Journal of Medical Virology 1:9–14 (1977)

Studies of an Outbreak of Acute Hepatitis A: II. Antibody Changes to Cytomegalovirus and Herpesvirus

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The acute and convalescent sera from 14 schoolchildren with acute hepatitis A were tested for antibody changes to 70 viral antigens. Marked decreases were noted in the levels of antibody to cytomegalovirus in 5 of the 14 children and in the levels of antibody to herpesvirus type 1 in 3. No such changes were noted in 9 sex- and age-matched healthy control children from the same classes.

Key words: hepatitis, cytomegalovirus, herpesvirus type I

INTRODUCTION

Sera from persons with chronic hepatitis have been tested on various occasions for evidence of antibody rises to a variety of bacterial antigens. Significant increases in antibody titers have been noted to such intestinal bacteria as Escherichia coli (Triger, Alp, and Wright, 1972: Bjørneboe et al., 1972), Bacteroides (Triger, Alp, and Wright, 1972) and Salmonella (Protell et al., 1971) but not to Haemophilus influenza (Triger, Alp, and Wright, 1972), bacterium not found in the digestive tract. The cause of these titer changes was attributed to the failure of the diseased liver to sequester the respective bacterial antigens. At least one related study in similar patients was carried out against viral antigens; increases in antibodies were detected to measles and rubella, but not to Mycoplasma pneumoniae, herpes simplex, and enteroviruses (Triger, Kurtz, MacCallum, and Wright, 1972). It was suggested that certain viruses, acquired through natural childhood infections may be continuously released in small quantities from the diseased liver. Presumably, the liver damage caused by the hepatitis infection depressed the sequestering ability of the Kupffer cells, freeing the antigens and making them available to antibody-producing cells in other parts of the reticuloendothelial system.

Received June 1, 1976
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Compared with the relative paucity of virus-related antibody studies in chronic hepatitis, many studies have shown an association between various respiratory and enetric viruses and outbreaks of acute hepatitis A (Davis, 1961; Liebhaber et al., 1965; Hatch and Siem, 1966; Hartwell et al., 1968; Zuckerman et al., 1968; Hatch and Swanson, 1969; Shulman and Barker, 1969; Berquist et al., 1972; Alwen, 1973). Moreover, recently Almeida reported seroconversions to several unidentified faecal antigens with virus morphology in patients with infectious hepatitis (Almeida et al., 1974). The early reporting of an outbreak of hepatitis A in a rural elementary school in Alabama in October and November 1972 permitted us to obtain acute and convalescent blood samples from 50 sick children and 24 healthy matched controls. Briefly, the children affected were between 6 and 12 years old, and the sharp "epidemic curve" suggested a common-source outbreak, one eventually traced to a contaminated water source. A more complete description of the details surrounding the outbreak is given in a companion paper (Baer et al., 1977). We noted a significant drop in the C′3 and C′4 components of complement in the acute specimens. Selected sera from this same outbreak were tested for possible antibody rises against a variety of viruses and mycoplasma, and the results of that testing are reported here.

MATERIALS AND METHODS

We tested paired sera from 14 randomly selected schoolchildren age 6–12 with acute hepatitis A (the definition of a case is given in the companion paper) and from 9 age- and sex-matched healthy children from the same classes. The acute sera were collected approximately one week after the peak of the outbreak (11/9/72), while the convalescent sera were drawn one month later (12/12/72).

Antibody Titer Determinations

The sera were tested for antibodies to the prototype strains of a large number of respiratory and enteric viruses by complement-fixation (CF), hemagglutination-inhibition (HI), indirect hemagglutination (IHA), and/or serum neutralization (SN) tests, as appropriate for each particular virus. The sera were heat-inactivated (56°C, 30 min) for all serologic tests. Antibodies to influenza A and B, parainfluenza 1, 2, and 3, the "soluble" and "viral" antigens of mumps virus, the group-specific hexon antigen of adenovirus, respiratory syncytial virus (RSV), Mycoplasma pneumoniae, herpes simplex 1, rubella, and cytomegalovirus (CMV) were assayed by the standardized CF test with overnight fixation of 5 units of complement (Casey, 1965). Antibodies to adenovirus types 1 to 33, influenza C, parainfluenza 4A and 4B, Newcastle disease virus (NDV), Coxsackievirus A-24, and coronavirus OC 43 were assayed by the standardized HI test with 0.01 M phosphate-buffered saline diluent and spectrophotometrically standardized 0.4% mammalian or 0.5% avian red blood cells (Hierholzer and Suggs, 1969; Hierholzer et al., 1969).

Hemagglutinating antigens of parainfluenza 4A and 4B were prepared for use in the HI test as described by Killgore and Dowdle (1970). Antibodies to herpes simplex 1 and CMV were determined by the IHA procedures of Bernstein and Stewart (1971a, b), and coronavirus 229 E antibodies were measured by a similar IHA test (Kaye et al., 1972). Neutralizing antibodies to the six Coxsackie B viruses, echovirus types 4 and 9, and Coxsackievirus A-10 were assayed by SN tests in monkey kidney (MK) tissue culture (Hierholzer et al., 1972). Neutralizing antibody titers to Coxsackievirus A-9, A-16, and A-21 and to four strains of enterovirus 70 were similarly assayed by SN tests in human diploid fibroblast (RU-1) tissue

culture, and to Coxsackievirus A-4 in suckling mice brain (Melnick and Wenner, 1969). Antibodies to the agent of infectious mononucleosis (Epstein-Barr virus) were measured by the ox cell hemolysin test with 2% erythrocytes (Mason, 1951).

Sera were tested by counterimmunoelectrophoresis for the presence of hepatitis B surface antigen (HBsAg) and antibody (anti-HBs) (Gocke and Howe, 1970).

RESULTS

All sera were negative for HBsAg and anti-HBs. Table I shows the variety of serologic tests performed on the selected sera, and the agents or antigens used. No significant increases in antibody titers were noted between the acute and convalescent specimens. Occasionally, low but stable complement-fixing antibody titers were noted for parainfluenza, adenoviruses, mumps, rubella and Coxsackie-viruses, but nothing beyond a twofold rise or fall was observed. It was seen, however, that marked falls (fourfold or greater) against cytomegalovirus and herpesvirus 1 occurred in sera of 5 (35%) and 3 (21%) of the sick children, respectively, whereas no such changes occurred in the matched healthy controls (Table II). There also appeared to be a relationship between the degree of illness and the antibody changes: Antibody changes were noted in 4 (67%) of the 6 icteric children whose sera we tested.

TABLE I. Serologic Tests Performed on Acute (11/9/72) and Convalescent (12/12/72) Serum Specimens, Hepatitis A Outbreak, Colbert County School, Alabama

Agent or Antigen	CF	IHA	HI	SN	Other
Adenovirus 1–33	•		•		
CMV	•	•			
Influenza A	•				
Influenza B	•				
Influenza C			•		
Parainfluenza 1, 2, and 3	•				
Parainfluenza 4A and 4B			•		
RSV	•				
Herpes 1	•	•			
Mumps (soluble and viral)	•				
Rubella	•				
Newcastle Disease Virus			•		
Coronavirus OC–43			•		
Coronavirus 229–E		•			
Coxsackie B–1, 2, 3, 4, 5, 6				•	
Coxsackie A–4, 9, 10, 16, 21				•	
Coxsackie A–24			•		
Echo 4 and 9				•	
Enterovirus 70 (4 subtypes)				•	
Mycoplasma pneumoniae	•				
Infectious mononucleosis					•
Hepatitis B					•

TABLE II. Complement-Fixing Antibody Titers Against Herpesvirus 1 and Cytomegalovirus, Hepatitis A Outbreak, Colbert County, Alabama

Specimen Number	Sick Individuals				Specimen Number	Healthy Control Individuals			
	Herpes		CMV			Herpes		CMV	
	Acute	Convalescent	Acute	Convalescent		Acute	Convalescent	Acute	Convalescent
4	32	8	64	8	12	0	0	0	0
15	32	32	32	32	17	8	16	32	32
32	0*	0	32	16	18	0	0	0	0
43	0	0	32	8	57	0	8	8	16
45	0	0	64	32	67	32	32	8	8
55	16	16	256	8	83	32	32	32	32
64	16	8	32	32	100	32	32	32	32
76	64	32	0	0	110	16	16	64	64
91	128	8	512	64	116	8	8	64	64
95	0	0	0	0					
96	16	16	16	16					
102	128	32	256	16					
128	32	32	64	64					
143	32	32	16	32					

*Indicates a level less than 1:8.

DISCUSSION

The fall in antibody titers to cytomegalovirus and herpesvirus that occurred in some of the hepatitis-A-infected schoolchildren was so dramatic as to demand a plausible explanation. The onset of illness in hepatitis A occurs after the beginning of virus excretion (Dienstag et al., 1975; Ward et al., 1958). In view of the usual preponderance of active CMV and herpesvirus infections in the young age groups represented here, natural infections probably developed years before this hepatitis A outbreak. It appears, therefore, that the stimulation of the already existing CMV and herpesvirus antibody titers in the affected children occurred a few weeks before the hepatitis symptoms appeared and that these levels were either at their peak or already beginning to fall when our acute samples were drawn. Such an occurrence could account for the titer decreases seen in this outbreak of acute hepatitis A in contrast to the titer increases reported in chronic hepatitis (Triger, Kurtz, MacCallum, and Wright, 1972).

Cytomegalovirus infection, common in childhood (Weller, 1970), has been increasingly diagnosed in the last few years and often involves the liver (Hanshaw et al., 1965). If temporary liver damage by acute hepatitis A infection stimulates Kupffer cells and liver macrophages, and this causes antibody rises to childhood viruses, CMV would likely be involved. Although the antibody titers to two other primarily childhood viruses, measles and rubella, were reported to be increased in chronic active hepatitis (Triger, Kurtz, MacCallum, and Wright, 1972) no examination for CMV antibody was made in that study; nor are we aware of acute and convalescent sera from acute hepatitis A cases being examined for antibody changes to such a variety of viral antigens as in the present study.

Of great interest are the comments recently made by Purcell et al. (1974) on the occurrence of CMV antibody in hepatitis B: "A relatively high proportion of patients with type-B transfusion hepatitis, or no hepatitis at all, have also developed antibody to CMV, (Prince et al., 1971; Purcell et al., 1971; Stevens et al., 1970), and it is difficult at present to evaluate the etiologic significance, if any, of this virus posttransfusion hepatitis." It also should be noted that we found no antibodies against Epstein-Barr virus, an agent recently incriminated in numerous cases of HBsAg-negative hepatitis in a dialysis unit (Corey et al., 1975).

Out data add more evidence that the critical liver changes that occur during acute hepatitis attacks may alter antibody levels to key antigens. Walker (1972) has reviewed two mechanisms that might affect antibody production: an increased processing of antigen in the proliferated Kupffer cells, which thus triggers antibody synthesis in immunocytes attracted to the liver (Paronetto et al., 1962; Hadziyannis et al., 1969); and, as mentioned earlier, a failure of Kupffer cells to sequester antigens absorbed from the gut. In addition, Popper et al. (1965) has shown that liver injury is associated with activation of the various components of the hepatic mesenchyma and some parts of the lymphoid system, and has suggested that this might explain the frequently encountered erratic false-positive Wasserman tests. The antibody rises to enteric bacteria noted by other workers (Triger, Alp, and Wright, 1972; Bjørneboe et al., 1972; Protell et al., 1971) during chronic hepatitis also suggest a failure in the sequestering ability of the liver.

Sera from other acute hepatitis A outbreaks should be examined to determine whether infectious childhood viruses can actually be recovered. It is again apparent, however, from the findings reported here that various antibody changes not due to the inciting virus may occur in infectious hepatitis A, and that such changes must be placed in proper perspective during the search for the precise etiologic agent of the disease.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Dr. Richard Levine, Dr. John Bryan, Mr. Bruce Wood, Dr. Harold C. Woodworth, and Mr. Roger Norris in these studies.

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