Interferon and Resistance to the Toxic Effects of Influenza Virus in vivo

By A. A. SMORODINTSEV, V. I. RUDENKO, S. A. MOSHKIN, D. A. GVOZDILOVA AND O. A. AKSENOV

All-Union Research Institute of Influenza, Leningrad, U.S.S.R.

(Accepted 20 May 1969)

SUMMARY

When mice were inoculated intravenously with Newcastle disease virus, interferon was formed, and the mice were protected against the lethal effects of a subsequent intravenous injection of a toxic strain of influenza A virus. Mice were similarly protected when injected intravenously with a suspension of mouse macrophages which had been stimulated to produce interferon by treatment in vitro with Newcastle disease virus. It is known that the reticuloendothelial system has a role in the production of interferon and in the development of non-specific resistance to virus infections. When this system was blocked completely by a thorotrast injection, or partially as the result of splenectomy, there was a sharp decrease both in the amounts of interferon formed in mice in response to an injection of Newcastle disease virus and in their resistance to the toxic effects of influenza virus.

INTRODUCTION

A number of workers have reported that various viruses (poxviruses, myxoviruses, arboviruses, etc.) cannot only infect cells but also produce toxic effects (Ginsberg, 1951; Davenport, 1952; Solovjev & Mastyukova, 1954; Pereira, 1958; Everett & Ginsberg, 1958; Mahnel, 1965). In particular, it has been shown that influenza virus contains a toxin (Shakhnazarova & Sacharov, 1946; Henle & Henle, 1946; Golbert & Peterson, 1948; Kato & Hara, 1961). It has been stated that in order to neutralize the toxic effects of viruses, including influenza virus, the infectivity must be neutralized (Zakstelskaya, 1958), but this seems not to be so. If animals are pretreated with various viruses, they can readily be protected against the effects of both virus multiplication and virus toxins. We have previously found that intravenous and intraperitoneal injections of Newcastle disease or mumps or influenza A virus can lead to an increase in the resistance of albino mice to the toxic effects of influenza virus. Since all these viruses can induce the formation of interferon, we have now investigated the relationship between production of interferon and the development of resistance in mice to the toxic effects of influenza virus.

METHODS

Viruses. Strain 32 of influenza A was isolated from man in Leningrad in 1949, and has recently been adapted to grow in mice. The preparation used contained 10⁸ egg ID₅₀, 10⁵ mouse LD₅₀ and 1280 haemagglutinin units/ml. The VICTORIA strain of Newcastle disease virus was used.
Interferon. Mouse interferon was harvested from cultures of mouse macrophages infected with $10^7$ to $10^9$ TCD$_{50}$ of Newcastle disease virus. The batch used had a titre of 640 units/ml.

Interferon assay. The quantitative haemadsorption method of Finter (1964) was used with mouse L cells, which were challenged with 0.2 ml. of Sendai virus containing $10^7$ egg ID$_{50}$.

Protection of mice against toxic effects of influenza virus. An acute intoxication was produced in test mice by intravenous injection of 1 ml. of undiluted strain 32 influenza virus. Mice were protected against this by induction of endogenous interferon by intravenous injection of Newcastle disease virus, or intravenous injection of a suspension of peritoneal macrophages and leukocytes treated with Newcastle disease virus, or intravenous injection of exogenous interferon. All these treatments were given 24 hr before intravenous injection of influenza virus. At intervals between 1 and 72 hr after injection of Newcastle disease virus or influenza virus serum was collected, and extracts of liver, spleen and thymus were prepared. These were assayed for interferon content after they had been centrifuged at 20,000 g, treated at pH 2 for 3 to 5 days at 4°C, and then returned to pH 7.

RESULTS

When mice were injected intravenously with strain 32 influenza virus, 70 to 80% died during the next 3 days, most dying between 18 and 48 hr after the injection. After 18 hr, virus could no longer be detected in the serum, but it persisted longer in the spleen and liver, and after 48 hr could still be detected in organ extracts tested at a dilution of 1/10. The influenza virus injection led to the appearance of foci of necrosis in the liver after 48 hr, and to marked degeneration of the lymphoid cells in the thymus cortex. Interferon at a concentration of 10 units/ml. was found in the serum 3 hr after injection of the virus, and it rose to a peak level of 40 units/ml. by the sixth hour, but none could be detected after 24 hr. Interferon concentrations in liver and spleen extracts increased and decreased at similar times, but none could be detected in the thymus (Table 1).

Table 1. Interferon concentrations (units/ml.) in organ extracts after treatment of mice with different inocula

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Source of Interferon</th>
<th>Hours after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>Serum</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>--</td>
</tr>
<tr>
<td>Newcastle disease virus</td>
<td>Serum</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>--</td>
</tr>
<tr>
<td>Newcastle disease virus, followed after 24 hr by influenza A virus</td>
<td>Serum</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>--</td>
</tr>
</tbody>
</table>
Interferon and resistance to the toxic effects of influenza virus in vivo

10^8 to 10^9 TCD₅₀, by intravenous injection 24 hr before injection of influenza virus, the mortality due to influenza intoxication was reduced to 5 to 10%. No foci of necrosis in the liver were seen, and there was no degeneration of the thymus cortical cells, although influenza virus disappeared from the serum and organs at the same rate as in control mice. After Newcastle disease virus, interferon was detected in serum, liver and spleen, but none was detected in the thymus (Table 1).

Mice could also be protected against the toxic effects of influenza virus by an intraperitoneal injection of Newcastle disease virus, and in one experiment 70% survived as compared with 20% of control mice. In the same experiment, other mice were first inoculated intraperitoneally with saline or nutrient broth, so that an exudate rich in macrophages and leucocytes was produced, and then with Newcastle disease virus. All these mice survived when challenged with intravenous influenza virus. Macrophages and leucocytes obtained from mouse peritoneal exudates were treated with Newcastle disease virus and then injected into other animals. Ninety per cent of these survived challenge with influenza virus. However, when the cells were first disrupted by ultrasonic disintegration and then injected, only 20% of the test animals survived challenge, the same proportion as in the control group. Mice were not protected when either intact or disrupted mouse macrophages and leucocytes had first been injected intravenously. When rabbit macrophages (5 x 10⁶ ml.) were treated in vitro with 20 TCD₅₀ of Newcastle disease virus, and then injected intravenously into mice, the animals were not protected against the toxic effects of influenza virus, and injection of rabbit serum or tissue culture interferon (280 units/ml.) was similarly ineffective.

The ability of macrophages to produce interferon and to phagocytose was next compared in in vitro experiments. Cultures of macrophages obtained from mice injected intravenously with influenza virus 24 hr before the cells were isolated produced significantly less interferon and had less phagocytic activity than macrophages obtained from normal mice, or from mice previously injected intravenously with Newcastle disease virus. When mice were first treated with Newcastle disease virus, however, and 24 hr later with influenza A virus, their macrophages retained the capacity to produce interferon and to phagocytose.

Since reticulo-endothelial cells provide a major source of interferon (Kono & Ho, 1965; Van Rossum & De Somer, 1966; Lackovic & Borecky, 1965; Fruitstone et al. 1966; Ho, Postic & Ke, 1968), the effects of blocking these cells completely with thorostrast and of reducing their number by splenectomy were tried. Thorostrast (0.2 ml.) was injected into the tail veins of mice 24 hr before they were challenged with influenza A virus. Ninety-nine per cent of these animals died, whereas the mortality among control challenged mice varied from 70% to 80%. The effects of thorostrast treatment were even more striking if mice were rendered ‘resistant’ to the toxic effects of influenza virus by pretreatment with Newcastle disease virus. An injection of thorostrast 24 hr before challenge with influenza virus completely abolished the protective effect of the Newcastle disease virus injection (final mortality 80%), whereas in control ‘resistant’ mice only 5% to 10% died. In addition, the amounts of interferon in the serum and organs of thorostrast-treated mice were reduced by about fourfold, and reached approximately the same levels as in control mice. When mice were splenectomized, there was a similar decrease in their resistance to the toxic effects of influenza virus inoculated 24 hr later.
These experimental results suggested the involvement of phagocytic cells both in the production of interferon and in the resistance which animals developed to the toxic effects of influenza virus. We therefore looked to see whether interferon prepared from macrophages could itself directly protect against the toxic effects of influenza virus. One ml. of macrophage interferon containing 640 units was injected into mice 24 hr before they were challenged with influenza virus. Twenty to 30% of these mice died, whereas the mortality among control mice challenged in parallel was 70% to 80%.

There was, therefore, a relationship between the production of endogenous interferon and the development of resistance to the toxic effects of an influenza virus injection. Mice could be protected against these toxic effects by a previous injection of preformed interferon. This suggests that interferon protects against the toxic effects of viruses as well as inhibiting their multiplication.

REFERENCES


(Received 9 December 1969)