Lectin histochemistry of gangliosidosis*
II. Neurovisceral tissues from patients with Sandhoff’s disease

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Summary. Lectin histochemical studies were performed on selected formalin-fixed, paraffin-embedded tissues of patients affected with the O variant of GM2-gangliosidosis (i.e., Sandhoff’s disease). The purpose was to identify specific sugar residues of undegraded “stored” substances in cytoplasm of affected cells. We studied neural tissues from 13 patients, visceral tissues from four patients, and placentae from three affected fetuses. Neurons in all 13 cases studied stained with Concanavalia ensiformis agglutinin (Con A) and with Ulex europaeus agglutinin-I (UEA-I). Succinylated wheat germ agglutinin (S-WGA) stained affected visceral cells and astrocytes and macrophages in the central nervous system. These results demonstrate that α-D-mannosyl and β-L-fucosyl residues, which bind Con A and UEA-I, respectively, are present in affected neurons. Furthermore, they revealed the affected non-neuronal cells and astrocytes contain complex carbohydrates with nonreducing terminal β-N-acetylgalactosamine, which binds S-WGA.

Key words: Hexosaminidase A and B deficiency — Lectin histochemistry

Sandhoff’s disease is a genetically determined lysosomal storage disease. It is an O variant of GM2-gangliosidosis, which is characterized by deficient activity of lysosomal hexosaminidase A and B [36, 37] due to a defect of their common B subunit [20, 38]. This deficiency results in massive neuronal accumulation of the GM2-ganglioside, Gα2 [33] visceral globoide (Gα4), and in the urinary excretion of oligosaccharides with terminal β-N-acetylgalactosaminyl (GalNAc) residues [40, 41]. Periodic acid-Schiff (PAS), and Sudan black stains on frozen tissues from affected patients show an accumulation of substances with 1,2 glycol groups or glycolipids. Until now no histochemical method has been used for identifying the presence of oligosaccharides in affected cells.

Recently, lectin reagents have been used to study glycoprotein [4, 6, 23] and glycolipid storage diseases [5, 18, 42]. Furthermore, oligosaccharides are retained in paraffin sections from individuals affected with glycolipid storage diseases and can be demonstrated with specific lectins [7]. Lectins are carbohydrate-binding proteins and glycoproteins, which have known sugar specificities [21] and are useful as histochemical probes for identifying and localizing carbohydrate residues [4]. In this study we used ten different lectins as probes, and avidin-biotin-peroxidase complex (ABC) as “visualant,” to determine the staining patterns of affected cells in patients with confirmed Sandhoff’s disease. We are now reporting that neurons from every patient affected with Sandhoff’s disease are stained with Ulex europaeus agglutinin-I (UEA-I) and Concanavalis ensiformis agglutinin (Con A). Furthermore, neurons were stained in varying degrees by other lectins in individual cases. In addition, macrophages, exocrine pancreatic cells and astrocytes stained with succinylated wheat germ (S-WGA).

Materials and methods

Formalin-fixed, paraffin-embedded brain and spinal cord sections were obtained from 13 cases of Sandhoff’s disease, visceral organs from four patients, and placenta from three therapeutic abortions of fetuses with Sandhoff’s disease. The clinical presentation, as well as the biochemical findings of some of these cases, have been reported elsewhere [11, 16, 17, 26, 29, 44, 45]. The absence of hexosaminidase A and B was documented in all cases. At least three corresponding normal tissues were used as controls. In addition, tissues were obtained from patients with
galactosylceramide lipidosis [7], from patients with glucosylceramide lipidosis (Alroy, unpublished observations) and from patients with glycoprotein storage disease [3].

Five-micrometer sections were stained with hematoxylin and eosin. For identification of astrocytes, neural tissues were stained with rabbit anti-glia fibrillary acidic protein (GFAP) [13], which was obtained from Dakopatts (Santa Barbara, Calif). Adjacent tissue sections were incubated with nine different lectins (Vector Laboratories, Burlingame, Calif) and controls for blocking lectin with specific sugars were prepared as described in the accompanying paper [9]. In addition, we used Lens culinaris agglutinin (LCA) which, like Con A, identifies α-mannosyl residues; however, the former is specific for fucosylated, N-glycosidically-linked complex oligosaccharides [15].

Results

Histopathological findings

Light microscopic examinations of neural tissue revealed lesions in all 13 cases, which are similar to those described earlier [2, 16, 17]. The lesions include various degrees of neuronal enlargement and vacuolization, as well as loss of Purkinje cells, diffuse demyelination, astrocytosis and infiltration by macrophages. They were most severe in the cerebral cortex and cerebellum, while in the brain stem and spinal cord the cytoplasm of neurons was only moderately enlarged and finely vacuolated. The morphology of the affected visceral organs, including liver, pancreas, kidney, spleen and lung, was similar to those reported previously [16, 17, 35]. The cytoplasm of hepatocytes and Kupffer cells, pancreatic exocrine cells, splenic, lymph nodes and pulmonary macrophages were enlarged and contained large vacuoles. Similarly, the cytoplasm of placental macrophages (i.e., Hofbauer cells) was enlarged and vacuolated. Corresponding normal control tissues did not display any of these lesions. When compared, the lesions in some storage diseases, such as fucosidosis or α-mannosidosis, revealed some similarities to those seen in Sandhoff’s disease, while the lesions observed in Krabbe’s or Gaucher’s disease were different.

Histochemical findings

Histochemical studies of affected neural tissue revealed consistent staining of neurons with Con A and UEA-I (Fig. 1). Affected neurons displayed either uniform (Fig. 1) or granular staining of the perikaryon with UEA-I (Fig. 2). Variation in staining intensity was observed between affected individuals. In most cases the vascular endothelium was also stained, but the erythrocyte membranes stained in less than 50% of the patients studied. Neurons were not stained by UEA-I in control brain sections and in neural tissue from patients affected with galactosylceramide lipidosis [7], glucosylceramide lipidosis (Alroy, unpublished observations) and in α-mannosidosis and sialidosis [3]. Similarly, the affected neurons stained with Con A, whereas the neurons in control sections and in sections from patients with galactosylceramide lipidosis, fucosidosis and sialidosis [3, 8] stained either weakly or not at all. Prominent and characteristic staining patterns of astrocytes, microgliosis, infiltrating macrophages and vascular endothelium was noted with wheat germ agglutinin (WGA), S-WGA, Ricinus communis agglutinin-I (RCA-I) and UEA-I (Table 1). Differential staining between various glial cells and neurons was best observed in the first layer of the cerebral cortex, since this layer contains few neurons but showed infiltration by macrophages and astrocytes. RCA-I stained the macrophages, endothelial cells and erythrocytes in all cases (Fig. 3), the astrocytes in half of the cases, and neurons of the deeper cortex in only three cases. As in a previous report, the microglia of control brains stained with RCA-I [28]. WGA stained the macrophages, astrocytes, endothelium and erythrocytes in all cases (Fig. 4) but neurons in only four cases. Similarly, S-WGA stained the macrophages and astrocytes in all cases (Fig. 5), the endothelium and erythrocytes in four cases and neurons in three (Fig. 6). In liver hepatocytes stained with Con A, peanut agglutinin (PNA), S-WGA and WGA, while the Kupffer cells stained with Con A, LCA, PNA (Fig. 7), RCA-I, S-WGA and WGA. Similar staining was observed in Hofbauer cells (i.e., stromal macrophages) in chorionic villi (Fig. 8) but not in corresponding normal placenta. Our findings in normal placentae confirm previous reports that Hofbauer cells did not stain with either PNA or S-WGA [27]. Pancreatic acinar cells, which appeared vacuolated on H&E, stained with Con A, Griffonia simplicifolia-I (GS-I), RCA-I, S-WGA (Fig. 9), UEA-I and WGA, while the islet cells stained only with Con A, RCA-I and with WGA. In normal control pancreas the acinar cells did not stain with S-WGA (Fig. 10), while in α-mannosidosis they did stain.

Discussion

Lysosomal storage diseases are a group of hereditary diseases, the majority of which are characterized by deficient activity of lysosomal hydrolase and result in the accumulation of undegraded substrates. Although it was first supposed that the stored substances were homogenous, recent biochemical studies of extracted substances from neural and visceral tissues from patients affected with glycosphingolipidosis, such as GM₁− and GM₂-gangliosidosis, demonstrated an accumulation of chemically diverse substances. Furthermore, each organ or cell type may have characteristic storage material [14, 34]. In the current study we used lectin histochemistry to demonstrate the presence of