

IMMUNITY IN EXPERIMENTALLY INDUCED INFECTIOUS HEPATITIS*

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In previous communications from this laboratory (1-3) and others (4) a distinction has been made between *infectious hepatitis* and *homologous serum jaundice*. Certain similarities and differences between these two conditions have been defined on the basis of clinical and epidemiologic data as well as from experiments in the transmission of virus to human volunteers (1-4). The exact relationship between these two conditions is not clearly understood. It is not yet known whether the apparent differences noted between them are representative of actually different viruses or of antigenic differences of various strains of a single virus.

The immunologic relationships between homologous serum jaundice and infectious hepatitis are also not clear. Both conditions probably belong to the same general group but the evidence for cross-immunity between them is contradictory. Oliphant (5) showed apparent protection in human volunteers convalescent from serum jaundice when reinoculated with a strain of infectious hepatitis. In contrast are reports from this laboratory (6) and by Neefe, Stokes, and Gellis (4) stating that patients convalescent from experimentally produced homologous serum jaundice are susceptible when reinoculated with two different strains of infectious hepatitis. Moreover, it has been observed that troops, convalescent from homologous serum jaundice induced by the inoculation of human blood products, contracted infectious hepatitis while occupying a zone where this disease was endemic (7, 8). Conversely, it has been reported that patients who have had infectious hepatitis are susceptible to homologous serum jaundice when inoculated with human blood products accidentally contaminated with the causative agent of this condition (9, 10).

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Homologous immunity has been shown to be present in both conditions. Planned experiments on human volunteers have demonstrated that subjects convalescent from both serum jaundice (4, 5) and infectious hepatitis (4) are apparently immune when reinoculated several months later with an homologous strain. Neefe, Stokes, and Gellis (4) have recently summarized the epidemiological data which suggest that one attack of infectious hepatitis is followed by some degree of immunity. Moreover, the demonstration of the protective effect of normal human gamma globulin when administered during the short incubation period of epidemic infectious hepatitis also suggests the presence of certain neutralizing substances in the blood of the normal adult population, possibly as a result of a previous clinical or subclinical attack of the disease (11-13). Attempts to demonstrate a similar protective effect of normal human gamma globulin when administered during the long incubation period of homologous serum jaundice have shown conflicting results (14, 15). In one study a significant prophylactic effect was observed when two injections of 10 cc. of gamma globulin were given a month apart, while in two other studies no benefit was found when a single injection of 10 cc. was given. The reasons for this discrepancy are not clear but it is possible that size of dose of gamma globulin, the long period of viremia in homologous serum jaundice, and the fact that viremia may be already established prior to the administration of gamma globulin may be important factors.

During the past two years in the course of experiments in the transmission of infectious hepatitis to human volunteers conducted by the U. S. Army Neurotropic Virus Commission, it has been possible to test the immunity of some of the convalescent subjects. It is the purpose of this paper to describe this work and to report the presence of homologous immunity in 9 human volunteers convalescent from infectious hepatitis, when reinoculated with the same strain of virus after a lapse of some months.

Materials and Methods

Subjects.—21 human volunteers, ranging in age from 19 to 29 years were employed. 9 of these men were convalescent 6 to 9 months from infectious hepatitis experimentally induced in this laboratory by the oral or parenteral administration of infectious feces or serum. Their incubation periods had ranged from 16 to 30 days and all 9 had been jaundiced, with courses of disease ranging from mild to very severe. The remaining 12 men had no previous history of infectious hepatitis and served as controls. Prior to the onset of disease these volunteers were housed in institutions in Middletown, Norwich, and New Haven, Connecticut. Each man was seen at least once a week and the period of observation extended for 3 to 4 months. Volunteers who became sick were hospitalized in the Isolation Pavilion of the New Haven Hospital.

Laboratory Observations.—The following tests of liver function were performed at weekly intervals throughout the period of observation: (1) quantitative serum bilirubin;¹ (2) brom-

¹ According to the method of Malloy and Evelyn (16) using the photoelectric colorimeter.

sulfalein dye retention test² (17); (3) cephalin-cholesterol flocculation (Hanger) (18); (4) thymol turbidity test (19); (5) determination of bilirubin (20) and urobilinogen (21) in the urine.

Virus.—The strain of infectious hepatitis virus used in this laboratory was originally obtained from the stool of a U. S. Army soldier (BE) who contracted *epidemic infectious hepatitis* in Sicily in 1943 (22). It has been through four passages in human volunteers to date. This agent is filtrable through an L2 Chamberland filter and withstands heating to 56°C. for 30 minutes (23). It has produced the disease in 27 out of 40 human volunteers (including those reported here) following parenteral or oral inoculation with incubation periods ranging from 15 to 34 days.

The inoculum employed in this experiment was serum representing the 2nd, 3rd, and 4th human passage of virus. The serum was obtained during the first 5 days of infectious hepatitis experimentally induced in human volunteers by feeding or parenteral inoculation of our strain of virus. This serum had been stored at dry-ice box temperature for periods ranging from 5 to 14 months. Before using, it was thawed at room temperature and heated to 56°C.

TABLE I

Results of Administration of Ictrogenic Serum (Infectious Hepatitis) to Normal Human Volunteers and to Human Volunteers Convalescent from Experimentally Induced Infectious Hepatitis

Subjects	Route	No. of volunteers		Incubation period
		Inoculated	Hepatitis	
(A) Convalescent 6-9 mos.....	Oral	5	0	—
(B) Normal controls.....	Oral	3	3	24-26
(C) Convalescent 6 mos.....	Par.*	4	0	—
(D) Normal controls.....	Par.	9	5	21-30

* Par. = parenteral

for 30 minutes to destroy bacteria. Serum in amount of 0.5 cc. was administered to the 9 convalescents by the same route (parenterally or orally) by which they had received the inoculum which produced their initial disease. The healthy controls were inoculated with the same serum in amounts ranging from 0.01 to 0.5 cc. A period of 1 to 3 months elapsed between the inoculation of the various groups of controls and convalescents. During this time the infectious serum was kept frozen at dry-ice box temperature.

RESULTS

All 9 men convalescent from infectious hepatitis resisted reinoculation with infectious material which induced infectious hepatitis in 8 out of 12 previously healthy control human volunteers (Table I). The convalescent subjects remained asymptomatic throughout the period of observation and their various tests of liver function were all within normal limits with the exception of 4 men who developed positive cephalin-cholesterol flocculation for several weeks during the period of observation. It is difficult to interpret the significance of

²10 per cent retention in the blood 30 minutes after the intravenous injection of 5 mg. bromsulfalein/kg. of body weight was considered the maximum normal level.

these positive cephalin-cholesterol flocculations. They may represent evidence of minimal disturbance of hepatocellular function. Similar border line disturbance of function was reported by Neefe, Stokes, and Gellis (4) in testing for immunity in patients convalescent from homologous serum jaundice.

SUMMARY AND CONCLUSIONS

1. 9 human volunteers, convalescent from infectious hepatitis experimentally induced by a strain of virus employed in this laboratory, were resistant when reinoculated with the same strain of virus, 6 to 9 months later.
2. 8 out of 12 previously healthy control subjects developed infectious hepatitis with jaundice following administration of this same material.
3. The demonstration of homologous immunity produced by this strain of virus is in agreement with the demonstration by others of homologous immunity produced by another strain of infectious hepatitis virus.

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