Acta virol. **26** : 86—90, 1982

ALBUMIN INTERACTION WITH HBsAg AS ONE OF THE POSSIBLE SOURCES OF THE POLYETHYLENE GLYCOL "IMMUNE COMPLEXES" TURBIDITY IN HEPATITIS B

J. PINTERA, J. JURÁK

Blood Transfusion Department, Faculty Hospital Brno-Bohunice, 657 20 Brno, Czechoslovakia

Received July 7, 1981; revised August 26, 1981

Summary. — The reported discrepancies between detection of the circulating immune complexes (CIC) by complement activation markers and by polyethylene glycol (PEG) turbidity tests suggested the possibility that PEG turbidity in hepatitis B is formed not only by HBsAg — immunoglobulin CIC but also by complexes formed on interaction of HBsAg with polymerized albumin. This hypothesis is supported by a correlation of HBsAg with the presence of albumin in the turbidities isolated from HBsAg positive and negative sera by the same procedure by which CIC had been detected. The existence of several sources of PEG turbidity specific for hepatitis B, e. g. HBeAg/anti-HBe; albumin/HBsAg; and others, makes these methods most appropriate for screening blood donors and testing for residual abnormalities in sera from apparently normal people.

Key words: hepatitis B; HBsAg-albumin binding sites; circulating immune complexes; polyethylene glycol turbidity test

Introduction

The circulating immune complexes (CIC) are abnormal serum components, products of the elimination of an undesired antigen by the antibody defense. If CIC molecules are sufficiently small, mostly due to antigen excess, they are not precipitated and remain in the circulation. The role of the complement system in the solubilization of CIC has been acknowledged (Miller and Nussenzweig, 1975). CIC may be detected in many ways — see Nydegger and Kazatchkin (1979) for review. Some tests are based on changes in configuration of the immunoglobulin component of CIC by which its domains, able to activate the complement system or the cellular receptors, become accessible. Another principle uses the precipitability of the CIC molecules by polyethylene glycol 6000 (PEG). At a 3.5% PEG concentration, less than 5% of free native IgG and IgA, and less than 8% of IgM precipitate, as compared with the precipitation of 80% of aggregated IgG or artificially formed complexes (Creighton *et al.*, 1973). Free HBsAg remains in the supernatant (Carella *et al.*, 1977). Albumin remains in the supernatant of 13%

PEG (Curling, 1980, p. 79). No albumin was detected by immunoelectrophoresis in HBsAg-CIC prepared by cryoprecipitation (Beutner *et al.*, 1978).

Detection of CIC in hepatitis B by different methods revealed some discrepancies. Carella *et al.* (1977) found a higher CIC incidence in HBsAg carriers, in haemodialysis subjects, in acute viral hepatitis, and in chronic aggressive hepatitis by the PEG test than by the Clq binding test. In our population of asymptomatic HBsAg carriers, tested by the Pegikem test (Hašková *et al.*, 1977), the incidence was still higher (28.1%) (Pintera, 1980, 1981*a*), and the prevalence of HBsAg among the CIC carriers amounted to 61.9%. The Pegikem test revealed 0.98% CIC carriers among normal blood donors (Pintera, 1981*a*). Anh-Tuan and Novák (1980) examined the anticomplementarity in the specific HBsAg-CIC, detected by the PEG test, and found very little correlation, i. e. C3 component concentration was normal or elevated in 88% of these PEG-detected HBsAg-CIC.

Binding sites for polymerized human albumin were discovered on some HBsAg particles, particularly on those in which HBeAg was also incorporated (Imai *et al.*, 1979). The bond between these two substances is immunochemically specific and correspondingly strong (O'Neill, 1979). There is little information available about the product of the reaction between HBsAg particle with a relative molecular weight of about 10^5 (Dreesman *et al.*, 1975) and an unknown number of molecules of the polymerized human albumin, which is an abnormal product appearing in some patients with chronic active hepatitis and cirrhosis. The polymerized albumin is absent in a 1% fresh albumin solution but appears in it after 21 days of storage at 4° C or after 4 days at room temperature (Lenkei *et al.*, 1974).

All these facts led us to assume that the CIC values determined by PEG tests in hepatitis B do not include only the actual immune complexes but also the products of the reaction between HBsAg and the polymerized human albumin. To test this hypothesis, we isolated the CIC by a procedure (Pintera, 1980, 1981*b*) equivalent to the Pegikem test and examined them for the presence of albumin in relation to HBsAg.

Materials and Methods

Isolation of CIC: 0.60 ml of serum diluted 1 : 3 with 0.1 mol/l borate buffer, pH 8.4 was added to 5.45 ml of a 4.17% PEG 6000 solution in the same buffer. The precipitate formed after 1 hr incubation at room temperature was spun off at 6000 × g for 10 min, the supernatant discarded and the sediment resuspended in 5 ml of the PEG solution. After centrifugation, the precipitated sediment was redissolved in 0.2 ml of borate buffer, corresponding to the starting volume of undiluted serum.

Screening procedure: 0.10 ml portions of serum diluted 1 : 3 with borate buffer were added to 0.91 ml borate buffer (blank) and to 0.91 ml of a 4.17% PEG 6000 solution in the same buffer. The absorbances of both mixtures were measured after 1 hr incubation at room temperature by an Eppendorf photometer at 436 nm and the differences between the PEG-precipitated material and the blank readings calculated. The numerical values for CIC were expressed (Hašková *et al.*, 1977) as absorbance units multiplied by 1000. As various PEG batches affect the turbidity differently, a CIC index has been introduced (Pintera, 1981*a*). This index represents the ratio of the test serum absorbance units to the value of the mean + 2 standard deviations determined for a normal population by the same PEG batch. CIC was thus considered as definitely proved if the index amounted to $\geqq 1$.

Table 1. Albumin reactivity in CIC, isolated from sera of HBsAg carriers and anti-HBs plasma donors

Source	Serum			Isolated CIC	
	HBsAG Ausria II ratio	Anti-HBs RIA units	CIC index ratio	HBsAg Ausria II	Albumin positivity by CIEP
1 HBsAg 1079	130.0	—	1.5	7.9	+
2 HBsAg 1038	120.8	—	0.5	6.2	+
3 HBsAg 1073	114.2	—	0.3	85.6	+
4 HBsAg 1187	104.3	—	0.7	10.9	+
5 HBsAg 1188	95.1	—	0.9	3.2	+
6 HBsAg 1067	88.3	—	0.4	15.9	+
7 HBsAg 1143	77.0	—	1.1	3.6	+
8 HBsAg 1119	3.4	—	1.1	5.2	+
9 Anti-HBs 46	1.3	412	1.5	2.0	+−
10 Anti-HBs 22	1.2	512	1.4	1.5	−
11 Anti-HBs 33	1.0	512	1.4	0.8	−
12 Anti-HBs 31	1.0	512	1.6	1.5	−
13 Anti-HBs 14	1.0	512	1.2	1.5	−
14 Anti-HBs 16	0.9	292	1.4	1.5	−

HBsAg was detected by Ausria II-125 Radioimmunoassay (Abbott). The Ausria II ratio significantly indicates presence of HBsAg if ≥ 2.1 and its absence if < 1.5.

The titre of anti-HBs was determined by Dr. J. Novák, Ing. M. Kselíková and J. Urbánková in the Institute of Haematology and Blood Transfusion, Prague; the limit for the purpose of HBIg production was 135 RIA units.

Albumin was assayed by counterimmunoelectrophoresis (CIEP) (Pintera *et al.*, 1974) with RAHu ALB SEVAC in the antibody well; Coomassie Brilliant Blue was used for staining.

Results

The results presented in Table 1 showed that the CIC isolated from the HBsAg-containing sera reacted positively in Ausria II. CIEP performed with a monovalent anti-human albumin antibody (RAHu ALB SEVAC) detected strongly reacting albumin determinants in all those CIC in which also HBsAg was proved, notwithstanding the fact that in some sera the presence of CIC was not unambiguously demonstrated. No albumin reactivity appeared in the Ausria II-negative CICs, isolated from anti-HBs positive sera lacking HBsAg. A slight albumin reactivity in one sample corresponded with a borderline Ausria II ratio, suggesting a possible simultaneous presence of HBsAg and anti-HBs. Albumin was thus correlated with HBsAg in the turbidity of the Pegikem test.

Discussion

The association of albumin with HBsAg in the CIC isolated by PEG supports the opinion that the turbidity in PEG assays for CIC is formed not only by immunoglobulin-containing CIC but also by the macromolecular complexes of HBsAg with human albumin.

The detection of CIC by PEG tests in hepatitis B is thus less specific for actual immunoglobulin-containing CIC but, at the same time, it has an increased importance for detection of states associated with the presence of HBsAg. The albumin-binding sites on HBsAg are known to be associated with a higher infectivity of the biological materials more than is HBeAg detection itself (Stevens *et al.*, 1978), and radioimmunoassay has been developed for their determination (O'Neill, 1979).

The Pegikem test, with which we had very good experiences in detecting HBsAg carriers among blood donors (Pintera, 1981*a*), offers no specific proof of either the actual HBsAg-Ig CIC or of the HBsAg-albumin complex. Both these kinds of molecules are able to participate in its positivity. There are further macromolecules, appearing almost exclusively in hepatitis B, which, due to its complex immunopathogenesis and metabolic deviations, can very likely participate in the Pegikem test positivity, e. g. HBeAg-CIC (Takahashi *et al.*, 1979), CIC of polymerized albumin with anti-albumin antibody (Lenkei *et al.*, 1974), CIC of Dane particles with anti-DP (Alberti *et al.*, 1978), perhaps also the polymerized albumin itself, CIC of anti-HBbrno antibody (Pintera and Kráčmar, 1981) which is alternatively presumed to be antibody to endogenous enterobacteria antigen, anti-EnEAg, playing an important role in chronic hepatitis and in the immune complex diseases, associated with hepatitis (Brzosko *et al.*, 1981). Some of these complexes can be visualised by electron microscopy, others can explain the cases of CIC without electron microscopic demonstration of Dane particles (Jelínková *et al.*, 1981). Plurality of the Pegikem-detected CIC sources in hepatitis B has been demonstrated also by long-term studies on CIC dynamics in acute hepatitis patients (Pintera *et al.*, 1981).

All these facts make the Pegikem test perhaps less specific for CIC but more apt to detect the HBsAg cariership and/or states affected by hepatitis B. This fact is important not only for blood donors screening but also for recognising more intimately the residual abnormalities in apparently normal people.

References

Alberti, A., Diana, S., Scullard, G. H., Eddleston, A. L. W. F., and Williams, R. (1978): Detection of a new antibody system reacting with Dane particles in hepatitis B virus infection. *Brit. med. J.* **2**, 1056—1058.

Anh-Tuan, N., and Novák, E. (1980): Detection and quantitation of hepatitis B surface antigen immune complexes (HBsAg-ICs) by an antigen-specific method. II. Circulating immune complexes (CICs) in patients with hepatitis B and asymptomatic HBsAg carriers. *J. immunol Meth.* **35**, 307—318.

Beutner, K. R., Tiku, M. L., and Ogra, P. L. (1978): Immune complexes and cellular immune reactivity in HBV infection, pp. 285—294. In G. N. Vyas, S. N. Cohen and R. Schmid (Eds): *Viral hepatitis*, Franklin Institute Press, Philadelphia.

Brzosko, W. J., Loch, T., Laskowski, Z., Janicki, P., and Kamińska, M. (1981): Role of enteric bacterial antigens in human pathology. I. Localization of enterobacteria endogenous antigen in organs of patients with chronic active hepatitis and liver cirrhosis. *Hepatitis scientif. Memo.* H-1903, April.

Carella, G., Digeon, M., Feldmann, G., Jungers, P., Drouet, J., and Bach, J. F. (1977): Detection of hepatitis B antigen in circulating immune complexes in acute and chronic hepatitis. *Scand. J. Immunol.* **6**, 1297—1304.

Creighton, W. D., Lambert, P. H., and Miescher, P. A. (1973): Detection of antibodies and soluble antigen-antibody complexes by precipitating with polyethylene glycol. *J. Immunol.* **111**, 1219—1227.

Curling, J. M. (1980): *Methods of plasma fractionation.* Academic Press, London.

Dreesman, G. R., Hollinger, F. B., and Melnick, J. L. (1975): Biophysical and biochemical properties of purified preparations of hepatitis B surface antigen (HBsAg). *Amer. J. med. Sci.* **270** 123—129.

Hašková, V., Kašlík, J., Matl, I., and Matějčková, M. (1977): Nový způsob stanovení cirkulujících imunokomplexů v lidských sérech. *Čas. Lék. čes.* **116**, 436—437.

Imai, M., Yanase, Y., Nojiri, T., Miyakawa, Y., and Mayumi, M. (1979): A receptor for polymerized human and chimpanzeee albumins on hepatitis B virus particles co-occurring with HBeAg. *Gastroenterology* **76**, 243—247.

Jelínková, A., Helm, R., Benda, R., Stránský, J., and Novák, M. (1981): Průkaz Daneových částic a cirkulujících imunokomplexů HBsAg u chronických forem hepatitidy typu B v elektronovém mikroskopu. *Čs. Epidemiol.* **30**, 148—155.

Lenkei, R., Mola, G., Dan, M. E., and Laky, M. (1974): The polymerized albumin and anti-albumin antoantibodies in patients with hepatic diseases. *Rev. roum. Biochim.* **11**, 271—275.

Miller, G. W., and Nussenzweig, V. (1975): A new complement function: solubilization of antigen-antibody aggregates. *Proc. natn. Acad. Sci. U.S.A.* **72**, 418—423.

Nydegger, U., and Kazatchkin, M. (1979): La détection de complexes immun solubles. *Nouv. Presse méd.* **8**, 203—209.

O'Neill, S. P. (1979): Interaction of hepatitis B surface antigen with polymerized human serum albumin. *J. med. Virol.* **4**, 177—185.

Pintera, J. (1980): Cirkulující imunní komplexy antigenu hepatitidy B. *Čs. Gastroenterol. Výž.* **34**, 1—11.

Pintera, J. (1981*a*): Screening of circulating immune complexes in blood donors as an effective method of further HBsAg carriers detection. *Folia haematol. (Leipzig)* **108**, 455—461.

Pintera, J. (1981*b*): HBsAg bound in circulating immune complexes as both an aid and a handicap for HBsAg detection. *Scipta med. (Brno)* **54**, 131—140.

Pintera, J., Vacl, J., Holland, P. V., and Alter, H. J. (1974): Some approaches to deciding HB Ag (Australia Antigen) positivity by counterimmunoelectrophoresis. *Blut* **29**, 165—171.

Pintera, J., and Kráčmar, C. (1981): Relations between the carriership of HBsAg, anti-HBc and anti HBrno, studied on the recipients of anti-HBrno-positive blood transfusion. *Scripta med. (Brno)*, in press.

Pintera, J., Ježek, P., Horecká, J., and Krajcová, J. (1981): Výskyt a dynamika kolujících imunních komplexů u akutní virové hepatitidy HBsAg positivní a negativní. *Čas. Lék. čes.* **120**, 513—517.

Stevens, C. E., Neurath, A. R., Szmuness, W., Beasley, R. P., and Ikram, H. (1978): Correlations between HBeAg/HBe, HBsAg titer, HBsAg-Associated albumin binding sites and infectirity, pp. 211—214. In G. N. Vyas, S. N. Cohen, and R. Schmid (Eds): *Viral hepatitis*, Franklin Institute Press, Philadelphia.

Takahashi, K., Miyakawa, Y., Gotanda, Mishiro, S., Imai, M., and Mayumi, M. (1979): Shift from free "small" hepatitis Be antigen to IgG bound "large" form in the circulation of human beings and a chimpanzee acutely infected with hepatitis B virus. *Gastroenterology* **77**, 1193—1199.