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Serum Interferon Assay as a Possible Test for Virus Infections of Man

By

T. H. J. Matthews and M. K. Lawrence

Clinical Research Centre,
Harrow, Middlesex, England

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Summary

Acute phase serum gave positive results in an interferon assay when collected from 17 out of 45 (38 per cent) patients with proven virus infection, and from none of 43 patients with other disease and none of 61 healthy subjects. Sera from 11 of 43 (26 per cent) patients with suspected virus infection were also positive. Interferon was detected in the sera of volunteers infected with respiratory viruses in strict isolation. It is suggested that the test might be used to supplement conventional tests for virus infections, and with modification may provide a useful diagnostic aid.

Introduction

It was noted many years ago that interferon was present in the serum in the acute phase of virus infections of man (7), and this has been subsequently confirmed (1, 5). Although interferon was not found in patients who were thought to have bacterial infections it is now known that bacterial infections can induce interferon (4). It is perhaps because of this knowledge that there has been no great enthusiasm for exploiting the idea of using a test for interferon in the blood as a rapid and general test for virus infection. We have therefore reinvestigated the problem to see if interferon is indeed found in virus infections and not in bacterial disease, and if so, whether it is found often enough to justify developing a better test for circulating interferon for use in diagnosis.

Materials and Methods

Blood was collected aseptically from children and adults within hours of admission to Northwick Park Hospital or the Central Middlesex Hospital. It was separated as soon as practicable, and the serum was stored at −20° C. Further acute phase sera were supplied by the Public Health Laboratory Service, Portsmouth. Interferon was detected using a modification of plaque inhibition technique (3), using a semi-micro method (8) in which V 3 cells, which are a vervet monkey kidney cell line responsive to human interferon, were grown in wells in a plastic tray. The serum was diluted 1:10 and added to at least two monolayers and held overnight at 37° C in a CO_2 incubator.

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The fluid was removed and the cells were challenged by adding 30—50 plaque forming units (PFU) of vesicular stomatitis virus (VSV) to each well, followed by an overlay containing carboxymethyl-cellulose. After incubation for three days the monolayers were fixed and sterilised with formalin and stained with crystal violet. A 50 per cent reduction in mean plaque count was regarded as indicating the presence of interferon. All positive results were confirmed by repeat tests. The serum was titrated for the presence of interferon and the titres ranged from 1:20 to 1:800. Some adult sera were brought to pH 2 and back to pH 7.4 after dilution but it was impractical to show that the inhibitor in these sera had other properties of interferon; but all showed the most important property of priming cells to resist virus infection and there is little doubt that the inhibitor was interferon. Parallel tests with dilutions of laboratory standard leucocyte interferon were included in all assays and showed that our test detected between 0.1 and 1.0 reference units of interferon per millilitre of serum dilution tested (reference standard B 69/19).

The patients were also subjected to routine bacteriological and virological diagnostic tests. They could be classified into those in whom a specific viral or bacterial diagnosis could be made, and those in whom a viral illness was suspected on clinical grounds. This latter group yielded no viruses on culture, usually of throat swabs and faeces, and most were tested by a standard serological screen.

Results

The results are summarised in the tables. Table 1 shows that no interferon activity could be found in the serum of normal subjects or of patients with unequivocal evidence of bacterial infection even if this was severe. The cases included bacterial pneumonia and meningitis, pyelonephritis, and abscesses in wounds, the liver, and the subphrenic space. In addition, no interferon was detected in the serum of patients with various other non-infective diseases. However, a substantial proportion of patients in whom a virus infection was proved had a positive serum interferon test. Table 2 shows that interferon tests were positive in a variety of patients with natural virus infections as well as in mild experimental infections with influenza B and rhinovirus. If herpes virus and rubella infections were excluded one half of the specimens from patients were positive.

Table 1. *Results of testing human sera for interferon*

Source of serum	No. tested	No. showing interferon-like activity
Healthy persons	61	0
Children and adults with metabolic malignant and inflammatory disease	21	0
Children and adult patients		
with proven bacterial infections	22	0
infected with mycoplasmas or Rickettsias	3	0
with clinically diagnosed virus infections	43	11 (26%)
with proven virus infection	45	17 (38%)
Volunteers		
infected with influenza B	19	9 (42%)
infected with rhinovirus type 4	24	3 (12.5%)

There were 43 children who suffered an illness which was clinically suspected to be due to a virus though none could be identified by the standard tests. Interferon was detected in the serum of 11 of these, whose symptoms and signs included

febrile convulsions, asthma, diarrhoea and vomiting, aseptic meningitis, skin rashes and lymphadenopathy. Some of the other patients may not have been infected or were infected by a virus which does not induce interferon such as the hepatitis viruses or cytomegalovirus.

The proportion of positive sera in volunteers (6) infected in strict isolation with influenza B was similar to that in hospital patients. Of 19 volunteers who were proved to be infected with an influenza B virus and who were bled when they developed symptoms, 9 were positive for detectable interferon and 10 were negative; the geometric mean clinical score (6) was 26.8 in the interferon positive group and 8.2 in the others and this indicates that the interferon positive volunteers had more symptoms and signs than the remainder. There was no such relationship in the volunteers given a rhinovirus, and the overall frequency of positive sera was lower than in influenza B virus infections.

Discussion

This reinvestigation seems to confirm the original idea of Wheelock and Sibley that interferon can be detected quite frequently in the serum of patients with definite virus infections which are serious enough to require medical attention. The sera were collected and handled like the "acute" serum for standard virus serology. We found no positive results in patients with other severe illness including bacterial infections, but about a quarter of patients in whom a clinical diagnosis of a virus infection was not confirmed nevertheless had a positive serum. It could be that some of the cases thought clinically to be virus infections were actually other infections or immunological in nature but they may have been infected with viruses—for example, coronaviruses and rhinoviruses would not be detected by the standard battery of diagnostic tests used. Virus infection was diagnosed by complement fixation tests on acute and convalescent sera and isolation by inoculation of the specimens into three lines of tissue culture cells. The correlation of a positive interferon test with virus infection is confirmed by the results in sera taken from volunteers who were infected in strict isolation (6). This result had been obtained previously in a study using a different method of interferon assay (5) which detected interferon by means of a rhinovirus and Sindbis virus in human fibroblasts and V3 cells respectively. Thus it is not necessary to use VSV in the assay—tests with viruses which are not restricted to approved laboratories could be used. Our results also follow the general tendency for interferon to be detected more often in more severe illnesses.

It is clear that interferon is likely to be found more often in some virus infections than in others. Herpes simplex and zoster seem to induce little interferon (Table 2) but this is not important as rapid clinical and laboratory diagnosis is usually possible for these organisms. Detecting interferon in the serum is no more difficult technically than isolating and identifying viruses and the results are available at least as quickly—in four days. We think it would be still more useful if the test could be simplified and speeded up to take, say, 36 hours; the results would then be available soon enough to influence the management of the case, though they would still not be as rapid as immunofluorescence applied to respiratory virus infections (2). Our results suggest to us that if an interferon assay on

the acute phase serum was added to the standard laboratory tests, the chances of recognising the presence of a virus infection would substantially increase. As in the case of any laboratory test the result would have to be interpreted cautiously as the test is not always positive in proved virus infections, and it is still theoretically possible for a non-viral inducer or an extensive immunological response to be the cause of a positive test result.

Table 2. *Results of testing human sera from cases of proven virus infection*

Virus infection diagnosed	No. tested	No. showing interferon-like activity
Rubella	6	1
Enterovirus	3	3
Influenza A and B	11	4
Adenovirus	5	2
Measles	5	3
Mumps	6	3
Herpes simplex or varicella zoster	8	0

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Authors' address: Dr. T. H. J. MATTHEWS, The London Hospital, Whitechapel, London, E. 1, England.

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