GLYCOCONJUGATE HISTOCHEMISTRY AND ULTRASTRUCTURAL STUDY OF MEMBRANOUS LIPODYSTROPHY. 
A CASE REPORT*

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A biopsy case of a 40-year old housewife, who was admitted to the hospital for bilateral elbow joint pain is examined. Light and electron microscopic examination of the biopsied material from the cystic lesion of her right humerus revealed a membranocystic lesion which was characteristic of membranous lipodystrophy (21).

Histological studies using lectin-HRP conjugates provided precise information on the carbohydrate components of the membranocystic lesion. MPA, specific for α-D-galactose residues, strongly stained the membrane of the membranocystic lesion, whereas, RCA-I and SBA, specific for β-D-galactose residues, did not bind the membrane. WGA and HPA failed to stain the membrane of the typical membranocystic lesion, but bound the degenerated adipose cells of the bone marrow.

The morphogenesis and the glycoconjugates histochemistry of the membranocystic lesion are discussed in the present paper.

Membranous lipodystrophy (21), of which the first autopsy case was reported by Nasu et al. as a new disease, is possibly caused by lipid metabolic disturbance and is characterized by peculiar arabesque profiles in various adipose tissues and accompanied by leukodystrophy of the brain. The biopsied specimen of this case was reported previously as a cystic bone disease revealing peculiar features at that time (26). Since then, approximately 60 cases have been found in Japan (1, 10, 11, 15, 16, 18, 19, 25, 27, 30), 20 cases in Finland, 9 cases in Sweden, whereas recently 5 cases were reported in USA (7, 8, 12, 29).

On the other hand, a characteristic membranocystic lesion unrelated to membranous lipodystrophy, has been found in the adipose tissues of patients suffering from various diseases including leukemia, cancer, dermatomyositis and limb necrosis (5, 13, 14, 20). Such nonspecific membranocystic lesion was designated by Nasu et al. as membranocystic degeneration due to environmental disturbance of the adipose tissue (20). In addition, experimental study of the membranocystic lesion provided morphogenetical evidence for the formation of this lesion (23). However,

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no lectin histochemical studies have hitherto been made on membranous lipodystrophy. We have recently obtained biopsied material from a patient diagnosed as membranous lipodystrophy. Histochemical studies using lectin-horseradish peroxidase (HRP) conjugates and ultrastructural examinations on the material provided interesting findings. The histochemical data obtained here are believed to be useful for the characterization of the membranocystic lesion.

**MATERIALS AND METHODS**

Case: A 40-year-old housewife was admitted to the Kagoshima University Hospital, because of bilateral elbow joint pain. Roentgenograms showed radiolucent, cystic changes in bilateral elbow and knee joints. Biopsied specimen from the right tibia showed characteristic membranocystic lesions in the adipose tissue of the bone marrow. Histochemical examination revealed that these lesions distinctly reacted with periodic acid Schiff (PAS), luxol fast blue (LFB), aldehyde fuchsin, Sudan III, Sudan black B and Nile blue. Histopathological examinations and X-ray findings were well correlated with membranous lipodystrophy (Nasu disease). However, she had no particular neuropsychiatric symptoms such as disorientation, dementia, epileptic convulsions or personality changes which were also characteristic of membranous lipodystrophy. Her family history showed no remarkable finding except that her parents were consanguineous. In addition, no significant abnormalities were observed in laboratory findings. Consequently, this case was believed to be an incomplete type of membranous lipodystrophy without expression of any neuropsychiatric symptoms at the present time.

Tissue preparation: Biopsied materials from the right humerus of the patient were divided into two parts; one was doubly fixed with half Karnovsky’s fixative and 1% buffered osmium tetroxide for morphological examination, whereas the other part was fixed with half Karnovsky’s fixative only for lectin histochemistry. Both the preparations were dehydrated through graded ethanol series, and embedded in Epon 812.

Light and electron microscopy: Thick sections cut with a Sorvall MT 2B ultramicrotome were stained with toluidine blue and examined light microscopically. Ultrathin sections cut with the same ultramicrotome were stained with a saturated aqueous solution of uranyl acetate (28) and then with a lead citrate solution (22) for 5 min each. The specimens were examined in a JEOL-100B electron microscope. Lectin histochemistry: Epon-embedded thick sections (thickness, 1.5 μm) were treated with 2.0 g KOH in 10 ml pure methanol and 5 ml propylene oxide to remove the epoxy resin for 5 min (16). After rinsing in 50% methanol and distilled water, sections were dipped in 0.3% hydrogen peroxide in pure methanol for 20 min to inhibit the activity of endogenous peroxidase, washed briefly in Dulbecco’s phosphate-buffered saline (PBS) (4), and then rinsed in 1% bovine serum albumin (BSA) in PBS for 10 min. Subsequently, sections were incubated in 25 μg/ml horseradish peroxidase (HRP)-labeled lectins for 60 min. The lectins used in this study were *Maclura pomifera* agglutinin (MPA), *Helix pomatia* agglutinin (HPA), *Triticum vulgaris* agglutinin (WGA), *Glycine max* agglutinin (SBA) and *Ricinus communis* agglutinin-I (RCA-I) and all these lectins were purchased from EY labo-
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in the dianaminobenzidine (DAB) hydrogen-peroxide medium, pH 7.6, described by Graham and Karnovsky (6). With or without hematoxylin-counterstaining, the sections were dehydrated, cleared, and mounted in Permount (Fisher Scientific Co., Fair Lawn, NJ). All these procedures were performed at room temperature. The treatment with methanol described above did not affect glycoconjugates in sections (24).

Controls: As a control, the HRP-labeled lectins were preincubated with their haptenic sugars (0.2 M D-galactose for MPA, SBA, RCA-I, 0.2 M Ga1NAc for HPA, and 0.2 M GlcNAc for WGA) for 30 min, and then applied onto sections.

RESULTS

Light microscopy: Epon-embedded thick sections distinctly disclosed foci of markedly undulating membranocystic lesions with arabesque profiles (Fig. 1). These lesions were scattered in the adipose tissues of the bone marrow. They were variable in size and complexity. There was less inflammatory cell infiltration, even though some macrophages were associated with these lesions. Less trabecular bones remained. There were no abnormalities in blood vessels of the bone marrow. Lectin histochemistry: MPA which specifically recognizes α-D-galactose residues (2), definitely stained all the typical membranous structures (Figs. 2, 3). The staining of membranocystic lesions with other lectin conjugates except for WGA and HPA, which specifically bind to N-acetyl-D-glucosamine (GlcNAc) (3) and N-acetyl-D-galactosamine (GalNAc) (9), respectively, was faint or negligible (not shown). Both the WGA and HPA conjugates did not bind to the typical membranous structures. However, they stained the plasma membrane of the bone marrow cells and degenerated adipose cells exhibiting no arabesque profile as well (Figs. 4–6).

The specificity of the lectin stainings observed was substantiated by the results of the control experiment using appropriate monosaccharides (not shown).

Electron microscopy: Well developed membranocystic lesions revealed the same profiles as those reported previously. The undulating membrane of the membranocystic lesion was composed of numerous minute tubular structures which were perpendicularly arranged to the inner surface of the cystic lumen (Fig. 7). These minute tubular structures showed communications with the cystic lumen of the membranocystic lesion. However, no definite trilaminar structure of the unit membrane was detected (Fig. 8). In addition to these well developed membranocystic lesions, other types of membranocystic lesions were observed. There were undulating thinner membranes without tubular structures forming a similar cystic lesion, whereas minute vesicular structures accumulated at the lipid-cytoplasmic interface of a degenerated adipose cell were observed (Fig. 9). Furthermore, electron opaque substances were seen at the lipid-cytoplasmic interface of a degenerating adipose cell. These electron dense substances irregularly extended into the extracellular matrix from a lipid-droplet of a degenerating adipose cell (Fig. 10).
DISCUSSION

Both the light and electron microscopic findings obtained in the present case coincide well with those of membranocystic lesions in membranous lipodystrophy (21). Although numerous histopathological studies on membranous lipodystrophy including experimentations have been performed, its etiology and morphogenesis are still under debate (1, 5, 8, 10, 11, 13–16, 18–21, 23, 25–27, 29, 30).

In the present case, there are several types of membranocystic lesions as previously reported. For instance, thicker membrane with well developed minute tubular structures and thinner membrane without minute tubular structures coexisted in these lesions (13, 14). Furthermore, electron dense substances were visualized...
FIG. 7. Electron microphotograph of the typical membranocystic lesion. Membranous structure is composed of an accumulation of numerous tubular structures. Cytoplasmic processes of a macrophage are noted. ×10,000, Bar=1.0 μm

FIG. 8. Electron microphotograph of the membranous structures of the membranocystic lesion. Numerous minute tubular structures are perpendicularly arranged to the inner surface. ×30,000, Bar=1.5 μm
Fig. 9. Electron microphotograph of degenerated adipose cells. Electron dense substances are observed at the lipid-cytoplasmic interface of the degenerated adipose cell (F1). On the contrary, numerous microvesicles are formed at the lipid-cytoplasmic interface of the degenerated adipose cell (F2). ×30,000, Bar=1.5 μm

Fig. 10. Electron microphotograph of a degenerated adipose cell. Irregular branchings of electron dense substances (arrows) connected with lipid content of a degenerated adipose cell are observed in the extracellular matrix. ×15,000, Bar=1.0 μm
not only at the lipid-cytoplasmic interface of a degenerating adipose cell but also in the extracellular matrix. Both of these were occasionally connected with each other. These findings strongly suggest that the electron dense substances at the lipid-cytoplasmic interface of a degenerated adipose cell could change into aggregations of minute vesicles, and eventually into minute tubular structures, which are characteristic of the membranocystic lesion. Concerning the morphogenesis of the membranocystic lesions, similar histological findings have been reported to exist in limb necrosis due to chronic arterial obstruction (13, 14) as well as membranous lipodystrophy (27). On the other hand, previous light and electron microscopic studies on experimentally induced membranocystic lesions in rabbit bone marrow adipose tissues have revealed that the minute tubular structure of the membranocystic lesion resulted from minute vesicles developed at the lipid-cytoplasmic interface of degenerated adipose cells (23). Thus, the interaction between the lipid and water-soluble components at the lipid-cytoplasmic interface of a degenerated adipose cell may play an important role in the formation of the membranocystic lesions.

Previous conventional histochemical studies have disclosed that membranocystic lesions exhibited autofluorescence and reacted for carbohydrates, proteins, lipids and in particular for phospholipids even in paraffin sections (5, 19). Therefore, the membranocystic lesions have been thought to be a stable complex consisting of lipid, carbohydrate and protein (5). The present investigation employing lectin-HRP conjugates has provided more precise information about saccharide residues involved in the lesions. The present electron microscopic examinations appear to indicate that the degenerated adipose cells stained with HPA and WGA are compatible with the initial or early stage of the membranocystic lesions. The lack of affinity of the membranocystic lesions towards HPA and WGA is taken to imply that GalNAc and GlcNAc residues are fundamental structures of saccharide moieties of the membranocystic lesions. MPA shows a high specificity for α-D-galactosyl residues (2). The affinity of the membranocystic lesions towards MPA suggests that a certain type of glycosylations would occur during the course of formation of the membranocystic lesion. Further investigations with lectin-colloidal gold conjugates at the ultrastructural level would provide more precise information as to the carbohydrate composition of the unique membranocystic lesion.

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