

## **The distribution patterns of hepatitis B antigen in the liver by immunofluorescence**

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### SUMMARY

The localization and the distribution patterns of HB-Ag in the liver of patients with various forms of hepatitis were examined by a direct immunofluorescent antibody technique.

Specific fluorescence was observed mainly in the cytoplasm of liver cells.

Four distribution patterns of fluorescence-positive cells were observed, namely, I. Solitary type, II. Focal type, III. Disseminated type, and IV. Lobular type. The patterns correlated well with the histological diagnosis of hepatitis. We are of the belief that the examination of HB-Ag in the liver by immunofluorescence may give important information in the course of progress of Hepatitis type B.

Some investigations<sup>1)</sup> have revealed that the HB-Ag was closely associated with the development of chronic forms of hepatic diseases. In order to investigate such a relationship, it seems important to know the intra-hepatic expression of HB-Ag as well as in serum. In this study, the role of HB-Ag in the development of chronic liver diseases was studied by examining the relationship between the cellular localization of HB-Ag in the liver by immunofluorescence and histological findings.

### MATERIALS AND METHODS

Forty-one liver tissues from patients with various forms of hepatic disease were studied (Table 1). A serum sample taken at the same time was tested for HB-Ag by counter-immunoelectrophoresis. Human antibodies to HB-Ag labeled with fluorescein isothiocyanate were used. The preparation, characterization and specificity control procedure was described previously<sup>2)</sup>. Immunofluorescent staining were controlled by blocking the reaction with unlabeled human antisera to HB-Ag.

**Table 1.** *Histological diagnosis and serum determination of HB-Ag and HB-Ab.*

	Ag		Ab
	positive	negative	positive
Acute hepatitis	5	3	
Chronic hepatitis active form	9	4	
"    inactive form	1	5	1
Liver cirrhosis	2	2	2
Primary hepatoma	4		
Asymptomatic carrier	2		
Fulminant hepatitis			1
	23	14	4

## RESULTS

A specific fluorescence was observed in 21 out of 23 HB-Ag seropositive cases and in none of 14 HB-Ag seronegative and in 4 anti HB-Ag antibody seropositive cases (Table 2). In the fluorescence positive cases, the distribution patterns of HB-Ag positive cells can be classified into four types.

**Table 2.** *Comparison of serologic and immunofluorescent determination of HB-Ag.*

Serum	Number	Immunofluorescence	
		positive	negative
HB-Ag positive	23	21	2
HB-Ag negative	14	0	14
HB-Ab positive	4	0	4
	41	21	20

- I Solitary type: one or more positive cells are seen in a liver lobule.
- II Focal type: small groups of positive cells exist sporadically.
- III Disseminated type: positive cells are scattered impartially.
- IV Lobular type: one entire liver lobule is positive.

Occasionally two or three types coexist in one specimen. Table 3 shows a comparison between the type of distribution pattern of fluorescence and the histologic feature of the liver specimen.

**Table 3.** *Classification of distribution patterns of HB-Ag positive cells in various liver diseases with seropositive for HB-Ag by immunofluorescence.*

Histological diagnosis	Number of patients with seropositive for HB-Ag.	Number with positive fluorescence in the liver.	Types of distribution patterns of HB-Ag positive cells in the liver by immunofluorescence			
			I (Solitary)	II (Focal)	III (Disseminated)	IV (Lobular)
Asymptomatic carrier	2	2	2			
Acute Hepatitis	5	4	1	2	1	
Chronic Hepatitis inactive form	1	1	1	1		
active form	9	8		2	7	3
Liver cirrhosis	2	2				2
Primary hepatoma	4	4	1		2	4

If more than one pattern were seen, the numbers are used twice or more.

All of the specimens examined showed fluorescence in the cytoplasm but not in the nucleus. In addition, 2 cases of asymptomatic carrier and 3 cases of chronic active hepatitis showed a specific fluorescence at a portion close to the cell membrane, and 3 cases of primary hepatoma showed an inclusion body like granule with fluorescence in the cytoplasm.

There was no difference in histological findings studied on hematoxylin-eosin and azan-mallory staining between the fluorescence positive and negative portions.

## DISCUSSION

In this study, four distribution patterns of fluorescence positive cells were observed. The patterns correlated well with the histological findings of liver specimens. The type of distribution pattern tended to change from I to IV as the hepatitis progressed to a more chronic and severer form. These observations lend further support to the concept that the HB-Ag in the liver plays an important role in the course of hepatitis.

The localization of HB-Ag observed in liver cells by immunofluorescence has been described as being either nuclear<sup>3)</sup> or cytoplasmic<sup>2,4,5,6)</sup> or both. The conflicting results might well be due to; 1. the difference in the stage of liver disease<sup>7)</sup>, 2. the difference in specificity of the antibody used<sup>8)</sup>, and 3. the difference in experimental conditions<sup>9)</sup>.

In our study, the specific fluorescence was observed mainly in the cytoplasm, but conclusive evidence has not been obtained as yet.

The significance of a specific fluorescence at a portion close to the cell

membrane and an inclusion body like granule in cytoplasm remains to be elucidated.

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