

History of U.S. Military Contributions to the Study of Viral Hepatitis

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Epidemic jaundice, although known by armies since ancient times, became a concern of the U.S. military only after outbreaks occurred during World War II. Early work by military investigators defined, for the first time, the existence of two different forms of hepatitis. Subsequently, investigators described the effective prevention of symptomatic hepatitis using immune serum globulin. Military researchers contributed to the isolation of and testing for the virus of infectious hepatitis, work that was then instrumental in the designing and fielding of a hepatitis A vaccine. Hepatitis B contributions included the elaboration of community-based epidemiology and description of the efficacy of immune serum globulin prophylaxis. Most recently, studies on hepatitis E defined the epidemiology, performed genomic sequencing, and developed a DNA vaccine currently being tested against the disease. Major research contributions to the understanding of and protection against viral hepatitis have been made by the military medical establishment over the past 60 years.

Hepatitis

U.S. Military Significance

Early Experiences

Epidemic jaundice has long been known to plague soldiers during times of war. It was a problem among Napoleon's troops during the Russian and Egyptian campaigns, and well-characterized outbreaks of jaundice were described during the U.S. Civil War, with approximately 70,000 falling ill to the disease.¹⁻³ The disease was common enough within troop populations to be recognized as "camp jaundice" (*jaunisse des camps*) and was known to have infected troops on both sides of the battles fought in the Mediterranean theater in World War I.³ Because U.S. troops were late entries into that war, however, and fought predominantly in France, the disease had little impact among our doughboys, and it received little or no attention by the U.S. military medical establishment at the time. This was to change in World War II.

Jaundice Following Yellow Fever Vaccination

Two events were to awaken the concern of the U.S. military medical establishment near the beginning of our involvement in World War II. The first was a striking epidemic of jaundice that occurred in the summer of 1942 among soldiers vaccinated with the yellow fever vaccine.⁴ Fifty thousand cases of jaundice were seen; probably 300,000 in total were infected, and 62 patients died. Not knowing the origin, investigators suspected that a viral agent had been transmitted from the combined lots of human serum used to manufacture the vaccine. The epidemic stopped

when those lots were destroyed, and a human serum-free process was substituted in the manufacture of the vaccine. "Look-back" investigations in later years confirmed that the responsible agent was hepatitis B virus.⁵

Epidemics in the Mediterranean Theater of Operations

The invasion of North Africa by Allied troops was soon followed by the appearance of jaundice among soldiers from all countries, including (as later realized) German troops in the opposite lines. A particularly interesting report published by Kirk, an Australian military physician, described an outbreak among his country's troops on the forward line at El Alamein.⁶ Of 7,500 men in two brigades that were moved up into the "box" on the line opposite the Germans, 1,059 developed jaundice within 30 to 45 days after arrival. Only 78 men of 3,900 belonging to a third brigade that remained in the rear became jaundiced. Ambulance drivers interacting with the front lines were not significantly affected. Kirk concluded that it was the occupation of recaptured land, amid numerous dead soldiers and accumulated feces, that was responsible for the outbreak of jaundice.

In 1943, following the invasion of Sicily, U.S. forces were faced with the second event that gained the attention of the medical establishment, i.e., their own notable outbreak of infectious jaundice. Among troops in Sicily and throughout the subsequent invasion of the Italian mainland, rates of illness reached 37 cases per 1,000 soldiers. More than 16,000 cases were recognized; each patient was hospitalized for an average of 6 weeks.³ Although mortality rates for jaundice were exceeded by those for malaria during this campaign, the former disease caused greater morbidity and more days of fighting strength lost than did any other illness in the Mediterranean theater.³ In fact, a total of 22,000 cases were reported before June 1944, and it is estimated that the drain in total fighting strength and training time delayed the D-Day invasion by at least 1 month.³

Hepatitis in Germany

Before the Allied drive through Germany was finished in early 1945, a total of 200,000 cases of hepatitis had been reported among U.S. troops worldwide, most occurring in Europe. Rates of this disease among U.S. troops continued to be alarming in Germany after the war, an observation consistent with the high endemic, and at times epidemic, rates of infectious jaundice that had been recognized in the German population for decades. Germany, like Italy, was proving to be fertile ground for the transmission of jaundice to occupation forces.

These high rates of jaundice allowed the first formal studies of the epidemiology of this disease by U.S. investigators. Older troops were less susceptible, as determined by age-specific rates of attack. Officers in many cases had higher rates of attack than did enlisted men; in retrospect, this phenomenon might have

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been attributable to the handling of officers' food by multiple, potentially infected cooks and stewards before service to the officers, compared with enlisted men who handled their own food.⁷ When the numbers of cases continued to increase during the occupation years,⁸ a hepatitis center was established at the 120th Station Hospital in Bayreuth, Bavaria. From this institution came early descriptive studies of the epidemiology of the disease and also an early investigation that suggested that exercise during convalescence only rarely caused worsening or prolongation of illness.⁹

U.S. Military Contributions

Context of Studies

Because of the sustained difficulties with this disease in occupied Germany after the war, the U.S. Army initiated investigations into the cause, epidemiology, and prophylaxis of this disease, as well as possible sanitation measures against it.^{7,8,10-14} It may be helpful to note that the following elements were not known by medicine at the time: whether the causative agent was a virus and, if so, how many viruses were responsible; the source, reservoir, and means of transmission of the agent; and patterns of immunity to the agent, with an eventual view toward prophylaxis or vaccination. It was suspected that, if a viral agent was responsible, it might be possible to use immune serum globulin in the prevention of clinical disease, an approach that had already been found to be useful against measles.¹⁵ The investigative tools available at the time were limited. There was no animal model of hepatitis known, and all studies had to be performed with human volunteer subjects who would be exposed to a disease that required weeks to months before clinical features were seen. The concept of subclinical hepatitis (elevation of transaminase levels only) was not yet a reality, and the measures of hepatic inflammation were limited to the rather insensitive assessments of total bilirubin levels and the brom-sulfalein retention test.¹⁶

As an interesting aside, Surgeon General-supported investigators at the University of Pennsylvania at this time studied the effects of various contemporary water purification technologies on the infectivity of fecally contaminated water, using the development of clinical jaundice among volunteers as the outcome measure.¹⁷ Those studies demonstrated that coagulation, filtration, and exposure to at least 1.1 ppm of total chlorine (0.4 ppm of free residual chlorine) were necessary and sufficient to totally prevent the transmission of disease.

Early Transmission Studies

In 1944, Havens et al.,¹⁸ working at Yale University with the support of the U.S. Army Surgeon General's Office, inoculated various materials obtained from soldiers with hepatitis in Italy and Sicily into human volunteers. Jaundice was induced in three of five volunteers through intracutaneous inoculation of frozen filtered serum obtained from jaundiced soldiers; the incubation period averaged 64 days. Jaundice was also induced in five of nine volunteers through feeding of frozen, filtered stool extracts and urine ($n = 6$) and serum ($n = 3$) obtained from jaundiced soldiers; the incubation period averaged 37 days, being shorter among volunteers fed excreta rather than serum. Although these body materials were therefore shown to be suf-

ficient for transmission by the indicated routes, the numbers were small and the efficiency of transmission by the different routes was undetermined. It was also still not known that there was more than one type of viral agent.

Further investigation using serum and stools obtained from volunteers at various intervals during their incubation periods following viral exposure demonstrated that these materials could transmit infection if collected at the onset of acute illness and through the 4th and 5th days of illness but not at the 25th or 26th day of illness.¹⁶ Urine and nasal washings were not infectious. Havens^{19,20} also demonstrated that there was no cross-immunity between short-incubation (or infectious) jaundice and long-incubation (or homologous serum) jaundice in challenge experiments, demonstrating for the first time that these two illnesses were immunologically and therefore pathogenetically distinct. A simultaneous line of investigation by Neefe et al.²¹ at the University of Pennsylvania, also supported by the Office of the Surgeon General, showed that the agents of infectious and homologous serum jaundice did, however, induce immunity to rechallenge with the identical strains. This investigation also carefully delineated the efficiency of parenteral transmission, the prolonged incubation, and the inefficiency of oral transmission of serum hepatitis, and the efficiency of oral transmission and short incubation of infectious hepatitis. These experimental data supported epidemiological observations made by Gauld²² during the Mediterranean troop epidemic, which suggested that infectious hepatitis and serum hepatitis were transmitted separately and immunity to each developed independent of the other.

Immunoprophylaxis against Infectious Hepatitis

It had previously been recognized that pooled plasma from immune individuals could protect against the development of measles after nonimmune individuals were exposed.¹⁵ In an experiment of opportunity, Stokes and Neefe²³ showed marked efficacy of pooled γ -globulin in the prevention of hepatitis in the setting of an outbreak at a summer camp for children in the summer of 1944. Subsequently, Gellis et al.²⁴ showed protective efficacy of γ -globulin when it was administered to the trial arms of two military organizations suffering epidemics of hepatitis in Europe (a bombardment group and several regiments of ground forces). Jaundice continued to be seen in the control arms of each of the two groups. Later, Stokes' group presented additional data generated from observations made at three separate civilian institutional facilities of different types, linked by a common high endemic rate of infectious hepatitis.²⁵ The use of γ -globulin at each institution allowed these workers to estimate that the duration of protection afforded by the injection of 0.06 mL/pound of body weight was approximately 5 months. They were able to epidemiologically postulate that both passive and active immunity occurred, as indicated by the lack of hepatitis at all for 5 months, followed by resumption of a lower than expected rate of hepatitis (rather than a rebound higher rate, if active immunity from interim exposure had not developed).

Willowbrook Experiments

Between the 1950s and the 1970s, a series of investigations to characterize the epidemiology of and immunoprophylaxis against hepatitis were performed at the Willowbrook State Hos-

pital, on Staten Island, New York, with support from the Office of the Army Surgeon General. These studies used opportunities presented by the extremely high rates of hepatitis that had been traditionally observed among severely mentally retarded patients (with most newly admitted children being infected within the first year after hospitalization).²⁶ Although they generated useful data and improved living conditions for participants, these studies were vulnerable to criticism as examples of potential conflicts of interest (because the principle investigator was an institutional authority, with control over inpatient conditions), as well as potential coercion of a vulnerable population.

In an early summary of one series of investigations starting in 1956, Krugman et al.²⁷ characterized the initial and subsequent attacks of hepatitis among inpatients. Between 4 and 8% of Willowbrook patients suffered second attacks. Postulates for this observation included reinfection by overwhelming doses of the same viral strain, the presence of unaffected serotypes of infectious hepatitis virus (similar to poliomyelitis strains), and the occurrence of viral latency and reactivation. Later studies suggested that hepatitis A and B circulated independently. In retrospect, these studies highlighted the ongoing difficulty of characterizing separate but highly similar syndromes caused by viruses for which there were no specific serological or direct viral detection assays. Additional information generated from these investigations included the demonstration that doses of 0.06 mL of γ -globulin/pound of body weight offered longer and more effective protection than 0.01 mL/pound. Finally, in a confirmation of the observation previously made by Stokes et al.,²⁵ the lack of rebound of clinical jaundice after 5 months of protection offered by γ -globulin suggested that active immunization from ongoing exposure was occurring during the period of passive prophylaxis. By comparing attack rates of overt hepatitis within the first 6 months of hospitalization with rates of inapparent hepatitis, Krugman et al.²⁷ were able to calculate the ratio of subclinical hepatitis to clinical hepatitis to be 12:1. These determinations were made possible by the recent addition of a sensitive hepatocellular enzyme assay (testing for serum glutamic oxaloacetic transaminase).

By the middle 1960s, the Willowbrook investigators had identified epidemiologically a virus (MS-1) characterized by a short transmission period (mean, 35 days) when fed to nonimmune (newly admitted) patients, a short period of abnormal liver function test results, and high attack rates with oral feeding, as well as a virus (MS-2) characterized by a longer transmission period (mean, 54 days), a longer period of elevated liver function test findings, and lower attack rates with oral feeding.²⁶ Immunity to challenge with the same strain was observed and cross-immunity to the other strain did not occur, confirming the earlier observations made by Havens.¹⁹ Ultimately, Willowbrook's MS-1 strain was recognized as the virus causing infectious hepatitis in the earlier studies and the MS-2 strain as that causing homologous serum hepatitis.²⁶

Effect of Exercise during Convalescence from Hepatitis

The time lost by military personnel during convalescence from hepatitis ran to many weeks, and it was an operational interest to determine how soon troops with jaundice could return to rigorous military duties without relapse of disease. As mentioned, Swift et al.⁹ initially generated data in Bayreuth suggesting that troops convalescing from hepatitis could "exercise"

without adverse effect. The quantification and duration of exercise in the control and trial arms of this study were difficult to interpret. Chalmers et al.²⁸ subsequently performed a study with clearer definitions of these parameters, evaluating patients admitted during the Korean War to the hepatitis center at the U.S. Army hospital in Kyoto, Japan. The investigators showed that, at least for patients in whom bilirubin levels had already decreased to 1.5 mg/dL or less, a program of vigorous exercise did not cause signs of clinical relapse of hepatitis. This massive study also suggested that dietary manipulations could provide a moderate and statistically significant benefit; with force-feeding of high-calorie (3,000 calories) and high-protein (150 g) diets, compared with ad libitum meals, illness was shortened by 22% or 6 days. Finally, in a well-controlled study of troops with hepatitis who were hospitalized in Vietnam, Repsher and Freebern²⁹ were able to demonstrate that there were no differences in the mean durations of illness for patients assigned to either a vigorous exercise group or a "rest" group, independent of patients' age, maximum bilirubin level, or serum glutamic oxaloacetic transaminase level.

Hepatitis B

Prophylaxis

Further characterization of the epidemiology of and strategies for protection against serum hepatitis were better realized only after more definitive work on the virus itself occurred in the 1960s. Following the discovery of the Australia antigen, i.e., the hepatitis-associated antigen (HAA), by Blumberg et al.,³⁰ its association with hepatitis was soon recognized, and assays became available to test whether clinical samples possessed HAA positivity.

Viral hepatitis was endemic among the populations of Asia (Korea, Vietnam, and Japan), and a high endemic rate had been recognized among U.S. troops assigned to the Far East. Prophylactic γ -globulin therapy had been used successfully to reduce the incidence of symptomatic hepatitis among personnel assigned to the Far East. The optimal dose and the necessary number of doses of γ -globulin were poorly defined, it was unclear which kind of hepatitis occurred among assigned troops, and it was unknown which type of hepatitis was prevented by the use of γ -globulin. In a massive, prospective, double-blind study that lasted for 2 years (1967-1969), taking advantage of the single port of aerial debarkation for troops entering Korea, 107,803 servicemen were randomized to receive various doses of γ -globulin on arrival.³¹ Personnel were monitored thereafter for the appearance of clinical hepatitis. Six months later, 65% of participants received a second injection of the same material. Effective detection of hepatitis cases occurred because of uniform admission of patients to only two Army hospitals in Korea. Prophylaxis was generally effective; among the 2-, 5-, and 10-mL doses of γ -globulin, protection was maximal at 5 mL. Protection lasted for 6 months. Protection was demonstrable for both HAA-positive and HAA-negative hepatitis. A later analysis of the stored sera from this study confirmed that the HAA-negative hepatitis that was successfully prevented included both hepatitis A and non-A, non-B hepatitis (probably but not definitively hepatitis C, because the γ -globulin was collected in the United States and therefore probably had no protective antibody against hepatitis E).³²

Epidemiology

The availability of tests for hepatitis B-related antigens and antibodies made possible the recognition and description of the epidemiology of hepatitis B among military personnel of all services over the past 30 years. The application of these tests for the first time in the 1970s was coincident with an interesting and troublesome phenomenon, the wave of intravenous drug abuse that appeared in U.S. forces both overseas and at state-side posts and bases. Early reports emerged from a Marine outbreak at Camp Lejeune, North Carolina, in 1970.^{33,34} This outbreak, like one described among Army soldiers at Fort Hood, Texas,³⁵ seemed to have been largely introduced into the respective communities by servicemen returning from Vietnam, where drug abuse had increased in prevalence. The incidence of hepatitis B also surged among U.S. forces in Europe, again related to intravenous drug abuse.³⁶ This incidence decreased markedly, as expected, after more aggressive screening and stringent rehabilitative and disciplinary measures were instituted by the middle 1970s.³⁷

A different epidemiology of hepatitis B was recognized among military personnel of all services stationed in the Far East (other than wartime Vietnam). Illicit drug abuse was rare. Occupational exposures among medical personnel were associated with risk, but even more commonly, in multiple studies, heterosexual exposure to the local population surfaced as a significant risk factor.³⁸⁻⁴⁴ This finding was consistent across multiple study methods, including surveys linking the appearance of hepatitis to antecedent behaviors as well as to concurrent or previous sexually transmitted diseases. Together, these data confirmed the ease with which hepatitis B was sexually transmitted, and they triggered the eventual strategy of mandatory hepatitis B vaccination for military personnel deploying to areas of high endemicity in the Far East (especially Korea).

Separately from the above efforts, investigators at the Walter Reed Army Institute of Research (WRAIR) generated data suggesting that hepatitis B virus is present in the saliva of patients with acute hepatitis with antigenemia or with the chronic carrier state.⁴⁵ Specimens of saliva from donors with visualized hepatitis B antigen particles were capable of parenteral transmission of hepatitis B in a primate model.⁴⁶ Although these data indicate the reasons why dental workers are at risk of occupational acquisition of hepatitis B, they also make it difficult to separate the effects of oral, vaginal, and seminal fluid exposures on the efficiency of sexual transmission of this virus.

Hepatitis A

Without specific identification or isolation of the virus responsible for infectious hepatitis, further work on developing a vaccine and specific tests for the agent did not progress; the prevention of infectious hepatitis remained the province of nonspecific immunoprophylaxis using pooled globulin fractions from U.S. blood donors. Workers at Merck, in 1979, finally reported the successful propagation of hepatitis A virus *in vitro* in infected marmoset liver cells.⁴⁷ Soon thereafter, investigators at WRAIR reported the development of a neutralizing antibody assay.⁴⁸ Finally, another team of WRAIR investigators reported the successful propagation of hepatitis A virus in an African Green monkey kidney cell culture, a line of primate origin that was suitable for the development of a vaccine for use in hu-

mans.⁴⁹ A prototype vaccine demonstrated immunogenicity in a phase I trial among soldiers at Fort Lewis, Washington, with as few as two or three doses (given reasonably far apart) providing excellent responses.⁵⁰ Further studies by WRAIR investigators showed that inoculation by jet injector (potentially useful for rapid mass inoculation in the military) provided antibody titers equivalent to those achieved with needle inoculation, as well as demonstrating that coadministration of the candidate hepatitis A vaccine with hepatitis B vaccine did not interfere with the immunogenicity of either preparation (reviewed by Hoke et al.⁵¹).

Following the first controlled trial of an inactivated hepatitis A vaccine (from strain CR326F) in Monroe, New York, which demonstrated protective efficacy in a setting of high endemicity,⁵² Innis et al.⁵³ performed a large-scale trial of the WRAIR vaccine made from the closely related strain HM175. This vaccine was given to 40,000 children in 148 primary schools in rural Kamphaeng Phet province in Thailand. Immunogenicity was high, the vaccine was safe, and the protective efficacy of at least two doses was at least 94%. These and related studies (reviewed by Clemens et al.⁵⁴) demonstrated that the hepatitis A vaccine was safe and effective (and probably would be for decades), prompting Food and Drug Administration approval in 1995.

Hepatitis E

To date, hepatitis E has not been widely recognized as having infected U.S. military personnel. Given the potential for U.S. military deployments to areas with endemic virus, however, and the known propensity of the virus to infect non-U.S. students, military personnel, and visitors to those areas,⁵⁵⁻⁵⁹ interest has been generated in diagnostic techniques for and prophylactic measures against this form of hepatitis. The pathophysiology and immune responses have been defined.⁶⁰ Animal reservoirs have been recognized,^{61,62} suggesting that hepatitis E (unlike other viral hepatitises of humans) may be considered a zoonosis.

Investigators from WRAIR sequenced and published the complete genome of a Nepali isolate.⁶³ The same group used restriction endonuclease analysis of multiple strains from different worldwide sources, indicating that there are at least two (and probably three) major genotypes that can be effectively discriminated and at least three subgenotypes of the Asian strain.⁶⁴ This group is currently pursuing the development of a hepatitis E DNA-based vaccine created by the insertion of DNA encoding the hepatitis E structural protein ORF-2 into a plasmid vector, which has proved immunogenic in an animal model.⁶⁵

Summary of Key U.S. Military Contributions to the Study of Viral Hepatitis

Key contributions include the following (Table I): (1) epidemiology of World War II epidemic described, with at least two modes of transmission suggested and at least two forms of transmissible disease; (2) transmission studies performed, with at least two separate agents recognized (with separate transmission patterns and natural histories); (3) cross-immunity studies performed, confirming at least two viral agents, and asymptomatic infection recognized; (4) effective prophylaxis against infectious hepatitis described (immune serum globulin); (5) exercise studies performed (showing early rehabilitation to be harmless);

TABLE I
TIME LINE OF KEY U.S. MILITARY CONTRIBUTIONS IN VIRAL HEPATITIS

1944–1950	Characterizations of World War II and occupation outbreaks of “infectious jaundice” (Mediterranean theater of operations during the war, postwar Germany) ^{3,6–8,10–14}
1944–1945	Development of human challenge model for transmission and immune studies of hepatitis; data indicated two different transmissible viral agents ^{18–21}
1944	After use of immune serum globulin for civilian summer camp outbreak, first use of this agent in trial to prevent infectious hepatitis among military personnel ²⁴
1950s–1960s	Willowbrook studies confirmed two separate modes of transmission, no cross-immunity, and efficacy of immune serum globulin prophylaxis ^{26,27}
1969	Controlled trial published during Vietnam War demonstrated lack of effect of exercise on convalescing patients with hepatitis, confirming two earlier trials during Korean War and German occupation ²⁹
1971	Large-scale trial demonstrated efficacy of immune serum globulin against both Australia-antigen positive (hepatitis B) and negative (hepatitis A) jaundice in Korea ³¹
1970s	Multiple reports described epidemiology of military community-based hepatitis B outbreaks (intravenous drug use association and heterosexual transmission described) ^{33–44}
1983	Neutralizing antibody assay developed for hepatitis A, allowing serological testing and quantification (WRAIR) ⁴⁸
1986	Prototype hepatitis A vaccine developed in candidate primate cell line (WRAIR) ⁴⁹
1994	Large-scale trial of hepatitis A vaccine demonstrated protective efficacy (WRAIR, in Thailand) ⁵³
1990s	Epidemiological description of hepatitis E (with reservoirs defined and transmission studies and serosurveys performed) in countries of expected deployment (WRAIR) ^{55–62}
1998	Hepatitis E genome defined, ⁶³ and genotyping characterized subtypes (WRAIR) ⁶⁴
2001	Candidate hepatitis E vaccine in development (WRAIR) ⁶⁵

(6) hepatitis B prophylaxis described (immune serum globulin); (7) hepatitis B epidemiology and sexual transmission described; (8) hepatitis A vaccine developed and largest field trial performed; and (9) hepatitis E epidemiology described, genome published, and vaccine developed.

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