CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY 10, 398–402 (1978)

Absence of a Liver-Specific Membrane Protein in a Strain of Chang Cells

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Received February 7, 1978

A strain of Chang cells, derived from a human liver line, was examined for the presence of a liver-specific membrane protein (LSP), as described by Meyer zum Büschenfelde. Indirect immunofluorescence and antisera to human, rat, and rabbit LSP were used to demonstrate that LSP was present on isolated human, rat, and rabbit hepatocytes but not on the Chang cells. The results indicate that these Chang cells do not possess the LSP. The use of Chang cells in the studies of liver immunopathology may not reflect a valid correlation with organ-specific events relevant to the LSP.

INTRODUCTION

Chang cells (1), an epitheloid, aneuploid human liver cell line, have been utilized to study membrane binding of hormones (2), amino acid utilization patterns (3), protein synthesis (4, 5), nucleic acid metabolism (6, 7), and drug toxicity reactions (8, 9). More recently, Chang cells have been used as target cells in systems designed to detect and to monitor humoral and cell-mediated immune (cytotoxic) reactions (10–14). Their derivation from a human liver cell line and their relative ease of cultivation have enhanced the acceptance of Chang cells in the study of liver immunopathology.

Since a liver-specific membrane lipoprotein (LSP) has been described (15), this study was designed to determine if a strain of Chang cells shares this organ-specific but not complete species-specific antigen. Indirect immunofluorescence using antisera directed against normal human, rat, or rabbit LSP followed by fluorescein isothiocyanate-conjugated (FITC) goat anti-rabbit or rat immunoglobulin was utilized.

MATERIALS AND METHODS

Preparation of Hepatocytes

Human liver tissue was obtained by needle biopsy and cut into small pieces. Isolation of hepatocytes was accomplished mechanically, as described previously (16). Briefly, small pieces of tissue in 1 ml of Hank's balanced salt solution (HBSS), deficient in calcium and magnesium and containing 10% fetal calf serum (FCS) was shaken in a water bath in an O_2 atmosphere at 37°C for 5 min. The cells were filtered through gauze and washed twice with veronal buffer solution, pH 7.2, containing 10% FCS. Isolated rat hepatocytes were obtained from Sprague–Dawley (175–200 g) rats by perfusion of the liver *in situ* with media containing collagenase (17). Isolated hepatocytes were obtained from young rabbits (300–400 g), as described previously (18). The dissociated liver cells were suspended in HBSS. Chang cells were obtained from the Microbiological Associates (Bethesda, Maryland) and were

0090-1229/78/0104–0398$01.00/0

subcultured for nearly 1 year in our laboratory prior to use in this study. Chang monolayer cells were dissociated with 0.125% trypsin–0.02% Versene solution or mechanically, using a rubber policeman. The cells were suspended in HBSS containing 10% heat-inactivated FCS.

Preparation and Absorption of Antisera

The liver-specific proteins (HLP = human liver-specific proteins; RLP = rabbit liver-specific proteins; and RALP = rat liver-specific proteins) used for immunization were isolated from normal human, rabbit, and rat livers according to the method described previously (15). The protein mixtures obtained from liver homogenates by ultracentrifugation and Sephadex G-100 chromatography contained 30 to 40% of the liver-specific lipoprotein, a membrane antigen (16), 20 to 30% of a liver-specific cytoplasmic protein, and 20 to 30% of three to four plasma proteins synthesized in the liver. The antisera to HLP, RLP, and RALP obtained from Meyer zum Büschenfelde were prepared and tested for specific reactions toward LSP, as described previously (15). The sera were absorbed with lyophilized human, rabbit, or rat plasma (1-ml aliquots of antiserum by 25 mg of protein, respectively). In addition, the antisera were absorbed with corresponding peripheral white blood cells or spleen cells (1 ml of antiserum with 10×10^6 cells) as well as with kidney cell homogenate.

Kidney preparations were obtained from minced tissue washed repeatedly with cold phosphate-buffered solution (PBS), pH 7.2, until the supernatant was essentially clear of blood and then mechanically separated with a Potter tissue grinder with a clearance of 0.2 mm. The resultant suspension was filtered through gauze, washed twice with cold PBS, and pelleted by centrifuging at 1500*g* for 10 min. Rabbit antiserum to HLP was further absorbed with rat and human kidney cell homogenates at a ratio of 1:1 (v:v) by incubating twice at 37°C for 1.5 hr, followed by a 24-hr incubation at 4°C. The suspension was centrifuged at 1500*g* for 20 min, passed through a 0.22-μm Millipore filter, and diluted 1:8 in PBS. Rat antiserum to RLP was absorbed in a similar manner with rabbit and human kidney cell suspension. Rabbit antiserum to RALP was absorbed with human and rat kidney cell suspension.

Immunofluorescence Studies

On isolated hepatocytes. One hundred microliters of human, rat, or rabbit hepatocyte suspension ($1-2 \times 10^5$ cells/ml) was incubated in 100 μl of rabbit antiserum to HLP, rabbit antiserum to RALP, and rat antiserum to RLP, respectively, for 20 min at 37°C. The suspensions were centrifuged for 6 min at 800 rpm at 4°C, and the supernatant was discarded. The suspension was washed twice in an excess of veronal buffer solution (VBS) plus 10% FCS. After the cells were pelleted, 0.2 ml of FITC goat anti-rabbit immunoglobulin (GIBCO) or FITC goat anti-rat immunoglobulin (Microbiological Associates) was added to the tubes, which were then incubated for 20 min. The mixture was centrifuged and washed twice with excess VBS plus 10% FCS. The cell suspensions were examined under ultraviolet light at a mangification of 400X.

On isolated Chang cells. Subcultures of Chang cells grown in monolayer were treated with trypsin and allowed to "recover" in the culture media for at least 6 hr before utilization. Similar preparations of Chang cells were obtained using a rubber policeman in the place of trypsin. Cell suspensions were prepared as outlined

above and incubated with each antiserum. Controls were performed by using sera from nonimmunized rabbits.

RESULTS

The results are summarized in Table 1. Rabbit antiserum to HLP detected the presence of LSP at the cell surface of isolated human hepatocytes, and a weak reaction was observed with rat and rabbit hepatocytes. Rabbit antiserum to RALP revealed positive fluorescence on rat liver cells and in lower intensity on human liver cells. Rat antiserum to RLP demonstrated the presence of LSP on rabbit hepatocytes (Fig. 1). The membrane fluorescence was linear. In contrast, none of the antisera used reacted with Chang cells (Fig. 2). The results with Chang cells were identical irrespective of using enzymatic (trypsin) or mechanical dissociation.

No fluorescence was observed on human or rat hepatocytes or on Chang cells when the serum of a nonimmunized rabbit was used. In addition, rabbit antisera to HLP and RALP did not stain isolated human or rat kidney cells, and rat antisera to RLP did not stain rabbit kidney. It should be noted that all the antisera yielded fluorescent Chang cells prior to absorption with the kidney cells.

DISCUSSION

The results indicate that the line of Chang cells utilized in this study does not possess LSP (15). In other studies, Hütteroth and Meyer zum Büschenfelde demonstrated the presence of LSP on approximately 20% of a Chang cell strain also obtained from Microbiological Associates and on 100% of another strain of Chang cells (19).

Evidence has been presented that lymphocytes from patients with chronic active hepatitis were cytotoxic to human Chang cells and toward autologous liver cells as target cells *in vitro* (12). Furthermore, in patients with CAH, mainly HBsAg-negative, cellular immunity has been demonstrated against the liver-specific lipoprotein (20, 21). The data suggest that in the described immune reactions of patients with chronic active hepatitis lymphocyte cytotoxicity against Chang cells and leukocyte migration inhibition by liver-specific lipoprotein may involve different antigenic sites.

The Chang cell strain used in our study appears to have lost the liver-specific membrane antigen by subcultivation. The alteration of the antigenic spectrum in

TABLE 1
IMMUNOFLUORESCENT DETECTION OF LSP ON HEPATOCYTES AND CHANG CELLS

Source of cells	Rabbit antisera to HLP	Rabbit antisera to RALP	Rat antisera to RLP
Human liver	++[a]	+	+
Rat liver	+[b]	++	ND[c]
Rabbit liver	+	ND	++
Chang cells	−[d]	−	−

[a] Strong fluorescence.
[b] Weak fluorescence.
[c] Not done.
[d] Negative fluorescence.

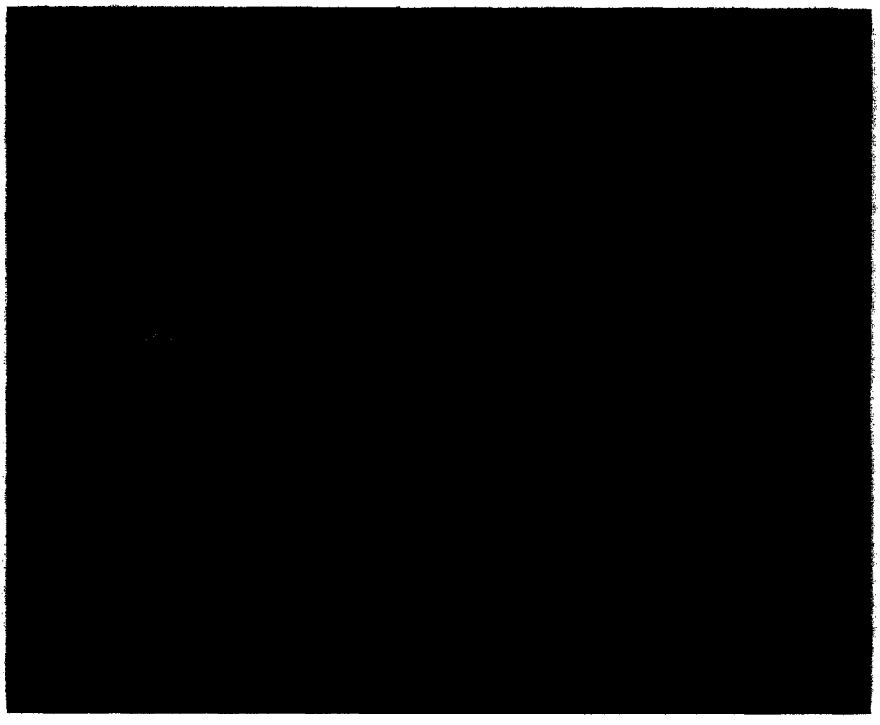

FIG. 1. Rabbit liver cells incubated with rat anti-rabbit LSP. (Magnification, × 400).

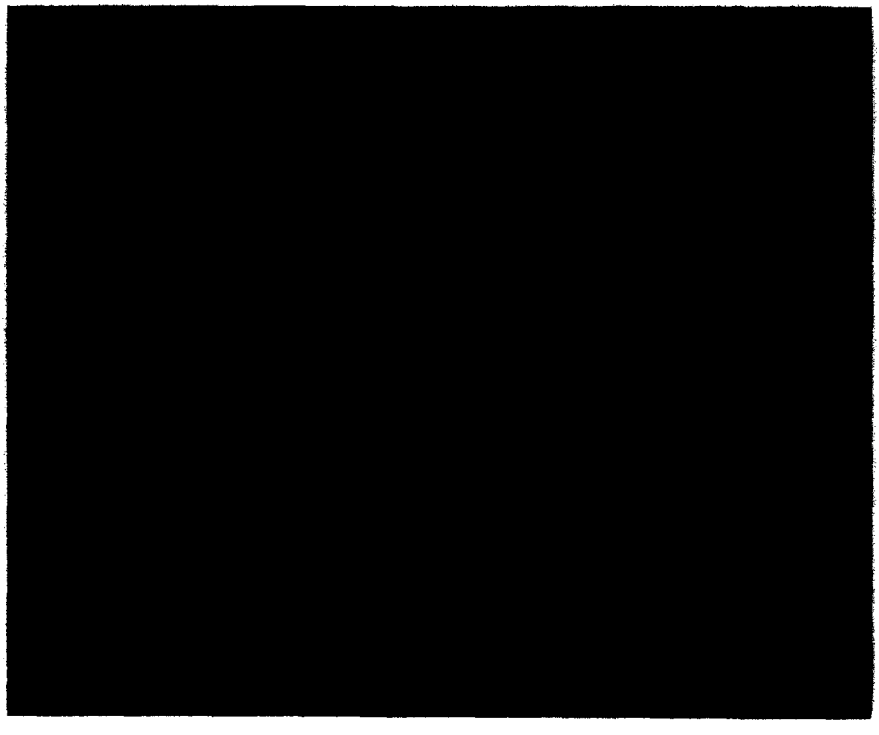

FIG. 2. Chang cells after incubation with rabbit anti human LSP. (Magnification, ×400).

cell cultures has been observed in different systems. It has been shown that kidney and liver cells undergoing neoplastic change can lose tissue-specific antigens (22, 23). In addition, kidney tubule cells lose their specific antigen during the first two cell generations of growth *in vitro*. Loss of organ-specific cell characteristics can be expected in long-term *in vitro* culture whereas retention of species-specific characteristics may yet be found (24). Other investigators (25), however, have shown that cultures of adult rat liver cells demonstrated the presence of organ-specific cell surface antigens as long as 6 months after *in vitro* culture.

The possible presence of other liver-specific membrane antigens in Chang cells is neither proved nor excluded. A liver-specific protein has been described (26) that appears to be different from the liver-specific lipoprotein described by Meyer zum Büschenfelde. This antigen, however, was not investigated in the present study.

It should be noted that both types of Chang cells, with and without the LSP, detectable by immunofluorescence, react with IgG in sera of patients with CAH in a granular fluorescence pattern (19, 27). This reaction has to be characterized by further studies.

The results of this study demonstrate that using Chang cells for *in vitro* immunological studies requires a further characterization of the antigenic pattern of the plasma membrane.

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