The localization of the Australia antigen in the liver by immunofluorescence

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SYNOPSIS The localization of Australia antigen in the liver by the fluorescent antibody technique (direct method) has been studied. Specific fluorescence was observed in the cytoplasm of liver cells. Two distribution patterns of fluorescent-positive cells, namely, a diffuse fluorescence in the liver lobules and a spotty distribution of fluorescent-positive cells, were observed.

Since the demonstration that the Australia antigen has been closely associated with a hepatitis virus, attention has mainly been directed towards the Australia antigen in serum together with its clinical and epidemiological associations and its localization in the tissues. The original studies using electron microscopy and the immunofluorescence technique (Nowoslawski, Brzosko, Madaliński, and Krawczyński, 1970; Coyne, Millman, Cerda, Gerstley, London, Sutnick, and Blumberg, 1970) showed that the Australia antigen was mainly in or on the surface of nuclei of liver cells, and recent reports (Scotto, Homberg, Stralin, Salmon, and Caroli, 1970; Stein, Fainaru, and Stein, 1971; Ahmed, Huang, and Spence, 1971) have suggested the presence of cytoplasmic Australia antigen.

The present report is an attempt to study the localization of the Australia antigen within the liver cells of patients with hepatic disease by the direct immunofluorescence method.

Materials and Methods

Antisera to the Australia antigen used were obtained from human and rabbit sera. The rabbit antiserum was prepared by hyperimmunization of rabbits with human serum containing the Australia antigen followed by absorption of the rabbit serum with normal human serum. The immunoglobulin fraction was separated by repeated ammonium sulphate precipitation. The presence of immunoglobulin was examined by immunoelectrophoresis and this fraction revealed a simple precipitin line with sera containing the Australia antigen and no precipitin line with sera in which Australia antigen was not recognized. The immunoglobulin was conjugated

with fluorescein isothiocyanate isomer 1 (Baltimore Biological Laboratories) by the modified method of Marshall, Eveland, and Smith (1958) of those methods of Goldstein, Slizys, and Chase (1961) and McKinney, Spillane, and Pearce (1964). The conjugate was purified by the procedure of Curtin (1961). The conjugated antisera were again confirmed to be acceptable for use by immunodiffusion which produced a single precipitin line with a serum containing Australia antigen and failed to precipitate with normal sera. Absorption of the conjugated antiserum with human liver powder was achieved by mixing the fluorescent antiserum with 1/10 of the amount of dried liver powder and incubating in a water bath at 37°C for one hour and at 4°C overnight. After centrifugation the supernatant was saved. In order to determine the specificity of a positive reaction, an inhibition test was performed on all specimens showing fluorescence by flooding replicate slides with unconjugated antiserum to the Australia antigen before fluorescein staining.

The liver specimens examined were taken immediately after death from two patients with cirrhosis and liver biopsy specimens were obtained from two cases of the Australia antigen carrier and one with subacute hepatitis. A blood sample from each patient was obtained at the same time. Liver biopsy specimens from five patients with chronic hepatitis in whose blood Australia antigen was not detectable were used as a negative control. The livers were processed immediately after they were obtained or they were stored in a deep freeze at -70°C for two or three days before they were processed. The liver specimens were sliced with a cryostat 4μ in thickness and fixed with acetone at 4°C for 30 minutes; one drop of conjugated antiserum was applied and slides were incubated for 30 minutes at 37°C in a

Received for publication 17 August 1972.

moist chamber then washed three times with phosphate-buffered saline. The stained slides were examined with the Nikon ultraviolet microscope. After the observation of the fluorescent staining the same slide was washed and processed by haematoxylin-eosin staining for orientation and histological examination.

Results

Two kinds of distribution pattern of liver cells with specific fluorescence were seen in the liver tissues: (1) a spotty distribution of liver cells with specific fluorescence and (2) diffuse fluorescence in the liver lobules. In the positive cells, apple-green specific fluorescence was observed only in the cytoplasm. The nuclei appeared dark. The cytoplasmic fluorescence showed a diffuse or fine granular appearance. All of the specimens examined showed cytoplasmic fluorescence and no nuclear fluorescence. In the

cirrhotic liver specimens obtained at necropsy the distribution of fluorescence was a combination of spotty and diffuse patterns. In specimens obtained by liver biopsy from hepatitis and carriers no such pattern was observed. There was no difference in histological findings studied on haematoxylin-eosin stained slides between the fluorescence-positive and -negative portions. In the liver from two carriers the fluorescence was more intense in the portion close to the cell membrane. No difference in the specific fluorescein staining of the liver specimens was found between the rabbit and the human antiserum. Five control specimens from seronegative patients showed no fluorescence (Table).

Discussion

It has been reported that specific fluorescein-tagged antibody to Australia antigen reacts with an antigen located in or on the surface of the nuclei of hepatic

Fig 1a

Fig 1b

Fig. 1 *Intrahepatic localization of Au-antigen with fluorescein-conjugated anti-Au.*

a *Diffuse fluorescence in the liver lobule.*

b *Diffuse fluorescence at the bottom half and a spotty distribution of liver cells with fluorescence at its right.*

Fig. 2a.

Fig. 2b

Fig. 2 Cytoplasmic localization of Au-antigen in hepatic parenchymal cells.

a and b The cytoplasmic fluorescence showed a diffuse or finely granular appearance and the nuclei appeared dark.

Histological Diagnosis	Number of Patients	Intracellular Localization of the Specific Fluorescence	Distribution Pattern of Positive Cells
Cirrhosis	2	Diffuse cytoplasmic (strong fluorescence)	Both spotty and diffuse
Healthy carrier	2	Diffuse cytoplasmic (strong at the portion close to the cell membrane)	Diffuse
Subacute hepatitis	1	Diffuse cytoplasmic (weak)	Diffuse
Control	5	Negative	

Table Localization and distribution of the specific fluorescence

cells from patients with viral hepatitis (Coyne *et al.* 1970). On the other hand, Nowoslawski *et al.* (1970) and also Edgington and Ritt (1971) demonstrated the specific fluorescence in the cytoplasm of liver cells. The discrepancy in the results may be due to the difference in specificity of the antibody used, the difference in the stage of the liver disease, and the difference in the reaction on different portions of specimens. Nielsen and Elling (1971) detected both cytoplasmic and nuclear fluorescence. The nuclear fluorescence was very labile when the specimens were

stored and it was also found in normal human liver cells. They supposed that antiserum to the Australia antigen might have two antigenic specificities, one precipitating with the Australia antigen in serum and another reacting by immunofluorescence with a labile antigen which appears to be a constituent of normal liver cell nuclei. The authors believe that storage at -70°C has no influence on the presence or absence of fluorescence, because no fluorescence was found in the nuclei even when the test was done immediately after biopsy. Nowoslawski *et al.* (1970),

who detected cytoplasmic fluorescence, observed Australia antigen-like particles in the nuclei by electron microscopy. They interpreted their observations to mean that the cytoplasmic fluorescence might be related to the presence of a non-particulate compound sharing immunochemical specificity with the particles in the nuclei, and cytoplasmic ribosomes would be considered as a possible site of synthesis of such an immunohistochemically identifiable component. More recently the presence of Australia antigen-like particles was observed in the cytoplasm by electron microscopy (Scotto *et al*, 1970; Stein *et al*, 1970; Ahmed *et al*, 1971). The fluorescence might be found both in the cytoplasmic and nuclear portion of the hepatic cells in certain experimental conditions. The conclusive evidence is still missing. However, in the present study the fluorescence was mainly found in the cytoplasm.

References

- Ahmed, M. N., Huang, S., and Spence, L. (1971). Australia antigen and hepatitis: an electron microscopic study. *Arch. Path.*, **92**, 66-72.
- Coyne (Zavatone), V. E., Millman, I., Cerda, J., Gerstley, B. J. S., London, T., Sutnick, A., and Blumberg, B. S. (1970). The localization of Australia antigen by immunofluorescence. *J. exp. Med.*, **131**, 307-319.
- Curtain, C. C. (1961). The chromatographic purification of fluorescein-antibody. *J. Histochem. Cytochem.*, **9**, 484-486.
- Edgington, T. S., and Ritt, D. J. (1971). Intrahepatic expression of serum hepatitis virus-associated antigens. *J. exp. Med.*, **134**, 871-885.
- Goldstein, G., Slizys, I. S., and Chase, M. W. (1961). Studies on fluorescent antibody staining. I. Non-specific fluorescence with fluorescein-coupled sheep anti-rabbit globulins. *J. exp. Med.*, **114**, 89-110.
- Marshall, J. D., Eveland, W. C., and Smith, C. W. (1958). Superiority of fluorescein isothiocyanate (Riggs) for fluorescent-antibody technic with a modification of its application. *Proc. Soc. exp. Biol. (N.Y.)*, **98**, 898-900.
- McKinney, R. M., Spillane, J. T., and Pearce, G. W. (1964). Factors affecting the rate of reaction of fluorescein isothiocyanate with serum proteins. *J. Immunol.*, **93**, 232-242.
- Nielsen, J. O., and Elling, P. (1971). Investigation of liver biopsies for Australia antigen by immunofluorescent technique. *Clin. exp. Immunol.*, **9**, 699-705.
- Nowoslawski, A., Brzosko, W. J., Madaliński, K., and Krawczyński, K. (1970). Cellular localisation of Australia antigen in the liver of patients with lymphoproliferative disorders. *Lancet*, **1**, 494-498.
- Scotto, J., Homberg, J. C., Stralin, H., Salmon, C., and Caroli, J. (1970). Observation ultrastructurale d'inclusions cytoplasmiques particulières dans des cas d'hépatites virales: rapport avec l'antigène australia? *Presse méd.*, **78**, 2061-2064.
- Stein, O., Fainaru, M., and Stein, Y. (1971). Virus-like particles in cytoplasm of livers of Au-antigen carriers. *Lancet*, **2**, 90-91.