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Virus interference. II. Some properties of interferon

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Interferon could be titrated by the amount of interference induced in fragments of chorio-allantoic membranes challenged with influenza A virus. Over a ten-fold range, inverse proportion between interferon concentration and haemagglutinin titre reached by the challenge virus was observed.

Interferon proved stable at 2°C for 2 weeks. Marked inactivation took place after 1 h at 60°C.

Interferon was not measurably sedimented by 100,000 g for 1/2 h. It was held back by gradocel filters of a.p.d. 0-6 μ. It was not dialyzable.

Interferon was active against influenza A, Sendai, Newcastle disease and vaccinia viruses. It was not neutralized by anti-MEL rabbit serum and only slightly inhibited by pooled human serum rich in complement-fixing antibody to influenza A soluble antigen.

INTRODUCTION

In the preceding paper, evidence has been presented pointing to the existence of a factor associated with interference among influenza viruses and for which the name ‘interferon’ is proposed. This factor is produced by fragments of chorio-allantoic membranes after they have been acted upon by heated-inactivated, non-infective influenza A virus. The present paper deals with some properties of interferon.

METHODS

The preparation of heat-inactivated influenza A virus (strain MEL) and the use of fragments of chorio-allantoic membranes in experiments on interference is described in the preceding paper (Isaacs & Lindenmann 1957).

Production of interferon

Twenty-four freshly prepared membrane fragments were suspended in 24 ml. of heated MEL virus in a tightly stoppered 250 ml. centrifuge bottle; the bottle was placed in a roller-drum at 37°C for 3 h and shaken every 15 min. The membranes were removed from the fluid, thoroughly washed (two changes of 4 ml. buffer per membrane) and each membrane placed in a stoppered test tube with 1 ml. of fresh buffer in a roller-drum at 37°C for 18 to 24 h. The membranes were then discarded and the culture fluid, which will be referred to as ‘standard interferon preparation’, or shortly ‘interferon’, tested for its interfering activity.

Assay for interfering activity

This was done in the manner described (Isaacs & Lindenmann 1957), adequate controls being included in each experiment. Unless otherwise stated, the challenge virus was a 1:1000 dilution of standard MEL seed. In some instances, other

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Challenge viruses were used: Sendai (Kuroya, Ishida & Shiratori 1953), Newcastle disease (strain HERTS) and vaccinia (an egg-adapted strain); the amount inoculated was in each case $10^{-8}$ ml. of standard seeds kept frozen in stock capillaries. Whilst the growth of all the Myxoviruses was estimated by haemagglutinin titration ($\log_2$, values of HA units/0.25 ml. are entered in the tables) the vaccinia virus was titrated as follows: 48 h after challenging the membranes belonging to the same group were pooled and extracted in a Ten Broeck grinder. Amounts of 0.05 ml. of dilutions $10^{-8}$ and $10^{-4}$ were inoculated on to the chorio of 12- to 13-day developing eggs and the resulting poxcs counted after 2 days’ incubation at 35°C.

Miscellaneous

Centrifugation was carried out in a Spino refrigerated analytical centrifuge.

Gradocal filter membranes with average pore diameter (a.p.d.) ranging from 1.8 to 0.052 $\mu m$ were used in the filtration experiments.

RDE was purified from Vibrio cholerae filtrates by absorption on fowl red cells and elution into saline (Burnet & Stone 1947).

Anti-MEL immune rabbit serum with haemagglutination inhibition titre of 1:1000 when tested against eight agglutinating doses of MEL and pooled human serum with a haemagglutination inhibition titre of 1:500 and a complement-fixing titre against influenza A soluble antigen of 1:120 by the method of Hoyle (1948) were used.

Results

Titration of interferon

In order to compare the interfering activity of various interferon preparations, a titration curve was obtained by diluting on different occasions three batches of standard interferon preparation in buffer and measuring the interference produced, using six or eight replicate membranes at each dilution. The results are plotted in figure 1. Thus, the undiluted standard interferon preparations reduced the haemagglutinin yield to less than 10% of the controls; ten-fold dilution almost entirely abolished interfering activity. Within this range, there seems to exist a linear relationship between interferon concentration and haemagglutinin suppression, a doubling in interferon concentration leading to a 50% reduction in the amount of haemagglutinin produced.

Stability

Table 1 shows the stability of standard interferon preparations at various temperatures. Interferon was stable for at least 2 weeks at 2°C. Complete inactivation at 60°C as shown in the table was found in three experiments, but in another experiment the inactivation was only partial. This is possibly due to variations in pH, a point not yet investigated. Rapid freezing at $-70°C$ followed by slow thawing at room temperature gave for some unknown reason erratic results ranging from strong inactivation to full preservation. When the heat stability of ribonuclease (kindly provided by Dr R. R. Porter) was tested in parallel with that of interferon, it was found that the interfering activity of ribonuclease (Le Clerc 1956) was unaffected by heating at 60°C for 1 h.

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*Attempts at revealing haemagglutinating activity*

Interferon is present in fluids rich in haemagglutination inhibitors; this can be readily shown by mixing heated MEL with buffer in which membranes have been kept for a few hours. The masking of heated MEL haemagglutinin by inhibitor, even after 24 h contact at 37°C, was quantitatively removed by treatment with purified RDE from *Vibrio cholerae* filtrate. Similar treatment of standard interferon preparation failed to unmask any trace of haemagglutinin. (Had the interference observed been due to heated MEL we should have expected to find at least 100 a.d./ml.) In addition, no interfering activity could be removed from interferon by absorption with 1% fowl red cells for 2 h at 2°C.

![Graph](image)

**Figure 1.** Titration of interferon. Geometric mean titres of groups of six to eight membranes with standard errors of means are shown. Symbols refer to three different experiments; the controls of all experiments are pooled.

**Table 1. Stability of Standard Interferon Preparations**

<table>
<thead>
<tr>
<th>Interfering Agent</th>
<th>Log&lt;sub&gt;4&lt;/sub&gt; HA Titre</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh interferon</td>
<td>1-0</td>
<td>3</td>
</tr>
<tr>
<td>24 h 2°C</td>
<td>2-0</td>
<td>4</td>
</tr>
<tr>
<td>1 week 2°C</td>
<td>1-0</td>
<td>1</td>
</tr>
<tr>
<td>2 weeks 2°C</td>
<td>2-4</td>
<td>2</td>
</tr>
<tr>
<td>24 h 37°C</td>
<td>1-2</td>
<td>3</td>
</tr>
<tr>
<td>1 h 58°C</td>
<td>5-5</td>
<td>21</td>
</tr>
<tr>
<td>1 h 60°C</td>
<td>7-7</td>
<td>147</td>
</tr>
</tbody>
</table>

*Centrifugation, filtration and dialysis*

Centrifugation of standard interferon preparations at 20000 g for 2 h or 100000 g for ½ h did not detectably reduce the interfering activity of the supernatant, although the same treatment removed all interfering activity from a preparation of heated MEL. From this result, we suspected interferon to be a rather small particle; however, filtration through a series of gradecool membranes
from 0.62 to 0.052 μ a.p.d. showed all interfering activity to be held back by the first membrane. Table 2 shows the results of a second filtration experiment with membranes of 1.8 to 0.6 μ a.p.d. All interfering activity passed the coarsest membrane, but a significant amount was held back by the 1.0 μ membrane and the 0.6 μ filtrate was devoid of measurable activity. After dialysis of 6 ml. of standard interferon preparation in a Visking cellulose casing against two changes of 500 ml. buffer for 48 h at 2°C the content of the dialysis tube showed unaltered interfering activity.

Electron microscopy of standard interferon preparations has not revealed any virus-like particles. It is hoped to investigate this point further with purified material.

Table 2. Filtration of Interferon

<table>
<thead>
<tr>
<th>Interfering Agent</th>
<th>Yield of Challenge Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control interferon</td>
<td>log₂ HA titre</td>
</tr>
<tr>
<td>filtered through 1.8 μ a.p.d.</td>
<td>1.5</td>
</tr>
<tr>
<td>filtered through 1.8 μ + 1.0 μ a.p.d.</td>
<td>1.7</td>
</tr>
<tr>
<td>filtered through 1.8 μ + 1.0 μ + 0.6 μ a.p.d.</td>
<td>4.4</td>
</tr>
<tr>
<td>filtered through 1.8 μ + 1.0 μ + 0.6 μ a.p.d.</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Table 3. Growth Inhibition of Four Viruses by Interferon

<table>
<thead>
<tr>
<th>Challenge Virus</th>
<th>log₂ HA Titre</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEL influenza A</td>
<td>3.0</td>
<td>3</td>
</tr>
<tr>
<td>NDV</td>
<td>1.8</td>
<td>6</td>
</tr>
<tr>
<td>Sendai</td>
<td>-1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Vaccinia</td>
<td>5.5 x 10⁴*</td>
<td>15</td>
</tr>
</tbody>
</table>

* Pock count per ml estimated by chorionic inoculation.

Activity spectrum

It was already known that heated MEL virus will interfere with the growth of many Myxoviruses (Henle & Henle 1945) and of vaccinia in fertile eggs (Depoux & Isaacs 1954a). Interferon was therefore tested against Sendai, Newcastle disease and vaccinia viruses, using the same system as for influenza A. The growth of the Myxoviruses was estimated by their haemagglutinin titres, that of vaccinia by a pock titration method (cf. Methods). Interferon showed clear-cut interference with all four viruses tested, resembling heated MEL in that respect.

Serological behaviour

The activity of interferon against viruses serologically completely unrelated to the original MEL strain gave us an opportunity to test the effect of anti-MEL sera against interferon. In these experiments, Newcastle disease virus was used as a challenge virus. Table 4 shows that a dose of type-specific rabbit serum sufficient to abolish all traces of interference by heated MEL was without effect on

interferon, while a similar dose of pooled human serum (containing large amounts of complement-fixing antibody to influenza A soluble antigen) had only a slight inhibitory action.

**Table 4. Effect of antisera on interferon**

<table>
<thead>
<tr>
<th>interfering agent</th>
<th>yield of challenge virus*</th>
<th>log₂ HA titre</th>
<th>% control</th>
</tr>
</thead>
<tbody>
<tr>
<td>heated MEL</td>
<td>2.2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>heated MEL + anti-MEL serum†</td>
<td>5.2</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>heated MEL + pooled human serum‡</td>
<td>6.1</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>interferon</td>
<td>1.8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>interferon + anti-MEL serum†</td>
<td>1.9</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>interferon + pooled human serum‡</td>
<td>3.2</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

* Newcastle disease virus.
† Immune anti-MEL rabbit serum 1:20.
‡ Pooled human serum 1:20.

**Discussion**

For interferon to be produced, both heated virus and membranes are necessary; interferon must therefore be derived either from the original virus or from the membrane or from both. Its site of action seems to be the membrane rather than the free challenge virus, since membranes brought in contact with interferon and thoroughly washed still show interference with a subsequent challenge virus.

The data presented are too scanty to allow even the crudest guess as to the interferon’s chemical nature; its low sedimentation rate together with the fact that it is held back by comparatively coarse filters might be explained by a low specific gravity or a filamentous shape. Pending further experimental results, we can compare interferon with some known interfering agents. Interferon cannot be identical with the original heated virus, the most conspicuous differences between the two being the interferon’s inability to agglutinate red cells, its resistance to the neutralizing action of viral antiserum and its lower sedimentation rate. For the first two reasons the interferon is also unlikely to be one of the surface constituents of the original virus. Apart from these, there is another reason to believe that interferon is not identical with receptor destroying enzyme, because this, while interfering with the growth of certain Myxoviruses, does not affect vaccinia (Depoux & Isaacs 1954b). Interferon might be some fragment from within the virus, set free in the course of breakdown of the heated virus particle by the cell; it is perhaps significant that the interfering activity of interferon and the heated virus have similar heat stability. The fact that interferon is only slightly neutralized by pooled human serum rich in complement-fixing antibody makes it difficult to identify it with influenza soluble antigen. If interferon is a reaction-product of the cell, it might be some cellular enzyme. Ribonuclease is known to produce interference (Le Clerc 1956), but an argument against interferon being ribonuclease is its heat-lability. A tempting hypothesis is the following: the heat-degraded virus feeds into the cell insufficient information for orderly virus
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synthesis to proceed, but sufficient for some kind of ill-organized reaction chain to start, leading sooner or later to a deadlock with piling up of distorted virus intermediates at a critical point in the chain. It is conceivable that large amounts of such substances—interferon—could block later synthesis of infective virus.

We wish to thank Dr C. H. Andrewes, F.R.S., for the interest he took in this work, Miss M. V. Mussett for help with computations and Mr V. G. Law for skilled assistance.

References