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Research Article

FURTHER EXPERIMENTAL ATTEMPTS TO TRANSMIT INFECTIOUS MONONUCLEOSIS TO MAN [1]

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The occurrence of definite but transient evidence suggestive of the experimental transmission of infectious mononucleosis to man obtained in prior experiments in this laboratory (1) as well as the reports of encouraging results in two instances abroad (2, 3) have prompted us to further attempts in this direction. Two of the possible factors bearing on the many failures with the experimental induction of this disease are: *1*) the partial or complete destruction of the hypothetical causative agent by freezing or other manipulations of the inocula; *2*) low individual susceptibility in the volunteers employed. In the work to be reported in this paper efforts were made to meet the first of these objections by better methods of preservation of the inocula and by the use of pools of material collected from several patients early in the course of illness. The second theoretical objection has been difficult to overcome but it was hoped that by the employment of subjects as young as possible and by the careful exclusion of all volunteers with histories suggestive of a prior illness with infectious mononucleosis some susceptible subjects might be encountered. The purpose of this paper is to present the results of these investigations.

MATERIALS AND METHODS

Donors: College students ill with infectious mononucleosis in the New Haven Hospital, Yale University Infirmary,[3] or the Mason Infirmary at Smith College [3] were used as donors.

The data concerning the laboratory aspects of these patients and the day after the onset of symptoms on which the inocula were obtained are shown in Table I. All donors with one exception had unequivocal evidence of infectious mononucleosis including an elevated heterophile antibody agglutination titer. This exception was a volunteer, designated in Table I as donor No. 6, who developed clinical signs suggestive of infectious mononucleosis at the time the inoculum was obtained, but whose subsequent course did not definitely support this diagnosis.

Subjects: The human beings used in these experiments were healthy male or female subjects of college age or younger. A careful history was taken and a physical examination with special regard to the lymphatic system was carried out on each subject prior to inoculation. Those with histories or records suggestive of prior illness with infectious mononucleosis were not used. During the course of the experiment all inoculated subjects were carefully examined at least once a week. Temperatures were taken as symptoms appeared in the first six ex-

TABLE I

Donors used for transmission experiments

Donor No.	Day inoc. obtained	Donors' signs of infectious mononucleosis		
		Day of disease	Per cent lymphs	Sheep Rbc titer
1	3	3	—	80
		9	70	640
2	5	5	35	80
		9	—	1280
3	4	4	39	40
		8	55	
		10		320
4	2	2	42	160
5	3	3	69	640
6*	29†	36	40	20
7	5	7	82	
		10		640
8	5	5	83	2560
9	6	6	53	320
		9	42	Pos.
10	4	4	58	320
11	5	4	69	40
		14	86	320
12	5	4	49	320
		11	50	320

* This was a volunteer who developed fever and adenopathy on the 27th day after inoculation, but who later showed no other evidence of infectious mononucleosis.
† Day after inoculation.

[1] Representing work done in part for the Virus and Rickettsial Disease Commission, Army Epidemiological Board, Office of The Surgeon General, U. S. Army, Washington, D. C.

[2] National Institutes of Health Postdoctorate Research Fellow, 1948–49.

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periments and daily in the others. No isolation precautions were carried out unless symptoms developed.

Laboratory work: Leucocyte counts done in duplicate, and differential counts enumerating 200 cells on smears stained with Giemsa were carried out at least three times prior to inoculation and at intervals of approximately three days thereafter. A heterophile antibody agglutination titer (4) and a Newcastle disease virus (NDV)-modified human red cell agglutination titer (5) employing the Australian strain of virus were determined prior to inoculation and at weekly intervals during the experiment. All subjects were followed for at least a month after inoculation.

Inocula were obtained from the donors listed in Table I. Sera were separated as soon as possible after obtaining and frozen at −70° C. Throat washings, obtained by repeated gargling with normal saline, were mixed with equal parts of nutrient broth containing 10 per cent normal rabbit serum, and frozen in lusteroid tubes or glass ampules at the bedside of the patient employing methyl alcohol and "dry" ice. The lusteroid tubes were sealed with a metal cap and the ampules by melting the glass tip. They were stored at −70° C. None of these materials were filtered. A penicillin-streptomycin mixture was added to some of the throat washings 30 minutes prior to inoculation (giving a final concentration of 1000 units of penicillin and 5 mgm. of streptomycin per ml.). In a few instances fresh, unfrozen throat washings were employed. The stool preparations were inoculated as a 50 per cent suspension in buffer (0.004 M citric acid-disodium phosphate, pH 7.2). Ultracentrifuged preparations of these stools had previously been inoculated intracerebrally into rhesus monkeys without producing evidence of poliomyelitis. In addition, the presence of pathogenic bacteria was excluded by suitable cultures.

Method of inoculation: Sera were inoculated intranasally and orally, or by parenteral injection intra- and subcutaneously. Throat washings were sprayed into the throat and nose with a hand atomizer in addition to which part of the inoculum was dropped directly into the nose with a pipette, and part was gargled repeatedly, then swallowed. The stool preparations were introduced into the stomach with a gastric tube after mixing with equal parts of skim milk.

RESULTS

Sixteen human subjects were inoculated, 10 with throat washings, four with serum, and two with stool preparations. The results are shown in Table II. It should be noted at this time that strict criteria of successful transmission would include the following features: *1*) symptoms compatible with the usual course of illness, *2*) objective physical signs, especially lymphadenopathy, *3*) a rise in lymphocyte count, *4*) the occurrence of atypical lymphocytes, and *5*) a rise in heterophile antibody titer. On this basis no subject developed

TABLE II

Results of attempts to transmit infectious mononucleosis to man, 1948–49

Subject No.	Donor(s)	Inoculum*					Changes in volunteers					Serology‡	Result
							Clinical signs†			Lymphocytes			
		Type	Frozen	P-S	Am't in ml.	Route	Fever	S.T.	Aden.	Per cent rise	Atypical forms		
1	1,2,3,4	T.W.	Yes	Yes	20.0	i.n., oral	0	0	0	16	+	0	Neg.
2	1,2,3,4	T.W.	Yes	Yes	20.0	i.n., oral	0	0	0	22	+	0	Neg.
3	1,2,3,4	T.W.	Yes	Yes	20.0	i.n., oral	0	0	0	0	0	0	Neg.
4	1,2,3,4	T.W.	Yes	Yes	20.0	i.n., oral	0	0	0	0	0	0	Neg.
5	1,2,3,4	Sera	Yes	No	5.0	i.n., oral	0	0	±	14	+	0	Neg.
6	1,2,3,4	Sera	Yes	No	5.0	i.n., oral	+	+	+	0	+	0	Uncertain
7	6	T.W.	No	Yes	10.0	i.n., oral	0	0	0	0	0	0	Neg.
8	6	T.W.	No	Yes	10.0	i.n., oral	0	+	0	22	+	0	Uncertain
9	5	T.W.	No	Yes	10.0	i.n., oral	±	+	+	0	+	0	Uncertain
10	5	T.W.	No	Yes	10.0	i.n., oral	0	0	0	0	0	0	Neg.
11	1,2,5,7,8,9	T.W.	Yes	No	10.0	i.n., oral	0	0	0	0	0	0	Neg.
12	1,2,5,7,8,9	T.W.	Yes	No	10.0	i.n., oral	0	0	0	0	0	0	Neg.
13	4,5,7,8,9,10	Sera	Yes	No	2.4	i.q., s.q.	0	0	0	20	0	0	Neg.
14	4,5,7,8,9,10	Sera	Yes	No	2.4	i.q., s.q.	§	§	§	§	§	0	Neg.
15	11,12	Stool	Yes	No	5.0	gastric	0	0	0	25	0	0	Neg.
16	11,12	Stool	Yes	No	5.0	gastric	0	0	0	0	0	0	Neg.

* T.W. = Unfiltered throat washings.
Frozen = Stored in frozen state until just before inoculation; others were used fresh.
P-S = Addition of penicillin and streptomycin.
i.n. = intranasal; i.q. = intracutaneous; s.q. = subcutaneous; gastric = intragastric by tube.
† S.T. = Sore throat; Aden. = cervical and/or axillary adenopathy.
‡ Serology = Heterophile antibody and NDV modified Rbc titers.
§ Varicella 20th day after inoculation.

unequivocal evidence of infectious mononucleosis, but a few showed three or more of these features, especially clinical and hematological changes, and the result has been termed uncertain in these instances. The details of these changes are as follows:

Subjects 1, 2, 3, and 4 were inoculated with pooled throat washings from Donors 1, 2, 3, and 4. Two developed an increase in lymphocytes with the appearance of a few atypical cells but without other evidence of infection, and two showed no changes.

Subjects 5 and 6 were given pooled sera orally and intranasally, also from Donors 1, 2, 3, and 4. Subject 5 remained asymptomatic following inoculation. On the 21st day enlarged left axillary lymph nodes were found but there were no other changes. Blood smears showed up to 5 per cent atypical lymphocytes and the total lymphocyte count increased 14 per cent over the preinoculation baseline. Serological tests were consistently negative. Subject 6 noted the onset of moderately severe, intermittent, frontal headache, malaise, chilliness, and tenderness of the glands on the right side of the neck, one week after inoculation. These symptoms persisted in mild form for a few days without objective physical findings. On the 27th day after inoculation the subject complained of a severe, pounding headache, pain on motion of the eyeballs, and feverishness. Temperature was found to be 101.0° F. Two days later physical examination disclosed tender and enlarged right cervical and bilateral axillary nodes and a moderately injected throat. Liver and spleen were not palpable but there was fist percussion tenderness over the liver. Glandular enlargement and low grade fever persisted for about a week. There were no other clinical manifestations. There were no appreciable changes in lymphocyte counts, heterophile antibody titers, or NDV modified red cell agglutination titers over a period of six weeks following inoculation but up to 6 per cent atypical lymphocytes were observed. Fresh, unfiltered throat washings obtained from this subject on the 29th day were passed immediately to Subjects 7 and 8 by oral and intranasal routes.

Subject 7 remained asymptomatic and showed no alteration in laboratory tests over a month's period of observation. Subject 8 experienced transient symptoms of feverishness, headache, and

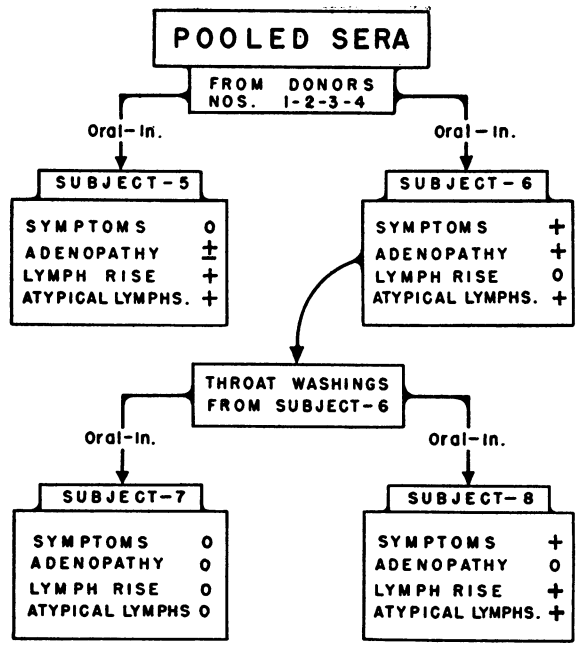

FIG. 1. DIAGRAMMATIC REPRESENTATION OF EXPERIMENTS SUGGESTIVE OF SERIAL EXPERIMENTAL TRANSMISSION OF INFECTIOUS MONONUCLEOSIS FROM MAN TO MAN
In = intranasal

anorexia on the seventh day following inoculation but there were no objective signs at this time. On the 28th day he complained of frontal headache, sore throat, burning eyes, malaise, and discomfort on swallowing. Physical examination revealed a somewhat injected throat and tender, but not enlarged, cervical lymph nodes. Subsequent course was uneventful. His lymphocyte count rose 22 per cent over preinoculation level and a few atypical lymphocytes were observed. There was no change in serological tests. The results obtained in Subjects 5 through 8 are presented graphically in Figure 1 as they represent the first instance in which evidence suggestive of serial experimental transmission from man to man of an illness exhibiting some of the features of infectious mononucleosis has been observed.

Subjects 9 and 10 were given fresh, unfiltered throat washings orally and intranasally which had been obtained on the third day of illness from Donor 5. On the 11th day after inoculation Subject 9 was found to have a flushed face, injected throat with a small amount of exudate, and tender, moderately enlarged cervical nodes bilaterally as well as tender but questionably enlarged axillary

nodes. There was no fever. Symptoms at this time included sore throat, mild chills, headache behind the eyes with discomfort on eyeball movements, and malaise. These symptoms and signs persisted without appreciable fever for a week at which time the tip of the spleen was felt 1 cm. below the costal margin. There was no significant rise in lymphocyte count although a few atypical forms were seen, and serological tests remained unchanged. Subject 10 remained asymptomatic and there were no changes in laboratory tests.

Subjects 11 and 12 were given pooled throat washings orally and intranasally from Donors 1, 2, 5, 7, 8, and 9. No evidence of illness developed.

Subjects 13 and 14 were inoculated intra- and subcutaneously with pooled sera from Donors 4, 5, 7, 8, 9, and 10. Subject 13 showed only a 20 per cent rise in lymphocytes consisting of normal forms. Subject 14 developed typical varicella on the 20th day after inoculation. Whether this illness was transmitted by the sera employed is not known. None of the donors developed varicella and the possibility of contact infection in the subject could not be excluded.

Subjects 15 and 16 inoculated by stomach tube with pooled stool suspension from donors number 11 and 12 remained well during the course of the experiment and the only alteration in laboratory studies was a 25 per cent rise in lymphocyte count in Subject 15 without the occurrence of atypical lymphocytes.

TABLE III

Summary of 41 attempts to transmit infectious mononucleosis to man carried out in this laboratory, 1946–1949

Year	Investigator	Inoculum	No. of subjects	Results	
				Neg.	Uncertain
1946	Havens	Serum	4	2	2
1947	Evans	Serum	8	7	1*
		Whole blood	6	6	0
		Throat washings	7	6	1*
1948–9	Evans	Serum	4	3	1
		Throat washings	10	8	2
		Stool	2	2	0

* In a previous report (1) these results were arbitrarily classified as negative in terms of unequivocal transmission of the disease.

DISCUSSION

The results of 16 attempts to transmit infectious mononucleosis to man presented in this paper combined with the 25 attempts previously reported from this laboratory (1), which include four unpublished experiments of Havens (6), are shown in Table III. Of these 41 experiments, a diagnosis of infectious mononucleosis was suggested by clinical and/or hematological changes in seven volunteers but there was insufficient evidence to term the result of transmission unequivocally successful. Of 40 experiments carried out in other laboratories, which have been summarized elsewhere (1), suggestive results have been obtained in two instances (2, 3). Yet neither of these two fully satisfies the strict diagnostic criteria outlined earlier: in one there was a typical clinical and hematological course as well as a compatible pathological picture in a biopsied lymph node but a negligible rise in heterophile antibody titer (2), and in the other a rise in heterophile antibody titer was accompanied by no clinical and transient hematological changes (3). It should be noted that other workers, and in some instances even the same investigators, using similar inocula have been unable to repeat these results. The difficulty encountered in the successful transmission of such a common disease is not easily explained. There is, however, evidence to suggest that the contagiousness of even the naturally occurring disease may be low. For instance, infectious mononucleosis does not appear to spread readily in the close confines of a college dormitory as shown by the lack of occurrence of either clinical or subclinical illness (7), and cross infections on open hospital wards have been notably absent (8, 9). Furthermore, it is very uncommon for infectious mononucleosis to involve roommates even though there may be several days of intimate exposure before the patient is hospitalized (7, 8, 10, 11). In light of this evidence, it is perhaps not so surprising that transmission of this disease under experimental conditions has not yet been established unequivocally.

SUMMARY

1. In an attempt to transmit infectious mononucleosis to human beings, 16 subjects of college age or younger were inoculated with serum, throat

washings, or stool preparations from patients with the disease.

2. Unequivocal evidence of successful transmission was not obtained but a few subjects showed suggestive clinical or hematological signs of the disease.

3. It is suggested that infectious mononucleosis may have a low contagiousness under either natural or experimental circumstances.

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