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Immunological aspects of cryoprecipitates from the sera of chronic HBsAg carriers

M. L. TIKU, K. R. BEUTNER & P. L. OGRA *Departments of Pediatrics and Microbiology, State University of New York at Buffalo and Division of Clinical Infectious Disease and Virology, Children's Hospital, Buffalo, New York, USA*

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SUMMARY

The sera of chronic hepatitis B surface antigen (HBsAg) carriers and seropositive controls were examined for the presence of immune complexes by cryoprecipitation. Cryoprecipitates (CP) were tested for HBsAg, antibody to HBsAg (anti-HBs), major classes of immunoglobulins, components of the complement system, rheumatoid factor and the ability to activate the alternative pathway of the complement system. For this analysis the methods employed included: radioimmunoassay, reverse passive haemagglutination, immunofluorescence, sucrose density gradient ultracentrifugation, agar-gel diffusion, immunoelectrophoresis, counterimmunoelectrophoresis, latex agglutination, and a haemolytic method for the detection of the activation of the alternative pathway of the complement system. HBsAg was frequently observed in the CP from chronic HBsAg carriers. No anti-HBs activity was detected in the serum of chronic HBsAg carriers. However, the CP from a number of chronic HBsAg carriers contained immunoglobulins and components of the complement system in the absence of rheumatoid factor, anti-HBs activity and were able to activate the alternative pathway of the complement system. On immunoelectrophoresis, a component of the CP reacting with anti-IgG, anti-IgA and anti-HBs antisera and demonstrating an altered (faster) electrophoretic mobility was observed. The nature of the CP strongly suggests the presence of circulating immune complexes in asymptomatic chronic HBaAg carriers. These immune complexes may be important in the eventual expression and outcome of clinical disease in apparently healthy carriers of HBsAg.

INTRODUCTION

The manifestation of acute or chronic type B viral hepatitis infection in man is known to vary from an asymptomatic state to clinically apparent liver involvement. In addition, hepatitis B virus (HBV) infection is also known to lead to polyarteritis (Gocke *et al.*, 1970), glomerulonephritis (Combes *et al.*, 1971), polymalgia rheumatica (Bacon, Noherty & Zuckerman, 1975), cryoglobulinaemia (McIntosh, Koss & Gocke, 1976) and serum sickness-like syndrome (Alpert, Isselbacher & Schur, 1971; Wands *et al.*, 1975). The spectrum of syndromes related to HBV may represent the dynamics and balance of interaction between virus and host. It has been suggested that the immunological response elicited against HBV in the host may be the basis for the pathogenesis of hepatic or non-hepatic disease (Edgington & Chisari, 1975). Immune responses, mediated either by T cells, B cells or both, maybe involved in this process (Edington & Chisari, 1975; Tiku *et al.*, 1978). The antibody or B-cell mediated response to various HBV related antigens notably against HBsAg has been shown to provide protection against infection (Iwarson *et al.*, 1975). On the other hand, the complexes of HBsAg and anti-HBs have been

Correspondence: Dr P.L. Ogra, Division of Infectious Disease and Virology, Children's Hospital, 219 Bryant Street Buffalo, New York, 14222, USA.

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incriminated in hepatic and extrahepatic injury (Nowoslawski *et al.*, 1975). Circulating and tissue-bound HBsAg/anti-HBs immune complexes have been demonstrated during acute and chronic type B hepatitis (Oldstone, 1975). In addition, these complexes have been shown to activate the complement cascade and are therefore thought to have potential for disease pathogenesis (Wands *et al.*, 1975; Wands, Alport & Isselbacher, 1975). The presence and characteristics of immune complexes in asymptomatic HBsAg carriers have so far received little attention.

Recent studies have failed to demonstrate anti-HBs in the sera of chronic HBsAg carriers (Vyas *et al.*, 1977). This has been interpreted as indicating that chronic HBsAg carriers do not produce anti-HBs. In the light of the massive antigenaemia present in these subjects it is also possible that anti-HBs exists in the form of HBsAg/anti-HBs immune complexes. In such a form anti-HBs would be difficult to detect in serum samples by conventional serological methods.

The present study was undertaken to evaluate the sera of asymptomatic chronic HBsAg carriers for the presence of immune complexes.

MATERIALS AND METHODS

Study population and specimen collection. Subjects were drawn from the resident population of an institution for mentally handicapped children and adults. The entire resident population of the institution was followed in an epidemiological programme to determine the characteristics of HBV infection in such an institutionalized setting. HBV infection is endemic in this population.

For the present study (Table 1) seventy-three chronic HBsAg carriers, two anti-HBs seropositive (HBsAg negative) and ten HBV seronegative subjects were selected at random from the resident population. Anti-HBs activity was not detected in the sera of the chronic HBsAg carriers. None of the subjects showed any signs of illness suggestive of acute or chronic hepatitis. The serological and clinical status of these subjects had been documented by repeated testing and observation over a 3–4 year period. Specimens of 6–50 ml of clotted blood were collected from each subject.

Cryoprecipitation. The method of cryoprecipitation has been described previously (as reviewed by Grey & Kohler, 1973). In brief, the sample of blood was allowed to clot at 37°C for 2 hr, was then centrifuged at 450 *g* at ambient temperature, and the serum incubated at 4°C for 7–10 days. Following this incubation, the serum was centrifuged at 12,000 *g* at 4°C for 15 min. The supernatant was decanted and the precipitate washed with ice cold phosphate buffered saline (PBS) by centrifuging as described above until the absorbancy at 280 nm of the supernatant was zero. The twenty cryoprecipitates (CP) obtained from 6–10 ml of blood were dissolved in 0·5 ml and the fifty-three CP obtained from 20–50 ml were dissolved in 1·5 ml of PBS pH 7·4, containing 0·1% sodium azide and 0·5% bovine serum albumin. The CP were then tested for the presence of HBsAg, anti-HBs and other immunological factors as described below.

Detection of HBsAg. Radioimmunoassay (RIA) (Ausria-II, Abbott Laboratory, North Chicago, Illinois) as described by Ling & Overby (1972) and a reverse passive haemagglutination (RPHA) system (Cayzer *et al.*, 1974) (kindly donated by Wellcome Research Laboratories, Beckenham, England) were employed for the detection of HBsAg. All serum samples were tested by RIA. The majority of CP were tested by RPHA and RIA; however, the quantity of CP available prevented all CP from being tested by both methods.

Detection of anti-HBs. Passive haemagglutination (PHA), RIA, immunofluorescence (IF), and a combination of sucrose density gradient ultracentrifugation and RIA were employed for the detection of anti-HBs. The PHA used commercially available HBsAg-coated human type O red blood cells (Vyas & Shulman, 1970) (Electronucleonics Laboratories, Inc., Bethesda, Maryland). The RIA for the detection of anti-HBs employed a commercially available solid phase RIA (Ausab, Abbott Laboratory, North Chicago, Illinois).

Immunofluorescence for the detection of anti-HBs activity was developed as follows. Normal horse serum was coupled by gluteraldehyde (Avrameas, Ternynck & Chuilon, 1969) onto turkey red blood cells (TRBC) forming HS-TRBC. HBsAg positive human serum, anti-HBs positive human serum, normal human serum (NHS) and fluorescein isothiocyanate (FITC) conjugated goat anti-human immunoglobulin were absorbed overnight at 4°C with an equal volume of packed HS-TRBC.

The absorbed HBsAg positive human serum, anti-HBs positive human serum, NHS and PBS in volumes of 25 μl were reacted in microtitre plates (Cook Laboratory Products) with 25 μl of a 6% suspension of TRBC coated with affinity column purified horse anti-HBs antibody (Ab-TRBC) (Wellcome Research Laboratories, Beckenham, England), and incubated at room temperature for 1 hr. The cells were then washed twice by centrifugation at 220 *g* with PBS and incubated with 25 μl of HS-TRBC absorbed anti-HBs positive human serum (PHA titre 128), for 1 hr at room temperature. The cells were again washed and reacted with 25 μl of HS-TRBC absorbed FITC-conjugated goat anti-human immunoglobulin for 1 hr. After two washes with PBS, a drop of the cell suspension was smeared onto a microscope slide. These preparations were read with a Leitz Ortholux microscope equipped with vertical illumination. Ab-TRBC, initially reacted with anti-HBs positive human serum, NHS or PBS, demonstrated no immunofluorescence. Irregular granular surface fluorescence was observed on Ab-TRBC initially reacted with HBsAg positive human serum.

For the evaluation of CP for the presence of anti-HBs by immunofluorescence, the following procedure was adopted. The

CP samples and PBS in volumes of 50 μl were absorbed with 25 μl of packed HS-TRBC in microtitre plates at 4°C. After overnight incubation, 25 μl of clear supernatant was reacted with 25 μl of a 6% suspension of Ab-TRBC for 1 hr at room temperature. The cells were washed twice by centrifugation at 220 *g* with PBS and incubated for 1 hr at room temperature with 25 μl of FITC-conjugated goat anti-human immunoglobulin. The cells were then washed twice with PBS and read as described above. CP demonstrating specific fluorescence were considered to contain anti-HBs as well as HBsAg-anti-HBs complexes.

The techniques of sucrose density gradient ultracentrifugation and RIA for anti-HBs were combined for the detection of anti-HBs in selected samples of CP. A 0·4 ml volume of CP was carefully layered onto a 7–40% pre-formed linear sucrose gradient prepared with glycine acetate buffer pH 2·8. The gradient was centrifuged in a SW 50-1 (Beckman Instrument Inc., Palo Alto, California) rotor for 18 hr at 165,000 *g* at 5°C. The gradient was then bottom punctured and twelve equal fractions were collected. Each fraction was then adjusted to a pH of 7·6 with NaOH and tested for anti-HBs by RIA.

Immunoelectrophoresis. Immunoelectrophoresis was performed as described previously (Williams, 1971). Antisera employed for developing included rabbit anti-normal human serum and anti-human heavy chain (anti-α, anti-γ and anti-μ antisera) (Behring Diagnostic). Following immunoelectrophoresis, the slides were washed in normal saline, air dried and stained with Ponceau Red, before being photographed.

Agar-gel diffusion. Agar-gel diffusion (Munoz, 1971) in 1% agarose in PBS with antisera specific for human heavy chains was employed for the detection of immunoglobulins in CP.

Detection of components of the complement system. Counterimmunoelectrophoresis (CEP) (Gocke & Howe, 1970) was employed to detect the third, fourth and fifth components of the complement system in the CP. For this assay rabbit antisera specific for C3 and C4 and a goat antiserum specific for C5 were obtained from Behring Diagnostic.

Detection of rheumatoid factor. A commercially available latex slide agglutination test (Hyland Laboratory) was used to detect rheumatoid factor in the test samples.

Activation of the alternative pathway of the complement system. The activation of the alternative pathway of the complement system was studied using two *in vitro* techniques. A CP demonstrating activity in either or both systems was considered capable of activating this pathway of the complement system.

The immunoelectrophoresis method as first described by Götze & Müller-Eberhard (1971; 1972), and subsequently modified (Fine *et al.*, 1972), was employed in the present study. The second method employed to demonstrate activation of the alternative pathway of the complement system was based on the haemolysis of glutathione-reduced fresh human type 0 erythrocytes as described previously (Arroyave, Valloto & Müller-Eberhard, 1974). The haemolysis induced by zymosan, a known activator of the alternative pathway, was assumed to indicate 100% activation. Haemolysis of 10% induced by a CP was considered indicative of activation of the alternative pathway of the complement system. Normal human serum induced less than 5% haemolysis.

RESULTS

All CP were tested initially for the presence of HBsAg and anti-HBs (Table 1). Of the seventy-three CP from the sera of chronic HBsAg carriers, fifty-two (71%) contained HBsAg.

Anti-HBs was detected by two different serological methods in eight out of seventy-three (11%) CP from the sera of chronic HBsAg carriers and by only one serological method in twenty of seventy-three

TABLE 1. Serological status of the study population and the distribution of HBsAg and anti-HBs in the cryoprecipitates obtained from the sera of chronic HBsAg carriers, anti-HBs seropositive subjects and seronegative subjects

		Number (%) positive for:						
				Cryoprecipitates				
		Serum						
Subjects	Number tested	HBsAg	Anti-HBs	HBsAg	Anti-HBs by 1 method	Anti-HBs by 2 methods	Anti-HBs by 1 method and HBsAg	Anti-HBs by 2 methods and HBsAg
Chronic HBsAg carriers	73	73 (100)	0	52 (71)	8 (11)	20 (27)	7 (9·6)	16 (22)
Anti-HBs seropositive	2	0	2 (100)	0	0	1 (50)	0	0
Seronegative	10	0	0	0	0	0	0	0

(27%) such CP. HBsAg and anti-HBs were detected in 9·6–22% of the CP, depending upon the criteria for designating a CP positive for anti-HBs. Thus 9·6% (seven out of seventy-three) of the CP contained HBsAg and anti-HBs detected by two serological methods. Anti-HBs, demonstrable by only one serological method, and HBsAg were present in 22% (sixteen out of seventy-three) of the CP. No anti-HBs activity was present in the seventy-three original serum samples from which the CP were prepared. The ten CP obtained from the sera of HBsAg seronegative subjects failed to manifest HBsAg or anti-HBs activity. One CP obtained from the serum of an anti-HBs seropositive control subject, seronegative for HBsAg but demonstrating a high anti-HBs titre, contained anti-HBs as detected by one serological method.

The detection of anti-HBs in CP by the various methods employed is shown in Table 2. PHA was the least sensitive test system detecting anti-HBs in only four of seventy-three (5%) CP tested by this method. RIA detected anti-HBs in thirteen of thirty-eight (34%) CP tested. Immunofluorescence and the combination of sucrose density gradient ultracentrifugation and RIA were the most sensitive methods for detecting anti-HBs in 57% and 55%, respectively, of samples tested.

TABLE 2. Anti-HBs and HBsAg in cryoprecipitates as evaluated by passive haemagglutination, radioimmunoassay, immunofluorescence, a combination of sucrose density gradient ultracentrifugation and radioimmunoassay and reverse passive haemagglutination, and various combinations of these methods

Test for:	Method	Number tested	Positive Number	Positive %
Anti-HBs	Passive haemagglutination	73	4	5·0
	Radioimmunoassay	38	13	34·2
	Immunofluorescence	20	11	55·0
	Sucrose density gradient ultracentrifugation and radioimmunoassay	14	8	57·1
	Radioimmunoassay and immunofluorescence	20	5	25·0
	Radioimmunoassay and passive haemagglutination	38	1	3·0
	Radioimmunoassay and sucrose density gradient ultracentrifugation and radioimmunoassay	9	2	22·0
HBsAg	Reverse passive haemagglutination	53	33	62·0
	Radioimmunoassay	56	39	70·0

A number of CP were tested by two serological methods. The combination of RIA and IF detected anti-HBs in 25% (five out of twenty) of the samples. Only one (3%) of thirty-eight CP tested by RIA and PHA was positive for anti-HBs. Two (22%) of nine samples tested by RIA and the combined method of sucrose density gradient ultracentrifugation and RIA demonstrated anti-HBs activity.

The methods of RPHA and RIA demonstrated a comparable ability to detect HBsAg in CP (Table 2). RPHA detected HBsAg in thirty-three out of fifty-three (62%) CP and RIA detected HBsAg in thirty-nine out of fifty-six (70%) CP.

Three HBsAg positive and seventeen HBsAg negative CP from the sera of chronic HBsAg carriers were pooled separately and concentrated. Using immunoelectrophoresis, the pooled CP were tested for reactivity against anti-normal human serum, anti-IgA, anti-IgG, anti-IgM and anti-HBs. The pooled concentrated HBsAg positive CP showed precipitin lines against anti-normal human sera in the β-2 region. It is important to note that no precipitin lines were observed in the albumin region, suggesting that there was no contamination of the CP with normal serum proteins. The pooled HBsAg negative CP demonstrated no reactivity with anti-normal human sera. The pooled HBsAg positive CP showed precipitin lines in the β-2 region with anti-IgA and anti-IgG antisera, as well as with anti-HBs (Fig. 1). No reactivity was observed with anti-IgM antiserum (Fig. 1).

The presence of IgG, IgA, IgM, rheumatoid factor and components of the complement system were valuated in fifty-three CP from chronic HBsAg carriers, two anti-HBs seropositive controls and ten

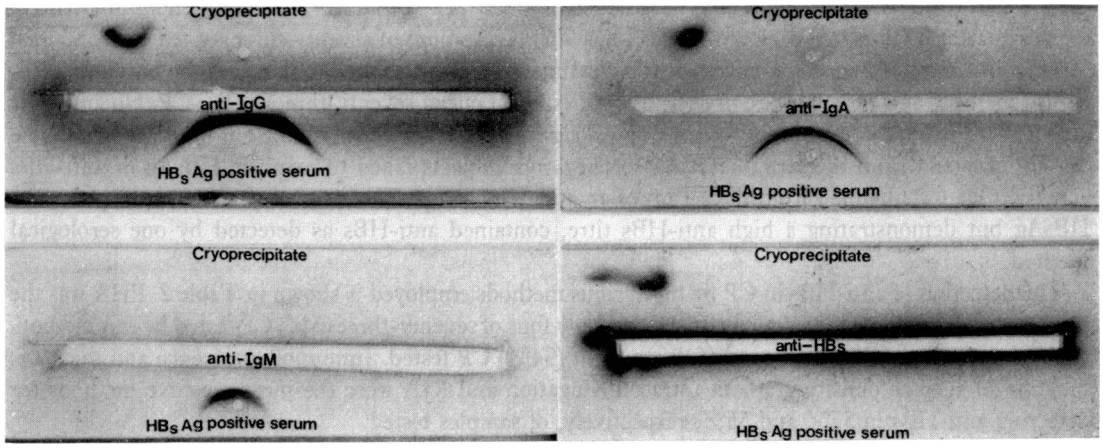

FIG. 1. Immunoelectrophoretic patterns of HBsAg positive human serum and concentrated HBsAg positive cryoprecipitate developed with anti-IgG, anti-IgA, anti-IgM and anti-HBs sera.

TABLE 3. Immunoglobulin, rheumatoid factor and complement components in the cryoprecipitates (CP) from the sera of chronic HBsAg carrier, anti-HBs seropositive subjects, and seronegative subjects

Serum source	Number tested	Number (%) of CP positive* for: IgG	IgA	IgM	Rheumatoid factor	C3	C4	C5
Chronic HBsAg carriers	53	22 (42)	7 (13)	18 (34)	17 (32)	11 (21)	19 (36)	16 (30)
Anti-HBs seropositive	2	1 (50)	0	1 (50)	2 (100)	2 (100)	0	1 (5)
Seronegative	10	4 (40)	1 (10)	1 (10)	4 (40)	2 (20)	1 (10)	2 (20)

* Immunoglobulins detected by agar-gel diffusion, rheumatoid factor detected by latex agglutination, and complement components detected by counterimmunoelectrophoresis.

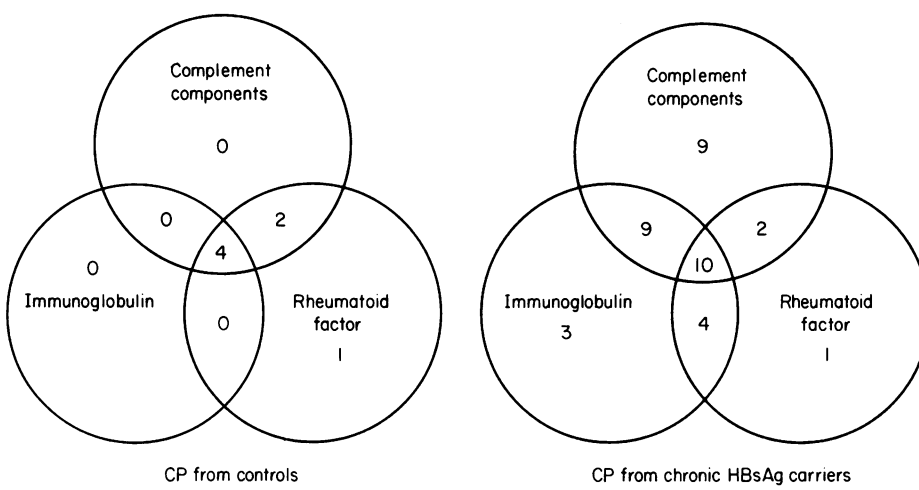

FIG. 2. Distribution of complement components, immunoglobulin and rheumatoid factor in cryoprecipitates (CP) obtained from the sera of seronegative and anti-HBs seropositive controls and chronic HBsAg carriers.

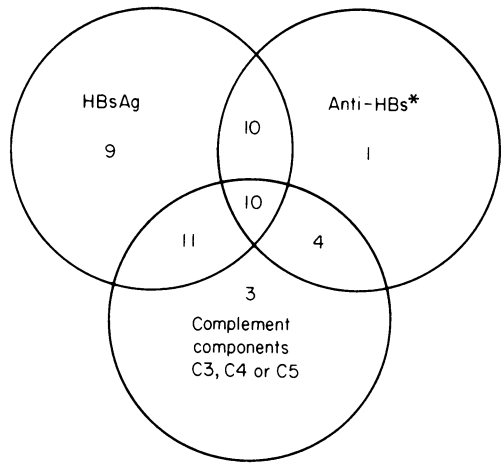

FIG. 3. The isolated and simultaneous presence of HBsAg, complement components and anti-HBs in the cryoprecipitates obtained from the sera fifty-three chronic HBs carriers. *Anti-HBs positive by one method.

seronegative controls (Table 3). IgG (42%), IgA (13%), and IgM (34%) were present in the CP from chronic HBsAg carriers. Immunoglobulins were also detected in the CP from the control groups. Thirty-two per cent of CP from chronic HBsAg carriers manifested rheumatoid factor activity, as did both of the CP from anti-HBs seropositive controls and 40% of the seronegative controls. C3, C4 and C5 components of the complement system were detected in 21%, 36% and 30%, respectively, in CP from chronic HBsAg carriers and from HBsAg seronegative controls.

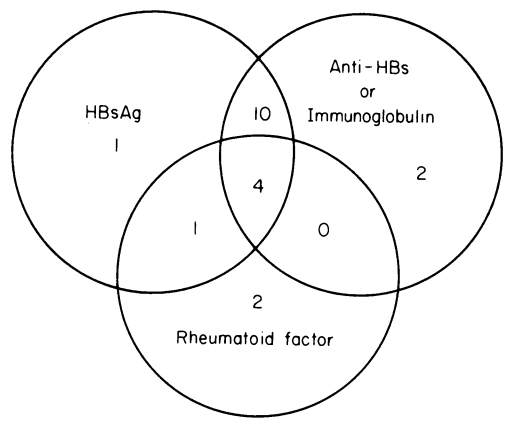

FIG. 4. The composition of twenty cryoprecipitates capable of activating the alternate pathway of the complement system. These cryoprecipitates were obtained from the sera of chronic HBsAg carriers.

All CP from the sera of anti-HBs seropositive controls and seronegative controls which contained immunoglobulin and/or complement components also manifested rheumatoid factor activity (Fig. 2). However, complement components and immunoglobulins were frequently detected in the CP from chronic HBsAg carriers in the absence of rheumatoid factor (Fig. 2).

The frequency of the simultaneous presence of HBsAg and anti-HBs detected by one or more methods, and the complement components C3, C4 and C5 observed in the sera of fifty-three chronic HBsAg carriers are presented in Fig. 3. Each of the reactants existed alone as well as in various combinations with other reactants. Ten of the fifty-three CP (19%) contained HBsAg, anti-HBs and complement components concurrently.

CP were also evaluated for their ability to activate the alternative pathway of the complement system. Twenty (66%) out of thirty CP tested were able to activate the alternative pathway of the complement system. No such activity was detected in four CP collected from seronegative controls and tested concurrently. The composition of the twenty CP capable of activating the alternate pathway of complement is summarized in Fig. 4. HBsAg, anti-HBs or immunoglobulin, and rheumatoid factor were all present in four of these CP. Ten CP capable of activating the alternative pathway of the complement system contained HBsAg and anti-HBs or immunoglobulin in the absence of rheumatoid factor. Two CP containing only anti-HBs or immunoglobulin, and two CP manifesting only rheumatoid factor activity, were able to activate the alternative pathway of the complement system.

DISCUSSION

Various techniques have been used to demonstrate virus-associated immune complexes (Oldstone, 1975). Cryoprecipitation is a simple technique that can be used to demonstrate immune complexes. Employing cryoprecipitation, the present study has demonstrated cryoprecipitates in the sera of chronic HBsAg carriers. The majority of CP from chronic HBsAg carriers contained HBsAg. Anti-HBs and the combination of anti-HBs and HBsAg were frequently observed in these CP.

The physicochemical nature of these CP strongly suggest the presence of immune complexes. A factor in HBsAg positive CP but not in HBsAg negative CP was identified by immunoelectrophoresis in the β-2 region. Reactions in this region were observed with anti-IgA, anti-IgG and anti-HBs sera. These immunoglobulins and HBsAg normally manifest distinct electrophoretic mobilities. IgG has been shown to carry anti-HBs activity (Lander, Giles & Purcell, 1971). Anti-HBs activity in the IgA class of immunoglobulins was first demonstrated at the mucosal surfaces by Ogra (1973) and has also been found in the serum by immunofluorescence techniques (Madalinski, Sztachelski-Budowsks & Brzosko, 1974). This indicates a close physical relationship between HBsAg and immunoglobulins in the CP and the presence of immune complexes. This simultaneous occurrence in CP of HBsAg, anti-HBs, complement components and immunoglobulins in the absence of rheumatoid factor adds additional evidence to suggest the presence of immune complexes in these CP. The presence of anti-HBs is particularly important since the serum of these chronic HBsAg carriers failed to manifest anti-HBs activity after repeated testing over a 3–4 year period.

Immunoglobulins and complement components were also demonstrated in the CP obtained from the control groups. However, all CP from the control groups which contained these factors also contained rheumatoid factor.

The detection of anti-HBs in some CP after, but not before, sucrose density centrifugation at pH 2·8 also suggests the presence of immune complexes. At pH 2·8, antigen–antibody complexes are known to disassociate into free antigen and free antibody. The immunofluorescent technique employed for the identification of anti-HBs by its design can only detect anti-HBs if it is complexed with HBsAg. The observed ability of a number of CP from chronic HBsAg carriers to activate the alternative pathway of the complement system also suggests the presence of immune complexes in the CP.

From the physicochemical nature of the CP it is suggested that chronic HBsAg carriers manifest a humoral immune response to HBsAg which may result in the formation of soluble immune complexes. The exact prevalence of immune complexes in chronic HBsAg carriers is difficult to estimate by cryoprecipitation. Although many properties of cryoprecipitation have been determined (Grey & Kohler, 1973), the mechanisms for the cryoprecipitation of proteins is not known. However, based on the frequency of HBsAg, anti-HBs and complement components in CP, the occurrence of immune complexes in asymptomatic chronic HBsAg carriers is not infrequent.

The observations of immune complexes reported here are consistent with previous reports which have detected immune complexes in acute and chronic type B hepatitis by various methods (Almeida & Waterson, 1969; Shulman & Barker, 1969; Millman *et al.*, 1970; Prince & Trepo, 1971; Alpert *et al.*, 1971; Coller *et al.*, 1971; Brzosko *et al.*, 1971; Bedarida, Zacchi & Tassi, 1972; Madalinski *et al.*, 1974; Wands *et al.*, 1975; McIntosh *et al.*, 1976). In a similar study, McIntosh *et al.* (1976) have recently

demonstrated immune complexes in cryoprecipitates from chronic HBsAg carriers. The content of these CP relative to immunoglobulin content, components of the complement system, HBsAg and rheumatoid factor was comparable to the findings reported here.

The occurrence of immune complexes in chronic HBsAg carriers may represent a meagre attempt of the host immune system to terminate the antigenaemia. Infusion of large quantities of anti-HBs into chronic HBsAg carriers results in a transient decrease in HBsAg titre (Reed *et al.*, 1973; Kohler *et al.*, 1974). Alternatively, the immune complexes in chronic HBsAg carriers may be significant as a factor which regulates the cell-mediated immune response. In subacute sclerosizing panencephalitis, a state induced by chronic measles virus infection, a factor which can block cell-mediated killing, migration inhibition factor activity and lymphocyte proliferation has been described (Allen *et al.*, 1973; Ahmed *et al.*, 1974; Valdimarsson, Agnarsdottir & Lachmann, 1974). Although measles virus antigen has not been demonstrated in this factor, it is precipitable by anti-immunoglobulin and anti-C3 sera. This suggests that the factor involved may be an immune complex. In a tumour system, a blocking factor composed of antigen and antibody has been shown to block T-cell function (Tamerius *et al.*, 1976). The possibility that immune complexes may regulate T-cell function is of particular interest in the light of recent reports of the absence of a detectable *in vitro* cell-mediated immune response to HBsAg in chronic HBsAg carriers (Dudley, Giustina & Sherlock, 1972; Lee *et al.*, 1975; Tong *et al.*, 1975; Ibrahim, Vyas & Perkins, 1975).

The ability of immune complexes from chronic HBsAg carriers to activate the alternative complement pathway is of interest since little is known about its role in viral infection. In a study of immune complexes in cryoprecipitates from the sera of patients with acute type B hepatitis, Wands *et al.* (1975) demonstrated the ability of these complexes to activate the alternative complement pathway. They associated this activity with the occurrence of viral-induced arthritis, while immune complexes from subjects with uncomplicated hepatitis failed to manifest such activity. In the present study the *in vitro* implications of the ability of CP to activate the alternative pathway of complement is not clear. This ability may contribute to clinically non-apparent hepatic or extra-hepatic tissue damage.

In experimental models it has been demonstrated that immune complexes bound to lymphocyte surfaces can be released by the activation of the alternative pathway of complement (Miller & Nussenweig, 1974; Miller, Saluk & Nussenweig, 1973; Miller *et al.*, 1975). Once released in this manner, the complexes lose their ability to bind to lymphocytes. This mechanism may have a regulatory effect on the immune response.

The results of this study suggest that HBsAg/anti-HBs immune complexes are present in asymptomatic chronic HBsAg carriers. The significance of these complexes is difficult to assess in the absence of clinically apparent extra-hepatic disease, although these complexes may have an immunoregulatory role.

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REFERENCES

AHMED, A., STRONG, D.M., SELL, K.W., THURMAN, G.B., KNUDSEN, R.C., WISTAR, R. & GRACE, W.R. (1974) Demonstration of a blocking factor in the plasma and spinal fluid of patients with subacute sclerosing panencephalitis. *J. exp. Med.* **139**, 902.

ALLEN, J., OPPENHEIMER, J., BRODY, J. & MILLER, J. (1973) Labile inhibitor of lymphocyte transformation in plasma from a patient with subacute sclerosing panencephalitis. *Infect. Immunity*, **8**, 80.

ALMEIDA, J.D. & WATERSON, A.P. (1969) The morphology of virus–antibody interaction. *Adv. Virus Res.* **15**, 307.

ALPERT, E., ISSELBACHER, J.K. & SCHUR, P.H. (1971) The pathogenesis of arthritis associated with viral hepatitis. *New Engl. J. Med.* **285**, 185.

ARROYAVE, C.M., VALLOTO, E.H. & MÜLLER-EBERHARD, H.J. (1974) Lysis of human erythrocytes due to activation of alternate complement pathway by nephritic factor. *J. Immunol.* **113**, 764.

AVRAMEAS, S., TERNYNCK, T. & CHUILON, S. (1969) Glutaraldehyde, cyanuric chloride and tetrazotized O-dianisidine as coupling reagents in the passive hemagglutination test. *Immunochemistry*, **6**, 67.

BACON, P.A., DOHERTY, S.M. & ZUCKERMAN, A.J. (1975) Hepatitis B antibody in polymyalgic rhematica. *Lancet*, ii, 476.

BEDARIDA, G., ZACCHI, T. & TASSI, G.C. (1972) Detection in serum hepatitis of Au anti-Au immune complex and of the antigen and antibody by means of the modified electrosyneresis technique. *Progr. Immunobiol. Standard*, **5**, 75.

BRZOSKO, W.J., MADALINSKI, K., KRAWCZYNSKI, K.,

SKWARSKA, H. & NOWOSLAWSKI, A. (1971) Australia antigen immune complexes in patients with different forms of hepatitis. *J. Infect. Dis.* **123**, 251.

CAYZER, I., DANE, D.S., CAMERON, C.H. & DENNING, J.V. (1974) A rapid haemagglutination test for hepatitis B antigen. *Lancet*, **i**, 947.

COLLER, J., MILLMAN, I., HALBHERR, T.C. & BLUMBERG, B.S. (1971) Radioimmunoprecipitation assay for Au antigen, antibody and antigen–antibody complexes. *Proc. Soc. exp. Biol. Med.* **138**, 249.

COMBES, B., STASTNY, P., SHOREY, J., EIGENBRODT, E.H., BARRERA, A., HULL, A.R. & CARTER, N.W. (1971) Glomerulonephritis with deposition of Australia antigen–antibody complexes in glomerular basement membrane. *Lancet*, **ii**, 234.

DUDLEY, F.J., GIUSTINO, V. & SHERLOCK, S. (1972) Cell-mediated immunity in patients positive for hepatitis associated antigen. *Brit. Med. J.* **iv**, 754.

EDINGTON, T.S. & CHISARI, F.V. (1975) Immunological aspects of Hepatitis B virus infection. *Amer. J. Med. Sci.* **270**, 213.

FINE, D.P., MANEY, S.R., JR., COLLEY, D.G., SERGENT, S. & DES PREZ, R.M. (1972) C3 shunt activation in human serum chelated with EGTA. *J. Immunol.* **109**, 807.

GOCKE, D.J. & HOWE, C. (1970) Rapid detection of Australia antigen by counterimmunoelectrophoresis. *J. Immunol.* **104**, 1031.

GOCKE, D.J., HSU, K., MORGAN, I., BOMBARDIERI, S., LOCKSHIN, M. & CHRISTIAN, C.L. (1970) Association between polyarteritis and Australia antigen. *Lancet*, **ii**, 1149.

GÖTZE, O. & MÜLLER-EBERHARD, H.J. (1971) The C3-Activator: An alternate pathway of the complement pathway. *J. Exp. Med.* **134**, (Suppl.), 90.

GÖTZE, O. & MÜLLER-EBERHARD, H.J. (1972) Paraxymal nocturnal hemoglobulinuria hemolysis initiated with C3 activator system. *New Engl. J. Med.* **286**, 180.

GREY, H.M. & KOHLER, P.F. (1973) Cryoimmunoglobulins. *Semin. Hematol.* **10**, 87.

IBRAHIM, A.B., VYAS, G.N. & PERKINS, A. (1975) Immune response to hepatitis B surface antigen. *Infect. Immunity*, **11**, 137.

IWARSON, S., AHLMEN, J., ERIKSSON, E., HERMODSSON, S., KJELLMAN, H., LJUNGGREN, L. & SELANDER, D. (1975) Hepatitis B immune serum and standard gamma globulin in prevention of hepatitis B infection among hospital staff: a preliminary report. *Amer. J. Med. Sci.* **270**, 385.

KOHLER, P.F., TREMBATH, J., MERRILL, D.A., SINGLETON, J.W. & DUBOIS, R.S. (1974) Immunotherapy with antibody, lymphocytes and transfer factor in chronic hepatitis B. *Clin. Immunol. Immunopathol.* **2**, 465.

LANDER, J.J., GILES, J.P. & PURCELL, R.H. (1971) Viral hepatitis type B (MS-2 strain) detection of antibody after primary infection. *New Engl. J. Med.* **285**, 303.

LEE, W.M., REED, W.D., MITCHELL, C.G., WOOLF, I.L., DYMOCK, I.W., EDDLESTON, A.L.W.F. & WILLIAMS, R. (1975) Cell-mediated immunity to hepatitis B surface antigen in blood donors with persistent antigenaemia. *Gut* **16**, 416–420.

LING, C.M. & OVERBY, L.R. (1972) Prevalence of hepatitis B virus antigen as revealed by direct radioimmunoassay with ^{125}I antibody. *J. Immunol.* **109**, 834.

MADALINSKI, K., SZATACHELSKI-BUDOWSKA, A. & BRZOSKO, W.J. (1974) DEAE-cellulose chromatography: A method for dissociation of soluble immune complexes of hepatitis B antigen. *J. Infect. Dis.* **129**, 371.

MCINTOSH, R.M., KOSS, M.N. & GOCKE, D.J. (1976) Nature and incidence of cryoprotein in Hepatitis B antigen (HBsAg) positive patients. *Quart. J. Med.* **45**, 23.

MILLER, G.W. & NUSSENZWEIG, V. (1974) Complement as a regulator of interactions between immune complexes and cell membranes. *J. Immunol.* **113**, 464.

MILLER, G.W., SALUK, P.H. & NUSSENZWEIG, V. (1973) Complement dependent release of immune complexes from the lymphocyte membrane. *J. exp. Med.* **138**, 495.

MILLER, G.W., STEINBERG, A.D., GREEN, I. & NUSSENZWEIG, V. (1975) Complement-dependent alterations in the handling of immune complexes by NZB/W mice. *J. Immunol.* **114**, 1166.

MILLMAN, I., LONDON, W.T., SUTNICK, A.I. & BLUMBERG, B.S. (1970) Australia antigen–antibody complexes. *Nature (Lond.)*, **226**, 83.

MUNOZ, J. (1971) Double diffusion in plates. *Methods in Immunology and Immunochemistry* (ed. by C.A. Williams and M.W. Chase), Vol. III, p. 146. Academic Press, New York.

NOWOSLASKI, A., KRAWCZYNSYI, K., NAZAREWICS, T. & CLUSARCZYK, J. (1975) Immunopathological aspects of Hepatitis B. *Amer. J. Med. Sci.* **270**, 229.

OGRA, P.L. (1973) Immunologic aspects of hepatitis associated antigen and antibody in human body fluids. *J. Immunol*, **110**, 1197.

OLDSTONE, M.B.A. (1975) Virus Neutralization and virus-induced immune complex disease. *Progr. Med. Virol.* **19**, 84.

PRINCE, A.M. & TREPO, C. (1971) Role of immune complexes involving SH antigen in pathogenesis of chronic active hepatitis and polyarteritis nodosa. *Lancet*, **i**, 1309.

REED, W.D., EDDLESTON, A.L.W.F., CULLENS, H., WILLIAMS, R., ZUCKERMAN, A.J., PETERS, D.K., WILLIAMS, G.W. & MAYCOCK, W.A. (1973) Infusion of hepatitis B antibody in antigen-positive active chronic hepatitis. *Lancet*, **ii**, 1347.

SHULMAN, N.R. & BARKER, L.F. (1969) Virus-like antigen, antibody, and antigen-antibody complexes in hepatitis measured by complement fixation. *Science*, **165**, 304.

TAMERIUS, J., NEPOM, J., HELLSTRÖM, I. & HELLSTRÖM, K.E. (1976) Tumor-associated blocking factors: Isolation from sera of tumor-bearing mice. *J. Immunol.* **116**, 724.

TIKU, M.L., BEUTNER, K.R., TIKU, K. & OGRA, P.L. (1978) Cell mediated immune response to liver tissue antigens and hepatitis surface antigen following hepatitis B virus infection in man. *J. Infect. Dis.* **138**, 587.

TONG, M.J., WALLACE, A.M., PETERS, R.L. & REYNOLDS, T.B. (1975) Lymphocyte stimulation in hepatitis B infection. *New Engl. J. Med.* **293**, 318.

VALDIMARSSON, H., AGNARSDOTTIR, G. & LACHMANN, P.J. (1974) Cellular immunity in subacute sclerosing pancephalitis. *Proc. Roy. Soc. Med.* **67**, 1125.

VYAS, G.N., ROBERTS, I., PETERSON, D.L. & HOLLAND, P.V. (1977) Nonspecific test reactions for antibodies to hepatitis B surface antigen in chronic HBsAg carriers. *J. Lab. clin. Med.* **89**, 428.

VYAS, G.N. & SHULMAN, N.R. (1970) Hemagglutination assay for antigen and antibody associated with viral hepatitis. *Science*, **170**, 332.

WANDS, J.R., ALPORT, E. & ISSELBACHER, K.J. (1973) Arthritis associated with chronic active hepatitis: complement activation and characterization of circulating immune complexes. *Gastroenterology*, **69**, 1286.

WANDS, J.E., MANN, E., ALPORT, E. & ISSELBACHER, K.J. (1975) The pathogenesis of arthritis associated with acute hepatitis B surface antigen positive hepatitis. *J. clin. Invest* **55**, 930.

WILLIAMS, C.A. (1971) Immunoelectrophoretic analysis in agar gels. *Methods in Immunology and Immunochemistry*, (ed. by C.A. Williams & M.W. Chase), Vol. III, p. 237. Academic Press, New York.