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CASE REPORT

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An autopsy case of hepatitis B (HB) antigen-positive polyarteritis nodosa, with reference to immunopathological studies of vascular lesions

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Abstract An autopsy case of an 11-year-old boy with polyarteritis nodosa is described in which the onset of the disease was associated with the presence of hepatitis B (HB) antigens (Ag) in the cytoplasm and nuclei of hepatocytes as detected by immunohistological methods. Deposits of HBsAg, HBeAg, IgG, IgM, C3, and C1q were demonstrated in systemic vascular lesions. It is considered that the arteritis was due to deposition in the arteries of immune complexes formed by HBAg and HB antibodies.

Key words Hepatitis B antigen · Immune complex · Polyarteritis nodosa

Introduction

Polyarteritis nodosa (PAN), first described by Kussmaul and Maier, is a well-known form of necrotizing angiitis, whose main manifestations are peripheral neuropathy, renal involvement, musculoskeletal and cutaneous manifestations, hypertension, gastrointestinal tract involvement, and cardiac failure. Systemic vasculitis has been found in association with chronic hepatitis B virus (HBV)-infected cases in the 20%–30% range in most studies, while among all patients with HBV infection, the frequency of clinically recognized PAN is very low.[1]

Kusakari and Hajikano,[2] from the Metropolitan Kiyose Children's Hospital, reported a clinical study of a case of HB surface antigen (HBsAg)-positive PAN in an 11-year-old boy.

In the present study, we performed further immunopathological studies of the autopsy materials of this case. We confirmed that HBAg-anti-HB immune complexes were directly involved in the immunopathogenesis of PAN in this case.

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Case report

The patient was an 11-year-old boy who complained of remittent fever and intermittent upper abdominal pain. He was born at fetal age 37 weeks at a birth weight of 2450 g and was the younger brother of identical twins. The twins were raised in an institution until the age of 10 years, and were in good health.

The family history showed that their mother had malignant hypertension. Moreover, she had a serological value of ×64 for HBs antibody. However, the elder of the identical twin brothers showed negative findings in tests for HBsAg and HBs antibodies.

The patient had suffered from increasing abdominal pain and persistent remittent fever (38–39°C) for about 3 months after reaching the age of 10 years, and was admitted to the Metropolitan Kiyose Children's Hospital in May 1978. Physical examination revealed a thin boy who appeared small in comparison with children of the same age and weighed 19.6 kg. His blood pressure was 120/70 mmHg, his pulse 120/min, and his body temperature 37.2°C. Abdominal tenderness and hepatomegaly palpable 5 cm below the costal margin was present. There was no splenomegaly. The following laboratory data were obtained: hemoglobin 8.1 g/dl, hematocrit 27%, platelets 340000/μl, white blood cell count 15300/μl, with a differential count of 86% neutrophils, 9% lymphocytes, 4% monocytes, and 1% eosinophils. Erythrocyte sedimentation rate (ESR) was 42 mm/h, GOT 18 KU, GPT 15 KU, LDH 240 U/l, ALP 14 KAU, sodium 139 mEq/l, potassium 3.9 mEq/l, chloride 108 mEq/l, BUN 14 mg/dl, creatinine 0.8 mg/dl, and total cholesterol 134 mg/dl. Urinalysis showed a 1+ test for protein, with 1–2 white blood cells per high power field. The C-reactive protein (CRP) test was strongly positive (6+). Rheumatoid factor was negative. IgG, IgA, and IgM were 1650, 456, and

288 mg/dl, respectively. C3 was low (22.2 mg/dl), while C4 was within normal limits (21.4 mg/dl). Serological tests for HBsAg and HBc antibodies were 1024 × (RPHA) and 131 072 × (IAHA), respectively, while HBs antibody and HBe antibody were negative.

From the 18th day after admission, the patient's blood pressure began to increase and from 2 weeks later, continuous hypertension (160–220/80–140) occurred. Hepatic intercostal and left renal arteriography showed multiple microaneurysms along the branches of these arteries. Biopsy specimens from a small artery in the subcutaneous tissue of the forehead region revealed arteritis with intimal thickening and disruption of the elastic lamina. The diagnosis of PAN was made based on clinical symptoms (fever and hypertension) and laboratory data (arteriography and biopsy), and prednisolone, 2 mg/kg/day, was started. The patient became afebrile after 1 day of therapy. Two months later, the abdominal pains subsided and CRP and ESR were within the normal range. However, in spite of the administration of cyclophosphamide, 2.5 mg/kg/day for 3 months, hypertension persisted, and in the eighth month of follow-up, the patient died from hypertensive encephalopathy due to malignant hypertension and cerebral hemorrhage.

Pathological findings

Formalin-solution-fixed paraffin-embedded sections of heart, liver, pancreas, intestines, mesentery, skeletal muscle, skin, and brain were obtained from postmortem materials preserved at autopsy. These were treated with hematoxylin–eosin (HE), phosphotungstic acid hematoxylin (PTAH), Masson trichrome, and elastica van Gieson (EVG) stains and examined. For immunohistochemical studies, serial sections (4μm) from selected specimens were deparaffinized with xylol, dehydrated with a graded alcohol series, and treated with 0.1% pronase (Protease type XIV; Sigma, St. Louis, MO, USA). Tissue sections were stained by the indirect immunofluorescence method or the avidin–biotin–peroxidase complex (ABC) system using antisera to HBs and HBcAg (Hybritex Co. Ltd., San Diego, CA, USA), HBeAg (Green Cross Co. Ltd., Osaka, Japan), IgG, IgM, C3, Clq, and fibrinogen (Cappel Research Products, Durham, NC, USA). The immunologically bound peroxidase was stained with 3-amino-9-ethylcarbazole (AEC). The specificity of the immunohistochemical staining was investigated by a blocking technique.

The morphologic changes in the liver were consistent with chronic inactive hepatitis. Immunohistochemical examination revealed variable amounts of HBsAg (Fig. 1A), HBeAg, and HBcAg in the cytoplasm and nuclei of hepatocytes.

The most prominent finding at autopsy was vascular lesions typical of "classic" PAN in varying stages of development. These were recent exudative lesions with fibrinoid necrosis accompanied by a mixed mononuclear and polymorphonuclear inflammatory reaction, and older lesions with focal or diffuse fibrous replacement of the media and obliteration of the lumen. Multiple areas of nodular lesions consisting of fibrosis along the anterior descending branch of the left coronary artery were visible on gross examination after removal of the fatty tissue in the pericardium. Microscopic examination revealed widespread intimal fibrosis, focal disruption of the elastic lamina, and fibrous replacement of the media of the coronary artery showing an aneurysm (Fig. 1B). The kidneys were slightly enlarged, their surface showing numerous foci of infarction or necrosis of parenchymal tissue. Microscopic examination of the kidneys revealed widespread acute (Fig. 1C) or older vasculitis of small and medium-sized arteries. The classification of vascular lesions was based on histologic criteria described by Arkin.[3] Morphologic features of vascular pathology in the kidneys corresponded to Stage II to Stage IV of PAN. Occasional glomeruli showed focal fibrinoid necrosis of the capillary tufts. In addition, there was arteriolar fibrinoid necrosis without inflammatory cells. Immunohistochemical examination revealed the localization of HBsAg (Fig. 1D and Fig. 2A), HBeAg (Fig. 2B), IgG and IgM (Fig. 2C), Clq (Fig. 2D), and C3 and fibrinogen (data not shown) in vascular lesions at various stages and in kidney glomeruli. Vascular lesions, including necrotizing angiitis in organs other than the kidneys, were observed in the heart, liver, pancreas, mesentery, skeletal muscle, and skin. There was no vasculitis in the brain, but there were arterioles showing fibrinoid necrosis, confirmed by PTAH, without inflammatory cells, and there was cerebral hemorrhage showing massive hematoma in the right hemispherium. In addition, marked edematous changes were seen throughout the tissue of the brain.

Discussion

Although vasculitides are commonly associated with hepatitis virus infection, the immunopathogenetic mechanisms are not well understood. The present patient was an individual with coexisting polyarteritis showing necrotizing angiitis and HBAg-positive hepatitis in whom HBAg in inflamed arteries was shown by immunological methods. The route of infection with HBV is unclear, but infection from mother to child (vertical infection) is most probable. The direct cause of the death appeared to be cerebral hemorrhage and hypertensive encephalopathy due to malignant hypertension (malignant nephrosclerosis) showing angionecrosis, with marked lesions of PAN in the kidneys.

Necrotizing angiitis is a term coined by Zeek in 1952[4] and is used "collectively for all vascular lesions that are characterized in their fully developed stage by fibrinoid necrosis and inflammatory reaction." Zeek classified five different entities under the category of "necrotizing angiitides," and PAN is one specific representative type of necrotizing angiitis. The diagnosis of PAN in the present case was also based on diastolic BP $>$ 90 mmHg, the presence of HBAg, an arteriogram showing aneurysms, and the findings of the biopsy, using the American College of Rheumatology 1990 criteria for PAN.[5] The histological lesions of PAN consist of

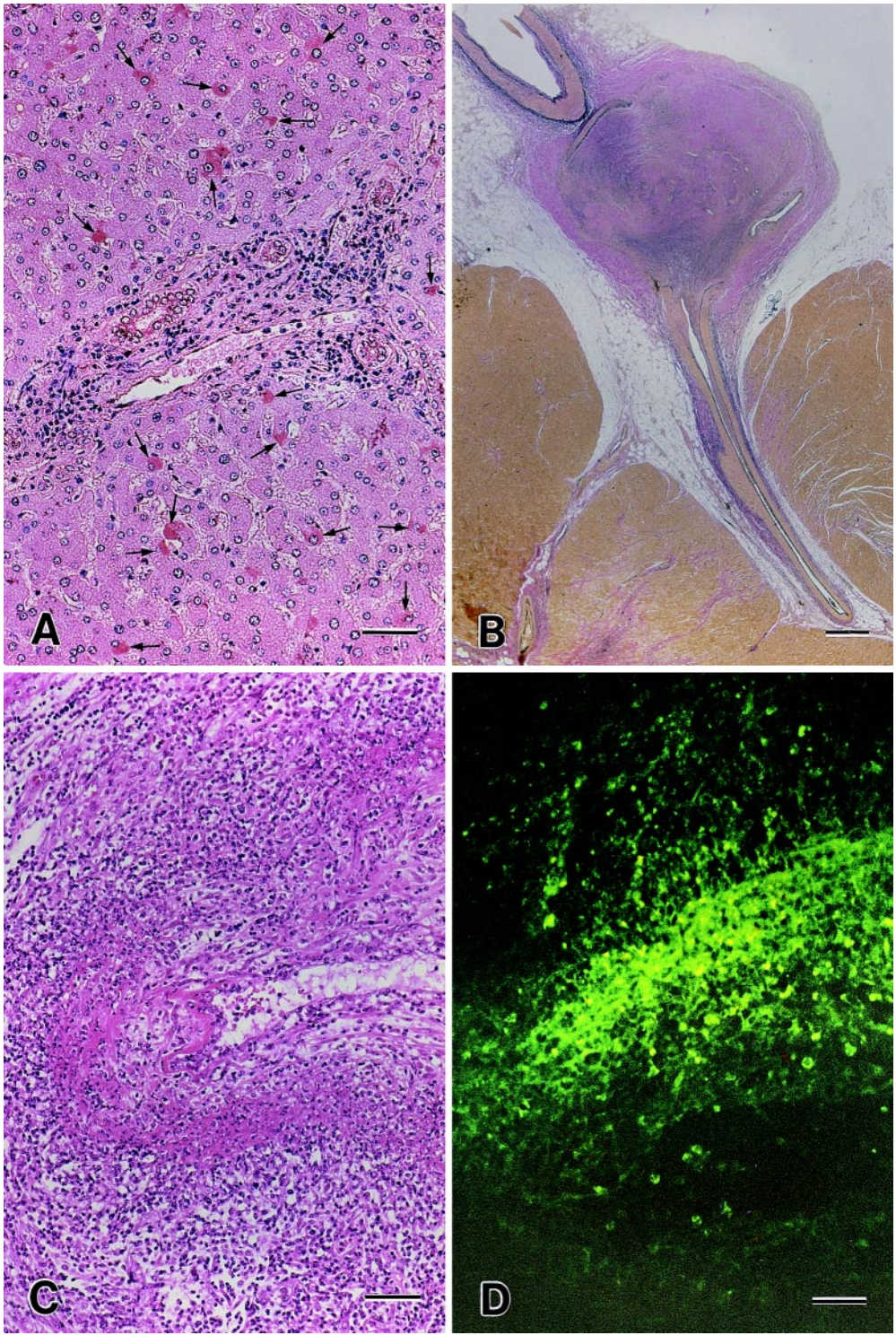

Fig. 1. **A** Immunoperoxidase-stained section from the liver. HBsAg is mainly localized in the cytoplasm of hepatocytes (*arrows*). *Bar* 60 μm. **B** Scar stage of PAN in the coronary artery, showing an aneurysm with obliteration of the lumen and focal disruption of the elastic lamina. EVG stain. *Bar* 400 μm. **C** Medium-sized renal artery showing complete fibrinoid necrosis with inflammatory cell reaction, indistinguishable from that seen in PAN. HE stain. *Bar* 60 μm. **D** Immunofluorescence-stained section from the same renal artery as in **C**. Minute granular deposits of HBsAg are seen in arterial media or around the damaged vessel wall. *Bar* 60 μm

extensive fibrinoid necrosis and inflammatory cell reaction, but lack extravascular granulomatous formation. Fibroblastic proliferation in the media is a prominent feature, and eventual destruction of the media results in small aneurysms. Lesions of the type described above were observed in this case. The histological changes in the liver were characteristic of chronic inactive hepatitis. However, the occurrence of HBsAg, HBcAg, and HBeAg in the cytoplasm and nuclei of the hepatocytes, as detected by immunohistological methods, indicated an ongoing infection with HBV. Trepo et al.[6] reported that the presence of HBeAg was correlated with a high titer of HBsAg, and with immunofluorescent detection of HBcAg in the nuclei of hepatocytes. The present patient, who lacked symptoms of hepatitis in

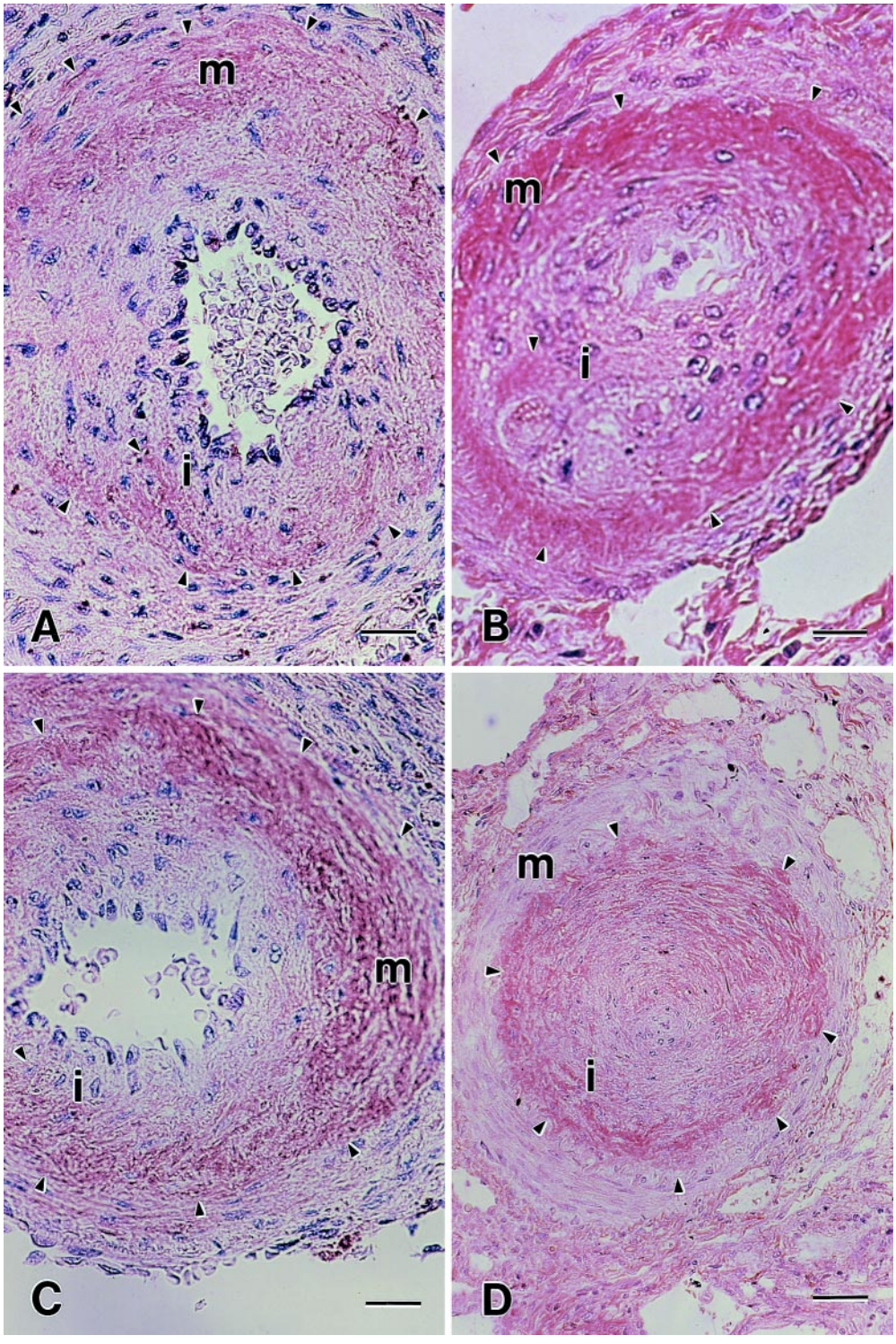

Fig. 2. Immunoperoxidase-stained sections from the kidney. Deposits of HBsAg **A**, HBeAg **B**, IgM **C**, and C1q **D** are seen in the arterial lesions (intima and media), corresponding to the granulation and scar stages of PAN (*arrowheads* indicate the stained region). i, intima; m, media. **A**, **B**, **C**, *bar* 40 μm. **D**, *bar* 80 μm

spite of strong positivity of HBsAg throughout his clinical course,[2] is considered to have been an asymptomatic carrier of HBsAg who had chronic hepatitis.

The report in 1970 by Gocke et al.[7] of four patients with HB-associated PAN was described as "the first recognition in man of a systemic vasculitis mediated by an immunologic reaction to a virus." Immunofluorescence studies of one of the patients revealed deposition of HBsAg, IgM, and βlc globulin in blood vessel walls.[7] In the present case, granular and lumpy deposits of HBsAg, HBeAg, IgG, IgM, Clq, and C3 were found in damaged arteries and kidney glomeruli by immunohistological methods. Immune pathogenesis in HB-associated PAN was further supported by immunohistological findings in the inflamed arteries of a similar patient

described by Gocke et al.[7] Persistent viral infections can cause chronic immune complex disease, as shown in experimental models.[8] Immune complexes under conditions of antigen excess are soluble, circulate in the blood, and accumulate in the vessel walls and glomerular basement membrane, subsequently leading to vasculitis and glomerulonephritis attributable to a type III hypersensitivity reaction.[9] During the early viremic phases of serum hepatitis, circulating immune complexes of HBsAg and HBs antibody have been recognized.[10] In the present patient, circulating HBAg-immune complexes were also thought to be present in the blood during the course of the disease. However, it is also thought that immune complexes may be formed in situ during the course of infections with HBV. In the present case showing deposition in the arteries of immune complexes formed by HBAg and HB antibodies, necrotizing arteritis appears to have been mediated by a type III hypersensitivity reaction. As revealed in the present case, HBV infection in patients with PAN suggests etiopathogenic significance as an initiating or accelerating factor. Similarly, the demonstration of HBAg-anti-HB immune complexes in vascular lesions provides substantial evidence for a primary pathogenic role for these complexes.

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