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Interferon Production in Human Mumps Infections* (32681)

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Interferon is now accepted as one of the important defenses against viral disease in animals (1). We have been interested in assessing its specific role in human illness, especially with reference to the severity and clinical course of the disease. The various clinical forms of mumps infection in man have been well documented and range from inapparent infection to severe meningoencephalitis, orchitis, or pancreatitis. However, knowledge of the precise factors conditioning these different individual responses to the infection is largely lacking. One possibility is that interferon, acting during early cycles of virus replication in a given host, is a determinant of the form which the infection takes. In the winter of 1966–1967, a number of children and adults with clinical mumps were observed, and interferon was demonstrated in samples of serum and saliva. In this communication we describe the results of our preliminary studies.

Methods. Interferon titers were assayed in human foreskin fibroblasts with bovine vesicular stomatitis virus (VSV) challenge, as previously described (2). Serum samples were stored at 4°C and assayed within 3 weeks. Bovine serum albumin (BSA), 0.05%, was added to urine samples which were stored at 4°C from within 1 hour after voiding and then dialyzed against Eagle's minimum essential medium (MEM) before interferon assay. After several studies demonstrated that only very low titers would be found, urines were concentrated by pressure dialysis 5-65X and then dialyzed against MEM. Previous studies with tissue culture interferon had shown this to be a satisfactory method of concentration.

In order to obtain samples of saliva, patients were asked to expectorate into sterile containers. Most specimens were collected within a few minutes, but a few were obtained over an interval of several hours. After collection, 0.05% BSA was added and the sample stored at 4°C. Before interferon assay, appropriate dilutions were centrifuged for 1 hour at 78,000*g*.

Most clinical specimens were obtained from subjects in the home. Diagnosis was based on the presence of parotitis, with or without other organ involvement, and an appropriate epidemiological history. If the diagnosis was questionable, as in cases of aseptic meningitis without parotitis or of parotitis occurring in the absence of exposure to mumps, paired sera were assayed for mumps complement fixation antibody titers.

Results. The results of studies on 64 sera, 12 urines, and 12 samples of saliva, obtained from 45 subjects, were analyzed. Figure 1 shows the range of serum levels found. Day 1 of illness is defined as that day on which any symptoms appeared, including fever, parotitis, or malaise. The highest titers occurred during the first 2 days of illness and then declined, although in a few patients very low or negative titers were noted on days 1 and 2.

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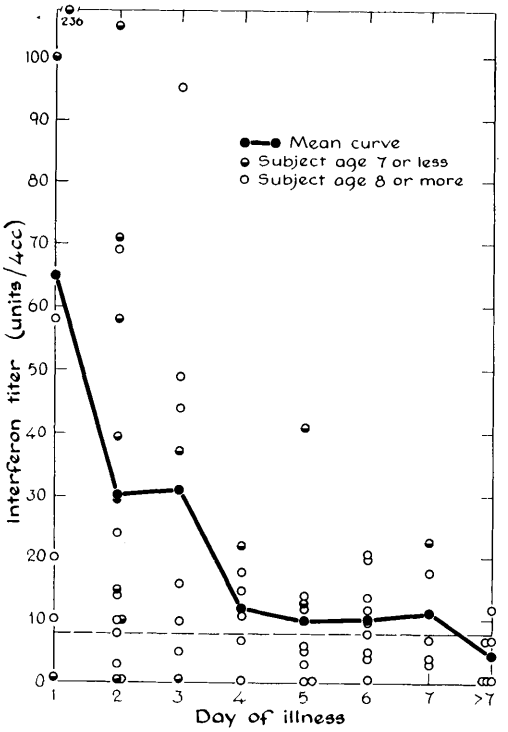

FIG. 1. Serum interferon titers in clinical mumps infection. Titers below 8 units are not considered to be of significance in this serum interferon assay.

The serum inhibitor in several different samples was demonstrated to be interferon by the usual criteria. It was not sedimented by ultracentrifugation at 78,000*g* for 1 hour but was trypsin sensitive (0.8 mg/ml, 38°C, 1 hour). It was cell species specific in that it was not active in either L_{929} or chick fibroblast cells against the same challenge virus in a plaque inhibition assay. It did not react directly with VSV on incubation for 1 hour at 38°C, and was ineffective when the cells were treated for shorter periods (1–2 hours) than used in the standard assay (6–12 hours). A 57% loss of activity after 1–6 hours incubation at pH 2 (4 assays) and an average fall in titer of 35% after a 1-hour incubation at 56°C (2 assays) are of note in that some interferons are stable under these conditions. However, a similar heat and acid lability has been described with human interferon found in cerebrospinal fluid (3, 4) and the Edmonston measles vaccine induced serum interferon was partially heat labile (2). In 5 of 11 samples of saliva interferon was demonstrable in titers ranging from 18 to 40 units. In all instances the specimen was spun for 1 hour at 78,000*g* and the supernatant studied. Only one specimen was obtained in sufficient quantity to characterize the inhibitor as interferon. Results of this characterization were the same as described above for the serum inhibitor except that, unlike the serum, the salivary interferon was stable at pH 2 for 12 hours and 56°C for 1 hour.

Of 7 urine specimens which were not concentrated, 3 had some antiviral activity with titers from 1 to 8 units. These levels were too low to characterize satisfactorily. However, the most likely source of false positive titers, namely viruria, which might itself cause viral interference in our assay, was virtually eliminated in 2 specimens by 1 hour of ultracentrifugation at 78,000*g*. None of the 5 concentrated urines showed any interfering properties.

Figure 2 shows the results of assays performed in urine and saliva relative to the day of illness. Concurrently obtained serum and saliva samples were roughly the same within the range of error of the assay. The urine interferon levels were consistently and significantly lower than those of concomittantly drawn serum levels. The highest titer noted

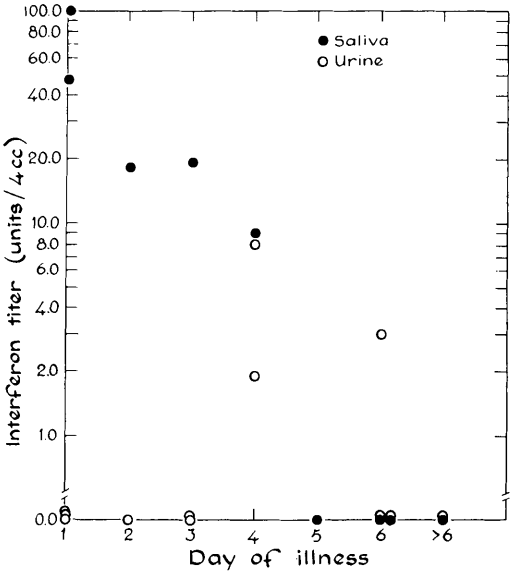

FIG. 2. Urine and saliva interferon titers in clinical mumps infection.

was only 1/5 that of the serum titer. It is possible that human interferon is reabsorbed by the kidny tubules or is relatively unstable in urine. Such instability could be due to the very low levels of protein normally present. For this reason bovine serum albumin (0.05%) had been added to each specimen, as it has been shown in this laboratory to stabilize interferon in protein-poor solutions (5), and the samples were assayed as soon as possible in order to avoid spontaneous loss fo activity. In spite of these precautions levels were never high enough to assess stability, although in 2 instances in which antiviral activity was present in urine on duplicate assays, a 3-fold fall in titer occurred over intervals of 6 and 28 days.

No significant correlations could be made between levels of interferon and severity of illness or age of these subjects. An early impression that unusually low titers were seen in adults, who generally had the most severe illness, could not be confirmed on the basis of the final data. It was clear that the presence of overt extraparotid involvement, with or without severe illness, did not necessarily result in high titers. An attempt was made to assess titers relative to the day on which clinical improvement occurred. Although titers tend to be highest before improvement and then to fall rapidly, not enough consecutive samples in single individuals were obtained to draw definite conclusions. In no instance did a titer rise during the illness.

The clinical course of several individuals illustrates these points. In two cases of aseptic meningitis other organ involvement was minimal and diagnosis was confirmed by a 4-fold rise in mumps CF antibody. A 20-year-old girl was ill with fever, headache, and photophobia, which slowly defervesced during a 5-day period. Five sera, taken from days 2–8 of illness had insignificant titers (3–7 units) which showed no fluctuation with changes in her clinical course. A 4-year-old child had a similar illness, with incapacitating symptoms for 2–3 days only, and titers of 37, 22, and 13 units on the third, fourth, and fifth days of illness, respectively, the days of the most severe but steadily declining symptoms.

The two children with the highest titers had relatively mild disease but the high titer was not necessarily followed by clinical improvement. One, a 4 year old, felt quite well on the first day of illness when her titer was 236 units, but on the following 2 days she had fever and malaise with mild abdominal pain. The other child had fever to 105°F and malaise on the first day of illness associated with a titer of 100. On the following day she was febrile but improved and had a titer of 58. She was essentially well by day 3 although the bilateral parotitis persisted over a week longer. Likewise two other children who had no demonstrable serum interferon on the second day of illness went on to have benign clinical courses.

Discussion. Our results demonstrated the presence of interferon in human serum and saliva during mumps infection. This was not unexpected in view of the prior description of interferon in cerebrospinal fluid (CSF) during mumps meningitis (3) and of the capacity of other myxoviruses (vaccine strains of measles (2, 6, 7), influenza (8), Newcastle disease virus,[4] and of clinical respiratory syncytial virus and influenza infections (9, 10) to induce interferon in man. The serum titers decreased steadily from the first day of symptoms and the rate of decrease resembled that noted with live measles vaccination in humans (2). The highest titer was observed in a child who had been mildly ill for only a few hours. These data suggested that interferon production may have started 2–3 days before the first measurements, which were always after the onset of symptoms. Such early production would be quite compatible with the fact that virus replication occurs at least in the parotid gland (11) prior to onset of symptoms and perhaps at other sites.

The wide scatter of titers even in the first days of illness emphasized the probability that any individual curve might be very different than the mean one drawn here (Fig. 1). In no instance when multiple samples were assayed from a single subject, however, did a titer rise during the course of the illness. There was no correlation between the magnitude of the titers and age or severity of

[4] Waddell, D. J., Walker, S., and Merigan, T. C., personal communcation.

illness. We were unable to obtain reliable records of temperature on all subjects and were therefore not able to comment on the correlation between fever and interferon titers observed in this laboratory (2) and elsewhere (3,12).

The presence of interferon in saliva was not surprising since the presence of replicating virus appears to be the stimulus for interferon formation, and interferon has been found in man in similar virus target organs such as the skin [varicella vesicle fluid (13) and vaccinia crusts (14)] and the cerebrospinal fluid (3). It is possible that the negative specimens did not represent material from the involved gland. The mouth is characteristically dry and the duct orifice may be swollen in mumps so that we had difficulty obtaining specimens of adequate size. When serum and saliva specimens were collected at about the same time, their titers were in the same range. However, even when serum levels were quite high, urine contained little if any interferon. This was disappointing in view of the hope that urine would provide a ready source of crude interferon for further studies.

The failure to find a relationship between the course of the clinical disease and serum interferon levels may be for any of several reasons. It is possible that the lack of precision of the interferon assay and the small number of patients with any given form of the clinical disease obscured differences that would be apparent with larger groups. It is also possible that interferon in the serum does not reflect the critical host virus interaction. This might only be assessed by study of a particular tissue site such as the portal of entry of the virus into the host or in a specifically involved organ. Finally, interferon may not be involved in determining the pattern of clinical response to virus infection. However, studies in animals suggest it is involved both in determining the relative virulence of different viruses (15) and in the attenuation of viruses for live virus vaccines (16, 17).

Summary. The production and variation in titer of interferon during clinical mumps infection was studied in 45 subjects. Serum titers in the clinical disease were highest at 1–3 days and then fell, but on occasion persisted as long as the eighth day of illness. The saliva also contained interferon in concentrations roughly parallel to those in sera taken at the same time. The titers could not be correlated with severity of illnes or observed clinical course.

Addendum. Since this work was completed, reports have appeared describing the presence of interferon in the urine of mice or rabbits (18–23), and one (24) has been summarized reporting failure to demonstrate interferon in the urine of children undergoing various common viral infections.

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Rhinoviruses: The Isolation and Characterization of Three New Serologic Types* (32682)

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The increasing number of reports concerning the isolation, characterization and epidemiology of a new group of viruses, the rhinoviruses, attests to the growing interest in them as causative agents of a significant proportion of mild illnesses affecting the upper respiratory tract of man. Rhinoviruses have been classified as a subgroup of human picornaviruses (1) and as such they posses an internal RNA component, are approximately 15-30 mμ in diameter, are ether stabile, and inactivated at low pH. On initial isolation rhinoviruses grow optimally at 33°C. The definition of the cultural conditions necessary for the detection of rhinoviruses in tissue cultures (2) and the concomitant use of human diploid cells (3) has led to the isolation of a large number of serologically distinct virus types. Through the efforts of a collaborative program conducted under the auspices of the Committee for Vaccine Development, National Institute of Allergy and Infectious Disease, and of the World Health Organization, a classification system was established (4) for those rhinoviruses which have been adequately characterized. Type numbers have been assigned to 55 serologically distinct rhinoviruses.

Three rhinoviruses, now established as new immunotypes, were isolated in this laboratory in 1962. They have been submitted as prototype strains to the Reference Laboratory of the Collaborative Study Program for rhinoviruses, and their isolation and characterization is the subject of this report.

Materials and Methods. Cell cultures. Diploid cell cultures of human fetal lung (HFD) were initiated and passaged according to the method of Hayflick and Moorhead (5). Tube cultures of HFD cells were seeded with 10^5 cells in Eagle's minimum essential medium with 10% fetal bovine serum and outgrowth was usually complete in 3–4 days.

Primary rhesus monkey kidney (MK) cell cultures were prepared by a modification of the method of Bodian (6). Outgrowth medium for MK cells consisted of 85 ml of Hanks' balanced salt solution, 10 ml of 5.0% lactalbumin hydrolyzate and 2 ml fetal bovine serum.

For maintenance of both cell types, Leibovitz Medium no. 15 with 2% fetal bovine

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