Acta Neuropathol (Berl) (1988) 75:385–390

Acta Neuropathologica

Reactions of vessel walls and brain parenchyma to the accumulation of Gaucher cells in the Norrbottnian type (type III) of Gaucher disease*

N.G. Conradi[1], H. Kalimo[2], and P. Sourander[3]

[1] Division of Neuropathology, Department of Pathology, University of Göteborg, Sahlgren Hospital, S-41345 Göteborg, Sweden
[2] Department of Pathology, University of Turku, SF-20520, Turku, Finland

Summary. Splenectomy in children with the Norrbottnian type of Gaucher disease is followed by increased blood levels of glucosylceramide and impaired neurological and mental status. High blood levels are associated with an increased accumulation of glucosylceramide in perivascular Gaucher cells in the brain compared to non-splenectomised cases. Surrounding the Gaucher cell infiltrates there is loss of neurons and slight demyelinaton in the brain parenchyma. The brains of four cases with the Norrbottnian type of Gaucher disease were examined by immunohistochemical stains in an attempt to further characterize the perivascular Gaucher cells and to examine the reactions of the vessel walls and brain parenchyma to the accumulation of Gaucher cells. The perivascular storage cells showed granular staining with antibodies to muramidase and α_1-antichymotrypsin confirming that they are blood-derived macrophages belonging to the monocyte-macrophage system. The Gaucher cells contained material positive for antisera to plasma proteins strongly suggesting that large molecules (including glucosylceramide) can escape from the blood and be taken up by the macrophages in Gaucher disease. The storage cells were surrounded by a reticulin network stained by antisera to collagen type III, type IV and laminin. The infiltrates were bounded from the brain parenchyma by a membrane strongly positive with antiserum for the basal lamina protein collagen type IV and laminin. The formation of a basal lamina around the Gaucher cell cuffs probably constitutes a protective phenomenon governing the brain parenchyma against the foreign cells. A focal loss of neurons but only minor loss of axons could be demonstrated with the antiserum to neurofilament. The brain parenchyma surrounding the Gaucher cell infiltrates showed marked astrogliosis in the anti-glial fibrillary acidic protein stain.

In the two cases previously shown to have higher blood levels of glucosylceramide there were astrocytes positive for plasma proteins indicating passage of plasma proteins into the brain, this was not seen in the non-splenectomised cases. The additive effect of low-grade tissue damage in the vicinity of the Gaucher cell infiltrates is probably enough to explain the increased neurological symptoms and mental retardation following splenectomy in the Norrbottnian type of Gaucher disease.

Key words: Gaucher disease – Brain – Immunohistochemistry – Macrophages – Astrocytes

Three types of Gaucher disease are generally recognized: type I (adult, non-neuronopathic), type II (infantile, acute neuronopathic) and type III (juvenile, subacute neuronopathic, including the Norrbottnian type). The three types differ not only with regard to neurological symptoms [2, 3, 5, 6] but also with regard to the amount and fatty acid pattern of the glucosylceramide accumulated in the brain [10, 22]. In type I, the glucosylceramide occurs only in perivascular phagocytic cells, so-called Gaucher cells [6, 13] and it has a fatty acid composition similar to that seen in glucosylceramide stored in spleen ("peripheral" type, [10]). In type II, glucosylceramide appears to be located in neurons as well as in phagocytic cells located both within the parenchyma and perivascularly [1, 13, 18]. The glucosylceramide of gray matter has a fatty acid composition indicating cerebral gangliosides as main precursor of the glucosylceramide ("central" type, [10]). In type III, glucosylceramide is found in neurons of gray matter and in

* Supported by grants from Swedish Medical Research Council (project nos. 03488, 07121, 07123)

Offprint requests to: N. Conradi (address see above)

perivascular Gaucher cells in gray and white matter [4, 19]. Generally, the fatty acid pattern of the glucosylceramide is of central type in gray matter and of the peripheral type in white matter [4, 10].

Splenectomy in the Norrbottnian type of Gaucher disease (type III) patients appears to speed up the disease process and to cause, eventually, a more severely impaired neurological status and intellectual function [2]. Biochemical studies have shown that splenectomy in type III cases leads to increased accumulation of glucosylceramide of the "peripheral" type in liver and brain indicating that when the material can no longer be stored in the spleen it is deposited in other organs [22]. The increased storage in the brain after splenectomy is restricted to the perivascular distribution [4, 19]. Surrounding the larger accumulations of Gaucher cells, there are signs of neuronal cell death and loss of myelin [4]. Hence, it was argued that, since the accumulation of Gaucher cells appear to affect the surrounding brain, it may constitute an important factor for the development of neurological disease.

The aims of the present study were (1) to further characterize the perivascular Gaucher cells and (2) analyze the reactions of the vessel walls and of the surrounding neural tissue to the accumulation of Gaucher cells.

Material and methods

Clinical material

Four of the five cases previously reported upon morphologically were included. Cases III:3 and III:6 had high blood levels of glucosylceramide during life and showed the most marked accumulation of glucosylceramide in the brain both biochemically and morphologically [4, 10, 19]. Case III:3 was subjected to splenectomy at the age of 2 years and 2 months and died at the age of 6 years and 10 months. Case III:6 was not subjected to splenectomy; the blood levels of glucosylceramide of this case were in the range of splenectomised patients. At death, the spleen was largely necrotic and fibrotic and there were high levels of glucosylceramide in the liver [11, 12]. The patient died at an age of 10 years and 5 months. Cases III:1 and III:4 were not splenectomised. Their blood levels of glucosylceramide were relatively lower [12]. They showed little accumulation of Gaucher cells in the brain and their brain glucosylceramide was probably derived from brain gangliosides [4, 10]. These patients expired at an age of 2 years 11 months and 7 years and 5 months, respectively.

Immunohistochemistry and histology

The following antibodies were used in this study: rabbit anti-human collagens type III and IV/7S domain, and anti-human laminin (kind gifts from Drs. Leila and Juha Risteli, [see 7], rabbit anti-human fibronectin (Dako A 245), anti-human albumin (Dako A 001, Dakopatts A/S, Glostrup, Denmark) and anti-human fibrinogen (Dako A 080), anti-human muramidase (Dako A 099), anti-human α_1-antichymotrypsin (Dako A 022), anti-bovine glial fibrillary acidic protein (GFAP, Dako Z 334) and monoclonal anti-neurofilament (200 kDa protein, Labsystems, Helsinki, Finland). Stainings were carried out on paraffin sections using either the peroxidase-antiperoxidase (PAP) method or avidin-biotin method (Vectastain ABC-kit) with diaminobenzidine or aminoetylcarbazole as chromagen. Non-immune rabbit serum was used as control. For histopathological examination sections were stained with Laidlaw's silver method for reticulin and silver-methenamine in addition to those prepared for the previous publication (including two different PAS-methods and luxol fast blue, Palmgren silver and Ranke stains).

Results

As reported previously [4, 19] the PAS-positive, perivascular Gaucher cells were most numerous in the cerebellar white matter and granule cell layer (Fig. 1). Their number varied considerably between the four cases. The greatest number (as also the amount of biochemically determined glucosylceramide [4, 10]) was seen in cases III:6 and III:3, who had been splenectomised.

The perivascular Gaucher cells were stained in sections treated with antibodies to muramidase and α_1-antichymotrypsin (Fig. 2). The cells showed slight difference in staining intensities. Mostly the staining was seen as well-defined granules in the cytoplasm of the cells (Fig. 2).

Staining with the silver stain for reticulin (Fig. 3) and with antiserum to collagen type IV, laminin and collagen type III revealed a similar network (Figs. 4–6) in the vessel walls. The anti-sera against the basal lamina proteins collagen type IV and laminin stained strongly all blood vessels corresponding to the basal lamina in their walls (Figs. 4, 5). The perivascular cuffs of Gaucher cells were always delineated from the surrounding brain parenchyma by a delicate positively-stained membrane and in their center the basal lamina of the vessel wall stained strongly, often appearing reduplicated (Figs. 4, 5). A delicate network staining weakly was seen also between the Gaucher cells. Staining for collagen type III was generally strongest in the sub-endothelial region and faded towards their periphery (Fig. 6). In some of the cuffs the staining between the Gaucher cells was more prominent for collagen type III than for the basal lamina proteins.

Antisera against plasma proteins gave positive reaction for the cells in the perivascular cuffs (Fig. 7). In the cases with high blood levels of glucosylceramide (III:3 and III:6), the brain parenchyma surrounding Gaucher cell accumulations contained positively stained astrocytes (Fig. 7). Occasional neurons also contained material staining for plasma proteins in these cases. Astrocytes or neurons with positive reaction for plasma proteins were not recorded in the non-splenectomised cases.

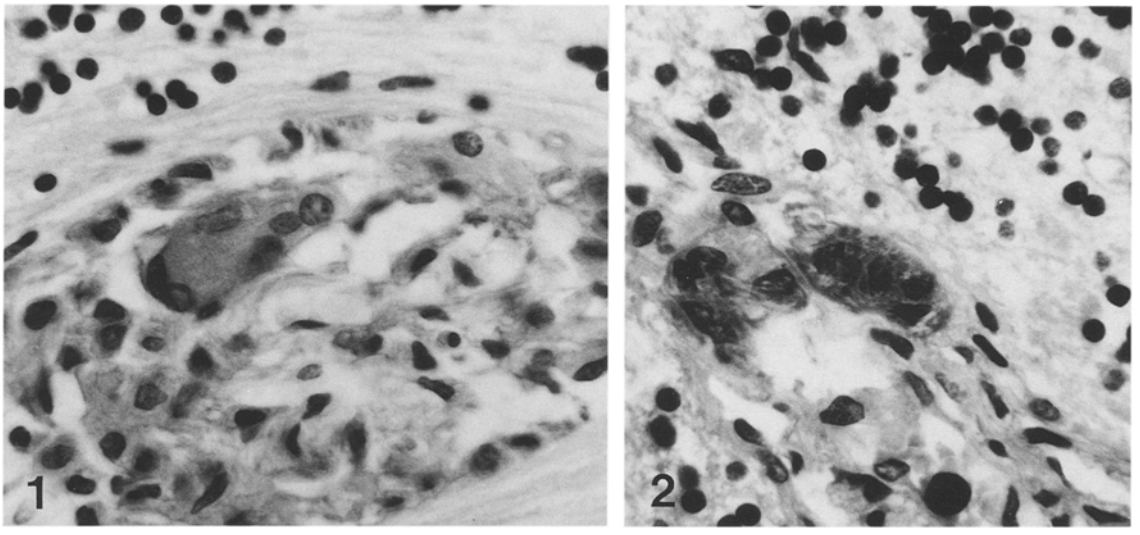


Fig. 1. Typical perivascular accumulation of Gaucher cells with abundant, strongly PAS-positive cytoplasm. PAS of McManus, cerebellum, case III:6, ×800

Fig. 2. The marker for lysosomal enzyme muramidase (lysozyme) gave granular positive staining of the Gaucher cell cytoplasm. The stored glucosylceramide itself obviously does not stain. Anti-muramidase + hematoxylin counterstain, cerebellum, case III:6, ×800

The brain tissue around the Gaucher cell infiltrates displayed strong gliosis as evidenced by accentuated reaction with anti-GFAP antiserum (Fig. 8). The staining was most prominent just outside the Gaucher cells, where a thicker glial limiting "membrane" was formed and it faded away from the Gaucher cell cuffs (Fig. 8). Axons positive for the anti-neurofilament serum as well as with the Palmgren silver stain were seen around the perivascular cuffs, usually in an approximately normal density and only rarely did we see a definite loss of axons. As reported previously, there was a marked loss of nerve cells in the vicinity of the cuffs, especially in the cerebellar granule cell layer.

Discussion

The exact origin of the perivascular Gaucher cells in the brain and the lipid stored in them is not clear. Passage of glucosylceramide from the blood into the brain was suggested from biochemical differences in fatty acid composition of the glucosylceramide in gray and white matter and between splenectomised and non-splenectomised cases [4, 10]. It has also been shown that the number of Gaucher cells in the brain increases after splenectomy [4, 19], probably in response to the increased concentration of glucosylceramide in blood [11, 12]. Several of the perivascular cells are multinucleate; the formation of multinucleate storage cells has been reproduced in cell culture of retinal tissue with glucosylceramide isolated from a case of Gaucher disease and galactosylceramide isolated from a case of Krabbe disease but not with sulfatide [20]. Monocytic cells with small Gaucher inclusions have been demonstrated in the blood [15] but we know of no report showing multinucleate cells in this compartment. On the basis of these and other previous results on Gaucher disease, the hypothesis was put forward that the perivascular Gaucher cells in the brain are transformed cells belonging to the monocyte/macrophage system, which take up glucosylceramide transported in the blood [4, 19]. The present study offers support for this hypothesis in relation to two questions: (1) do the perivascular cells exhibit the characteristics for macrophages in other locations? and (2) is it possible for large molecules transported in the blood to reach the perivascular compartment?

The Gaucher cells reacted positively with antisera both to muramidase and to α_1-antichymotrypsin, two commonly used markers for macrophages. These antisera are not definitely specific for macrophages but rather demonstrate cells active in phagocytosis, as both substances are lysosomal enzymes. It has been questioned whether endogenous phagocytic cells do exist at all in brain parenchyma and suggested that the brain macrophages are blood-derived and belong to the monocyte/macrophage system [8, 17, 21]. Major phagocytic function has not been ascribed to pericytes [16]. Therefore, it seems most likely that the Gaucher cells are transformed monocytic cells derived from the blood.

Presence of anti-albumin- and -fibrinogen-positive material in the Gaucher cells gives evidence for pas-

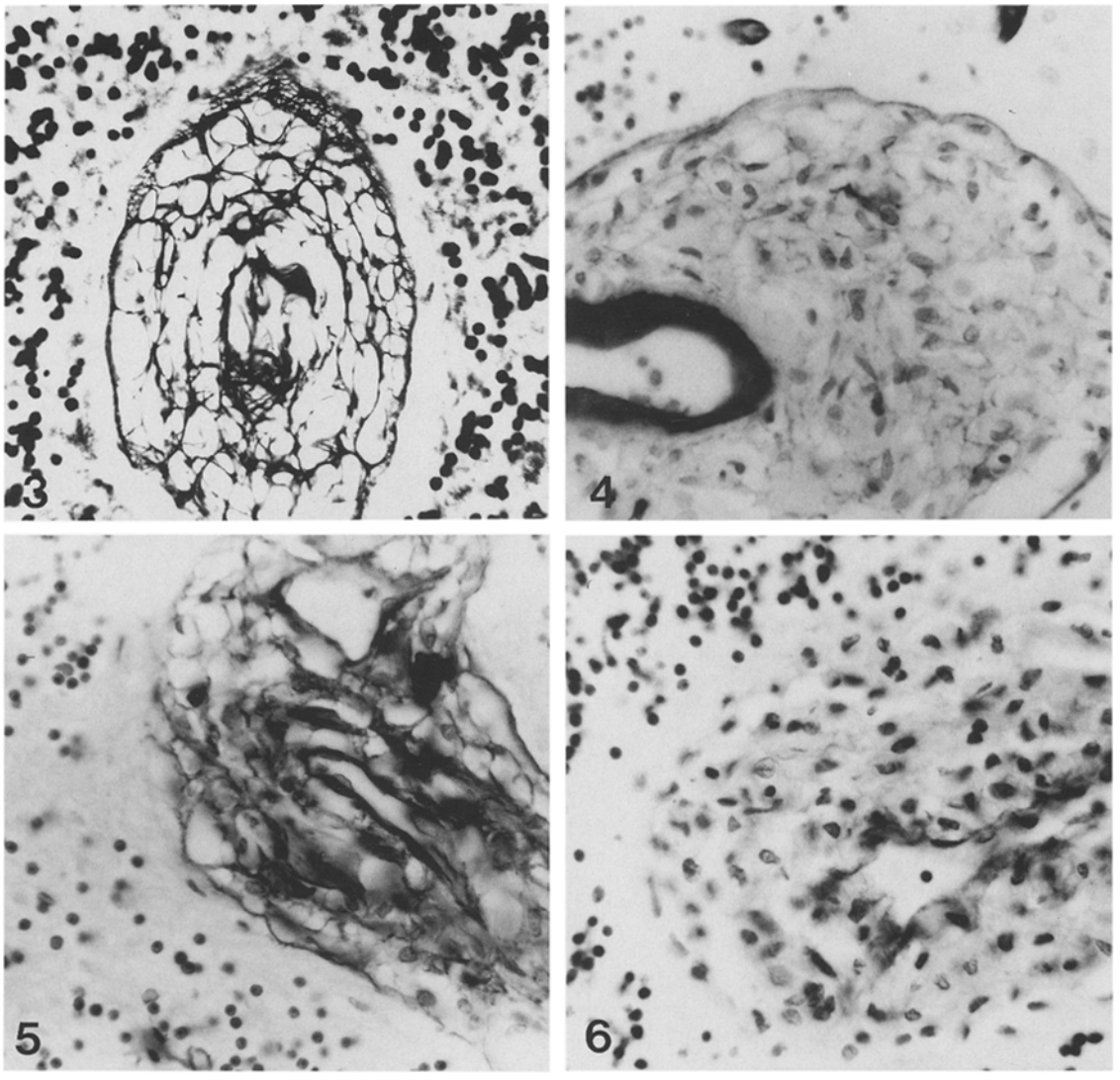


Fig. 3. Silver stain for reticulin revealed a network which bounded the perivascular cell infiltrate and in the interstices of which the Gaucher cells reside. Laidlaw's silver for reticulin, cerebellum, case III:6, ×400

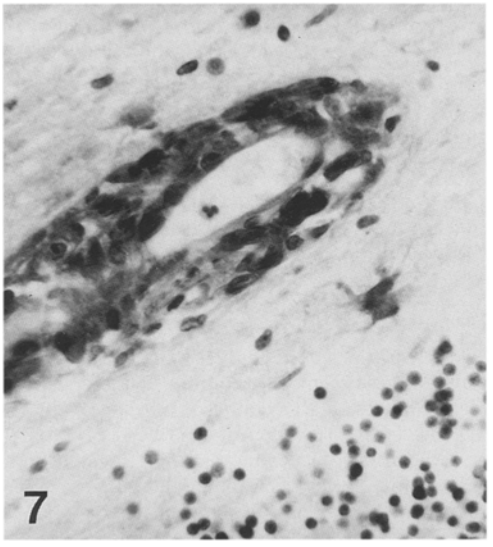
Fig. 4. The Gaucher cell infiltrates were surrounded by basal lamina as indicated by positive staining with antiserum to the basal lamina specific collagen type IV. The subendothelial basal lamina of the blood vessel (inner ring) appeared thickened. A delicate network of weaker staining was visible also between the Gaucher cells. Anti-human collagen type IV + hematoxylin, cerebellum, case III:6, ×400

Fig. 5. Antiserum to another basal lamina-specific glycoprotein, laminin, gave similar staining as for collagen type IV with somewhat stronger positive reaction between the Gaucher cells. Laminin apparently forms a perivascular network which corresponds to that obtained with silver staining for reticulin. Anti-human laminin + hematoxylin, cerebellum, case III:6, ×400

Fig. 6. Immunohistochemical staining for collagen type III (the classical "reticulin") was most evident in the subendothelial region and between the innermost Gaucher cells. Anti-human collagen type III + hematoxylin, cerebellum, case III:6, ×400

sage of large molecules from the circulation. This indicates that glucosylceramide molecules may also leak from the blood and be taken up into Gaucher cells. Interestingly, there were signs of passage of plasma proteins into the surrounding brain tissue only in the splenectomised cases. This suggests that the accumulation of Gaucher cells itself enhances the passage of larger molecules over the blood-brain barrier thereby possibly increasing the potential for uptake of glucosylceramide from blood.

Formation of basal lamina around the perivascular Gaucher cell cuffs is an interesting reaction at the interphase between the neuroectodermal brain parenchyma and the mesenchymal macrophages.



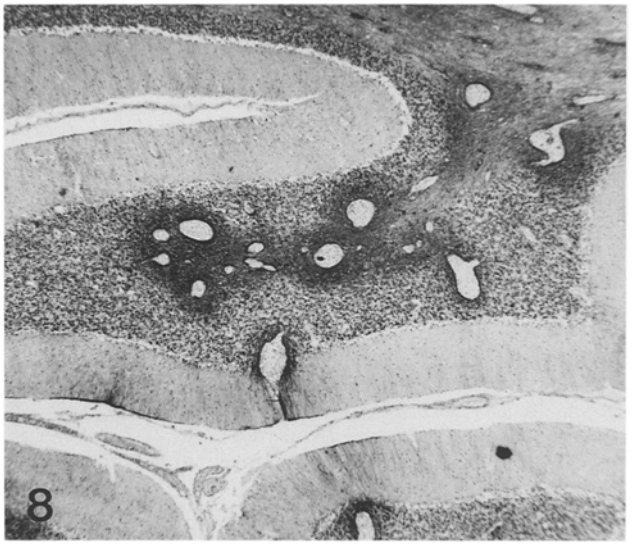


Fig. 7. Positive staining with antisera to plasma proteins (here fibrinogen) is present in the perivascular Gaucher cells. These proteins spread also into the surrounding brain parenchyma as demonstrated by the positive staining of nearby astrocytes. Anti-human fibrinogen + hematoxylin, cerebellum, case III:6, ×400

Fig. 8. There was a marked accentuation of GFAP-positivity in the brain parenchyma around the perivascular Gaucher cell infiltrates. Anti-GFAP + hematoxylin, cerebellum, case III:6, ×25

Similar reaction has been described in cerebral trauma, where the mesenchymal scar becomes limited from the brain by a newly formed glia limitans with basal lamina [9]. Similarly, a reticulin network, rich in the two basal lamina proteins collage type IV and laminin, is formed around the perivascular infiltrates of neoplastic cells in primary brain lymphoma [7]. This has been suggested to be a protective phenomenon to reestablish the integrity of the neuroectodermal "realm" against the connective tissue. This also appears to be a reasonable explanation for the production of a thickened astrocytic glia limitans with basal lamina around the foreign cells in Gaucher disease.

There was a vigorous astrogliosis around the perivascular cuffs and the decreasing density with increasing distance from the vessels of this gliosis strongly suggests diffusion of some gliosis-inducing factor. Since there were signs of astrocytic uptake of plasma proteins, the gliosis could be caused by extravasated plasma components. However, release of toxic molecules from the Gaucher cells could also be the inductive factor. Such molecules may include the tentatively neurotoxic substance glucosylsphingosine (psychosine) [10] and lysosomal enzymes, which may be released from macrophages during phagocytosis [14].

Clinical findings strongly relate impaired neurological and mental status and splenectomy [2]. It has been shown that splenectomy leads to increased accumulation of glucosylceramide in the brain [10]. This glucosylceramide has a fatty acid pattern similar to that in the spleen and the accumulation can only be demonstrated in the perivascular Gaucher cells [4]. In a previous study it was noted that areas of slight demyelination and neuronal loss surround the Gaucher cell infiltrates in white and gray matter, respectively [4]. The present results indicate that only minor loss of axons accompany the accumulation of perivascular Gaucher cells. There is, however, a marked astrogliosis in these locations. The additive effect of low-grade damage to the brain tissue in the vicinity of Gaucher cell accumulations may well be enough to explain the aggravated neurology and mental retardation following splenectomy. The definite mechanism by which the brain becomes damaged by the increased accumulation of Gaucher cells still awaits its explanation. Since the glucosylceramide accumulated in the brain following splenectomy is of the "peripheral" and not of the "central" type [4] an extrinsic toxic mechanism is strongly advocated.

Acknowledgements. Our warm thanks are due to Drs. Leila and Juha Risteli, Ms. Lisbeth Gustavsson, Kristina Müntzing and Christina Jacobson.

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Received June 2, 1986/Accepted July 10, 1987