

The role of circulating hepatitis B antigen/antibody immune complexes in the pathogenesis of vascular and hepatic manifestations in polyarteritis nodosa

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SYNOPSIS To investigate further the role of hepatitis B antigen (HBs Ag) and specific immune complexes in polyarteritis, sera from 55 histologically confirmed cases were tested for the presence of hepatitis B antigen-associated particles and hepatitis B-antibody (anti HBs) by solid phase radioimmunoassay, electron microscopy, and passive haemagglutination. Results of these findings have been correlated with the clinical course of the disease.

HBs Ag was detected in 30 patients (54.5%) and anti HBs in 13/45 (28%). Subtyping in 20 patients revealed that 11 were Y and 9 D. Thirty-seven cases (69%) demonstrated either HBs Ag or anti HBs and 5/45 (11%) had both. Electron microscopic examination showed 20 nm spherical and tubular particles in sera of 20/27 patients with 42 nm particles in 11 cases and clumped particles in 12 (60%).

No correlation was found between detection of immune complexes and liver disease whereas the presence of coexisting hepatitis B antigen and antibody or aggregated particles was restricted to cases of active vasculitis. Seroconversion or the presence of hepatitis B antibody alone was associated with improved prognosis. Circulating hepatitis B antigen antibody complexes may be responsible for vasculitis or polyarteritis but do not appear to be pathogenic for the liver.

Studies by several authors have suggested that chronic hepatitis B infection could cause a proportion of cases of polyarteritis nodosa (Trepo and Thivolet, 1970a and b; Gocke, Hsu, Morgan, Bombardieri, Lockshin, and Christian, 1970; Baker, Kaplan, Benz, Sidel, and Wolfe, 1972; Martini, Strohmeyer, and Sodomann, 1972). Hepatitis B antigen has now been detected in 22 (40%) of 53 cases examined for the presence of this antigen in four different centres (Trepo, 1972; Gocke, Hsu, Morgan, Bombardieri, Lockshin, and Christian, 1971; Ziff, 1971; Kohler, 1973). However, even if the frequency of an association between polyarteritis and persistent HBs antigenaemia is thus now established, the immunopathogenic mechanism involved still remains obscure. The simplest hypothesis would be to consider HBs Ag-associated polyarteritis nodosa as an immune complex disease involving HBs Ag/anti HBs circulating complexes (Trepo and Thivolet, 1970a and b; Gocke *et al.*, 1970).

Gocke *et al.* (1970) reported immunofluorescence and density gradient experiments which were interpreted as consistent with this type of mechanism. However, Prince and Trepo (1971) failed to detect circulating immune complexes in six out of seven cases analysed by sensitive haemagglutination, haemagglutination inhibition, and ultracentrifugation techniques. Furthermore, these authors emphasized the fact that circulating HBs Ag/anti HBs complexes could be found in many chronic HBs Ag carriers without evident vasculitis or liver disease. Similar conflicting data have also been reported by others (Gerber, Brodin, Steinberg, Vernace, Yang, and Paronetto, 1972; Heathcote, Dudley, and Sherlock, 1972; Nowoslawski, Krawczynski, Brzosko, and Madalinski, 1972; Couleru, German, Bousquet, and Sarrazin 1972). In the present study we have further investigated these questions by the use of electron microscopy as well as the more sensitive of the serological assays now available, and have attempted to correlate our findings with the clinical course of the disease.

Materials and Methods

PATIENTS

Sera were obtained from 55 histologically confirmed cases of polyarteritis nodosa observed in Lyons or referred to this centre. Detailed clinical features of many of these patients have been described elsewhere (Trepo, 1971; Trepo, Thivolet, and Lambert, 1972). Patients found to be positive for HBs Ag or anti HBs were subsequently followed and serial serum specimens were obtained. The presence or absence of liver disease was determined by routine clinical and liver function tests, as well as by liver biopsy examinations in 18 cases.

The activity of the vascular disease in the cases studied was classified as follows:

1 Active

Twenty-three patients with at least five of the following signs or symptoms: fever, weight loss ≥ 5 kg, arthralgia, myalgia, polyneuritis, glomerulonephritis, hypertension, leucocytosis (greater than $10\,000$ wbc/mm³ with more than 75% polynuclear leucocytes), relapse of symptoms when steroids were withdrawn.

2 Quiescent

Six patients without immunosuppressive treatment seen more than one year after onset with stabilized disease characterized by the absence of fever, arthralgia, myalgia, or recent significant weight loss and with an erythrocyte sedimentation rate less than 25% and a leucocyte concentration ≤ 9000 mm³.

3 Apparently recovered

This group consisted of three patients in whom all clinical signs or symptoms as well as significant laboratory abnormalities had disappeared for 16 months or more (16 to 64 months) without treatment.

SEROLOGICAL METHODS

Serum specimens were shipped frozen in dry ice to The New York Blood Center where they were tested by haemagglutination for the presence of anti HBs (Prince, Brotman, and Ikram, 1972) and by radioimmunoassay for HBs Ag (Prince, Brotman, Jass, and Ikram, 1973) using Ausria kits supplied by Abbott Laboratories. The specificity of positive results obtained only by radioimmunoassay was confirmed by repeating the test after neutralization with known human anti-HBs-positive sera (Prince *et al.*, 1973). Subtyping of HBs Ag for Y (ay) or D (ad) specificity was performed by haemagglutination inhibition using red cells coated with either ad or ay antigen and tested versus adsorbed antiserum

that is more than 90% monospecific for Y or D (Prince *et al.*, 1972).

Forty-five serum samples from 27 patients with polyarteritis nodosa were sent frozen in dry ice to London for electron microscopic studies. Specimens were processed and examined after negative staining by methods which have been described elsewhere (Zuckerman, 1970). Negative stain preparations were made in carbon and formvar-coated smethurst no. 400 copper grids using 4% ammonium molybdate (pH 5.3) as the contrast stain. They were viewed in a GEC EM 801 microscope.

Results

SEROLOGY

HBs Ag was found in 30 out of 55 patients. Chronic HBs antigenaemia remained detectable in all serial specimens except in two cases, in which disappearance of HBs Ag was followed by the appearance of a low level of HB antibody (titres 1:8). Anti HBs only was detected in eight more cases at a titre of 1:8 or greater by haemagglutination. The simultaneous presence of HBs Ag and anti HBs was detected transiently in five of 45 cases in which tests for both antigen and antibody were carried out. Subtyping performed by haemagglutination inhibition in 20 cases revealed that 11 patients were Y and nine others D.

Finding	Technique ¹	No. of Cases Tested	No. of Cases Positive
HBs Ag	RIA	55	30 (54.5%)
Anti HBs $\geq 1/8$	HA	45 ²	13 (28%)
HBs Ag or anti HBs	RIA HA	55	38 (69%)
HBs Ag + Anti HBs $\geq 1:8$	RIA		
Anti HBs $\geq 1:8$	HA	45 ²	5 (11%)
without HBs Ag	RIA		
	HA	45 ²	8 (17.7%)

Table I Serological results obtained in 55 cases of polyarteritis nodosa

¹RIA = radioimmunoassay; HA = haemagglutination.

²In 10 cases no specimen was available for anti HBs detection.

ELECTRON MICROSCOPY

Electron microscopic studies performed on 45 serum samples from 27 patients revealed particles suggestive of HBs Ag in 34 specimens from 20 cases. The 20 patients in whom the particles were seen had all been found to have circulating HBs Ag. No particles were detected in any of the patients with antibody only. All three morphological types of particle (21) were observed, 20 nm spherical particles, filaments, and 42 nm Dane particles (fig 1). Twenty nm spherical

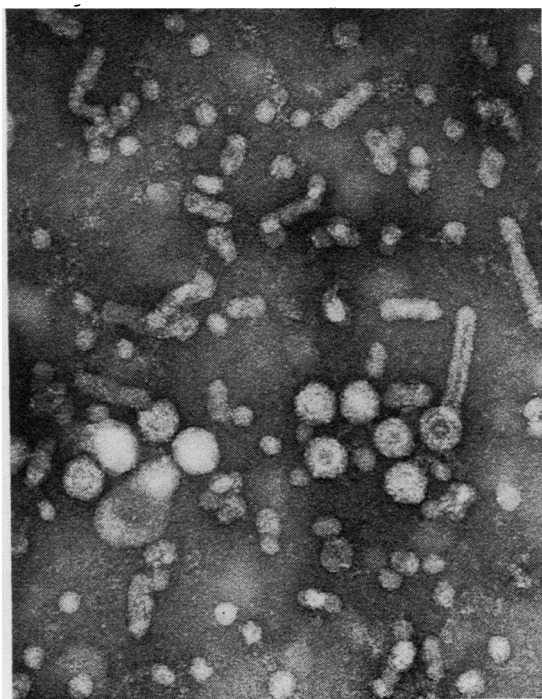


Fig 1 A typical area of a grid square prepared from serum LSH 16131-A/10872. All three morphological types of particles are present: 20-30 nm particles, filaments varying in length, and 42 nm Dane particles, some with attached filaments. $\times 126\ 000$

Findings	No. Cases Positive	No. Sera Positive
20-30 nm particles and filaments	20 (74%)	34 (75%)
Above and 42 nm particles	11 (41%)	17 (37%)
Scattered particles only	8 (29%)	20 (44%)
Scattered particles and clumps	12 (44%)	14 (31%)

Table II Electron microscopic observations in 45 sera from 27 cases of polyarteritis nodosa

particles and filaments were always found together, filaments being less numerous than 20-30 nm particles. The 42 nm particles were present in 11 of 20 cases in which the other particles were found.

Careful study of the distribution of particles revealed that only scattered particles could be observed in 20 sera from eight patients (figure 2) whereas aggregates of different size, many of them including 42 nm particles (figure 3) were observed in 14 samples from the 12 other patients studied. The three serum samples examined by electron microscopy in which both HBs Ag and anti HBs (haemag-

glutination titre $\geq 1:8$) were present revealed clumped particles; however, only three out of 12 sera with clumps had demonstrable antibody.

CORRELATION OF SEROLOGICAL AND ELECTRON MICROSCOPIC DATA WITH THE COURSE OF THE DISEASE (TABLES III AND IV)

Both high and low HBs Ag titres were found associated with severe polyarteritis and no difference in the course could be observed between patients bearing either Y or D specificity. Serial samples were examined from 10 cases with a prolonged course and recurrent illness. In three cases the titre of HBs Ag progressively increased from the onset of the disease to a constant high titre under combined steroid and azathioprine therapy with concurrent clinical improvement. In three others seen later in the course a steady high titre of HBs Ag was observed. In two patients the HBs Ag titre dropped during a period of clinical exacerbation being only detectable by radioimmunoassay in one.

Dramatic clinical improvement leading to complete clinical remission was followed by the total disappearance of HBs Ag with the appearance of a low level of anti HBs in two patients.

Seven of the eight patients with anti HBs were seen either during a quiescent phase of the disease (four patients' titre 1:64, 1:128, 1:512, 1:32 000) or after complete clinical remission for more than two years (three patients' titres 1:8, 1:8, and 1:16) (table III).

Out of 23 patients with active illness 18 were found to have circulating HBs Ag (78.2%), one anti HBs, and four (17.3%) both HBs Ag with transient anti HBs at a titre $\geq 1:8$, at the onset of vasculitis (table III). Electron microscopic examination revealed clumped particles in 12/18 (66.6%) (table IV).

Stage of Disease	No. of Cases Studied	Cases with HBs Ag only	Cases with Anti HBs $\geq 1:8$ Only	Cases with HBs Ag and Anti HBs $\geq 1:8$
Active	23	18 (78.2%) ¹	1 ² (4%)	4 ³ (17.3%)
Quiescent	6	2 (33%) ¹	4 ³ (66.6%)	0 ³ (0%)
Apparently recovered	3	0 (0%)	3 (100%)	0 (0%)

Table III Relationship between serological findings and stage of disease in 32 cases associated with HBs Ag and/or anti HBs

¹ $\chi^2 = 1.687$, *P*—non-significant

² $\chi^2 = 3.712$, *P*—on the borderline of significance

³ $\chi^2 = 0.036$, *P*—non-significant

Of six stabilized patients seen during a quiescent phase of the disease, four had demonstrable anti HBs (table III). No clumps were observed in this group and only one had scanty Dane particles (table IV).

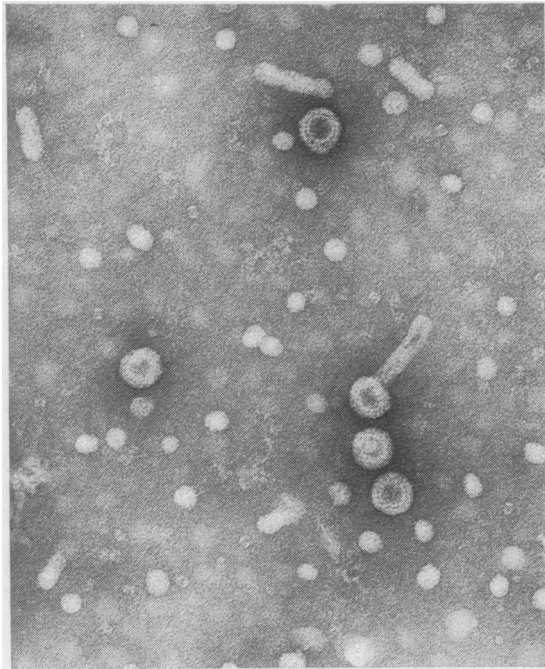


Fig 2 Scattered particles only were observed in 20 sera from eight patients—serum LSH 16133-A/10556. $\times 126\ 000$

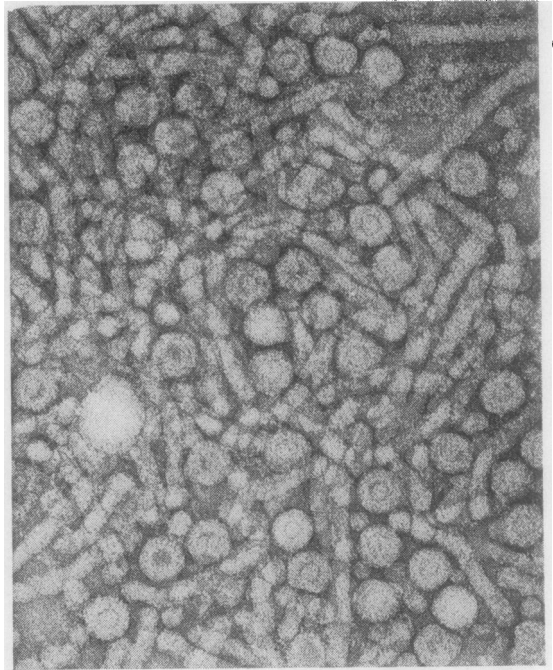


Fig 3 Part of a large clump composed of all three morphological types of particles as seen transiently in serial serum samples of two cases. $\times 126\ 000$

Stage of Disease	No. of Cases with 20 nm and Filaments	Proportion of Cases with Particles	Proportion of Cases with Clumps
Active	18	10/18	12/18
Quiescent	2	1/2	0/2
Apparently recovered	0	0	0

Table IV Relationship between electron microscopic observations and stage of disease in 20 cases of polyarteritis nodosa associated with HBs Ag

All the three apparently recovered patients were found to have anti HBs at low titre (1:8). Clumped particles were observed in association with histologically confirmed chronic hepatitis in seven cases (five persistent and two aggressive), liver cirrhosis in one, and no clinical or laboratory evidence of liver dysfunction in four, with histologically confirmed normal liver in two of these. Transaminases, bilirubin, and alkaline phosphatase were within normal limits in seven of 12 of the patients with clumped particles.

Discussion

The detection of HBs Ag in 54.5% of cases of poly-

arteritis nodosa clearly indicates a significant relationship, since this is an even higher frequency than that found by the same radioimmunoassay technique in 216 cases (47%) of acute hepatitis in adults from Lyons (Trepo, Trepo, Ortiz, Monier, and Sepetjian, 1973).

Both Y and D subtypes were equally frequent in the cases of polyarteritis nodosa tested, and were not associated with differences in clinical course, suggesting again that subtype antigens may represent sero-epidemiological markers devoid of specific biological properties (LeBouvier, 1973).

The significance of anti HBs in the absence of detectable HBs Ag in eight of 45 (17.7%) of cases of polyarteritis nodosa is more difficult to interpret since a similar frequency of anti HBs has been observed in several population groups (Lander, Alter, and Purcell, 1971).

However, it is noteworthy that the presence of anti HBs alone was shown to follow termination of HBs antigenaemia with dramatic improvement in two of these cases and to be associated with quiescent disease in five others. It may thus be suspected that anti HBs in those cases reflect seroconversion and is beneficial consequences in HBs Ag-associated

polyarteritis rather than past and unrelated hepatitis B virus infection.

It is likely that the 42 nm particles represent the virion of type B hepatitis. Their high proportion in these patients is striking, since in the more usual HBs Ag-containing sera the number of 42 nm particles is just within the range of detectability in the electron microscope (Almeida, 1971).

Since most of our patients received immunosuppressive therapy (steroid with or without azathioprine) a possible effect of this therapy on the proportion of 42 nm particles cannot be ruled out.

Almeida and Waterson (1969) emphasized that electron microscopic examination of HBs Ag-associated particles in serum may provide a useful tool for the detection of circulating HBs Ag/anti HBs immune complexes. They also suggest that the presence of such complexes as well as their composition could account for the different clinical patterns of hepatitis type B. Clumps of HBs Ag-associated particles have been considered by most authors to represent immune complexes.

In this series clumps were found in all three samples examined by electron microscopy in which both HBs Ag with anti HBs were present. If we accept clumps as an index of HBs Ag/anti HBs complexes, electron microscopy would appear to be more sensitive for the detection of such immune complexes than the serological techniques presently used. In the present study clumps as well as co-existing HBs Ag with anti HBs were restricted to cases with signs of active vasculitis.

Although the numbers in the quiescent and apparently recovered groups are too small to provide statistically significant results, these findings suggest that HBs Ag/anti HBs complexes may be related to the pathogenic mechanism.

The observation that the HBs Ag titre may drop during exacerbation of illness and that 'recovery' may coincide with the disappearance of HBs Ag and the appearance of anti HBs is also compatible with the hypothesis of an immune complex mechanism for the pathogenesis of polyarteritis nodosa.

The presence of vasculitis with fibrinoid necrosis, polymorphonuclear infiltrate, and the identification by immunofluorescence of HBs Ag with immunoglobulins and complement in recent exudative hyaline and fibrinoid lesions supports this suggestion (Nowoslawski *et al.*, 1972). It is not clear why circulating HBs Ag complexes are not found in a greater proportion of cases with the techniques used, and why only a small proportion of patients with circulating HBs Ag/anti HBs immune complexes develop polyarteritis.

The factors that determine the localization and inflammatory effects of immune complexes respon-

sible for the development of vasculitis or glomerulonephritis are only beginning to be appreciated. It appears that complexes are pathogenic only if of a certain size and definite antigen/antibody ratio. The nature as well as the amount of antibody is critical in this regard (Cochrane and Koffler, 1973).

We failed to find a significant association between the presence or absence of aggregated particles and evidence of liver disease. In our cases of polyarteritis nodosa, hepatitis was usually found to be present at the onset of the disease but was mild and resolved almost totally in more than half of the cases despite continued active vasculitis. This dissociation suggests that hepatitis and vascular lesions may be mediated through different immunopathogenic mechanisms. The same dissociation is observed in the serum-sickness-like syndrome which precedes the onset of liver injury in acute hepatitis type B. Sequential complement analyses suggested that this syndrome could result from the formation of HBs Ag/anti HBs complexes (Alpert, Isselbacher, and Schur, 1971). Thus circulating immune complexes may be crucial pathogenic factors in hepatitis B-associated polyarteritis, glomerulonephritis (Knieser, Jenis, Lowenthal, Bancroft, Burns, and Shalhoub, 1974), and vasculitis; however, the available evidence suggests that they are not able to induce active liver disease.

A cell-mediated immune response to so far undefined antigenic specificities, possible membrane antigens on the surface of virus-infected liver cells, may be involved (Prince and Trepo, 1971; Dudley, Fox, and Sherlock, 1972; Popper and MacKay, 1972).

Since this paper was submitted for publication we have investigated the sera of polyarteritis patients for the presence of the antibody directed against the recently identified hepatitis B core antigen. This antibody (anti HBC) was detected in 35/38 of the cases with either HBs or anti HBs by a modified indirect immunofluorescence technique confirming actual or recent replication of hepatitis B virus in those cases.

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