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Biologic Significance of the Detection of HBsAg and HBcAg in Liver and Tumor from 204 HBsAg-Positive Patients with Primary Hepatocellular Carcinoma

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Hepatitis B virus surface and core antigens (HBsAg, HBcAg) were examined in the resected primary hepatocellular carcinoma from 204 patients who had HBsAg in serum. Ninety patients had small (<5 cm) and 114 had large hepatocellular carcinoma (>5 cm). HBsAg was detected in hepatocellular carcinoma in 65 cases (32%) and HBcAg in 30 cases (14.7%); hepatitis B virus antigens were more frequently detected in small (HBsAg in 42.2% and HBcAg in 20%) than in large hepatocellular carcinoma (HBsAg 23.7% and HBcAg 10.5%). These results suggest that replicative forms of hepatitis B virus DNA may exist in hepatocellular carcinoma more frequently than previously believed and that the malignant hepatocytes can support hepatitis B virus replication. A lymphocytic infiltration in hepatocellular carcinoma was more often observed in hepatocellular carcinoma expressing HBsAg (71%) or HBcAg (63%) than in hepatocellular carcinoma with no detectable HBsAg (26%) or HBcAg (37%), $p < 0.01$. The reaction was mild in the majority (85%) of the cases. These findings suggest that hepatitis B virus antigen expression in hepatocellular carcinoma can provoke a local immune response. The most striking finding was that patients with hepatitis B virus antigens in small hepatocellular carcinoma had a 5-year survival rate (13%) lower than that (50%) of the antigen-negative patients ($p < 0.05$). In contrast, patients with a marked local immune response in hepatocellular carcinoma, regardless of the viral antigen status, had significantly better 5-year survival rates (43%) than those with no or a mild lymphocytic reaction (18%). These findings indicate that a marked immune response in hepatocellular carcinoma is a favorable prognostic sign. The unfavorable course of the patient with viral antigen-expressing hepatocellular carcinoma may in fact reflect the inability of the host immune response to clear the viral infection and the antigen-expressing tumor cells.

Both retrospective and prospective epidemiologic studies have shown an etiologic relationship between chronic hepatitis B virus (HBV) infection and hepatocellular carcinoma (HCC) (1, 2). This relationship has been strengthened by the discovery that HBV DNA is integrated to the host cellular genome in a majority of HCC cases (3–6).

Previous reports have claimed that HBV antigens are rarely detectable in tumor tissue, even in cases in which HBV DNA is present in the tumor and HBsAg is detectable in serum. In this report, we describe the results of testing for HBV antigens in the liver and tumor from a large number of patients with HCC. We found that HBV antigens were more frequently detectable in small than in large HCC and that presence of HBV antigens in HCC was commonly associated with a lymphocytic infiltration in the tumor parenchyma.

MATERIALS AND METHODS

Patients. From May, 1979, to December, 1987, 258 resected specimens of primary HCC were examined; 115 had a maximal diameter of less than 5 cm (small HCC), and 143 had a diameter of greater than 5 cm (large HCC). HBsAg was detectable in serum in 222 cases (86%). However, only 204 cases (92%) had tumor tissue available for histologic detection of the HBV antigens to form the basis of this study; these included 90 small and 114 large HCC. The 18 cases not analyzed included 16 with extensive tumor necrosis due to transcatheteric hepatic arterial embolization (nine cases), intratumoral alcohol injection (six cases) or spontaneous necrosis (one case) and two cases from which there was inadequate tissue for study.

Detection of HBV Antigens in Tissue. HBsAg and HBcAg were detected in formalin-fixed, deparaffinized sections of liver and tumor tissue by the peroxidase-antiperoxidase (PAP) method (7–9) using rabbit anti-HBs (Behringwerke, Marburg, Federal Republic of Germany) and anti-HBc (Dako Corp., Santa Barbara, CA). Two to four sections taken from the tumors and two sections from the nontumorous liver were examined. For the large HCC which had heterogeneous histologic features, representative sections were taken from different areas of the tumor.

Host immune response to the tumor or tumor antigens was assessed by the degree of lymphocytic infiltration in the HCC and was graded as none, mild (focal to +) and marked (++ to ++++).

Tissue specimens from 34 patients with HCC who had no detectable HBsAg in serum were included for comparison.

HBV serological markers, HBsAg, HBeAg and anti-HBe,

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were measured in serum by radioimmunoassays (Abbott Laboratories, North Chicago, IL).

RESULTS

HBV Antigens in the Liver. HBsAg was detected in either the tumorous or nontumorous areas of the liver in 98% of the 204 HBsAg-positive cases. In two cases, HBsAg was detectable in the tumorous portion only. HBcAg was detected in liver from 53% of cases and was significantly more frequent among the HBeAg-positive (80%, 28/35) than among the negative patients (50%, 69/138) ($p < 0.002$).

HBV Antigens in Hepatocellular Carcinoma. HBsAg was detected in the tumorous tissue in 65 cases (32%) and was present significantly more frequently in small than in large HCC (Figs. 1 and 2, Table 1). HBsAg was also detected more often in HCC tissue from patients who were HBeAg positive (55.6%, 20/36) than in those who were HBeAg negative (29.5%, 41/139). Histologically, the proportion of HBsAg-positive cells in the tumors varied considerably; in some cases the majority of cells were positive (Fig. 3).

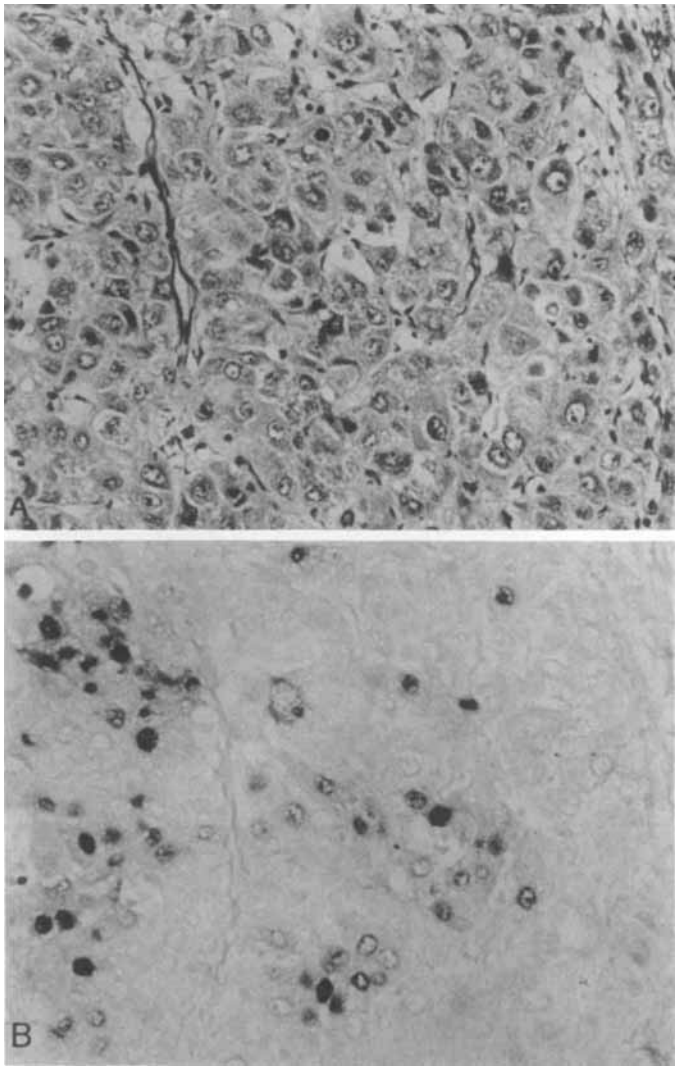

FIG. 1. Grade II small HCC of solid pattern and with mild lymphocyte infiltration (A) shows hepatitis B core antigen (HBcAg) in many tumor cells in the adjacent sections (B). A: H & E stain, × 300; B: PAP stain for HBcAg, × 300.

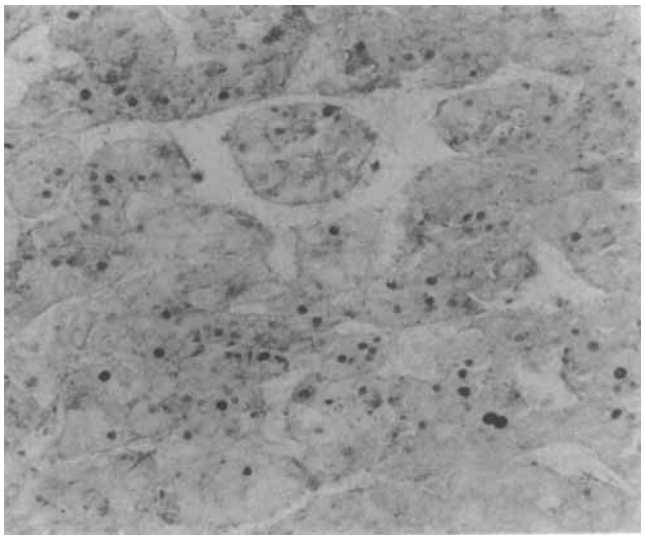

FIG. 2. Grade II trabecular HCC which was 6 x 4.5 cm in size reveals nuclear HBcAg and cytoplasmic HBsAg (not shown) in many tumor cells. PAP stain for HBcAg, × 300.

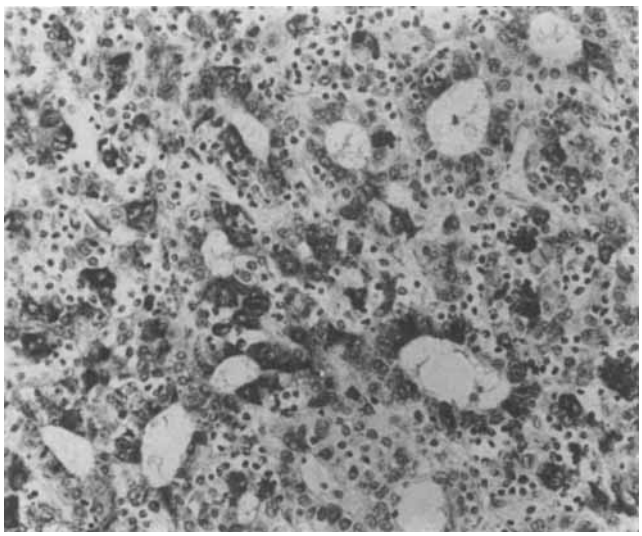

FIG. 3. Acinar-type small HCC reveals HBsAg in numerous tumor cells associated with marked lymphocyte infiltration and tumor cell degeneration. PAP stain for HBsAg, × 300.

HBcAg was detected in HCC cells in 30 cases (14.7%) and was demonstrated more frequently in small than in large HCC (Table 1). As with HBsAg, HBcAg was found more often in HBeAg-positive (33%, 12/36) than in HBeAg-negative cases (12%, 17/139) ($p < 0.005$). Furthermore, HBcAg in HCC was detected more frequently in cases with HBcAg in the nontumorous liver (24%, 26/108) than in cases without (4%, 4/93) ($p < 0.0001$). Tumors larger than 15 cm in diameter had the lowest rates of HBsAg (6%, 1/17) and HBcAg positivity (6%, 1/17). HBcAg was present in the nucleus and cytoplasm of malignant cells in 15, in the nucleus alone in 2 and in the cytoplasm alone in 13 HCC cases (Figs. 1B and 4). HBcAg and HBsAg were present usually in the same area and even in the same tumor cells. The distribution

TABLE 1. Detection of hepatitis B surface and core antigens in HCC in relation to tumor size

Tumor size	No. of cases	% of cases with		
		Serum HBeAg	HBsAg in HCC	HBcAg in HCC
Small	90	27	42	20
Large	114	14[a]	24	10.5
p values[b]		<0.04	<0.004	<0.05

[a] Only 85 cases tested.
[b] Fisher's exact test.

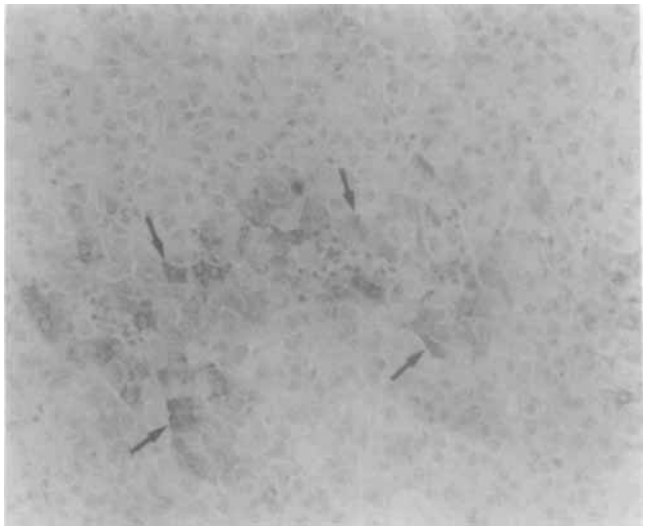

FIG. 4. Grade II HCC of acinar pattern shows patchy distribution of HCC cells containing cytoplasmic HBcAg (*arrows*), accompanied by mild focal lymphocyte infiltration. HBsAg was also positive (not shown). PAP stain for HBcAg with hematoxylin counterstain, × 300.

of the HBcAg-positive tumor cells also varied considerably from being focal to diffuse (Figs. 2 and 4).

Correlation of Local Host Immune Response in HCC with HBV Antigen Expression and Survival. Lymphocytic infiltration in the tumor parenchyma was regarded as a local host immune response which ranged from a focal (Fig. 4) to a marked and diffuse pattern (Fig. 3). The presence and degree of lymphocytic infiltration correlated well with HBV antigen expression in the tumor, being twice as common in the HBV antigen-positive than in the antigen-negative HCC cases (Table 2). However, the degree of infiltration was usually mild or focal in degree (85%); the prevalence of marked infiltration did not differ between the HBV antigen-positive (10/65, 15%) and -negative HCC cases (12/139, 9%).

Regardless of HBV antigen expression, cases of HCC with a marked degree of lymphocytic infiltration had significantly better survival rates than those with no or a mild degree of infiltration (Table 3).

In contrast to the findings for lymphocytic infiltration, survival rates for patients with tumors expressing HBV antigens were significantly lower than those for patients with antigen-negative tumors (Table 4). The difference in survival did not correlate with the presence of HBeAg

TABLE 2. Local host immune response and tumor HBV antigen expression in HCC

HBV antigens in HCC	Lymphocyte infiltration in HCC (+/total)	p value
HBsAg (+)	46/65 (71)[a]	<0.00001
HBsAg (−)	36/137 (26)	
HBcAg (+)	19/30 (63)	<0.006
HBcAg (−)	63/172 (37)	
All (−)[b]	8/34 (24)	

[a] Numbers in parentheses are percentages.
[b] Negative for both serum and liver HBsAg.

TABLE 3. Local host immune response in HCC and patient survival

Local host immunity[a]	Crude survival rate (no. surviving/no. followed)	
	4-year	5-year
Marked	9/15 (60)[b]	6/14 (43)
None or mild	32/119 (27)	20/111 (18)
p value[c]	<0.02	<0.05

[a] Degree of lymphocytic infiltration graded as marked (+2 to +4) or mild (focal to +1).
[b] Numbers in parentheses are percentages.
[c] Fisher's exact test.

TABLE 4. HBV antigen expression in HCC and patient survival

Tumor size	HBsAg		Survival (no. surviving/no. followed)	
	Serum	HCC	2 years	5 years
Small	+	+	16/30 (53)[a,b]	3/23 (13)[c]
	+	−	28/37 (76)[b]	16/32 (50)[c]
	−	−	7/11 (64)	1/7 (14)
Large	+	+	5/15 (33)	0/12 (0)
	+	−	19/68 (28)	7/58 (12)
	−	−	3/9 (33)	2/8 (25)

[a] Numbers in parentheses are percentages.
[b] Fisher's exact test: $p < 0.05$.
[c] Fisher's exact test: $p < 0.005$.

in serum, liver cirrhosis or tumor invasiveness, but the prevalences of HBeAg positivity (35% vs. 18%), cirrhosis (70% vs. 52%) and tumor invasion (76% vs. 66%) were all slightly higher in the antigen-positive patients. Survival rates in patients with large HCC were poorer than in patients with small HCC and showed a similar, although not statistically significant, inverse correlation with HBV antigen expression.

DISCUSSION

HBsAg is reported to be frequently demonstrable in nontumorous portions of liver from patients with HCC, but to be rarely detectable in the tumorous part (8, 10–16). Similarly, HBcAg has been reported to be only rarely detectable in HCC (10, 13, 15). Based on these findings, investigators have proposed that the switch-off of the HBcAg gene expression may be essential to the growth of HCC (17). These studies on HBV antigen, however,

have often been based upon autopsy studies of large tumors using insensitive means of viral antigen detection.

In this study of a large number of resected HCC, we could detect HBsAg in 32% and HBcAg in 15% of tumors. The two viral antigens were more frequently detected in small than in large HCC specimens (HBsAg in 42% vs. 24% and HBcAg in 20% vs. 10.5%). These findings suggest that detection of HBV viral gene products in HCC is related to the tumor size and hence may be related to the stage of tumor growth. The frequent detection of both HBcAg and HBsAg in small HCC, regarded as the earlier stage of HCC development, also suggests that replicative forms of HBV DNA may exist in HCC more frequently than previously believed, as has been recently suggested by others (18). The correlation of HBcAg detection in tumor cells with serum HBeAg positivity suggests that malignant hepatocytes can support HBV replication and that expression of viral gene products in tumor cells probably relates more to viral replication than expression of integrated HBV DNA in the transformed hepatocytes.

We noted that HBV antigen expressing HCC frequently had an associated local immune response, as shown by the degree of lymphocytic infiltration. Interestingly, cases of HBsAg-negative HCC had the lowest degree and frequency of lymphocyte infiltration in the tumor. These results suggest that during the course of tumor growth and cell diversification, the antigen-expressing tumor cells are preferentially eliminated, resulting in a selective expansion of the antigen-negative cells, perhaps with integrated viral sequences only.

Although viral antigens have been demonstrated in HCC in previous studies, the clinical correlates of their presence have not been explored. The demonstration of an unfavorable outcome for patients with HBcAg- and/or HBsAg-expressing cells in HCC was the most striking finding in this study but is difficult to explain. Several factors may contribute to the unfavorable prognosis associated with antigen-expressing tumor cells. Previous studies (16, 19) have demonstrated that tumor invasiveness, as manifested by local invasion, portal vein tumor thrombi and satellite lesions, is the most decisive factor in duration of patient survival. Well-established cirrhosis, which may lead to hepatic failure, also plays a role in survival statistics. In the current study, the prevalences of tumor invasion, liver cirrhosis and serum HBeAg positivity were all slightly higher in patients with HBV antigen-positive HCC than in those with antigen-negative HCC. The reasons for this are not known, but the persistence of HBV antigens in HCC and the higher serum HBeAg positivity may reflect the inability of the host immune response to clear the viral infection and the antigen-expressing tumor cells. This same deficient immune response may account for the lack of containment of the tumor and thus tumor invasion. This suggestion is partly supported by the mild degree of lymphocytic response in the majority of antigen-positive HCC and the frequent persistence of viral antigens in the intrahepatic tumor recurrences after resection of the HBcAg-positive (4/5, 80%) or HBsAg-positive (4/7, 57%) primary HCC (20).

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