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Absence of Detectable Interferon in Jejunal Biopsies, Jejunal Aspirates, and Sera in Experimentally Induced Viral Gastroenteritis in Man (39031)

RAPHAEL DOLIN AND SAMUEL BARON

Medical Virology Section, Laboratory of Clinical Investigation and Cellular Virology Section, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

Acute infectious nonbacterial gastroenteritis (AINBG) is a widespread, common, disease whose manifestations consist chiefly of vomiting and/or diarrhea accompanied by low grade fever (1, 2). Illness occurs following an incubation period of 18–72 hr, and lasts 24–48 hr, with a characteristically rapid recovery (3). Etiologic agents appear to be viral in character (4, 5), and antibody rises to those agents have been detected in convalescent sera taken 3–6 weeks after illness (6, 7). However, because of the short incubation period and duration of illness, it has been suggested that nonimmunologic host defense mechanisms, particularly interferon, may be responsible for this rapid recovery (8). To investigate this question, we examined sera, jejunal secretions, and jejunal mucosal biopsies from normal volunteers inoculated with Norwalk or Hawaii agents of AINBG for the presence of interferon during experimentally induced infection.

Materials and methods. Experimental subjects were 13 healthy adults, ages 19–21, who participated in the normal volunteer program at the National Institutes of Health. Stool filtrates containing Norwalk or Hawaii agents were administered orally as previously described (2), and volunteers were observed for the development of clinical illness (4). The Norwalk-containing filtrate (designated 8FIIa) (9) had induced gastroenteritis in 23 of 44 volunteers to whom it had been administered, and the Hawaii-containing filtrate (designated 21KI) (9) induced gastroenteritis in 15 of 26 volunteers to whom it had been administered. Serum samples, jejunal aspirates, and peroral jejunal biopsies were obtained 48–96 hr after inoculation (24–48 hr after the onset of illness in ill volunteers). Jejunal biopsy specimens were obtained employing the Rubin tube (10) positioned fluoroscopically at the ligament of Treitz. Jejunal aspirates were obtained either through the Rubin tube or through an additional per oral polyethylene tube. Within 15 min after biopsy, specimens were transported to the laboratory, washed three times in cold Minimum Essential Medium (MEM), homogenized in a Virtis 45 homogenizer at 40,000 rpm for 10 min, and made up to 20% suspensions in MEM supplemented with 20% fetal calf serum. Jejunal aspirates were obtained undiluted except for the addition of 20% fetal calf serum. Transport, homogenization, and dilutions were carried out at 4°, and all samples were frozen at −70° until examined. Interferon was assayed by inhibition of cytopathology (CPE) of vesicular stomatitis virus (VSV) in cultured human foreskin fibroblast cells (HFS-1) (11). Interferon titers were defined as the reciprocal of the maximum dilution which would result in 50% inhibition of CPE when 400 PFU of VSV/0.1 ml were inoculated. The human reference interferon, NIH G-023-901-527, titered 12,000 units/ml in this system.

In addition, biopsies were examined by light and electron microscopy for morphologic changes, and paired sera (preinoculation and three weeks postinoculation) were examined by immune electron microscopy for antibody rises by the methods previously described (6, 7).

Results. The Norwalk agent was administered to seven volunteers, four of whom became ill. The mean incubation period was 22 hours, and illness lasted 24–48 hr. Three of the ill volunteers experienced vomiting, diarrhea, and low grade fever (p.o. temperatures 38.0–38.5°), and one ill volunteer experienced only vomiting.

The Hawaii agent was administered to six volunteers, three of whom became ill.

TABLE 1. SPECIMENS ASSAYED FOR INTERFERON IN VIRAL GASTROENTERITIS INDUCED BY NORWALK AND HAWAII AGENTS.

Agent	Volunteers	Clinical status	Serum[a] Number	Serum[a] Interferon titer (units/0.1 ml)[b]	Jejunal aspirates[a] Number	Jejunal aspirates[a] Interferon titer (units/0.1 ml)[b]	Jejunal biopsies[a] Number	Jejunal biopsies[a] Interferon titer (units/0.1 ml)[b]
Norwalk	7	Ill	4	<1	3	<1.3	3	<5
		Well	3	<1	2	<1.3	3	<5
Hawaii	6	Ill	3	<1	2	<1.3	3	<5
		Well	3	<1	2	<1.3	2	<5

[a] Samples obtained 24–48 hr after onset of illness or 72–96 hr after inoculation in well volunteers.

[b] Interferon titer defined as the reciprocal of the maximum dilution which will result in 50% inhibition of CPE by vesicular stomatitis virus in human foreskin fibroblasts (11).

The mean incubation period was 37 hr, and illness lasted 24–48 hr. One volunteer experienced vomiting and diarrhea, one experienced vomiting alone, and the third experienced diarrhea alone. All three had low grade fever (p.o. 37.8–38.4°).

Three of the four volunteers with Norwalk-induced illness, and all three volunteers with Hawaii-induced illness manifested serum antibody rises to the homologous particle by immune electron microscopy. Paired sera were examined from five of the six volunteers who remained well, and no serum antibody rises were noted.

Diarrheal stools from acutely ill individuals failed to reveal Salmonella, Shigella, enteropathogenic E. coli or protozoal agents.

All ill volunteers recovered spontaneously, completely, and were asymptomatic and afebrile by 48 hr after the onset of illness. The other six volunteers remained clinically well throughout the study.

Interferon assays were performed with undiluted serum, undiluted biopsy homogenates (these represented 20% suspensions or a 1:5 dilution), and undiluted jejunal aspirates (with 20% fetal calf serum added or a $\frac{1}{5}$ dilution). No interferon was detectable in serum, jejunal aspirates, or jejunal biopsy specimens taken 24–48 hr after the onset of illness or 72–96 hr after inoculation of the volunteers who remained well (see Table I).

Discussion. The inability to detect interferon in serum, jejunal aspirates, or jejunal biopsy specimens has several possible interpretations. First, interferon may have been present but was not detected because of: (1) insensitivity of assay, (2) inappropriately timed specimens, (3) sampling of uninfected areas, or (4) rapid degradation of interferon prior to assay.

The interferon assay which was employed detected the full unitage of the standard reference human interferon and was thus of sufficient sensitivity to detect interferon induced by other viral infections (12). The specimens above were obtained either at the height of illness or shortly after when maximum interferon levels are present in a number of other viral infections (12, 13). In addition, histologic examination of biopsy specimens from ill volunteers demonstrated pathologic changes previously described in infection with Norwalk and Hawaii agents (14, 15); so that aspirates and biopsies were performed in affected sites, although no virus particles have been detected in these or previously reported biopsy specimens. Circulating interferon has generally not been detected in viral infections which only involve superficial mucosal surfaces (16), and since histopathology in viral gastroenteritis is limited to the superficial mucosa without evidence for viremia or spread to deeper structures (14, 15), the presence of interferon in serum would not necessarily be anticipated.

The search for interferon in intestinal secretions has been hampered by the rapid inactivation of interferon by proteolytic enzymes present in the gut (17). The addition of 20% fetal calf serum was intended to inhibit this proteolysis, but it has been recently reported that human duodenal secretions inactivated 500 U of interferon/1.0 ml in the presence of 16% fetal calf serum or 0.67% soybean trypsin inhibitor (8). It has, therefore, been suggested that cell to

cell spread of interferon may occur, in sites protected from enzymatic degradation (8). However, assay of jejunal mucosal homogenates in this study failed to detect cell associated interferon.

A second possible interpretation of these findings is that interferon was not present because the intestine is a poor producer of interferon. Despite the fact that the intestine contains a variety of cellular elements, including lymphocytes and fibroblasts which can synthesize interferon, gastrointestinal interferon production in man has not been demonstrated. Human fetal intestine in organ culture did not synthesize interferon despite the application of a variety of inducers, including both synthetic polynucleotides and infectious viruses (8).

A third alternative explanation is that the viruses employed here, the Norwalk and Hawaii agents, may be poor inducers of interferon, themselves. These two antigenically distinct agents (9) appear to have properties most closely resembling the parvovirus group of animal viruses, though they do not appear to be antigenically related to known parvoviruses. It is of interest that two rodent parvoviruses, the Kilham rat virus and H-1 virus, appear to be poor inducers of interferon in vitro, though they are highly sensitive to its antiviral action (18). Since neither Norwalk nor Hawaii agents have been conclusively cultivated in vitro, their ability to induce interferon, as well as their sensitivity to interferon, awaits development of suitable in vitro assay and culture systems.

The above studies in which appropriately timed and collected specimens were examined with a sensitive assay failed to detect interferon production in viral gastroenteritis. The role of other host defense mechanisms in the rapid recovery from this illness is currently under investigation.

Summary. Seven of thirteen normal volunteers inoculated with either the Norwalk of Hawaii agent of viral gastroenteritis demonstrated clinical illness, with typical rapid onset, short duration, and rapid recovery. Examination of sera, jejunal aspirates, and jejunal biopsy specimens obtained 48–96 hr after inoculation, failed to reveal the presence of interferon in samples from either ill or well subjects.

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