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CLINICAL STUDIES OF INTERFERON*

(I) INTERFERON PRODUCTION IN CHILDREN WITH VIRAL DISEASES

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ABSTRACTS

In this paper children with acute viral illnesses were treated with a view to determining the mutual relationship, if any, between the presence of circulating interferon antibody status and the clinical courses of the children.

The patients treated were as follows:
29 patients suffering from mumps,
14 patients suffering from rubella,
27 patients suffering from measles, and
8 patients suffering from chickenpox.

Circulating interferon was detected in the sera of 61 acute patients treated. The positive sera were all those obtained within the first seven days of the acute illness, but no ihnibitor was detected in those sera which were obtained later.

Sixteen samples of saliva from patients with mumps were tested for the sole aim to detect interferon in them.

No correlation was observed to exist between the age of patients or the severity of the clinical courses, and the presence of interferon; however, all patients with detectable interferon were found febrile at the time of sampling.

INTRODUCTION

The synthesis of interferon, which is a protein inhibitor working against virus recurrence, has long been thought to be a major factor in modifying the outcome of viral infections in man and animals[1)~11)]. Interferon seems to be particularly important during the acute phase of primary viral infections[10)].

Several recent reviews[5)7)10)12)] have pointed out that viral agents vary from

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one another in their capability to stimulate the endogenous formation of interferon as well as in their sensibility to the inhibitory effects of the same.

However, with a few exceptions[13)~18)], the majority of these studies have been carried out with animals[2)3)6)19)~25)], chick embryos[26)], or tissue cultures[27)~30)], and not with the human host.

The present study was undertaken with the aim of ascertaining if there were any differences in the responses of circulating interferon to acute viral infection in children, with respect to the type of virus causing the infection. Measles, mumps, chickenpox and rubella were selected for this study, because they occurred frequently in children.

MATERIALS AND METHOD

Patients selected. Patients living in Nagoya and its vicinity, whose primary diagnosis was measles, mumps, chickenpox or rubella in 1966 and 1967, were selected as given above.

Samples of serum and saliva were obtained on the first day when the patients visited hospitals.

Complement-fixation tests were carried out for determination of antibody against mumps and rubella.

Assay of interferon. Serum and saliva samples were centrifuged at 1,000 × g, and the supernatant fluids were dialyzed in the ice box for 24 hours, first against pH 2.0 glycine buffer, and then against pH 7.4 saline to be neutralized. Precipitated materials were removed by centrifugation, and the supernatant fluids were then ultracentrifugazed at 100,000 × g for one hour; the top three-fourths of the resulting supernatant fluids were stored at −20°C, until assayed for detection of interferon. Serum and saliva samples were assayed in FL cells cultured in tubes. The cultures of FL cells were rinsed once in a phosphate-buffered saline (PBS) and inoculated in duplicate with 0.5 ml of serial twofold dilutions of the serum and saliva samples in Eagle's medium[31)]. After the incubation in tubes at stationary racks for 18 hours at 37°C, the samples were removed, and the cultures, rinsed once in PBS, were inoculated with 100 tissue culture infective dose ($TCID_{50}$) of Vesticular Stomatitis Virus (VSV) in 0.2 ml of Eagle's medium, titrations of virus and normal serum controls was included in this test.

The tubes inoculated with VSV were placed in a stationary rack at 37°C for two days, and at the expiration of the period the viral controls were found to show degeneration by at least 80%. Those cultures which showed degeneration by less than 20% were considered "protected". A sample was considered to contain inhibitor when it reduced the titer of VSV by 80% or more. Because interferon activity is a quantitative function, the inhibtor titers were expressed as units per ml of the sample (that is, the reciprocal of inhibitor

titer per 0.5 ml multiplied by 2 is equal to units per 1.0 ml of serum).

The inhibitor found in these samples was further tested, the result being that it was not dialyzable, nor sedimented at 100,000 × g for one hour, and was trypsin-labile. The substance was not considered to be an antibody: it did not neutralize 25 $TCID_{50}$ of VSV when, after being incubated for 45 minutes at 37°C, it was inoculated into tube cultures of FL cells. Furthermore, no inhibition forces against VSV replication was observed in these cells when compared with the control.

Five of the samples were tested in their heat stability at 56°C for 30 minutes, with the result that the inhibitor in these samples were not heat-stable. It must be, however, added that sufficient materials were not available for further charcterization of the inhibitor.

RESULTS

1. Mumps:

Twenty-nine samples of serum and 16 of saliva from patienis with mumps were tested with respect to interferon, and 19 of the 29 samples of serum were again used in the test for Complement fixing (CF) antibody. Fig. 1 shows the relation between interferon and CF antibody production in children suffering from mumps (Table 1).

Interferon titer in the serum was high directly after the onset of the illness, but gradually decreased until it disappeared entirely by the 6th day. Interferon titer in the saliva rose later than that in the serum did, reaching the peak by the third day from the onset of the illness. The peak of interferon titer in the saliva was lower than that of interferon in the serum. Both serum

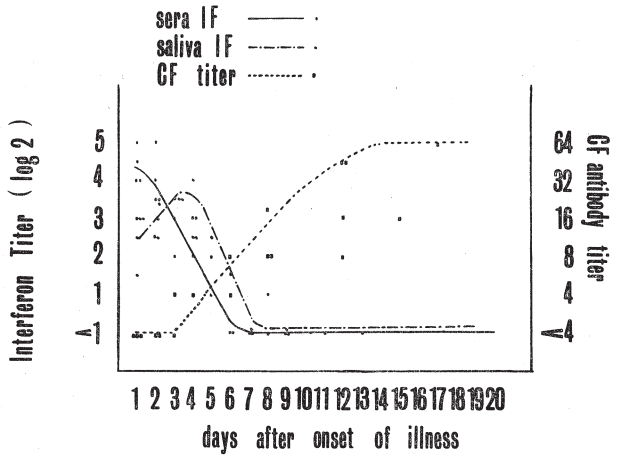


FIG. 1. Interferon titer in sera and saliva and CF antibody titer of mumps patients.

TABLE 1. Interferon titer in sera and saliva and CF antibody titer of mumps patents

Case No.	days after onset	IF titer (log 2) in sera	IF titer (log 2) in saliva	CF antibody titer in sera
1	1	3	1.5	<4
2	1	4	2.5	—
3	1	4	3	<4
4	1	4.5	3	<4
5	1	5	—	—
6	2	2.5	2.5	—
7	2	3.5	3	<4
8	2	3.5	3.5	—
9	2	4	4	<4
10	2	5	—	—
11	3	2	—	<4
12	3	3	3.5	4
13	3	3.5	3.5	—
14	4	2	2.5	4
15	4	3	2.5	—
16	4	3	4	—
17	5	1	—	—
18	5	2	2.5	—
19	6	<1	—	4
20	6	<1	—	8
21	6	1.5	—	—
22	7	<1	—	—
23	7	<1	<1	—
24	8	<1	—	16
25	8	1	—	8
26	8	—	—	8
27	9	<1	<1	—
28	9	<1	—	—
29	11	<1	—	—
30	12	—	—	8
31	12	—	—	16
32	12	—	—	32
33	12	—	—	32
34	13	<1	—	—
35	15	—	—	16
36	17	—	—	64

and saliva interferons disappeared by approximately the 6th day. When interferon in the serum flags, CF antibody began to develop and reached the maximum titer in 2 to 3 weeks.

2. Rubella:

Fourteen samples of serum from patients with rubella were tested for interferon, and 10 of these samples were also used in testing for CF antibody. Interferon in the serum began to lose strength earlier than any other three kinds of viral infections, and disappeared by fourth to fifth day after onset. The CF antibody rose earlier than that in the serum from patients with mumps (Fig. 2), (Table 2).

3. Measles and chickenpox:

Twenty-seven samples of serum from patients with measles and 8 smples

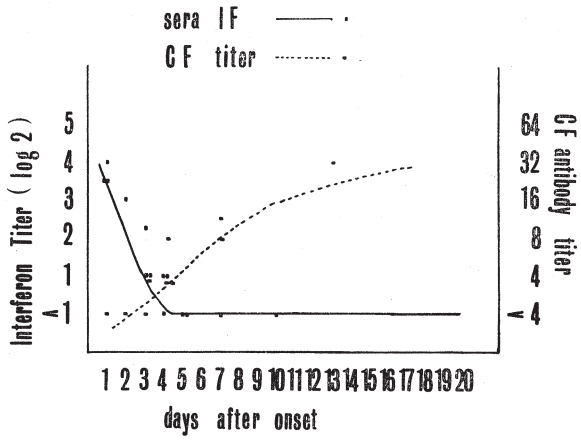


FIG. 2. Inteferon titer and CF antibody titer of rubella patients.

TABLE 2. Interferon titer and CF antibody titer of rubella patients

Case No.	days afte ronset	IF titer (log 2) in sera	CF titer
1	1	3.5	—
2	1	3.5	<4
3	1	4	—
4	2	3	<4
5	3	1	<4
6	3	1	4
7	3	2	—
8	4	<1	4
9	4	1	4
10	4	1	8
11	5	<1	—
12	5	<1	—
13	7	<1	8
14	7	—	16
15	10	<1	32

of serum from patients with chickenpox were tested for interferon (Table 3 and 4). Fig. 3 summarizes the variations of interferon titer in the sera from patients respectively with mumps, rubella, measles and chickenpox; it shows how in terms of time the circulating interferon loses strength the sequence being rubella, chickenpox, mumps, and measles patients in their respective clinical courses.

DISCUSSION

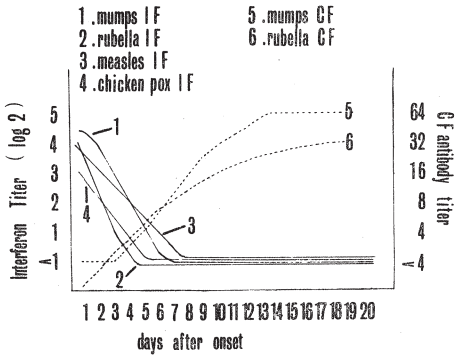
Although the *in vivo* induction of human interferon has been shown in patients infected with respiratory viruses, it is only patients with influenza A 2 infections that have specifically been studied, interferon being detected in

TABLE 3. Interferon titer in sera from patients with measles

Case No.	days after onset	IF titer
1	1	4.5
2	1	5
3	1	5
4	1	5
5	2	4
6	2	4.5
7	2	4.5
8	3	2.5
9	3	4
10	3	4
11	4	2
12	4	3
13	4	3
14	4	3
15	4	4
16	5	2
17	5	2
18	6	<1
19	6	1
20	7	<1
21	7	<1
22	8	<1
23	8	<1
24	8	<1
25	9	<1
26	11	<1
27	14	<1

TABLE 4. Interferon titer in sera from patients with chickenpox

Case No.	days after onset	IF titer
1	1	2.5
2	1	2.5
3	2	2.5
4	3	2
5	3	2
6	4	<1
7	4	1
8	5	<1



FGI. 3. Interferon titer and CF antibody titer of mumps, rubella, measles and chicken-pox patients.

nasopharyngeal washings[14)32)], and in the sera[14)]. Wheelock and Sibley[11)] have reported of the detection of circulating interferon in 7 out of 23 hospitalized patients with clinical evidences of an acute respitory illness. All but one of these had an interferon titer of 1 : 10 or greater, but the patients treated were all adults except one, and specific viral etiologies were not determined in their patients. Ray and his associates[8)] have found circulating interferon in their patients infected with RS, influenza A 2 and influenza B viruses. However, in their studies no inhibitor has been detected in the patients infected with the virus of parainfluenza or adenovirus. And at any rate, the small number of infection cases that were studied have not enabled them to come to any conclusion as to the something that induces the circulating interferon to generate.

In the course of the present study, the writer detected not only circulating interferon generated in patients with mumps, measles, chickenpox and rubella, but also interferon in the saliva from patients with mumps. It was, however, found that the pattern of interferon production varied according to the respective human viral diseases of whatever kind. So the writer has come to

assume that viruses vary considerably in their respective efficiency as interferon inducers, as described by Isaacs[7] and Ruiz-Gomes[32].

The studies of various animal hosts by Baron and his associates[3] indicate that the appearance of interferon in the serum takes place during the viremic phase of infection. The observations by Wheelock and Sibley[18] during their studies with yellow fever vaccines suggest that a similar situation exists in the human host.

The relationship of age with the interferon responsiveness, however, was not clarified in this study.

The only clinical finding which appeared to correlate with the demonstion of the circulating inhibitor was that all the inhibitorpositive patients were feverish at the time of sampling. This fact has already been noted in the studies by Petralli and his associates[16] and also in those by Wheelock and Sibley[11].

The inhibitor discovered in the course of this study shows several of the major criteria by which an interferon is conceived[7,33]. It was not dialyzable, nor sedimented at 100,000 × g, and was trypsinlabile. What is more important, this inhibitor was observed not to be an antibody or nonspecific inhibitor against an extracellular virus. Furthermore, it seemed unlikely for this inhibitor to generate owing to nonspecific inhibitors casually acting at the cellular level; it was detected only in those sera which were collected during the acute phase of the illness concerned, and not found in 17 samples of serum collected during the convalescent stage. Inactivation was not observed to occur with interferons of some animal species when they were heated at 56°C for 30 minutes[5,33], but a significant partial inactivation was observed with the interferons of human origin when treated in the same procedure[11,18].

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