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Splenic "Regeneration" after Partial Splenectomy for Gaucher Disease: Histological Features

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ABSTRACT: Partial splenectomy for Gaucher disease is often followed by reenlargement of the splenic remnant. It remains unclear if this process is due to tissue regeneration or to continued deposition of glucocerebroside in the reticuloendothelial system or both.

We compared the splenic architecture before and after reenlargement in three cases of failed repeated partial splenectomy after two, six and five years. Using the number of lymphoid follicles per hundred low power fields (LF/LPF) as an arbitrary index, we found that prior to the first operation 18, 20 and 27 lymphoid follicles were present per one hundred low power fields, while at the second operation, the corresponding rates were 11, 15 and 17; in control spleens, an average of 712.5 lymphoid follicles were present in one hundred low power fields. The difference in the LF/LPF ratio before and after reenlargement, led us to speculate that splenic re-enlargement in Gaucher disease is mainly the result of the continued deposition of the glucocerebroside in the reticuloendothelial system of the splenic remnant, though some degree of true regeneration as well cannot be completely ruled out. These findings are compared with animal studies and results for partial splenectomy on humans, performed for trauma. Further studies in patients with Gaucher disease are warranted to better define the underlying mechanism of splenic reenlargement.

Keywords: Gaucher disease, splenic regeneration, partial splenectomy

INTRODUCTION

Before enzyme replacement therapy for Gaucher disease with alglucerase (1,2) was widely instituted, total splenectomy had been replaced by partial splenectomy in the belief that this would prevent overwhelming postsplenectomy sepsis and prevent osteoarticular destruction and the problem of hypersplenism (3,4). However, the remaining spleen tends to reenlarge after surgery and bone destruction, if present before splenectomy, is not stopped. However, because overwhelming postsplenectomy sepsis is indeed prevented, this is by itself a sufficient

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reason for contemplating splenic preservation (5-7), provided that the remaining splenic mass fulfills a normal function. This indication seems to be accurate for other hematologic diseases in childhood as well (8,9).

The reason for the reenlargement of the splenic remnant after partial splenectomy remains unclear. It may be due to tissue regrowth, continued deposition of the glucocerebroside in the reticuloendothelial system, or both. To help answer this question, we present three failed attempts at repeated partial splenectomy, which provided us with a unique opportunity to examine the splenic architecture before and after reenlargement and to compare the findings to splenic architecture of normal spleen resected for trauma and other Gaucher patient spleens at first resection.

The data are also analyzed in view of earlier clinical and experimental findings.

Table 1. Number of Lymphoid Follicles per 100 Low Power Fields in Gaucher Patients

Patient	Age at 1st Surgery	NF/100 LPF at 1st Surgery	Age at 2nd Surgery	NF/100 LPF at 2nd Surgery
I	10	18	12	11
II	6	20	12	15
III	12	27	17	17
IV	11	33		
V	13	10		
VI	15	8		
VII	16	33		

Table 2. Number of Lymphoid Follicles/100 Low Power Fields in Control Patients

Patient	Age (yrs)	NF/100 LPF
I	8	692
II	9	725
III	9	712
IV	12	686
V	14	736
VI	16	724

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MATERIALS AND METHODS

Three patients, all the offspring of unrelated Ashkenazi Jews, presented independently with Gaucher disease type I at ages 5, 7½ and 5 years. First partial splenectomy was performed at 10, 6 and 12 years of age (patients I, II, III, Table 1).

In case 1, two years later, total splenectomy was performed within six hours of repeated partial splenectomy because of the immediate, massive reenlargement of the remnant due to insufficient venous drainage. In case 2, partial splenectomy was followed by total splenectomy six years later, when the spleen reached 18 cm below the costal margin, and no salvageable remnant could be identified. In case 3, a second partial splenectomy was contemplated after five years, when the spleen reached 10 cm below the costal margin but no splenic vein was detected on preoperative angiography, and in addition huge areas of infarction were found at laparotomy. Size of the splenic masses removed at total definitive splenectomy was 20 x 17 x 15 cm (1600 g), 14 x 15 x 6 cm (1100 g), and 26 x 22 x 13 cm (4800 g).

Splenic tissue from four other Gaucher patients who had undergone splenectomy was examined. These patients were aged 11, 13, 15 and 16 years (patients IV - VII, Table 1). The control group consisted of six patients aged 8, 9, 9, 12, 14 and 16 years who had undergone splenectomy because of trauma (Table 2).

Histologic Study

The available material for this retrospective study consisted of the original hematoxylin and eosin, Periodic acid Schiff- stained slides and the respective paraffin blocks obtained at partial and total splenectomy. The interval between the two procedures was two, six and five years in cases 1,2 and 3, respectively.

To define the difference in general pattern and/or degree of infiltration of the disease, the number of lymphoid follicles/low power field (x40) - (LF/LPF) was selected as the arbitrary index. For each specimen, 100 LPF were examined. We based this method on the assumption that Gaucher disease affects primarily the red pulp, while the white pulp is reduced later, by pressure, and conserves the normal relationship of its lymphoid components (T and B cells). To verify this last point, immuno-peroxidase stains with LCA (leukocyte common antigen, Dako, California, USA), UCHL-1 (Dako, California, USA) and B (L26, Dako California USA) were performed. No functional aspects were investigated because of the nature of the available material.

RESULTS

The ages and number of follicles per one hundred low power fields (LF/LPF) are presented for the three patients as well as patients undergoing first splenic resection (this incudes the first operation of our three patients) and the control group. The spleens of the Gaucher patients had significantly less lymphoid follicles compared to the control patients (8 - 33 compared to 686 - 736, respectively) (Tables 1, 2). There was no change with age. The second splenic resection specimens showed an even lower number of lymphoid follicles per hundred low power fields examined (Table 1).

DISCUSSION

There is an extensive discussion in the literature as to whether the remaining spleen after partial splenectomy undergoes an abnormal increase in the number of normal cells in a normal tissue arrangement (hyperplasia) or a regeneration or renewal of the lost tissue (10,11).

The ability of the spleen to regenerate after partial splenectomy is controversial. Some studies have shown hypertrophy of missed residual splenunculi after total splenectomy for hematological disorders (12), suggesting the possibility of regeneration, and experimental studies have found evidence of regeneration even in as little as 5% of the spleen maintained on the native artery (11). However, functional regeneration after auto-transplantation has not been proven (13,14).

Partial splenectomy, when performed because of trauma, is not usually followed by an enlargement (in size or weight) of the remnant spleen, as indicated by isotope scanning studies (10), though this is not true for diseased spleens. In one extreme case, subtotal splenectomy in a 19-month-old girl with Gaucher disease and massive splenomegaly (12% of body weight) was followed only three months later by splenic mass growth to the pelvic edge (15).

The mechanism of splenic remnant enlargement in Gaucher disease is unclear. Many authors have attributed it to glucosylceramide deposition with or without some degree of true splenic tissue regeneration. The variable rates of splenic remnant enlargement, though still not satisfactorily explained, may be related to unequal rates of glucosylceramide deposition resulting from the different enzyme activities in the different Gaucher disease subtypes (4). Further long-term follow-ups are needed to determine if such splenic regrowth is universal or characteristic of only certain types of enzyme defects.

To determine the influence of blood flow on the regenerative capacity of the spleen, Pabst et al. (16) resected 75% of piglet spleens using three different techniques. They found that when the splenic remnant adjacent to the main vessels was left intact, there was no obvious regrowth after six months; when the splenic tissue at the smaller vessels was left, however, an increase in size was observed, but this may also have been due to the high number of infarctions noted.

Pouche et al. (17), using a rat model, reported postsurgical inflammatory changes after 30 days and an essentially new structure consisting of lymphoid mantels of contiguous follicles, hyperplasia of prepenicillary lymphoid micronodules, and expansion of the lymphoid mantel enveloping ramifications of centrofollicular arteries, after 90 days. However, since the functional capacity of this follicular morphology was not established, the authors were unable to conclude if the observed histologic changes were true regeneration (5-12).

In a more recent work, Hayari et al. (18) quantified the DNA content and incorporation of 3H-thymidine into the remaining splenic tissue in rats, as indicators of cellular proliferation. Both factors showed significant differences from controls by day 1 ($p<0.001$), while the calculated weight gain of the splenic remnant was five fold greater than that for controls at one week. However, by three months, all parameters had returned to control levels, and the authors concluded that the changes were due to a postsurgical inflammatory reaction and not splenic regeneration. Likewise radioisotope studies in dogs six months after partial splenectomy also yielded no evidence of regeneration (19).

Levy et al (20) recently demonstrated better neovascularization and regeneration of autotransplanted splenic chips in dogs which were treated with an omental angiogenic factor (OAF) before their implantation in the omentum.

An interesting observation was recently published by Westerman et al (20) who investigated whether splenic regenerates in the rat are re-innervated and whether this depends on the donor and host age. No innervation of splenic regenerates was observed after implantation into old hosts, correlating with poorly develop splenic compartments. In contrast, almost normal re-innervation occurred in young hosts, in which, after the implantation of young tissue, the final size of splenic regenerates was three times larger.

It is noteworthy that the present study was performed in humans with Gaucher disease type I, which is characterized by expansion of the red pulp area and reduced white pulp area (Figures 1,2). We observed enlargement of the remnant spleens in both weight and size and a change in the histological picture in general and in relationship between the red and white pulp areas in particular. All three patients showed greater expansion of the red pulp at the second operation compared with the first one, with preserved distribution of the T and B cells in the remaining lymphatic tissue of both specimens. Apparently, then, the same mechanism was responsible for both enlargements; i.e. the Gaucher cells. All three patients had a significantly lower LF/LPF index from controls and in all cases this index was found to be even lower at the second operation. The results may indicate that after partial splenectomy, the density of lymphoid follicles was minor in the "regenerated" spleen, in which case the infiltration of splenic tissue was greater than any possible growth of normal splenic tissue. Had both components of the re-enlarged spleen, i.e., the splenic tissue and the infiltrating Gaucher cells, maintained their previous growth rate, there would have been no differences in the LF/LPF index before and after splenic re-enlargement, while if splenic enlargement were secondary only to Gaucher cell infiltration, the LF/LPF index would have been much lower. Therefore, we suggest that splenic re-enlargement in Gaucher disease is a combined process, resulting principally from the continuous infiltration of glucocerebroside, but also affected to some degree by true regeneration.

The observation that the number of lymphoid follicles in type I Gaucher disease is reduced 30-90-fold times has not been previously described and warrants further investigation.

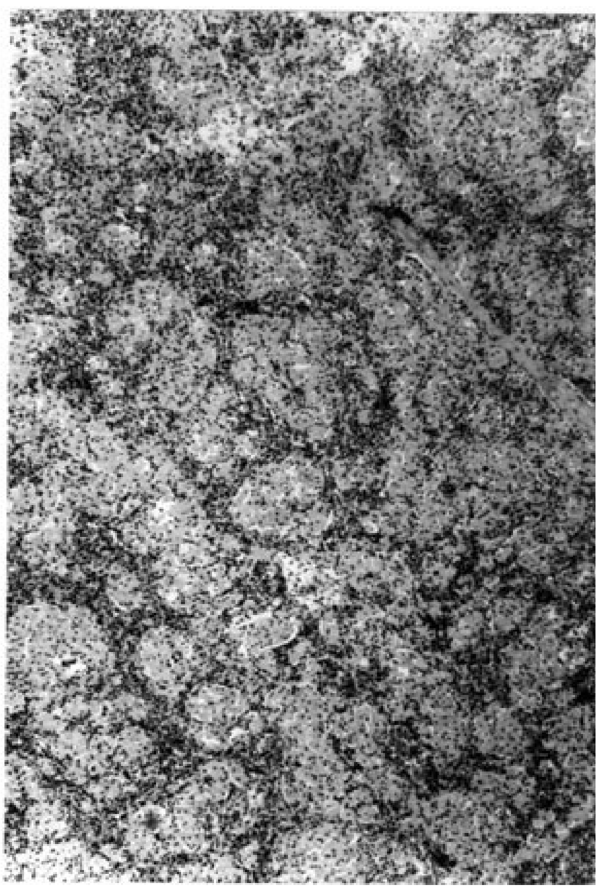

Figure 1. A) Clusters of Gaucher cells expanding the red pulp with nearly total loss of the white pulp (H&E x 27).

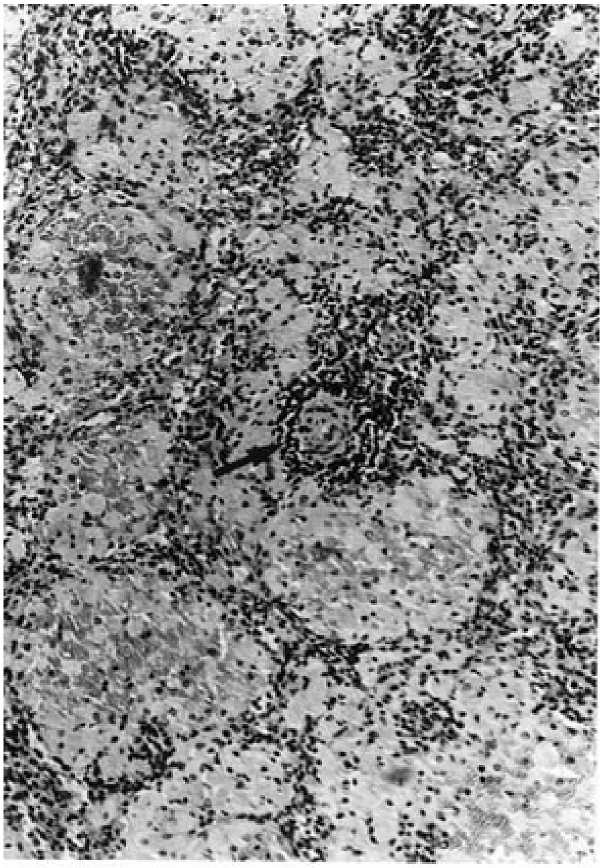

Figure 1. B) Remaining penicillar artery of a nearly completely blown out white pulp follicle (arrow) (H&E x 75).

Figure 2. A) Control case showing well-preserved white pulp. (H&E x 30)

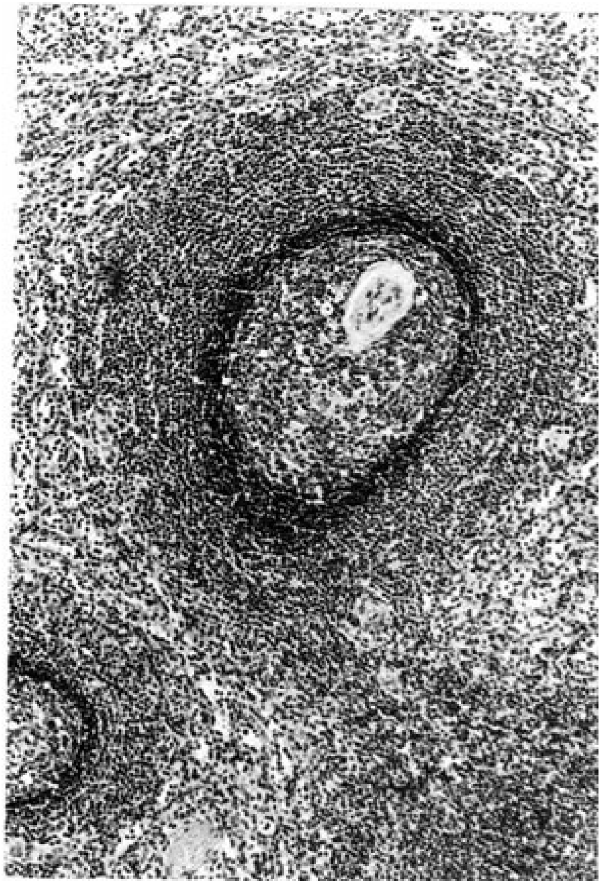

Figure 2. B) Control case showing well-preserved white pulp. (H&E x 72)

REFERENCES

1. Beutler E. Enzyme replacement therapy for Gaucher disease. *Baillieres Clin Haematol* 10:751-763, 1997.
2. Beutler E, Demina A, Laubscher K, et al. The clinical course of treated and untreated Gaucher disease. A study of 45 patients. *Blood Cells Mol Dis* 21:86-108, 1995.
3. Rodgers GM, Tribble MD, Joef A. Partial splenectomy in Gaucher disease. *Ann Surg* 205:693-699, 1987.
4. Guzzetta PC, Ruley J, Merrick HFW, et al. Elective subtotal splenectomy. *Ann Surg* 211:34-42, 1990.
5. Cohen IJ, Katz K, Freud E, Zer M, Zaizov R. Long-term follow-up of partial splenectomy in Gaucher disease. *Am J Surg* 164:345-347, 1992.
6. Singer DB. Postsplenectomy sepsis. *In* Rosenberg HS, Bolande RP (eds): Perspectives in Pediatric Pathology, Chicago, IL, Year Book Med Publishers, pp 285-311, 1973.
7. Pabst R, Reilmann H. The overwhelming postsplenectomy sepsis problem. *World J Surg* 4:423-432, 1980.
8. Banani SA, Bahador A. Management of thalassemia major by partial splenectomy. *Pediatr Surg Int* 9:350-352, 1994.
9. Tchernia G, Gauthier F, Mielot F, et al. Initial assessment of the beneficial effect of partial splenectomy in hereditary spherocytosis. *Blood* 81:2014-2020, 1993.
10. Bowen J, Gough D. Splenic regeneration does not occur after sub-total splenectomy. *Pediatr Surg Int* 9:423-424, 1994.
11. Michalski S, Blankenhorn P, Lepsein G, et al. Fatal infection after splenectomy despite reimplantation of splenic tissue. *Klin Wochenschr* 69:375-378, 1991.
12. Ludtke FE, Mack S, Schuff-Werner P, et al. Splenic function after splenectomy for trauma. Role of autotransplantation and splenosis. *Acta Chir Scand* 155:533-539, 1989.
13. Van Wyck DB, Witte MH, Witte CL. Compensatory spleen growth and protective function in rats. *Clin Sci* 71:573-579, 1986.
14. Holdsworth RJ. Regeneration of the spleen and splenic autotransplantation. *Br J Surg* 78:270-278, 1991.
15. Fleshner PR, Astion DJ, Ludman MD, et al. Gaucher disease: Fate of the splenic remnant after partial splenectomy. A case of rapid enlargement. *J Ped Surg* 24:610-612, 1989
16. Pabst R, Kamran D, Creutzing H. Splenic regeneration and blood flow after ligation of the

splenic artery or partial splenectomy. *Am J Surg* 147:382-386, 1984.
17. Pouche A, Chiodera P, Bosio P. Allevi G, Tiberio G. Experimental splenic regeneration. *Surg Gynecol Obstet* 162:25-29, 1986.
18. Hayari L, Livne E, Bar-Maor JA. Does the spleen regenerate after partial splenectomy? A morphological, biochemical, and radiocyto-graphic study in rats. *Pediatr Surg Int* 9:35-40, 1994.
19. Bar-Maor JA, Sweed Y, Shoshany G. Does the spleen regenerate after partial splenectomy in the dog? *J Pediatr Surg* 23:128-129, 1988.
20. Levy Y, Miko I, Hauck M, Mathesz K, Furka I, Orda R. Effect of omental angiogenic lipid factor on revascularization of autotransplantation spleen in dogs. *Eur Surg Res* 30:138-143, 1998.
21. Westerman J, Michel S, Lopez-Kostka S, et al. Regeneration of implanted splenic tissue in the rat: Re-innervation is host age-dependent and necessary for tissue development. *J Neuroimmunol* 88:67-76, 1998.