Localisation of e-antigen in nuclei of hepatocytes in HB_sAg-positive liver diseases

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SUMMARY By the direct immunofluorescence technique 19 liver biopsies were examined for the presence of the e-antigen associated with the hepatitis B virus infection. With the use of double incubation and blocking experiments with FITC and Rhodamine labelled antisera against hepatitis B core antigen and e-antigen, evidence is presented that the e-antigen is localised in the nuclei of the hepatocytes. We conclude that the e-antigen and the core antigen are not identical, although they are often present simultaneously in the nucleus of the hepatocyte.

It is well established that the e-antigen is closely associated with the hepatitis B virus infections (Nielsen *et al.*, 1974). However, the exact biological nature of the e-antigen and its intracellular localisation is still obscure.

Using direct immunofluorescence technique on liver sections Trepo *et al.* (1976) have demonstrated cytoplasmic binding of human anti-e serum samples. In contrast with this, we now have evidence for an intranuclear localisation of the e-antigen on the basis of double incubation studies with monospecific fluorescein- or rhodamine-labelled anti-HB_c-, anti-HB_s- and anti-e-conjugates.

Methods

PATIENTS

Liver biopsies from 13 patients with HB_sAg and e-antigen positive chronic active hepatitis (CAH) were investigated. All the patients in this group had a highly active chronic liver disease as judged by liver function tests and liver histology. Furthermore, four HB_sAg and e-antigen positive patients on chronic haemodialysis were included in the study. These patients had either a mild form of chronic persistent hepatitis (CPH) or a normal liver histology. Two patients with HB_sAg positive and e-antigen negative CAH served as controls.

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The e-antigen was determined by the Ouchterlony technique as described by Magnius and Espmark (1972a, 1972b). Each positive sample was tested for identity with reference sera previously described (Nielsen *et al.*, 1974; Hess *et al.*, 1977).

Immunofluorescence studies were performed to demonstrate HB_sAg, HB_cAg and e-antigen by the direct immunofluorescence technique. The conjugates were self-prepared and purified according to a method described elsewhere (Arnold and Mayersbach, 1972; Arnold, 1976). The following conjugates were used in this study: (1) human anti-HB_s-FITC (titre (RIA): 1:4096, mol F/P-ratio 2.8; negative for anti-HB_c and anti-e); (2) human anti-HB_c-FITC (titre (IAHA): 1:64 000, mol F/P-ratio 2.5; negative for anti-HB_s and anti-e); (3) anti-HB_c-Rhodamine (mol F/P-ratio 3.5); (4) anti-e-FITC (2+ positive by immunodiffusion, mole F/P-ratio 2.9, negative for anti-HB_s, weakly positive for anti-HB_c: 1:100 in the immune-adherence-haemagglutination-test); anti-e-Rhodamine (mol F/P-ratio 3.2). Two FITC conjugated anti-e sera of human origin were used simultaneously with identical results. The anti-e serum samples were taken from healthy HB_sAg carriers—that is, asymptomatic, without biochemical and histological abnormalities. The two anti-e sera had specificities against e₁ as well as e₂. The anti-HB_c used for conjugation were taken from two HB_sAg positive patients with chronic active hepatitis. These sera were negative for anti-HB_s, anti-e, and immuno-complexes. Furthermore, anti-human-IgG-FITC and normal human-IgG-FITC were used for negative controls and the respective unlabelled antisera of all mentioned conjugates for blocking studies.

Table 1 Cellular localisation of HB_cAg and e-antigen in HB_sAg-positive CAH and patients treated with dialysis

	n	e-antigen (serum)	HB _s Ag (cytopl.)	HB _c Ag (nucleus)	e-antigen	
					(nucleus)	(cytopl.)
CAH HB _s Ag-positive	13	13	9	13	12	0
Patients with dialysis (HB _s Ag-positive)	4	4	2	4	4	0
CAH HB _s Ag-positive	2	0	2	2	0	0

Table 2 Demonstration of e-antigen and HB_cAg in liver biopsies by single and double staining procedure including blocking studies

Incubations			Results	
I		II	Nucleus	Cytoplasm
1	Anti-HB _c -FITC	—	++ 80%	0
2	Anti-e-FITC	—	++ 80%	0
3	Anti-e-Rhodamine	—	++ 80%	0
4	Anti-human-IgG-FITC	—	(+) (10%)	0
5	Normal H-IgG-FITC	—	0	0
6	Anti-HB _c (serum)	anti-HB _s -FITC	0	0
7	Anti-HB _c (IgG-fract.)	anti-HB _s -FITC	0	0
8	Anti-e (serum)	anti-HB _s -FITC	0 - (+)	0
9	Anti-e (IgG)	anti-HB _s -FITC	0 - (+)	0
10	Anti-e (serum)	anti-e-Rhodamine	0	0
11	Anti-HB _c (serum)	anti-e-FITC	++	0
12	Anti-HB _c (serum)	anti-e-Rhodamine	++	0
13	Anti-HB _c -FITC	anti-e-Rhodamine	FITC ++ Rhod. ++	0
14	Anti-e-Rhodamine	anti-HB _c -FITC	0 - (+) ++	0

Results

Table 1 summarises the results in the 17 HB_sAg and e-antigen positive patients and in the two HB_sAg positive and e-antigen negative patients. All patients had a high percentage of hepatocytes with intranuclear HB_cAg. The e-antigen was demonstrated in 13 out of 14 cases with HB_sAg/e-antigen positive CAH and in all of the four HB_sAg/e-antigen positive patients on haemodialysis. The e-antigen was constantly found in the liver cell nuclei, and was never detected in the cytoplasm of the hepatocytes.

Preincubation with unlabelled anti-HB_cAg before incubation with either anti-HB_c-FITC or anti-HB_c-Rhodamine showed a complete blockage of HB_cAg-specific fluorescence. On the other hand, after preincubation with unlabelled anti-HB_cAg before incubation with anti-e-FITC or -Rhodamine, a bright fluorescence for intranuclear e-antigen was revealed (Table 2). The immunofluorescent pattern of both HB_cAg and e-antigen after double incubation with anti-HB_c-FITC and secondly anti-e-Rhodamine is demonstrated in the Figure. Also, in this case, there was no evidence for e-antigen in the cytoplasm, although almost 20% of the hepatocytes had HB_sAg

in their cytoplasm after incubation with anti-HB_s-FITC. With the use of serial sections and double staining for e-antigen and core antigen, a number of nuclei were exclusively stained only with the e-antigen conjugates and a fewer number only with anti-core conjugates.

The anti-e conjugates did not show any reaction with the biopsies from the HB_sAg-positive, e-antigen-negative patients.

Discussion

In the present study an intranuclear localisation of the e-antigen in hepatocytes is clearly demonstrated by a double staining procedure as well as by the blocking studies. In contrast with the observations of Trepo *et al.* (1976) a cytoplasmatic fluorescence was never seen. This difference is probably due to different specificities of the respective antisera and unrelated to the e₁/e₂ difference as both anti-e conjugates used in the present study had specificities against e₁ and e₂ without showing any cytoplasmatic fluorescence.

It was most remarkable in the present study that HB_cAg and the e-antigen were very often present

Figure Immunofluorescent demonstration of intranuclear HB_cAg and e-antigen in a 7 µm cryostat section of a patient with HB_sAg-positive chronic active hepatitis. A: incubation with anti-HB_c-FITC. B: same section after double incubation with anti-e-Rhodamine. Original magnification × 250.

simultaneously in the same nuclei. However, in a few cells only the e-antigen was present and in others only the HB_cAg. A close association between the e-antigen in serum and HB_cAg in the liver cell nuclei has previously been described (Hess *et al.*, 1977); however, the present study clearly demonstrates that this association is also present on the cellular level. On the basis of these findings and the present blocking and double incubation studies the existence of three different intranuclear antigenic complexes associated with hepatitis B virus may be postulated. That means that some cells have only HB_cAg in the nuclei, others have only the e-antigen, but the majority have the HB_cAg/e-antigen complex. This hypothesis may be elucidated further by absorption studies of different anti-e-sera with HB_cAg and the use of heterologous experimental anti-e-sera raised in rabbits.

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