ECHO Virus Type 25, Infection in Adult Volunteers. (29849)

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Although ECHO virus, type 25, has been recovered from children with a variety of illnesses(1-4), the relationship of this virus to disease in adults is still uncertain. Moreover, the frequency of neutralizing antibody in inmates of correctional institutions(5) and civilian populations(1,6) indicates a high incidence of infection with this serotype. In an attempt to gain additional data on the human response to this agent, adult volunteers were inoculated with a strain of ECHO virus, type 25, which was serologically related to the prototype strain. This report describes the results of these experiments.

Materials and methods. Clinical procedures. Participants in this study were male inmates of federal correctional institutions between the ages of 22 and 36 years. The men were admitted to the Clinical Center where history and physical examination, urine analysis, complete blood count, chemical tests of liver function, X-rays of chest and paranasal sinuses, electrocardiogram, and nose, throat and rectal cultures for bacteria were done prior to inoculation. The laboratory studies were repeated at weekly intervals after inoculation for 2 weeks.

The volunteers were housed and kept under isolation conditions as previously described (7). Oral temperature, pulse and respiratory rates were determined 4 times daily. At least once daily a physical examination was performed by a team of 2 physicians, and this, with a record of any complaints of illness, was recorded before leaving the room. The examining physicians were unaware of the type of inoculum or route of administration. No antibiotics or aspirin were given to the volunteers.

Inoculum. The virus strain used to prepare the inoculum was obtained from a rectal specimen of a child resident in Junior Village, Washington, D. C., during the outbreak from which the prototype strain was isolated (2). Fluid from a bottle culture of Macaca kidney cells maintained with Medium No. 199 which had been inoculated with the rectal specimen was prepared and safety-tested by the procedures previously described(8).

Volunteers were administered 2.5 ml of virus suspension: 0.5 ml of undiluted inoculum was instilled in each nostril by pipette; 0.5 ml of a 1:10 dilution of the inoculum was administered in each nostril and the oropharynx by a DeVilbiss No. 127 atomizer. Immediately following administration of the virus, a sample of the inoculum was titrated in rhesus monkey kidney cell cultures. After a 7-day incubation period in a horizontal position at 36.6°C, the titration endpoint which was determined by the method of Reed and Muench(9) was $10^{-5.0}$ $TCID_{50}$/ml.

Virus isolation and serologic procedures. Throat and rectal specimens were collected as shown in Table I. All specimens were stored at —20°C and thawed 6 months later for testing. Virus isolation attempts were done using rhesus monkey kidney cell cultures by a procedure previously described (10). When obtained, at least 3 isolates from each man were identified by neutralization tests against prototype immune serum by a procedure described elsewhere(11).

Neutralization tests were done with sera collected before and 28 days after inoculation; sera were tested against 100 $TCID_{50}$.

Results. The illness, virologic and serologic results for the 11 men who were given ECHO virus, type 25, are summarized in Table I. None of the volunteers with preinoculation neutralizing antibody became ill, whereas 4 of 8 men without neutralizing antibody developed illness.

Volunteer E.T., on the second day postinoculation, had a temperature elevation to 38.2°C accompanied by rhinitis and cough. The third day, his maximal temperature elevation was 37.7°C. Physical examination re-

TABLE I. Illness, Virologic and Serologic Responses of Volunteers Following Inoculation with ECHO Virus 25.

Pre-inoculation antibody status	Volunteer	Illness	Virus isolation* — Throat 1	Throat 2	Throat Total	Rectum 1	Rectum 2	Rectum Total	Neutralizing antibody titer
Antibody-negative	E.T.	+	2/7†	0/7	2/14	1/7	0/7	1/14	<4‡ 16
	D.D.	+	3/7	0/7	3/14	0/7	0/7	0/14	<4 64
	E.C.	+	0/7	0/6	0/13	1/7	2/4	2/11	<4 16
	J.A.	+	0/7	0/6	0/13	0/7	2/4	2/11	<4 32
	F.H.	0	4/7	1/6	5/16	6/7	1/4	7/11	<4 16
	C.G.	0	2/7	0/7	2/14	4/7	1/7	5/14	<4 64
	S.W.	0	0/7	1/7	1/14	3/7	0/7	3/14	<4 16
	J.H.	0	0/7	0/7	0/14	1/7	1/7	2/14	<4 32
Antibody-positive	R.C.	0	1/7	0/7	1/14	0/7	0/7	0/14	8 64
	R.A.	0	0/7	0/7	0/14	0/7	0/7	0/14	16 16
	A.W.	0	0/7	0/7	0/14	1/7	1/7	2/14	16 16

* Weeks post-inoculation.
†Numerator indicates No. of positive specimens and denominator indicates No. of specimens tested.
‡ Reciprocal of dilution; first line indicates titer of pre-inoculation serum and second line indicates titer of post-inoculation serum.

vealed an injected pharynx, tender anterior cervical nodes, and nasal erythema and discharge. Subjective complaints included sore throat, nasal discharge and productive cough. The following day, the patient was afebrile, wheezes were heard in his right chest and he complained of a productive cough. The next day he was asymptomatic.

Volunteer D.D., on the third day after inoculation, developed a febrile illness with a temperature of 37.6°C. Subjective complaints included headache, nasal discharge and obstruction, sore throat and cough. Physical examination revealed an injection of the left tympanic membrane, slight palpebral conjunctival injection bilaterally, nasal injection and discharge, tender anterior cervical nodes and tenderness over the trachea. The following day he again had a temperature elevation to 37.5°C and complained of increased malaise. He continued to have nasal and pharyngeal signs and symptoms accompanied by cough, until the ninth day after inoculation, although he was afebrile after the fourth day.

Volunteer E.C. complained of anorexia, chills, headache, nasal discharge and sore throat 24 hours after inoculation. At that time he had nasal obstruction and discharge, lymphoid hyperplasia of the tonsils and granularity on the soft palate. On the second day after inoculation the volunteer had a temperature elevation to 37.5°C. He complained of cough and nasal discharge, and on physical examination he had a boggy nasal mucosa and anterior cervical adenopathy. By the third day after inoculation his upper respiratory signs and symptoms had completely cleared.

Volunteer J.A. had an afebrile illness characterized by nasal symptoms and pharyngitis. On the day following inoculation, the patient complained of sore throat and on physical examination had inflammation of the posterior pharynx and tender left anterior cervical nodes. By the second day, the pa-

tient had developed marked inflammation and follicular hyperplasia of the tonsils with tender cervical nodes bilaterally. By day 3, the pharyngitis was accompanied by symptoms and physical findings of nasal obstruction. After day 4, there was absence of upper respiratory illness.

These illnesses were not accompanied by any significant change of the white blood cell count and differential except in volunteers E.T. and D.D. The admission white blood cell count of E.T. was 8,000 with 52% polymorphonucleocytes. The maximum elevation of his white blood cell count was on the second day after inoculation when the white blood cell count was 14,200 with 60% polymorphonucleocytes. The leucocyte of D.D. rose to 10,200 with 79% polymorphonucleocytes the third day after inoculation. There was no significant change in the bacterial flora of the nose and throat. X-rays of the chest and sinuses also remained normal.

Virus was isolated from either the oropharynx or rectum of all volunteers except one antibody-positive man. Isolations were infrequent after day 10 and in those instances where virus was recovered from the throat, it usually preceded isolation from the rectum. Additional rectal specimens from 3 to 6 weeks after inoculation obtained from most volunteers were virus-negative (not shown).

With the exception of 2 antibody-positive volunteers, the other volunteers developed 4-fold or greater rises in neutralizing antibody.

Discussion. The illnesses which developed in one-half of the antibody-negative volunteers experimentally infected with ECHO virus, type 25, suggests that this agent may play a role in respiratory disease in adults. Despite the presence of diffuse involvement of the upper respiratory tract and evidence of mild tracheobronchitis in 2 men the illnesses were characterized by the predominance of pharyngitis and cervical adenitis. Three of the illnesses were associated with low-grade fever. The absence of disease in the other antibody-negative individuals despite evidences of viral infection suggests that this agent may produce apparent illness irregularly in the population. These findings reported here, of course, do not exclude the possibility that ECHO virus, type 25, may be responsible for other clinical syndromes which may be more severe than that which occurred in these volunteers. In this connection, the etiological role of this serotype in an outbreak of aseptic meningitis which occurred mostly in children in Ontario, Canada, has been recently reported(4).

Although the role of neutralizing antibody was not fully defined in these experiments, a suggestion of its effectiveness was indicated by the failure to cause illness in the antibody-positive volunteers following type 25 inoculation.

The isolation rate of virus in antibody-negative volunteers was low but was in agreement with a study reported for naturally-occurring infection in children(2).

Summary. Eight antibody-negative and 3 antibody-positive adult volunteers were inoculated with ECHO virus, type 25. Infection of antibody-negative individuals resulted in respiratory illness in 4 volunteers. The illnesses which were associated with low-grade fever in 3 men were characterized by pharyngitis and cervical adenitis. Inoculation of antibody-positive individuals failed to cause illness but was associated with limited virus shedding in 2 of 3 subjects.

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Messenger RNA for Interferon Production. (29850)

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Heller(1) has reported that Actinomycin D inhibits the induction of interferon production in mammalian cells even when the inducing virus used was an RNA virus whose growth would not be inhibited by actinomycin. These results have been confirmed in our laboratories(2) and elsewhere(3). These observations imply that host cell DNA contains the information for interferon production. In uninfected cells, this DNA function is repressed, but some event in virus function acts as a derepressor. The expression of this DNA as template for messenger RNA for interferon is blocked by Actinomycin D. This report is concerned with the time during which the messenger RNA for interferon is synthesized.

Experimental. The virus used, the relevant methods for interferon production and assay, and the procedures with radioactive metabolites in chick embryo tissue cultures (CE) have been described(4).

Experiments on the time course of production of interferon showed that monolayers of CE cells infected with Chickungunya virus at a multiplicity of 10 produced maximum titres of interferon at about 5 hours after infection, with the first detectable interferon being found after 2 hours. To determine whether host DNA was needed for interferon production throughout the course of infection the following type of experiment was done: Monolayers of CE cells in BME without serum were exposed to Chickungunya virus at a multiplicity of 10. At the indicated times after virus addition, actinomycin, at a concentration of 0.2 μg/ml was added. The cultures were allowed to incubate for a total of 6 hours after virus addition, and the interferon titres of the fluids determined. Controls received no actinomycin. Fig. 1 shows the results of a typical experiment of this type.

It will be seen that if actinomycin is added at any time up to 1½ hours after virus, there is no production of interferon even 5 hours later. After approximately 1½ hours of infection, the production of interferon is no longer subject to control by actinomycin, indicating that practically all the messenger RNA for interferon is made by this time.

Since a delay between time of addition of actinomycin and onset of inhibition of RNA synthesis in these experiments would necessitate a correction to the data of Fig. 1, the following experiment was performed: Actinomycin, at a concentration of 0.2 μg/ml was added to CE cells at various times, from 60 minutes before to 5 minutes after addition of H^3-uridine for 15 minutes, and the amount of incorporation into RNA determined. When actinomycin was added 60 minutes before the H^3-uridine there was an 85% inhibition in RNA synthesis (30% inhibition of protein synthesis). Even when actinomycin was added 5 minutes after the beginning of the H^3-uridine pulse, there was still a 40% inhibition. Therefore no large time correction would appear necessary for the data of Fig. 1.

Discussion. Actinomycin inhibits the synthesis of RNA(5), and also prevents migration from the nucleus to the cytoplasm of already formed RNA(6,7,8). Since by 1½ hours after infection actinomycin no longer affects the ultimate production of interferon, one can say that the messenger RNA for interferon has already been made at that time, and if interferon is made in the cyto-