Relationship between HBV-specific DNA polymerase and HBe antigen/antibody system in chronic HBV infection: factors determining selection of patients and outcome of antiviral therapy*

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SUMMARY The sera of 23% of HBe antigen positive patients with chronic hepatitis are HBV-DNA polymerase negative. These patients are probably undergoing spontaneous seroconversion from a state of high to low viral replication and do not require antiviral therapy. In chronic HBV infection rapid changes in viral replication as a result of antiviral therapy are reflected by changes in HBV-DNA and HBV-DNA polymerase but not by changes in HBe antigen concentrations. Disappearance of HBe antigen from serum may be delayed for 180 days after permanent inhibition of HBV replication with adenine arabinoside or its monophosphate derivative.

It is established that during chronic HBV infection there is a phase of high-level viral replication when the patient is HBe antigen (HBeAg) and HBV-DNA polymerase (DNAp) positive, and this is followed by a phase of chronic HBs antigenaemia, without detectable viral replication, when there is antibody to HBeAg (anti-HBe) in the serum. These phases are of clinical significance in that during the period of HBe antigenaemia a patient is highly infectious,[1] and there is more rapid progression of hepatic inflammatory activity.[2 3] Spontaneous clearance of HBeAg occurs relatively infrequently (3–15% of cases per annum)[4 5] and thus both natural and synthetic antiviral agents are used to accelerate this process.[6–9]

In this study we have examined a group of HBeAg positive patients to determine whether the level of HBV-DNA polymerase activity is useful in predicting whether a patient will convert

spontaneously to a state of low-level replication and therefore not require antiviral therapy. In order to do this, the HBeAg/anti-HBe status and level of DNAp were determined in a group of patients with chronic HBV infection, and selected patients followed to determine whether clearance of HBeAg occurred. In addition, we examined the sequence of virological and serological events occurring during and after antiviral therapy, to determine the most useful techniques for monitoring such therapy.

Methods

Part 1 DETERMINATION OF HBeAg/ANTI-HBe AND HBV-DNAp STATUS OF PATIENTS WITH HBSAg POSITIVE CHRONIC HEPATITIS OR CIRRHOSIS

Patients
Seventy-four patients with HBsAg positive chronic liver disease were studied. Their histological diagnoses, at the time of the study were: chronic persistent hepatitis (19 patients), chronic active hepatitis (20 patients), active cirrhosis (17 patients), and inactive cirrhosis (18 patients). Four patients who were HBeAg positive and DNAp negative when first studied and who had received no steroid treatment throughout their course were followed serially.

Part 2 DETERMINATION OF HBeAg TITRE AND
HBV-DNAp DURING AND AFTER ANTIVIRAL
THERAPY

Patients

Fifteen patients with HBsAg/HBeAg/DNAp positive non-cirrhotic chronic active hepatitis underwent antiviral therapy with adenine arabinoside (ARA-A) or its highly soluble monophosphate derivative (ARA-AMP) (Table). All the patients had been HBeAg and DNAp positive for at least six months and had not received steroids and/or immunosuppressants during this period. The exception was case 6 in whom steroids were stopped two months before treatment.

Patients were classified at the end of a follow-up period of 360 days as 'non-responders' to treatment if they had only temporary inhibition of DNAp and did not lose HBeAg and as 'responders' if they had permanent inhibition of DNAp and eventually lost HBeAg. The mean duration of treatment with ARA-A (MP) was 20±3·5 days (SEM) for the responders and 13±1·5 days for the non-responders. Samples were taken at day 0 (before starting treatment), one, seven, 14, 21, 28, and thereafter every 30 days until 360 days. Sera were stored at −20°C until the end of the observation period, when they were thawed once for the determination of all viral markers.

VIROLOGY AND SEROLOGY

HBsAg was detected by radioimmunoassay (Ausria II, 125 Abbot) and quantified by 'rocket' immuno-electrophoresis[10] (data expressed as a percentage of a laboratory standard). HBeAg was detected by radioimmunoassay (Abbot Laboratories) and quantified by serial dilution, the titre expressed as the lowest dilution at which the ratio between sample and mean negative control dropped below 2·1. Data were expressed as Log_2 reciprocal titre. HBV-DNAp was measured by the method of Marion *et al.*[11] The titrated nucleotide used was methyl-thymidine 5′-triphosphate. The upper limit of the normal range was 850 dpm/200 μl (2 standard deviations above the mean of 50 negative controls), and levels above this were regarded as positive. Data are expressed as dpm × 10^3/200 μl. Serum HBV-DNA in case 3 was measured by hybridisation with ^{32}P cloned HBV-DNA.[12] The autoradiograph spots were quantified photometrically and HBV-DNA expressed as area in arbitrary units under the peak after 11 days' exposure. Student's *t* test was used for statistical analysis.

Results

Part 1 DETERMINATION OF HBeAg/ANTI-HBe AND
HBV-DNAp STATUS OF PATIENTS WITH HBsAg
POSITIVE CHRONIC HEPATITIS OR CIRRHOSIS

The relationship between HBeAg/anti-HBe and HBV-DNAp status and histological diagnosis is shown in Fig. 1. Patients in the non-cirrhotic groups (chronic persistent hepatitis or chronic active hepatitis) were significantly younger (mean age 33 and 34 years) than those with cirrhosis (active

Table *Details of antiviral therapy*

Case	Drug	Dose (mg/kg/day)	Route	Duration of therapy (days)
Responders				
1	ARA-A	10	IV	14
2	ARA-AMP	5	IM	34
3	ARA-AMP	10–5	IM	24
4	ARA-AMP	10–5	IM	21
5	ARA-AMP	15	IM	11
6	ARA-A	10	IV	14
Non-responders				
7	ARA-AMP	10–5	IM	13
8	ARA-A	10	IV	14
9	ARA-AMP	10	IV	10
10	ARA-A	10	IV	10
11	ARA-A	20–5	IV	15
12	ARA-AMP	10	IM	10
13	ARA-AMP	10	IM	10
14	ARA-A	10–5	IV	20
15	ARA-AMP	15	IM	14

Dose (mg/kg/day): 10–5 = 10 mg for 5–7 days followed by 5 mg;
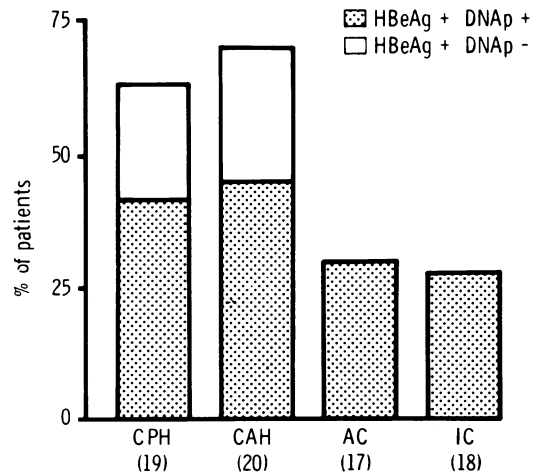20–5 = 20 mg for 6 days followed by 5 mg.

Fig. 1 *HBeAg, DNA polymerase, and histology.*

cirrhosis or inactive cirrhosis) (mean age 47 and 47 years) (p<0·05). The percentage of patients with HBeAg was significantly higher (p<0·05) in the non-cirrhotic compared with the cirrhotic groups. Twenty-three per cent of the patients in the chronic persistent hepatitis and chronic active hepatitis groups, however, but none of the cirrhotics were HBeAg positive and DNAp negative. HBsAg concentrations were higher in chronic persistent hepatitis (58·8±19 units: mean ± SEM) than in chronic active hepatitis (32·7±13 units) and significantly lower (16·3±7 and 19·3±7 units) (p<0·01) in active cirrhosis or inactive cirrhosis.

Two patients with chronic persistent hepatitis and two with chronic active hepatitis, who were HBeAg positive and DNAp negative when first seen were studied prospectively (Fig. 2). In two patients, HBeAg disappeared from the serum during follow up. DNAp remained negative and anti-HBe appeared. In one patient HBeAg disappeared and anti-HBe appeared after two months; this, however, was followed by reappearance of HBeAg, which was still present, albeit together with anti-e, two months later. The fourth patient, whose DNAp had always been at the upper limit of the normal range, did not show evidence of seroconversion from HBeAg to anti-HBe throughout a two-year period.

Part 2 DETERMINATION OF HBeAg TITRE AND HBV-DNAP DURING AND AFTER ANTIVIRAL THERAPY

The titre of HBeAg and the level of DNAp in serum were similar before starting ARA-A (MP) in the 'responder' and 'non-responder' patients (Fig. 3).
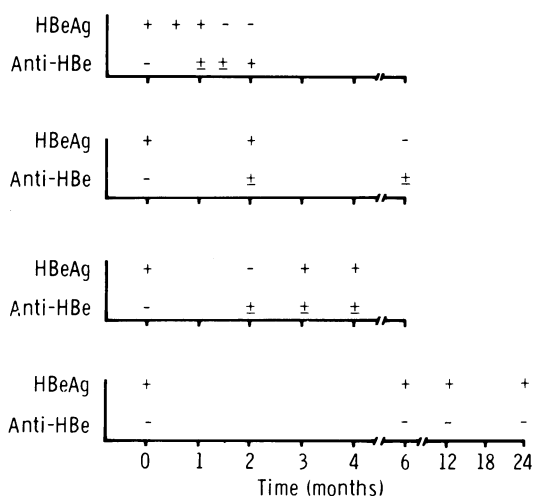

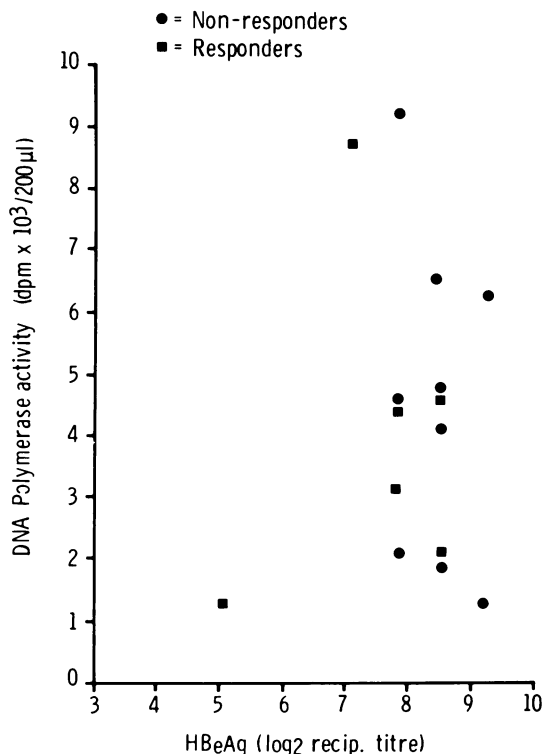Fig. 2 *HBeAg DNAp patients: prospective study.*

Fig. 3 *HBeAg titre and DNA polymerase before antiviral therapy.*

The only patient with a low HBeAg titre before treatment (case 6) had had a fourfold decline in DNAp during the previous two months: he was the only subject who had recently received steroids. There was no correlation between HBeAg and DNAp at this stage (r = 0·069).

In the 'non-responders' there was a peak inhibition of DNAp seven days after starting treatment (Fig. 4) but enzyme activity remained constant thereafter. In contrast the 'responders' showed a longer lasting inhibition of DNAp – because of the longer period of treatment – then, after therapy, a moderate rebound to levels approximately 50% lower than pretreatment values. Within 90 days of starting treatment, all the patients in the 'responder' group showed a permanent DNAp negativity. After the 21st day the difference in DNAp activity between the two groups was statistically significant (p<0·01). In the 'non-responders' the HBeAg titre remained at pretreatment levels for the entire period of follow-up of 360 days. There was no fall in titre which could be related to the temporary fall in DNAp activity. In
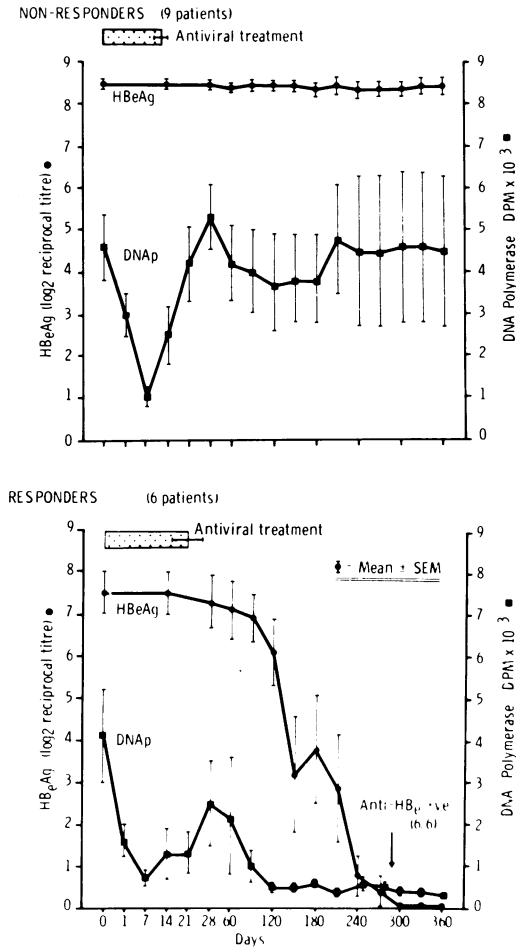
Fig. 4   *HBeAg and DNA polymerase during and after antiviral therapy. Hatched area = negative range for DNAp (up to 0·85 dpm × 10³). The wide SEM during the later follow-up of the 'non-responders' is accounted for by one case with an extremely high DNAp activity (more than five times the mean of the other cases).*

contrast, in the 'responders', HBeAg titre began to decline at 28 days, reached a 50% reduction at 95±9 days, and disappeared after 235±24 days. The mean delay between DNAp and HBeAg disppearance from the serum in this group was 178±20 days. Anti-HBe appeared after 262±23 days in all the 'responders' and was always absent in the 'non-responders'.

Serum HBV-DNA concentration in case 3 parallelled the changes in DNAp (Fig. 5).

None of the treated patients studied lost HBsAg. While the concentration of HBsAg in the 'non-
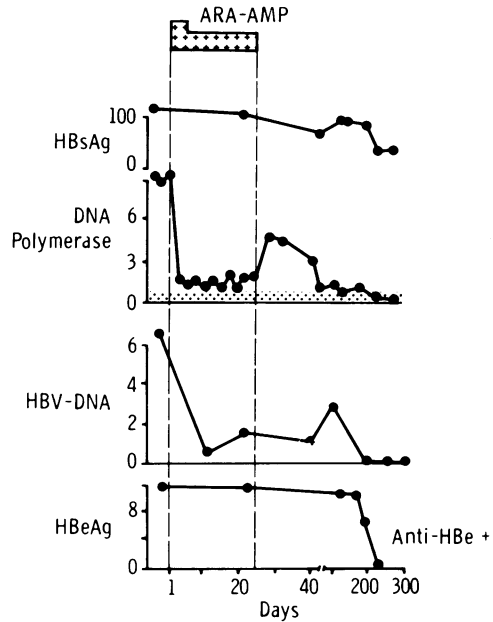

Fig. 5   *Serum HBV-DNA and DNA polymerase in response to treatment. HBeAg expressed as percentage laboratory control, DNAp as dpm × 10³, HBV-DNA as area in arbitrary units × 10² under the peak and HBeAg as log₂ reciprocal titre.*

responders', however, stayed constant, the 'responders' showed a fall in concentration representing a 44–85% reduction from pretreatment values by 360 days.

Discussion

High-level viral replication – HBV-DNAp positivity and high serum concentration of HBsAg – was found more frequently in patients with chronic persistent or active hepatitis than in those with active or inactive cirrhosis. As the patients with chronic hepatitis were younger than those with cirrhosis it can be argued that their infection had been acquired more recently and they therefore had had less time to spontaneously convert from high to low-level viral replication.[4][5] The existence of a group of HBe antigen positive patients who were negative for HBV-DNAp among those with chronic hepatitis but not among those with cirrhosis is consistent with the suggestion that the HBeAg positive/DNAp negative status represents an inter-medite stage of seroconversion, eventually ending in the loss of HBeAg and acquisition of anti-HBe. In this study three out of four HBeAg positive/DNAp

negative patients who were studied prospectively cleared HBeAg and developed anti-HBe. The fourth patient, who had failed to clear HBeAg over a two-year period, has always had 'borderline' values of DNAp and apparently represents a stable state of minimal viral replication.

In the group of patients in whom viral replication had been inhibited with ARA-A and ARA-AMP (responders), there was a time interval of around 180 days between the disappearance of DNAp and HBeAg. If a similar time course of clearance of HBeAg occurs during spontaneous seroconversion, the patient who is HBeAg positive/DNAp negative may be expected to become HBeAg negative within 180 days. These patients should not, therefore, be treated with antiviral compounds but observed over a period of six months to see if seroconversion occurs. The long delay before HBeAg is lost in chronically infected subjects is in contrast with the temporal sequence observed in acute type B hepatitis, where HBeAg and DNAp disappear within 30 days of the onset in those that recover.[13]

The delay in clearance of HBeAg after inhibition of viral replication with antiviral compounds, makes assay of this viral protein of little use in monitoring the immediate effects of such therapy. In contrast, changes in HBV-DNA polymerase and HBV-DNA occur rapidly and assays of these components of the virus are useful. If HBeAg concentrations are the sole technique available for measuring the effects of antiviral therapy, observations must be continued for several months.

No patients in the 'responder' group lost HBsAg from the serum. The continued production of this viral protein after cessation of detectable HBV replication is highly suggestive of integration of viral DNA into the host cell genome even at this relatively early stage of the chronic infection.[14–16]

In conclusion, we have shown that HBeAg cannot be considered as a reliable marker of high level viral replication because of the delay in its disappearance when replication is inhibited. HBeAg positive/DNAp negative patients with chronic HBV infection represent a group of subjects undergoing spontaneous cessation of high level viral replication and such patients should not be treated with antiviral drugs.

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