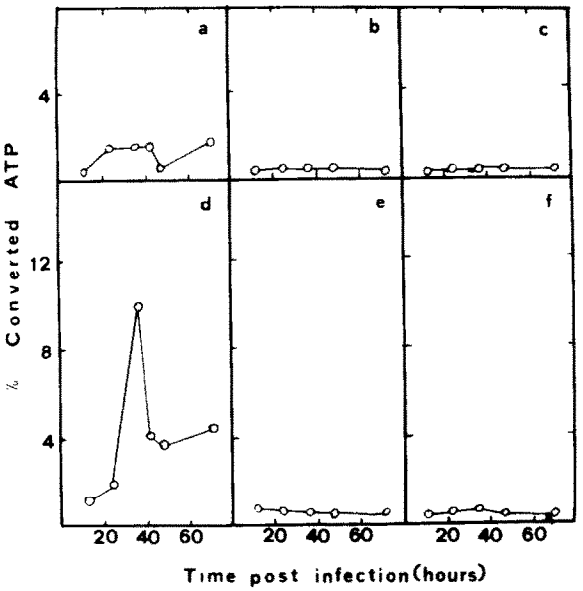


Fig.2. 2′,5′ Oligo(A) synthetase activity in C_3H mice at different times after EMC virus infection. Top row, healthy animals; bottom row, animals with hepatomas. Homogenates prepared from: liver (a,d), spleen (b,e) and plasma (c,f).

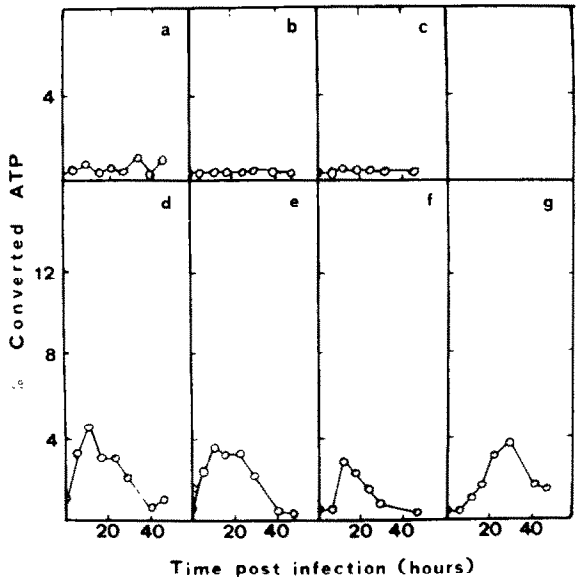


Fig.3. 2′,5′ Oligo(A) synthetase activity in C_3H mice at different times after inoculation of the mice with poly(I)poly(C) (10 mg/kg boty wt). Top row, healthy animals; bottom row, animals with breast cancer. Homogenates prepared from: liver (a,d), spleen (b,e), plasma (c,f) and neoplastic tissue (g).

(ii) 2′,5′ Oligo(A) synthetase in tumor-bearing animals (breast cancer or hepatomas) is detected at elevated levels only after infection with the virus (fig.1d–g, fig.2d–f).

(iii) The increased activity of the enzyme after infection is detected in the homogenates prepared from liver, spleen, plasma, as well as the tumor cells of the breast cancer-bearing animals, whereas the same enzymatic activity is observed only in the liver cells (neoplastic cells) of animals with hepatomas.

Similar results were obtained when C_3H mice were inoculated with poly(I)poly(C), a good interferon inducer [10]. Poly(I)poly(C) results in the induction of synthetase only in C_3H mice which bear spontaneous tumors (fig.3,4). Inoculation of healthy animals with poly(I)poly(C) has no effect

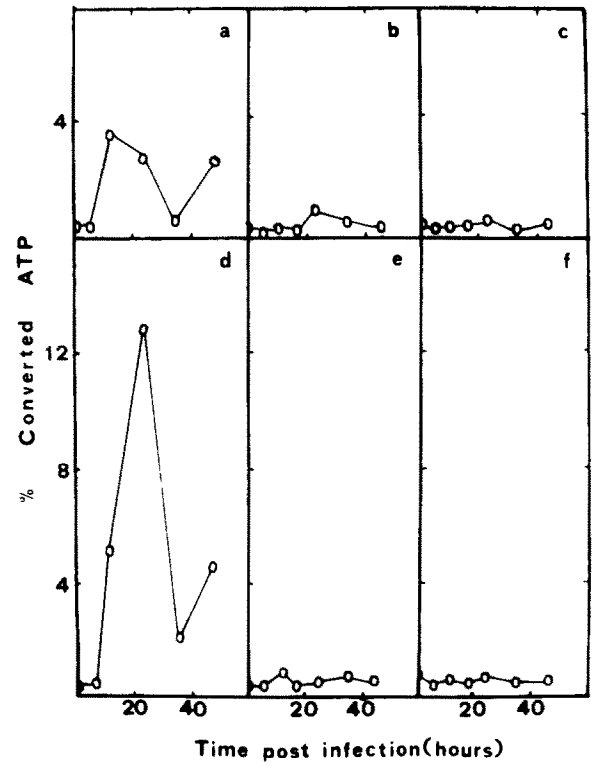


Fig.4. 2′,5′ Oligo(A) synthetase activity in C_3H mice at different times after inoculation of the mice with poly(I)poly(C) (10 mg/kg body wt). Top row, healthy animals; bottom row, animals with hepatomas. Homogenates prepared from: liver (a,d), spleen (b,e) and plasma (c,f).

on the levels of the synthetase except that of liver where again the enzyme is induced but in low levels (fig.4a).

4. DISCUSSION

The aim of these experiments is to show the presence of 2′,5′ oligo(A) synthetase in experimental animals, its distribution in various animal organs, its induction with virus or dsRNA and finally the demonstration of the effect, if any, of the tumor on the synthetase system.

With the synthetase assay used here we were unable to detect significant levels of the enzyme in the organs tested in control animals (virus non-infected of dsRNA non-inoculated). An exception to this finding are the levels of the enzyme in liver cells. Homogenates from livers of healthy females or males show detectable levels of 2′,5′ oligo(A) synthetase after virus of poly(I)poly(C) treatment. Considering that this assay detects enzyme quantities which convert 1% of the ATP present in the assay mixture [4], we conclude that except for liver homogenates, the level of the 2′,5′ oligo(A) synthetase in the various tissues of 'our C_3H mice' is very low and in this respect the C_3H mice used in this work are different from those used by others [6].

The fact that the results obtained with either EMC virus or poly(I)poly(C) were similar shows that synthetase induction is probably a consequence of the interferon synthesized by the animals in response to virus infection or dsRNA inoculation. This finding is in agreement with all the published work [4–6]. What is puzzling is our finding that the induction of the synthetase with interferon inducers is possible only in animals with spontaneous tumors. It appears that the presence of the tumor plays an essential role in the induction of this enzyme. This is in contrast to previously published works [4–6] in which the induction of high levels of 2–5A synthetase has been shown in different organs of normal mice injected with poly(I)·poly(C) or infected with EMC virus. The controversy between these and our results requires further studies in order to be clarified. It is generally accepted that the induction of the synthesis of various proteins in the cell by interferon is the result of a derepressive procedure of certain genes which in the normal cell are under tight negative control [11] (i.e. these genes are not expressed in the normal cell). It seems, for some unknown reason, that the presence of the tumor in the animal might have an auxiliary effect on this 'depressive' function of interferon. However, the more effective induction of the synthetase in the tissues of tumor bearing animals could be simply the result of a greater amount of interferon synthesized in these animals. Recent preliminary experiments on synthetase induction with interferon in both healthy and neoplastic animals show that the production of interferon cannot alone account for the differences observed on synthetase induction since we find that the same amount of exogenous interferon induces higher levels of synthetase in the animals with tumors. Both possibilities described above, i.e. (a) the function of interferon (derepression) is more effective in animals with tumors and (b) interferon inducers produce larger amounts of interferon in neoplastic mice, support the observations we report. These possibilities will be further clarified when the measurement of circulating interferon in all the experimental mice becomes possible. The fact that the same phenomenon is observed with totally different tumors (breast cancer and hepatomas) leads to the conclusion that this might be a general situation which accompanies neoplasia. It would be of interest to determine whether the induction of 2′,5′ oligo(A) synthetase is also found in animals bearing chemically induced tumors.

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