IMMUNOLOGICAL CROSS-SPECIES REACTIVITY OF DESMIN IN FAT-STORING CELLS (ITO CELLS) OF VERTEBRATES

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In avian smooth muscle, desmin is a major protein composed of intermediate filaments and is localized at the Z-line of myofibrils. It has been reported that in liver tissue, desmin is restricted to certain smooth muscle cells of the large blood vessels in the portal triads. We have recently identified desmin in fat-storing cells (Ito cells) of the Wistar rat liver by means of immunocytochemical techniques using rabbit IgG antibody to chicken gizzard desmin. We studied the immunologic cross-reactivity of this desmin antibody in fat-storing cells of vertebrates. Fresh liver tissues were obtained from a cyclostome (lamprey), fish (carp, eel, young yellowtail, saurel), amphibians (bullfrog, newt), reptiles (striped snake, viper, soft-shelled turtle), birds (quail, linnet) and mammals (guinea pig, mouse, rat, pig, cat, dog, rhesus monkey, man). Desmin was identified in fat-storing cells by indirect immunofluorescent microscopy of frozen tissue sections. Fat-storing cells of a cyclostome, amphibians, reptiles and birds clearly reacted with this desmin antibody. In mammals, however, fat-storing cells of the rhesus monkey and man were not stained by immunoperoxidase and immunofluorescent techniques. Similarly, the fat-storing cells of fish were not stained.

Essentially, these immunocytochemical studies using chicken gizzard desmin antibody provide evidence of the immunologic cross-species reactivity to the desmin found within fat-storing cells of many vertebrates. However, the fat-storing cells of fish, the rhesus monkey and man do not appear to demonstrate cross-reactivity. This finding would suggest either that desmin is immunologically heterogeneous or that there are differences in the accessibility of the antigenic determinants of desmin within the fat-storing cells of the latter species.

The fat-storing cell (Ito cell) was first discovered in the human liver by Ito (13). Subsequently, Ito and his coworkers demonstrated the existence of fat-storing cells within the livers of mammals, birds, reptiles, amphibians, fish and cyclostomes by light microscopy (14-16, 18).

The ultrastructure of the fat-storing cell was described in the rabbit (37), mouse (24), man (2, 17), rat (36), bat (30), kitten (31) and in some reptiles (29).

In avian smooth muscle, desmin is a major protein composed of intermediate filaments (4, 12, 19, 23, 28) and is localized at the Z line of myofibrils (21, 23).

In non-muscle cells, it has been demonstrated that desmin exists in chicken embryo fibroblasts and baby hamster kidney (BHK) cells (10, 32). We have identified desmin in the fat-storing cells of Wistar rats by means of indirect immunoperoxidase and immunofluorescent methods using chicken gizzard desmin antibody (38, 39).

In the present study we report the immunological cross-species reactivity of the antibody to the desmin found within the liver's fat-storing cells of selected vertebrates.

MATERIALS AND METHODS

Desmin was extracted from chicken gizzard smooth muscle with 1 M acetic acid according to the method of Hubbard and Lazarides (12). This extract was analyzed by preparative polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate (SDS-PAGE) (20), and two-dimensional electrophoresis (isoelectric focusing/SDS-PAGE) of the extract as described by O'Farrel (26). Antibody against this extract was raised in New Zealand white rabbits. The specificity of this antibody has been previously described (38, 39).

The following kinds of animals were used for this study: a cyclostome (lamprey), fish (carp, eel, young yellowtail, saurel), amphibians (bullfrog, newt), reptiles (striped snake, viper, soft-shelled turtle), birds (quail, linnet) and mammals (guinea pig, mouse, rat, pig, cat, dog, rhesus monkey and man). Lamprey and reptiles were purchased from a chinese medicine shop. Rhesus monkey liver tissues were donated by Dr. Shikata (Nihon University). Other animals were purchased from a pet shop. The human liver tissues were obtained during surgical procedures. We performed the study during the winter, from October 1983 to February 1984.

Liver tissues were rapidly removed from freshly killed animals, cut into small pieces, embedded in OCT compound (Tissue Tek II, Lab Tek, Miles Lab, Naperville, U.S.A.), immediately frozen by acetone dry ice and preserved at -80°C . Four micrometer cryostat sections of unfixed frozen tissue were cut on a cryostat (IEC. CTF. microtome-cryostat, International Equipment Co., U.S.A.), air dried and treated with acetone at 4°C for 20 min before being used for immunoperoxidase and immunofluorescent studies.

The cryostat sections were washed several times for 5 min each, in 6 mM phosphate buffer (PB), pH 7.2, containing 0.1 M KCl and 5 mM MgCl_2 . The sections were incubated in a moist chamber for 1 h with PB which contained either desmin antibody (0.032 mg/ml) or immunoglobulin from the appropriate pre-immune serum, and were washed three times in PB for a total of 30 min. The sections were then incubated in a moist chamber for 1 h with the N-(7-dimethylamino-4-methylcoumarinyl)maleimide (DACM)-conjugated swine anti-rabbit IgG (25), or horseradish peroxidase-conjugated goat-anti-rabbit IgG (HRP conjugate: Miles Lab., Elkhart Ind., U.S.A.) as the second antibody. For the immunoperoxidase study, endogenous peroxidase was inactivated according to Brown *et al.* (3). Specimens were examined with a Nikon Apophoto M-19 fluorescence microscope or an Olympus BH-2 microscope.

RESULTS

1. Cyclostome

In the sections of lamprey liver stained by the indirect immunoperoxidase method using chicken gizzard desmin antibody, stained cells were found between the parenchymal cells in the hepatic lobules (Fig. 1). By the indirect immunofluorescent method the cytoplasm of stained cells showed brilliant fluorescence, and nuclei and fat-droplets were translucent (data not shown). The preimmune rabbit serum did not produce noticeable staining.

2. Fish

Carp and eel, representing fresh-water fish, and saurel and young yellowtail, representing seawater fish, were studied. No cell staining was identified in the hepatic lobules of eel, saurel and young yellowtail. Faint, slender or oval fluorescent structures were scattered within the hepatic lobules of carp, but the staining was not considered to be desmin because it was different from the staining reactivity of other animals with this antibody (Fig. 2).

3. Amphibians

In the bullfrog liver, large cells were stained with this antibody. These cells bore cytoplasmic processes that spread between parenchymal cells (Fig. 3). Simi-

FIG. 1. Cyclostome-lamprey (immunoperoxidase procedure and poststained with methyl green). Numerous scattered cells (arrows) are stained with the HRP conjugate of desmin antibody. The cytoplasm appears dark brown due to the presence of HRP conjugate, and contains a few fat droplets (arrowhead). $\times 380$

FIG. 2. Fish-carp (immunofluorescent procedure).

Faint, slender or oval fluorescent structures are scattered within the hepatic lobules; no staining is observed in the other fish. $\times 380$

FIG. 3. Amphibian-bullfrog (immunofluorescent procedure).

Large cells (arrows) are specifically stained, and cytoplasmic processes can be seen to spread between parenchymal cells. $\times 380$

FIG. 4. Reptile-striped snake (immunoperoxidase procedure).

The cytoplasm contains a few fat droplets and cytoplasmic processes extend along the sinusoidal walls (arrows). $\times 380$

FIG. 5. Bird-quail (immunofluorescent procedure).

Star-shaped cells (arrows) are stained and dendritic processes can be seen to spread along the sinusoidal walls. $\times 380$

FIG. 6. Rodent-rat (immunoperoxidase procedure). $\times 380$

FIG. 7. Ungulate-pig (immunofluorescent procedure). $\times 380$

Extended cytoplasmic processes of the stained cells (arrows) branch out along the sinusoidal walls and between the parenchymal surfaces.

FIG. 8. Carnivore-cat (immunoperoxidase procedure).

Numerous stained cells (arrows) are scattered in the lobules. Cytoplasmic extensions are well developed, branched out and pass between adjacent parenchymal cell surfaces. Spindle- or star-shaped cells are also present along the sinusoidal walls and between the parenchymal cells. The cytoplasm of each cell contains a few fat droplets. $\times 380$

larly shaped cells were also seen in the newt liver, although they were fewer in number than in the bullfrog.

4. Reptiles

Stained cells were observed within the hepatic lobules of reptiles. The liver of the striped snake was stained with this antibody as shown in Fig. 4. Cytoplasmic processes were identified as those extending along the sinusoidal wall.

5. Birds

Quail liver lobule demonstrated staining of star shaped cells with dendritic processes which spread along the sinusoidal walls (Fig. 5). In the liver of the linnet, cells were stained in a similar manner with this antibody.

6. Mammals

Studies were conducted in rodents (rat, mouse and guinea pig), an ungulate (pig), carnivores (cat and dog), and primates (rhesus monkey and man). In the mouse liver, stained dendritic cells were seen along the sinusoidal walls and between the parenchymal cells. Also, spindle- or star-shaped cells, stained with this antibody, were found in guinea pig liver and rat liver (Fig. 6). Similar cells were found in pig liver (Fig. 7) and livers of carnivores (Fig. 8). Hepatic lobule cells of the rhesus monkey and man failed to stain with this antibody (Fig. 9).

FIG. 9. Primate-rhesus monkey (immunofluorescent procedure).

Cells are not stained with this antibody, but non-specific fluorescence can be seen. $\times 380$

DISCUSSION

Intermediate filaments are divided into five major classes, both biochemically and immunologically: namely, cytokeratin, vimentin, desmin, neurofilament and glial filament (21). In the liver, cytokeratin exists in hepatocytes (7) and vimentin is especially present in Kupffer cells (9). The localization of desmin in liver tissues was reported by Denk and Franke (6) who demonstrated desmin in smooth muscle cells of the arterial wall in bovine liver by means of indirect immunofluorescent techniques. Details about antigenic proteins and animals were not known.

The fat-storing cells discovered by Ito (13) are identical to the stellate cells ("STERNZELLEN") described by von Kupffer (33, 34). The fat-storing cells contain characteristic lipid droplets (16) and have the function of storing vitamin A (24, 35). The size and number of lipid droplets vary with the different seasons and the nutritional conditions, and furthermore, species-specific differences exist (14). Recently it was suggested that fat-storing cells are involved in liver fibrosis (1, 8). We have reported that fat-storing cells in the liver of Wistar rats can be stained with antibodies against chicken gizzard desmin by using indirect immunocytochemical (39) and immunoelectron microscopic techniques (38). We have also described the relationship between fat-storing cells and hepatic fibrosis produced in the rat by administration of CCl_4 or pig serum (40).

In this paper, we studied the immunologic cross-reactivity of antibodies to desmin located in the fat-storing cells of twenty vertebrate species ranging from a cyclostome to mammals using immunocytochemical methods. Fat-storing cells of a cyclostome, amphibians, reptiles, birds and mammals (except the rhesus

monkey and man) were stained with desmin antibody; however, the fat-storing cells of fish and primates did not react with this antibody. Lazarides and Balzer (22) reported the interrelationship of desmin in various muscle and non-muscle cells using immunologic techniques. Antibodies to chicken gizzard desmin cross-reacted with chick, duck and quail skeletal myofibrils, but did not cross-react with rat or guinea pig myofibrils, and similarly did not cross-react with avian or mammalian primary, secondary or established fibroblastic or epithelial cells grown in tissue culture, despite the fact that these cells contained cytoplasmic 10 nm filaments. They concluded that there were biochemically and immunologically specific forms of desmin for avian and mammalian muscle cells. The cross-reactivity of neurofilament proteins also has been studied and it has been shown that mammalian neurofilaments are distinguishable from those of avian, fish and amphibian origin (5). Our results indicate that antibodies raised against chicken gizzard desmin have a wide cross-reactivity to fat-storing cells, except in fish and primates. Since the desmin antibodies showed a different specificity for the fat-storing cell, the antigenic determinant exposed on desmin might be different between fish and primates, and other species. Recent investigations have yielded new information about the primary structure of chicken desmin (11). Furthermore, the homology of structure between hamster desmin and chicken desmin has been reported using cloned cDNA probes (27). It is evident from our observations and those of other investigators that further studies are required firstly to identify the structural characteristics of desmin, perhaps by means of antigenic sites exposed by antibodies, and secondly, to define the genetic variations of desmin found between different species and possibly even within a species.

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