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Antibodies against adeno-, cytomegalo- and rubella viruses in Australia-antigen-negative sera from patients with infectious hepatitis

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SUMMARY

Antibodies neutralizing adenovirus type 5 were found in all of 50 pairs (100 %) of sera from patients with acute icteric infectious hepatitis. The incidence in sera from the general population was 57 %. No differences in mean titre or in proportion of positive sera were found in the same sera tested for complement-fixing antibodies to cytomegalovirus and for antibodies to rubella virus haemagglutinin. The results can be interpreted as supporting the involvement, either direct or indirect, of adenovirus in the aetiology of infectious hepatitis; but could also be due to a non-specific anamnestic enhancement of the production of antibody to adenovirus, or to coincidental infection with adenovirus and the agent of infectious hepatitis.

INTRODUCTION

There have been a number of reports of raised antibody titres to a variety of bacterial and viral antigens in the sera of patients with liver disease (Bjørneboe, Prytz & Ørskov, 1972; Triger, Kurtz, MacCallum & Wright, 1972; Triger, Alp & Wright, 1972). Alwen (1968) reported that in a small group of sera from patients with acute icteric infectious hepatitis the proportion containing antibody neutralizing adenovirus type 5 was higher than in a group of normal control sera. No such increase was found in sera from patients with jaundice caused by infection due to *Leptospira* spp. Adenovirus has long been popular as one of the so-called 'candidate' viruses in infectious hepatitis (Hersey & Shaw, 1968). It was therefore of interest to extend the earlier work to a much larger group of sera from patients with acute icteric infectious hepatitis; and when possible to test sera from the same patients 3 months after the illness. Two other common viruses, cytomegalovirus and rubella were included in the survey.

MATERIALS AND METHODS

Sera

Sera from patients in the acute icteric phase of infectious hepatitis were obtained from Public Health Laboratory Service laboratories and from general practitioners throughout the country in response to a request published in the Communicable Disease Report through the courtesy of Sir James Howie of the Public Health Laboratory Service. Sera were also obtained from the same patients 3 months later. Specimens consisted of separated serum or whole blood without additives. The serum was removed from clotted blood and centrifuged at 1000 *g* for 10 min. to remove blood cells.

Through the courtesy of Dr S. J. Starkie, of St Mary's Hospital Medical School, London, W.2, control sera were obtained from a large collection obtained for tissue-typing from the siblings and relatives of patients with kidney disease, and from members of the general public. No medical histories were available for the donors of the control sera: but none of the donors were acutely ill when the specimens were taken.

Sera were stored frozen at $-70°$ C in sealed glass ampoules and inactivated at 56° C for 30 min. before use.

The mean age of the 27 men and 23 women from whom infectious hepatitis sera were obtained was 20 yr.; and of the 25 men and 25 women in the control group, 22·6 yr.

Adenovirus neutralization test

The adenovirus type 5 used throughout the neutralization tests came from the same batch grown in HeLa cells and stored at $-70°$ C. The virus was originally obtained from Dr H. G. Pereira of the National Institute for Medical Research, London, N.W.7. HeLa cells were originally obtained from Flow Laboratories Ltd. The cells were cultured in Eagle's minimum essential medium; subcultured twice a week, and always used for neutralization tests 2 days after subculture. A rapid neutralization test was used in which cultures of HeLa cells were inoculated with mixtures of adenovirus type 5 and dilutions of the test serum. Cytopathic effect caused by unneutralized adenovirus was assessed 2 days later by counting cells microscopically. The neutralization titre was calculated and expressed as the reciprocal of the dilution of serum that reduced by 50 % the proportion of cells showing characteristic nuclear cytopathic effect (Boyer, Denny, Miller & Ginsberg, 1960) in control cultures inoculated with fetal bovine serum in place of the test serum. Titres of 1/5 or greater were considered positive. Sera were coded before testing: 2 sera from patients in the acute phase of hepatitis, sera from the same patients 3 months later, and 2 control sera were tested at one time.

Complement-fixation test for cytomegalovirus

Antibodies to cytomegalovirus were measured in complement-fixation tests by a technique similar to that of Bradstreet & Taylor (1962). The tests were done in disposable micro-titre trays (Flow Laboratories Ltd). The antigen and positive reference serum were obtained from Dr C. M. Patricia Bradstreet, Standards

Laboratory, Central Public Health Laboratories, Colindale, London, N.W. 9. The complement, sheep erythrocytes and haemolytic serum were obtained from Burroughs Wellcome & Co., Beckenham, Kent. Paired sera were tested in parallel. Titres of 1/2 or greater were considered positive.

Haemagglutination-inhibition test for rubella virus

Antibodies to rubella haemagglutinin were measured by a micromethod (Stewart *et al.* 1967). The lyophilized antigen was obtained from Flow Laboratories Ltd. Non-specific haemagglutination inhibitors were removed from test and control sera with manganous chloride and heparin (Feldman, 1968). Antigen controls and known positive and negative serum controls were included in each group of tests. The end-point was taken as the reciprocal of the highest dilution of serum showing complete inhibition of agglutination. Titres of 1/4 or greater were considered positive.

Australia antigen

All sera were tested for Australia antigen and antibody by immunodiffusion (W.H.O., 1970) using an Australia antigen detection kit supplied by Behringwerke AG. Two of the sera from patients with infectious hepatitis were positive for Australia antigen, one for antibody, and one for both; they were discarded from the series. None of the control sera were positive for either Australia antigen or antibody.

RESULTS

The distribution of the titres of the sera from patients with acute infectious hepatitis, of sera from the same patients three months later, and of the controls, is shown in Fig. 1. All patients with hepatitis had antibody that neutralized adenovirus in their sera. Two of the acute phase sera had titres of less than 1/5; but 3 months later the serum from each of these patients contained antibody. One convalescent serum had a titre of less than 1/5 though serum from the same patient during the acute phase of the illness was positive.

Antibody that neutralized adenovirus was found in only 57 % of the sera in the control group.

The geometric mean titres were 1/84 for the acute phase sera, 1/81 for the convalescent phase sera and 1/14 for the control sera. The mean titres of the acute phase and control sera, omitting negative results, were 1/103 and 1/93 respectively, and did not differ significantly by Student's *t*-test. The mean titres of the acute phase sera from patients with hepatitis and the control group were 1/9 and 1/11 respectively, and did not differ significantly. Two of the convalescent sera showed a twofold and 3 a fourfold rise in titre; and 2 of the convalescent sera showed a twofold and 1 a fourfold drop in titre.

In the haemagglutination test for rubella virus, the proportion of positive sera (titres > 1/4) in patients with hepatitis was 82 %; and 86 % in the control group. The mean titres for the acute, convalescent and control sera were 1/52, 1/52 and 1/78 respectively. The mean titres of the hepatitis sera and the control sera were

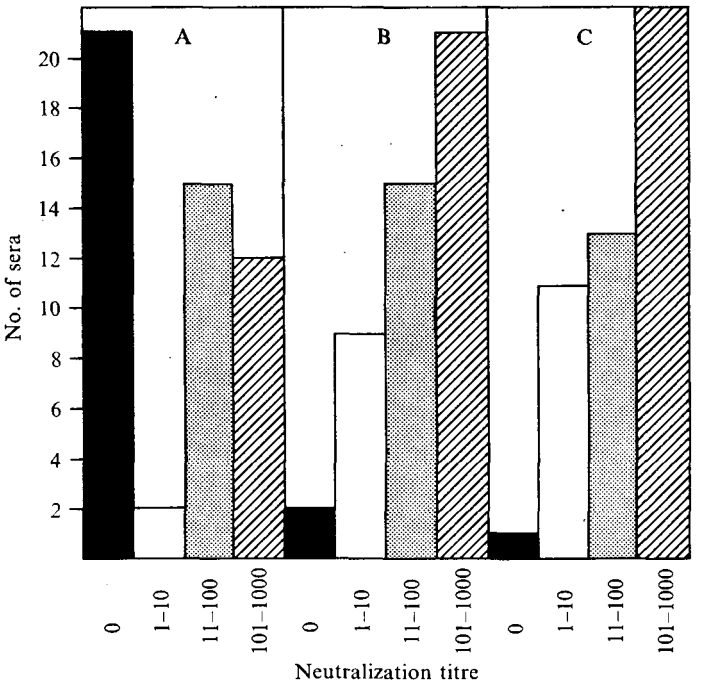

Fig. 1. Distribution of titres of antibody neutralizing adenovirus type 5. A, control sera; B, acute phase sera; C, convalescent phase sera.

not significantly different. Four convalescent sera showed twofold increases and 2 fourfold increases in titre; and 8 of the convalescent sera from patients with hepatitis showed a twofold drop and 6 a fourfold drop in titre.

DISCUSSION

These results demonstrate an increase in the proportion of sera from patients with acute icteric infectious hepatitis that are positive for antibody neutralizing adenovirus type 5. Antibody was detected in all of 50 pairs (100 %) of infectious hepatitis sera; but in only 28 of 50 control sera (57 %). The latter figure agrees well with published estimates of the prevalence of antibody to adenovirus type 5. For example Huebner *et al.* (1954) reported 53 % for a large group of sera from the general population between the ages of 16 and 34 years. However, Potter, Shedden & Zachary (1963) reported only 20 % in a survey of the general population. Twelve sera in our test group showed a twofold or greater rise in titre compared with 5 that showed a twofold or greater drop. However, in 76 % of paired sera there was little or no difference in titre between the first and second specimens.

Although it cannot be excluded, the possibility of a direct involvement of adenovirus type 5 in the aetiology of infectious hepatitis, – as for example, in canine infectious hepatitis in which the liver damage is caused by the multiplication of an adenovirus – appears unlikely despite the various isolations of adenoviruses from cases of the disease (Davis, 1961; Hatch & Siem, 1966; Hartwell, Love & Eidenbock, 1966). An indirect action of adenovirus is perhaps more

probable; this could arise from adenovirus multiplication elsewhere than in the liver, with consequent damage by adenovirus antigen(s), or antigen(s)/antibody complexes.

Non-specific heterologous enhancement was first described by Weil & Felix (1916), who described a non-specific enhancement of antibodies to *Salmonella typhi* during infection with typhus. Our results could be interpreted as heterologous enhancement of antibody against adenovirus by acute infectious hepatitis without involvement of adenovirus in pathogenesis. The icteric phase of acute infectious hepatitis, though the major clinical symptom, may be a sequel to the main phase of the disease, so that antibody stimulation might have preceded the time at which our first samples were taken, possibly by up to 3 weeks. This could explain why in 76 % of the hepatitis sera the titres of antibody neutralizing adenovirus were similar in the acute and convalescent phases of the disease. It would be of great interest to measure the neutralizing antibody levels in a group of pre-icteric sera. After stimulation it would be expected that the adenovirus-neutralizing antibody titres would remain raised for some time (Sohier, Chardonnet & Prunieras, 1965).

Nuromskaya, Potulova & Fedenko (1968) suggest that patients with infectious hepatitis are predisposed to simultaneous infection with an adenovirus. Closs (1972) has also reported a rather unusual association between an outbreak of acute infectious hepatitis and rubella virus in which the two diseases caused simultaneous outbreaks with exactly similar incubation periods. The high proportion of sera from infectious hepatitis patients positive for adenovirus neutralizing antibody may be due to simultaneous infection with the agent of infectious hepatitis and adenovirus. Infectious hepatitis frequently begins with a sore throat (Glover & Wilson, 1931) and lymphadenopathy (Finks & Blumberg, 1945), which could be due to infection with adenovirus rather than to the multiplication of the agent of infectious hepatitis.

The possibility that the high titres of antibody neutralizing adenovirus type 5 are due to impairment of the normal mechanism for removing continuously released antigens with consequent enhancement of antibody production, is of particular interest in relation to recent reports concerning raised antibody titres to a number of unrelated antigens in infectious hepatitis. Triger, Kurtz *et al.* (1972) demonstrated raised titres to measles and rubella viruses, though not to coxsackie B1 and B5, *Herpes simplex*, parainfluenza type 1, or *Mycoplasma pneumoniae*, in chronic active hepatitis. Bjørneboe *et al.* (1972) reported an increased frequency of agglutination reactions with 10 different antigens of *Escherichia coli* group O in the serum of patients with cirrhosis of the liver; and Triger, Alp *et al.* (1972) described an increase in antibody titre to *Escherichia coli* and *Bacteroides* in patients with liver disease. Patients with acute viral hepatitis were included in the group and showed increased incidence of antibody to *E. coli* and *Bacteroides*; but not to *Haemophilus influenzae* B which is not commonly found in the gastrointestinal tract. Protell *et al.* (1971) demonstrated an increased proportion of sera positive for agglutinins to *Salmonella* antigens in patients with chronic active liver disease. It therefore appears that antibodies to a number of antigens present in the

gastrointestinal tract in health are increased in titre, in incidence, or in both, during certain diseases of the liver including acute viral hepatitis. Both Bjørneboe (1971) and Triger, Kurtz *et al.* (1972) suggest that in some diseases of the liver its ability to sequester antigens is reduced; or that antigen already sequestered and rendered temporarily non-antigenic is released and becomes able to stimulate antibody production; and that such increases may largely account for the hyperglobulinaemia common in liver disease. In support of this hypothesis are the observations that after the establishment of a portacaval shunt in cirrhosis the level of immunoglobulins is greater than in cirrhotics without a shunt, and that the incidence of agglutinins to intestinal microbes is highest (Bjørneboe *et al.* 1972).

The hypothesis could also explain the increased proportion of sera positive for antibody neutralizing adenovirus in acute viral hepatitis, as adenovirus infections may persist in an occult form associated with low levels of circulating antigen and antibody.

Most of the micro-organisms to which antibodies are increased in liver diseases are commonly found in the gastrointestinal tract. Although adenovirus infections are most commonly encountered in the upper respiratory tract they can also affect the gastrointestinal tract, which is the usual route of vaccination against them. Neither rubella nor cytomegalovirus commonly affect the gastrointestinal tract. In this survey the proportion of hepatitis sera positive for antibody to rubella haemagglutinin and cytomegalovirus was no higher than in the control group.

Titres of antibodies fixing complement with adenovirus type 5 were not measured in this survey. Hatch & Swanson (1969) investigated the incidence of complement-fixing antibodies to adenovirus type 4 and to the San Carlos agent No. 8 (an atypical adenovirus type 3, isolated from a case of infectious hepatitis). The incidence varied from 61 to 67 % in acute and convalescent hepatitis sera, and from 52 to 56 % in normal controls. The titres in the two groups were not significantly different. Alwen (1968) also found no increase in complement-fixing antibodies to adenovirus type 5 in a small group of sera from patients with acute viral infectious hepatitis; though the proportion of sera positive for neutralizing antibody was increased.

We think it probable that the increased proportion of sera positive for antibody neutralizing adenovirus type 5 from patients with infectious hepatitis is the result either of a heterologous immunological enhancement, or a specific stimulation of the production of antibody facilitated by impairment of the process by which continuously released antigen from a chronic infection is sequestered. Although sera from patients with acute icteric Weil's disease due to *Leptospira* spp. do not show increased titres of antibody neutralizing adenovirus (Alwen & Fulton, 1968), and increases in antibody to adenovirus may not be a general feature of diseases of the liver causing jaundice, it would be of great interest to extend the survey of antibody neutralizing adenovirus to include sera from patients with liver diseases other than infectious hepatitis, and particularly to sera from patients with Australia antigen.

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