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IgG subclasses in circulating immune complexes with hepatitis B e antigen in chronic hepatitis B

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SUMMARY

IgG subclasses of antibodies to hepatitis B e antigen (anti-HBe) complexed to HBeAg were determined in 126 HBsAg-positive sera. In the assay HBeAg complexes were bound to microtitre plates by monoclonal anti-HBe and indicated by biotinylated monoclonals to each of the four human IgG subclasses. To evaluate the specificity of the complexed IgG, serum dilutions were also tested for HBeAg and for subclasses of anti-HBe IgG. Two groups of sera were investigated: (i) 64 sera from 64 HBsAg carriers; and (ii) 62 sera from 13 HBeAg-positive patients, of whom five seroconverted to anti-HBe. At least four sera were available from each of these patients. Complexed anti-HBe IgG was detected in 22 of 30 HBeAg-positive, and in three of HBeAg-negative carrier sera. There was no significant association between presence of complexed anti-HBe and levels of HBeAg in these sera. Complexes with multiple subclass composition were found in 13 of the 25 sera with complexed anti-HBe. The most common IgG subclasses found complexed to HBeAg were IgG1 (75%) and IgG4 (67%). A significant association ($P < 0.05$) was found between the presence of free and complexed anti-HBe IgG1 in the carrier sera, indicating that the IgG1 antibodies, complexed to HBeAg, were specific for HBeAg. In the five patients who seroconverted to anti-HBe, anti-HBe IgG1 was detected in the HBeAg-positive phase before seroconversion. In the eight patients with persistent HBeAg antigenemia, free anti-HBe IgG1 was detected in only two sera from two different patients. In one patient, complexed anti-HBe IgG1/IgG4 was detected in all serum samples drawn during a period of 111 months. In conclusion, complexed anti-HBe might be detected several years before apparent seroconversion to anti-HBe in conventional anti-HBe assays. In contrast 'free' anti-HBe IgG1, when detected in HBeAg-positive sera with our anti-HBe subclass assay, seemed to signal ensuing apparent seroconversion to anti-HBe.

Keywords IgG subclasses hepatitis B

INTRODUCTION

During acute and chronic hepatitis B (HB), circulating immune complexes (CIC) containing hepatitis B surface antigen (HBsAg) have been reported and are proposed to have a pathogenetic role in the disease (Brown *et al.*, 1983). The reports are conflicting as to whether HBsAg complexed to IgM in acute HB is an early marker for progression to a chronic hepatitis B virus (HBV) infection (Careoda *et al.*, 1982; Grangeot-Keros *et al.*, 1988). Little is known about CIC with hepatitis B core antigen (HBcAg), or about the nature of the plasma proteins complexed to hepatitis B e antigen (HBeAg) in serum of patients with chronic HB. A molecular relationship has been shown to exist between HBeAg and HBcAg by denaturation of native HBcAg or recombinant HBcAg (rHBcAg), thereby revealing antigenic epitopes with HBeAg specificity (Ohiro *et al.*, 1980). Despite their close relationship, HBcAg and HBeAg are dealt with differently by the immune system (Milich *et al.*, 1988). HBcAg has been reported to have one epitope reactive to B cells (Ferns & Tedder, 1986), whereas at least two epitopes have been demonstrated on HBeAg (Ferns & Tedder, 1984). Anti-HBe has recently been shown to consist mainly of the IgG subclasses IgG1 and IgG4 (Sällberg *et al.*, 1989; Sällberg, Norder & Magnius, 1990) and anti-HBe isotypes other than IgG were not detected.

IgG has been demonstrated to be complexed with HBeAg in patients with chronic HB (Takahashi *et al.*, 1978). CIC containing HBeAg and IgA, IgG and IgM were found in a patient with glomerulonephritis (Takekoshi *et al.*, 1979). They suggested that HBeAg CIC may play a pathogenetic role in this condition.

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The investigators did not determine whether the complexed IgG was anti-HBe, or was just IgG unspecifically bound to HBeAg. The present study was undertaken to analyse the isotype composition and specificity of IgG complexed to HBeAg.

MATERIALS AND METHODS

Materials

Two groups of sera were investigated. The first group consisted of 64 sera from the same number of chronic HBsAg carriers, negative for antibody to hepatitis delta virus (anti-HD) and described previously (Sällberg *et al.*, 1990). These sera were found HBsAg positive throughout consecutive serum samples sent to the Department of Virology during three periods, January to July during the years 1986, 1987 and 1988. Apart from HBsAg, all sera had been tested for HBeAg/anti-HBe, anti-HBc, anti-HBc IgM, anti-HBs, and anti-HD using commercial radioimmunoassays (RIAs; Abbott Laboratories, IL).

The second group consisted of 62 sera from 13 patients all negative for anti-HD, with the clinical and serological diagnosis of chronic HB, followed and sampled at Roslagstulls Hospital, Stockholm. Of these sera 22 derived from five patients who seroconverted to anti-HBe during follow-up. The patients were followed during periods ranging from 10 to 62 months (mean 32 months). The remaining 40 sera derived from eight patients, who were all persistently positive for HBeAg during follow-up lasting from 13 to 111 months (mean 46 months).

HBV-DNA was determined in all patient sera using a dot-blot procedure according to the method described previously (Norder, Brattström & Magnius, 1989).

Determination of IgG subclasses complexed to HBeAg

The enzyme immunoassays (EIAs) for the determination of IgG complexed to HBeAg were all performed as follows. A 96-well polystyrene plate (Nunc 96F, Copenhagen, Denmark), was coated overnight at 4°C with 50 μl monoclonal antibody (MoAb) to HBeAg (clone 57/8; Behringwerke A.G., Marburg, Germany), diluted to a final concentration of 500 ng/ml in 0·05 M $NaHCO_3$, pH 9·6. The sera to be tested were diluted 1/100 in phosphate-buffered saline (PBS) with 0·05% Tween 20 and 1% bovine serum albumin (BSA; PBS-TA). Between each step in the assay, the plate was washed 10 times in PBS with 0·05% Tween 20 (PBS-T). To reduce possible unspecific binding, 100 μl of PBS with 2% BSA were added to each well and the plate was incubated for 20 h at 4°C. The serum dilutions were added and the plate was incubated overnight at 4°C. After washing, biotinylated MoAbs to human IgG subclasses were added and the plate was incubated for 90 min at 37°C. The plate was washed and 50 μl horseradish peroxidase (HRPO)-conjugated streptavidin (HRPO-SA; Zymed, San Francisco, CA), diluted 1/6000 in PBS-TA, was added. After another incubation for 30 min at ambient temperature and washing, the plate was developed by adding 50 μl orthophenylene diamine (OPD; Sigma Chemicals, St Louis, MO). After 30 min incubation at ambient temperature, the reaction was terminated with 50 μl 0·5 M H_2SO_4 and the absorbancies were read at 492 nm in a Kontron SLT 210 spectrophotometer.

The specificities and designations of the MoAbs were as follows: anti-IgG1, clone HP6069, diluted 1/1000; anti-IgG2, clone HP6002, diluted 1/2000; anti-IgG3, clone HB6047, diluted 1/4000; and anti-IgG4, clone HP6025, diluted 1/1000 in PBS-TA. All clones were purchased from Zymed, CA. All sera were tested for HBeAg diluted 1/100, using a RIA described previously (Norder *et al.*, 1989), and also for subclasses of anti-HBe IgG, anti-HBe IgM and anti-HBe IgA1, using EIAs described previously (Sällberg *et al.*, 1989).

Standardization of results and calculation of end-point titres

In each assay at least 15 sera, negative for all HBV markers, were used for the calculation of the mean and s.d. of the absorbancies for negative samples. The mean +3 s.d. was used as cut-off in all assays, except for the anti-HBe RIA where 50% inhibition was used as cut-off.

RESULTS

In Table 1 results are given from testing the 64 HBV carrier sera for subclasses of free and complexed anti-HBe IgG. Complexed anti-HBe IgG was found in 22 of 30 sera positive for HBeAg, and in two out of 33 sera with anti-HBe. Twenty-four out of 30 carrier sera found positive for HBeAg, when tested undiluted,

Table 1. Outcome from testing 64 carrier sera for subclasses of IgG in circulating immune complexes with HBeAg by EIA, and for HBeAg/anti-HBe by RIA (Abbott)

Per cent inhibition in anti-HBe RIA	HBeAg/anti-HBe status in RIA	No. of sera positive for HBV-DNA	No. of sera positive by EIA for IgG of indicated subclass complexed to HBeAg			Total out of tested sera
			IgG1	IgG3	IgG4	
<25	+/−	17	10	2	9	12/18
25–50	+/−	11	7	3	6	10/12
25–50	−/−	0	1	0	0	1/1
51–70	−/+	2	1*	0	0	1/2
71–85	−/+	0	0	0	0	0/1
>85	−/+	2	0	0	1*	1/30
Total		32	19	5	16	25/64

* One sample found negative for HBeAg but positive for HBV-DNA and anti-HBe.

were also found positive for HBeAg at a dilution of 1/100. No correlation was found between the sample/negative ratio (S/N) in HBeAg RIA and absorbancy levels in any of the subclass-specific EIAs for complexed anti-HBe ($P > 0.05$).

A significant correlation ($P < 0.05$; Fisher's test) was found between the presence of free and complexed anti-HBe IgG1 in these 24 sera, since all seven sera positive for free anti-HBe IgG1 contained complexed anti-HBe IgG1, while only nine of the 17 sera without free anti-HBe IgG1 contained complexed anti-HBe IgG1. No such relation was found for free and complexed anti-HBe IgG3 or anti-HBe IgG4 among these 24 sera.

In Table 2 the results of testing the patient sera for HBV-DNA and HBeAg at a serum dilution 1/100 are given.

The results of testing the patient sera for complexed and free anti-HBe IgG1 and IgG4 are shown in Table 2 and Figs 1 and 2, respectively. Complexed anti-HBe IgG3 was detected in one

Table 2. Background data, histopathology, relative time for serum sampling, and HBV-DNA in serum in (a) eight patientrs with chronic HB, who showed no change in HBeAg/anti-HBe status, and (b) five patients who seroconverted to anti-HBe during the follow-up period

(a) Patient	Sex/age (years)	Epidemiological background	Histopathology	Months from first serum	HBV-DNA	S/N in RIA for HBeAg at 1/100	Complexed anti-HBe IgG1	Complexed anti-HBe IgG4	Free anti-HBe IgG1	Free anti-HBe IgG4
1	M/24	Intravenous drug abuser	CAH	0	—	0·9	–	–	–	–
				11	–	0·5	–	–	–	–
				36	–	0·6	–	–	–	–
				43	–	0·9	–	–	–	–
2	M/28	Homosexual	CPH progressing to CAH	0	+	5·3	+	+	–	–
				2	+	4·7	+	+	+	–
				10	+	6·2	+	+	–	+
				22	+	6·6	+	+	–	+
				28	+	7·7	+	+	–	+
3	M/29	Homosexual	No biopsy	0	+	4·4	+	+	–	+
				16	+	4·4	+	+	–	+
				38	+	4·4	+	–	–	–
				57	+	2·5	–	–	–	–
4	M/33	Immigrant from Turkey	CAH	0	–	0·7	–	–	–	–
				1	–	2·0	–	–	–	–
				4	–	2·8	–	–	–	–
				34	–	4·1	–	–	–	–
5	M/38	Transfusion	No biopsy	0	+	3·9	–	–	–	–
				3	+	4·5	–	–	–	–
				7	+	8·2	–	–	–	–
				9	–	4·8	–	–	–	–
				15	–	3·2	–	–	–	–
				19	–	0·5	+	+	–	–
				22	+	4·2	+	+	–	–
				39	+	4·7	–	–	–	–
6	M/42	Homosexual	CAH progressing to cirrhosis	0	+	5·2	–	–	–	–
				4	+	4·9	–	–	–	–
				15	+	3·0	–	–	–	–
				27	+	4·8	–	–	–	–
				40	–	3·1	–	–	–	–
7	M/47	Homosexual	CPH	0	+	8·4	+	+	–	+
				24	+	5·9	+	+	–	+
				60	+	6·7	+	+	–	+
				81	+	8·3	+	+	–	+
				111	+	7·2	+	+	–	+
8	F/22	Immigrant from Korea	No biopsy	0	–	4·0	+	–	+	+
				2	–	1·2	–	–	–	–
				4	–	3·1	–	–	–	–
				7	–	1·5	–	–	–	–
				13	–	1·4	–	–	–	–
Cut-off (mean + 3 s.d.)				—	—	2·1	–	–	–	–

Table 2. *Continued.*

(b)

Patient	Sex/age (years)	Route of infection	Histopathology	Months from first serum	HBV-DNA	S/N in RIA for HBeAg at 1/100		Percentage inhibition in RIA for anti-HBe	Complexed anti-HBe		Free anti-HBe	
						1/1	1/100		IgG1	IgG4	IgG1	IgG4
9	M/27	Intravenous drug abuser	No biopsy	0	+	8·3	6·4	15	+	−	+	+
				1	+	7·3	2·3	30	+	−	+	−
				2	ND	1·6	1·4	53	−	−	+	−
				7	+	1·1	0·9	93	−	−	+	+
				10	−	0·7	0·6	100	−	−	+	+
10	M/33	Homosexual	No biopsy	0	+	11·2	4·6	23	−	−	+	−
				12	−	2·2	1·0	65	−	−	+	−
				24	−	1·1	1·1	46	−	−	−	−
				27	−	1·2	0·9	67	−	−	−	−
11	M/36	Homosexual	CAH progressing to cirrhosis	0	+	8·0	1·7	42	−	−	+	−
				7	+	0·9	0·7	46	−	−	+	−
				12	+	1·3	0·6	47	−	−	+	−
				26	+	1·3	0·7	56	−	−	+	−
12	M/49	Homosexual	CAH/cirrhosis	0	+	10·2	4·1	34	+	+	+	+
				1	+	8·9	4·5	42	+	+	+	+
				8	+	0·8	1·0	68	−	−	+	+
				11	−	0·7	0·7	84	−	−	+	+
				14	−	0·7	0·8	80	−	−	+	+
13	F/20	Immigrant from Korea	CAH with early cirrhosis	0	+	3·8	1·9	63	−	−	+	+
				8	−	2·2	0·7	67	−	−	+	−
				13	−	0·6	0·9	92	−	−	+	−
				37	−	0·7	0·9	94	−	−	+	−
Cut-off (mean + 3 S.D)				—	−	1·8	1·4	50	−	−	−	−

S/N, sample to negative ratio. CAH, chronic acute hepatitis; CPH, chronic persistent hepatitis; ND, not done.

serum from patient no. 5 and in four sera from patient no. 9. Free anti-HBe IgG3 was detected in three sera from patient 11 and in two sera from patient 13.

In the eight patients who were persistently HBeAg positive, there was a positive relation between the presence of HBV-DNA in serum and complexed anti-HBe (Table 1). Among these eight patients, three homosexual men (nos 2, 3, and 7) were positive for HBeAg at a serum dilution 1/100 and for HBV-DNA in all sera. All these had demonstrable complexed anti-HBe in serum and, in one (no. 7), complexed anti-HBe IgG1/4 persisted up to 111 months. One patient (no. 1), who was negative for HBeAg at a serum dilution 1/100 and for HBV-DNA in all sera, lacked demonstrable complexed anti-HBe. One patient (no. 6), who lost HBV-DNA in his last serum also lacked demonstrable complexed anti-HBe. Out of two patients with fluctuating HBeAg levels (nos 4 and 8), who were negative for HBV-DNA in all sera, one (no. 8) had detectable complexed anti-HBe in a sample with the highest recorded level of HBeAg. The remaining patient (no. 5), who did not seroconvert and who was positive for HBeAg at a serum dilution 1/100 and for HBV-DNA during two periods, showed complexed anti-HBe in sera preceding the HBV reactivation.

In all the five patients who seroconverted, anti-HBe IgG1 was detected before seroconversion to anti-HBe. Only two sera from two different patients who did not seroconvert were positive for free anti-HBe IgG1. The results obtained from testing all sera for complexed anti-HBe are given in Table 3. As shown, anti-HBe IgG1 and IgG4 were the predominating isotypes complexed to HBeAg in chronic HB. Multiple IgG isotype composition of complexed anti-HBe was detected in 13 out of 25 carrier sera and in 16 out of 22 patient sera with complexed HBeAg.

DISCUSSION

As described previously, HBeAg occurs both in free and complexed forms in chronic HB (Takahashi *et al.*, 1978). We have demonstrated that the IgG1 subclass is the most common IgG isotype complexed to HBeAg, and since a positive relation was found between complexed and free anti-HBe IgG1, that these antibodies should be regarded specific for HBeAg. The finding, that IgG1 is the dominant subclass of anti-HBe IgG complexed to HBeAg, corroborates what is known for free anti-HBe IgG (Sällberg *et al.*, 1989, 1990). IgG1 has also been reported to be the predominant IgG subclass of anti-HBs (Skvaril & Joller-Jemelka, 1984), and of the anti-HBc in chronic and acute HB (Sällberg *et al.*, 1988; Sällberg & Magnius, 1989). Reports dealing with the IgG subclass response to other viruses have shown similar results (Coleman *et al.*, 1985; Linde *et al.*, 1988; Skvaril, 1986). Anti-HBe IgG2 was not detected, either free or complexed. Similarly IgG2 to other viral antigens seems to be of low importance (Rossi *et al.*, 1988; Skvaril, 1986).

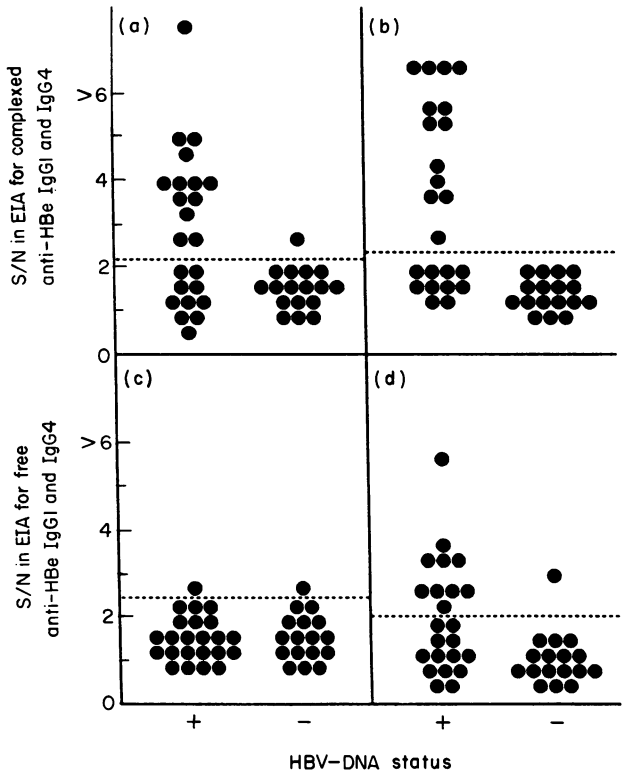

Fig. 1. Prevalence of complexed anti-HBe IgG1 (a) and IgG4 (b), and free anti-HBe IgG1 (c) and IgG4 (d) in relation to presence of HBV-DNA in 40 sera from eight patients, who showed no change in HBe status. Values are given as sample to negative ratio (S/N) when tested by EIA.

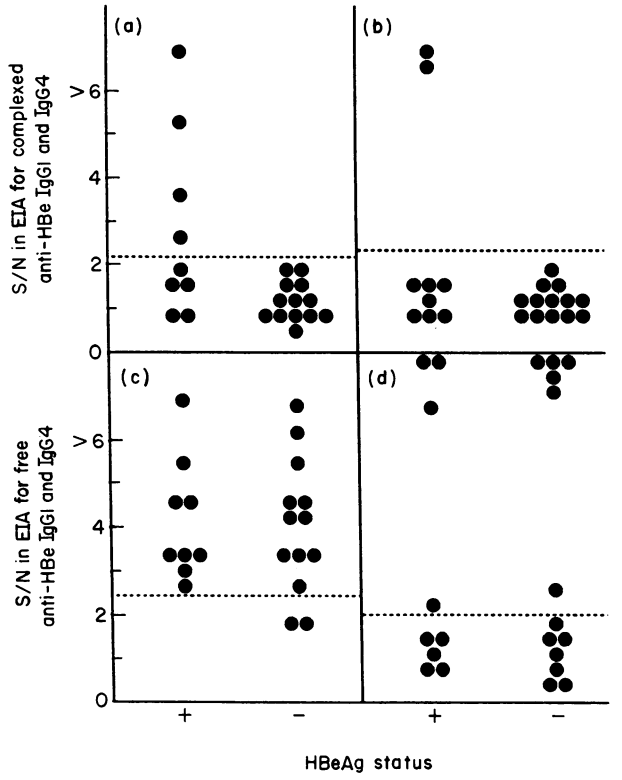

Fig. 2. Prevalence of complexed anti-HBe IgG1 (a) and IgG4 (b), and free anti-HBe IgG1 (c) and IgG4 (d) in relation to HBeAg status in 22 sera from five patients who cleared HBeAg. Values are given as sample to negative ratio (S/N) when tested by EIA.

It is likely that the non-significant association between free and complexed anti-HBe IgG3 might be explained by complexes containing IgG3 being more efficiently cleared from the circulation (Rath & Devey, 1988). The low prevalence of complexed anti-HBe IgG3 is in agreement with IgG3 being a minor anti-HBe IgG subclass (Sällberg *et al.*, 1989, 1990). It is worth noting that this does not correlate with what is known from the other HBV antigens, HBsAg and HBcAg, where IgG3 next to IgG1 has been found to be the predominating IgG isotype (Skvaril & Joller-Jemelka, 1984; Sällberg *et al.*, 1988, 1989).

Regarding IgG4, the next most common IgG isotype found complexed to HBeAg, it is notable that Neurath & Strick (1977) proposed that HBeAg had the properties of an immunoglobulin, in particular IgG4. In the light of our findings, this assumption might be explained by the high level of complexed anti-HBe IgG4, as detected in some of our patient sera. This finding is similar to that of Rath & Devey (1988), who reported high levels of IgG4 complexed to HBsAg. We found that anti-HBe IgG4 often occurred complexed to HBeAg in combination with anti-HBe IgG1, similar to the reported co-occurrence of anti-HBs IgG4 and IgG1 in complexes with HBsAg (Rath & Davey, 1988). In fact, among the consecutive carrier sera, IgG1 and IgG4 are found to co-occur in 79% of the complexes with multiple isotype composition. The possibility that the complexed IgG4 might represent an anti-idiotype should be kept in mind.

As shown, when testing sera from the five patients who seroconverted to anti-HBe, and sera from the eight patients with persistent HBeAg antigenemia, HBeAg-IgG CIC was found to be common in HBeAg-positive sera. These complexes may persist in serum of chronic HB patients for up to 9 years. This suggests that the detection of complexed anti-HBe does not predict ensuing conversion to anti-HBe in serum. However, the finding of anti-HBe IgG1 in chronic HB patients seems to predict apparent seroconversion to anti-HBe, implying that the development of anti-HBe IgG1 might signal immune clearance of this antigen.

With current techniques, we were unable to test for HBeAg-CIC containing IgM or IgA. Though both of these isotypes have been reported complexed to HBeAg by Takekoshi *et al.* (1979); we have previously failed to detect these isotypes as uncomplexed anti-HBe in either acute or chronic HB (Sällberg *et al.*, 1989, 1990).

The lack, or undetectable levels, of anti-HBe IgM and IgA1 is consistent with a report by Bruderer & Heusser (1988). When immunizing primed mice with immune complexes containing T cell-dependent antigens they found a smaller increase in the IgM levels than the increase found when immunizing with free antigen. This result was not affected by the immunoglobulin isotype in the complex. Since HBeAg has been reported to be complexed and also strictly T cell dependent (Milich *et al.*, 1988), this supports the lack of detectable anti-HBe IgM despite a persistent antigenic stimulation.

Our results indicate that IgG1 complexed to HBeAg during chronic HB is specific for HBeAg, since if IgG were unspecifically bound to HBeAg a direct relationship would be anticipated between levels of HBeAg and complexed IgG. Our

Table 3. Prevalence of IgG subclasses and subclass composition of circulating immune complexes containing HBeAg in all sera tested irrespective of HBeAg/anti-HBe status

Source of HBsAg-positive sera	No. of sera with indicated single IgG isotype complexed to HBeAg			No. of sera with multiple IgG subclasses complexed to HBeAg of indicated composition				No. of sera with complexed anti-HBe IgG out of tested sera
	IgG1	IgG3	IgG4	IgG1+3	IgG1+4	IgG3+4	IgG1+3+4	
Carrier sera	7	1	4	1	9	2	1	25/64
Patient sera								
Non-seroconverting	2	1	1	0	12	0	0	16/40
Seroconverting	0	2	0	2	2	0	0	6/22
Total	9	4	5	3	23	2	1	47/126

results also show that complexed anti-HBe IgG1 might occur up to 10 years before the apparent seroconversion from HBeAg to anti-HBe. In contrast, the finding, of 'free' anti-HBe IgG1 in our subclass assay in sera from patients with HBeAg-positive chronic HB seems to be an indicator for ensuing seroconversion in generally employed anti-HBe assays. The presence of 'free' anti-HBe IgG1 in HBeAg-positive might be due to different HBeAg epitopes being involved or that bound anti-HBe dissociates from HBeAg complexes allowing anti-HBe to be detected in the anti-HBe subclass assay. Seroconversions mediated by antiviral treatment are worth studying in the future with regard to 'free' and complexed anti-HBe isotypes.

REFERENCES

BROWN, S.E., STEWARD, M.W., VIOLA, L., HOWARD, C.R. & MURRAY-LYON, I.M. (1983) Chronic liver disease: the detection and characterization of circulating immune complexes. *Immunology*, **49**, 673.

BRUDERER, U. & HEUSSER, C. (1988) Regulation of immunoglobulin isotype expression in mice by antibodies in immune complexes. *Immunol. Lett.* **17**, 235.

CAREODA, F., DE FRANCHIS, R., D'AMINIO-MONFORTE, A., VECCHI, M., ROSSI, E., PRIMIGNANI, M., PALLA, M. & DIOGUARDI, N. (1982) Persistence of circulating HBsAg/IgM complexes in acute viral hepatitis type B: An early marker of chronic evolution. *Lancet*, **ii**, 358.

COLEMAN, R.M., NAMHIAS, A.J., WILLIAMS, S.C., PHILLIPS, D.J., BLACK, C.M. & REIMER, C.B. (1985) IgG subclass antibodies to Herpes Simplex *Virus. J. infect. dis.* **151**, 929.

FERNS, R.B. & TEDDER, R.S. (1984) Monoclonal antibodies to hepatitis B e antigen (HBeAg) derived from hepatitis B core antigen (HBcAg): Their use in characterization and detection of HBeAg. *J. gen. Virol.* **65**, 899.

FERNS, R.B. & TEDDER, R.S. (1986) Human and monoclonal antibodies to hepatitis B core antigen recognise a single immunodominant epitope. *J. med. Virol.* **19**, 193.

GRANGEOT-KEROS, L., PELLETIER, P., GILLES, M., BRIANTAIS, M.J. & PILLOT, J. (1988) Prognostic value of HBsAg/IgM complexes in hepatitis B patients: Nature of the proteins involved. *J. med. Virol.* **25**, 309.

LINDE, A., SUNDQVIST, V.A., MATHIESEN, T. & WAHREN, B. (1988) IgG subclasses to subviral components. *Monogr Allergy*, **23**, 27.

MILICH, D.R., MCLACHLAN, A., STAHL, S., WINGFIELD, P., THORNTON, G.B., HUGHES, J.L. & JONES, J.E. (1988) Comparative immunogenicity of hepatitis B virus core and e antigens. *J. Immunol.* **141**, 3617.

NEURATH, A.R. & STRICK, N. (1977) Host specificity of a serum marker for hepatitis B: evidence that e antigen has the properties of an immunoglobulin. *Proc. natl Acad. Sci. USA*, **74**, 1702.

NORDER, H., BRATTSTRÖM, C. & MAGNIUS, L.O. (1989) High frequency of HBV DNA in anti-HBe positive sera on longitudinal follow-up of patients with renal transplants and chronic hepatitis *B. J. med. Virol.* **27**, 322.

OHIRO, H., YAMAKI, M., ONODERA, S., YAMADA, E. & ISHIDA, N. (1980) Antigenic conversion of HBcAg to HBeAg by degradation of hepatitis B core particles. *Intervirology* **13**, 74.

RATH, S. & DEVEY, M.E. (1988) IgG subclass composition of antibodies to HBsAg in circulating immune complexes from patients with hepatitis B virus infection. *Clin. exp. Immunol.* **72**, 164.

ROSSI, M.A., AZZARI, C., RESTI, M., APPENDINO, C., PEZZATI, P. & VIERUCCI, A. (1988) Selectivity in IgG subclass response to hepatitis B vaccine in infants born to HBsAg-positive mothers. *Clin. exp. Immunol.* **72**, 196.

SÄLLBERG, M., NORDER, H., LINDE, A. & MAGNIUS, L.O. (1988) Subclass pattern of anti-HBc IgG in chronic hepatitis B: Relation to HBeAg/anti-HBe and HBV-DNA. In '*Viral Hepatitis and Liver Disease*' (ed. by A. J. Zuckerman) p. 244. Alan R. Liss, NY.

SÄLLBERG, M. & MAGNIUS, L.O. (1989) An enzyme immunoassay for anti-HBc IgG1: Significance of low level results in competitive assays for anti-HBc. *J. clin. Microbiol.* **27**, 849.

SÄLLBERG, M., NORDER, H., WEILAND, O. & MAGNIUS, L.O. (1989) Immunoglobulin isotypes anti-HBc and anti-HBe, and HBV DNA elimination in acute hepatitis B with rapid and slow clearance of HBsAg. *J. med. Virol.* **29**, 296.

SÄLLBERG, M., NORDER, H. & MAGNIUS, L.O. (1990) Comparison of class and subclass distribution of antibodies to the hepatitis B core and e antigens in chronic hepatitis B. *J. med. Virol.* **30**, 1.

SKVARIL, F. & JOLLER-JEMELKA, H. (1984) IgG subclasses of anti-HBs antibodies in vaccinated and non-vaccinated individuals and in anti-HBs immunoglobulin preparations. *Int. Arch. Allergy appl. Immunol.* **73**, 330.

SKVARIL, F. (1986) IgG subclasses in viral infections. *Monogr. Allergy* **19**, 134.

TAKAHASHI, K.M., IMAI, M., MIYAKAWA, Y., IWAKIRI, S. & MAYUMI, M. (1978) Duality of hepatitis B e antigen in serum of persons infected with hepatitis B virus: evidence for the nonidentity of e antigen with immunoglobulins. *Proc. natl. Acad. Sci. USA*, **75**, 1952.

TAKEKOSHI, Y., TANAKA, M., MIYAKAWA, H., YOSHIZAWA, K., TAKAHASHI, K. & MAYUMI, (1979) Free "small" and IgG associated "large" hepatitis B e antigen in the serum and glomerular capillary walls of two patients with membranous glomerulonephritis. *N. Engl. J. Med.* **300**, 814.