Foamy Histiocytes in Spleens Removed for Chronic Idiopathic Thrombocytopenic Purpura

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FOAMY HISTIOCYTES were found in three of 47 spleens removed for chronic idiopathic thrombocytopenic purpura (ITP). Fat stains demonstrated the absence of lipid. These cells have never been reported in ITP except for a brief mention by Carpenter et al. Somewhat similar histiocytes have been described in thalassemia²,³ and in an ill-defined condition characterized by splenomegaly.⁴ The morphologic and histochemical characteristics of these cells will be described and an analysis made of the clinical and hematologic findings of the three patients.

MATERIALS AND METHODS

Sections of 65 spleens removed for various hematologic disorders were studied. These included ITP (47 cases), thalassemia (6 cases), splenomegaly of undetermined origin (5 cases), hypersplenism (3 cases), hereditary spherocytosis (3 cases), myelofibrosis with extramedullary hematopoiesis (1 case). Fifty normal spleens served as controls.

Tissues were fixed in buffered formalin or Zenker's solution. All sections were stained with Harris' hematoxylin and eosin. The paraffin blocks of three spleens with ITP and foam cells, one spleen of thalassemia with similar cells, and spleens from the control series were stained with the following additional stains: Gomori's iron reaction,⁵ periodic acid Schiff (PAS) with and without digestion by diastase,⁶ alcian blue with and without digestion by hyaluronidase,⁶ toluidine blue with and without digestion by ribonuclease,⁷ Mallory's aniline blue,⁶ and Gomori's methenamine silver nitrate with and without digestion by diastase.⁸ Frozen sections from ITP case no. 1 were stained with Scharlach R,⁶ oil red O,⁶ nile blue sulfate,⁶ osmic acid,⁵ PAS with and without digestion by diastase, and toluidine blue with and without digestion by ribonuclease.

CASE REPORTS

Case No. 1: B. A., a 57 year old white man of Italian extraction, was admitted on July 16th, 1960, because of hematuria. A diagnosis of ITP was first made in 1916 when prolonged bleeding followed a dental extraction. During the intervening years he continued to bruise easily. A diagnosis of diabetes insipidus was made in 1943 because of marked polyuria and polydypsia which was controlled by pitressin. This therapy was discontinued in 1948 without recurrence of symptoms. Physical examination was negative with the exception of numerous brownish pigmented areas on both lower extremities. Blood studies showed: RBC 5.2 million, hemoglobin 17.4 Gm., hematocrit 51. MCV 99, CI 1.0, reticulocytes 0.3 per cent, platelets 56,000, WBC 9750 with 65 per cent segmented polymorphonuclear leukocytes and 35 per cent lymphocytes. The peripheral blood smear showed slight anisocytosis, poikilocytosis, hypochromia and minimal polychromatophilia. Platelets

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were markedly diminished. A sternal bone marrow biopsy revealed a normocellular marrow. Megakaryocytes were present in increased numbers and platelet formation was diminished. Erythropoiesis was normoblastic. Granulopoiesis was orderly in maturation. A normal E/M ratio was present. No foreign cells were seen. Impression: Idiopathic thrombocytopenic purpura. Other laboratory studies showed: serum bilirubin 1.0 mg. per cent, FBS 94 mg. per cent, BUN 11 mg. per cent, serum albumin 3.6 Gm. per cent, serum globulin 3.5 Gm. per cent, L.E. test negative, platelet agglutinins negative, bleeding time 7 minutes, Lee-White clotting time 9-12-15 minutes, clot retraction poor, tourniquet test positive, prothrombin time 14.5 seconds (normal control 12.0 seconds), prothrombin consumption time 63 seconds. Chest and skull x-rays were normal. A diagnosis of chronic ITP was made and after a two-month course of steroid therapy, splenectomy was performed on 9-30-60. The platelet count reached 300,000 postoperatively and has remained normal.

The spleen weighed 178 Gm. An accessory spleen, 2 cm. in diameter, was also removed. The capsular surface was wrinkled and shiny, the cut surface red to brown. Microscopic sections showed groups of clear cells averaging 18 μ in diameter with bluish granules in the cytoplasm. The nuclei were vesicular and did not contain prominent nucleoli. These cells were located in the red pulp outside the sinusoids (figs. 1 and 2). Most sinusoids were empty and were lined by prominent endothelial lining cells. The lymphoid tissue was abundant but not hyperplastic. Germinal centers were not prominent and there was no marginal zone around the individual lymphoid follicles. Very few isolated megakaryocytes were seen. The vessels showed thickening and hyalinization of the walls. Similar findings were seen in the accessory spleen. A subsequent liver biopsy performed on October 24, 1960, failed to demonstrate similar cells.

Case No. 2: I. M., a 36 year old white man, was admitted on January 11, 1960, with a history of bright red blood in the stool, intermittently present for two years and associated with easy bruising. The past medical history, family history, and systemic review were

Fig. 1.—Case 1. Large numbers of foamy nonlipid histiocytes in splenic red pulp outside of sinusoids. Arrows point to two of the cells in a large cluster. x 100 (enlarged 5 times) hematoxylin-eosin.
Fig. 2.—Case 1. Arrows point to two foamy histiocytes in a cluster of cells located in splenic red pulp. x 420 (enlarged 5 times) hematoxylin-eosin.

non-contributory. Physical examination revealed no abnormalities besides petechiae on both lower extremities. Blood studies showed: RBC 4.9 million, hemoglobin 14.2 Gm., hematocrit 47, MCV 99, CI 0.9, platelets 20,000, WBC 7300 with 66 per cent segmented polymorphonuclear leukocytes, 32 per cent lymphocytes and 2 per cent monocytes. Sternal bone marrow biopsy revealed a hypocellular marrow. Megakaryocytes were present in normal numbers and platelet production was inadequate. Erythropoiesis was normoblastic in nature and diminished. Granulopoiesis was diminished and orderly in maturation. No foreign cells were seen. Impression: idiopathic thrombocytopenic purpura. Other laboratory studies showed bleeding time 5 minutes, Lee-White clotting time 9–12–15 minutes, clot retraction poor, tourniquet test positive, FBS 80 mg per cent, BUN 11 mg per cent, cholesterol 240 mg. per cent with 73 per cent esters, serology negative, L.E. Test negative, serum uric acid 3.5 mg. per cent, serum albumin 4.2 Gm. per cent, serum globulin 3.1 Gm. per cent. Sigmoidoscopy and gastrointestinal x-ray survey were normal. A diagnosis of chronic ITP was made and prednisone 20 mg. q.i.d. instituted. A splenectomy was performed on May 2, 1960, because the platelet count remained at 29,000. The platelet count rose to 200,000 postoperatively and has since remained normal.

The spleen weighed 210 Gm. and had a smooth gray capsule. The cut section was deep reddish brown. Microscopic sections showed a small number of clear cells scattered throughout the red pulp outside the sinusoids, identical to the cells described in Case No. 1 (fig. 3). The spleen was markedly congested and the sinusoids could not be made out because of the engorgement with blood. Lymphoid follicles were prominent with large germinal centers and marginal zones. Very few isolated megakaryocytes could be demonstrated. The arteries showed thickening of the walls and narrowing of the lumens.

Case No. 3: H. W., a 37 year old white woman developed menorrhagia and easy bruising in 1957. A diagnosis of idiopathic thrombocytopenic purpura was made in 1958 and the patient was given methyl prednisolone for one year with no change in the blood platelet count. The past medical history and systemic review were non-contributory. Her paternal...
Fig. 3.—Case 2. Arrows point to two histiocytes within a cluster in splenic red pulp. x 420 (enlarged 5 times) hematoxylin-eosin.

grandmother died of leukemia. Physical examination was negative except for petechiae on both lower extremities. Blood studies showed: RBC 4.0 million, hemoglobin 12. Gm., platelets 4,000, WBC 7600 with 1 per cent bands, 74 per cent segmented polymorphonuclear leukocytes and 25 per cent lymphocytes. A peripheral blood smear showed slight anisocytosis, poikilocytosis, minimal hypochromia and polychromatophilia. No abnormal RBC or WBC were seen. Platelets were diminished. A sternal bone marrow biopsy revealed a normocellular marrow. Megakaryocytes were present in normal numbers but platelet production was diminished. Erythropoiesis was normoblastic. Granulopoiesis was orderly in maturation. No foreign cells were seen. Impression: idiopathic thrombocytopenic purpura. Platelet agglutinins were present in the patient's serum. The L.E. test was consistently negative. A splenectomy was performed in December 1959, but the platelet values never rose above 40,000. Up to the present time no new signs or symptoms have developed.

The spleen weighed 123 Gm. The capsule was gray with small pearly gray areas. The cut surface was dark red brown and homogeneous. Numerous pinpoint-sized follicles were apparent, the largest measuring 0.1 cm. Microscopic sections showed numerous groups of clear cells identical to those seen in Cases No. 1 and No. 2 scattered within the red pulp outside the sinusoids (fig. 4). In contrast to these histiocytes, typical lipid globules were scattered in the red pulp outside the sinusoids and in the lymphoid follicles (fig. 5). Sinusoids were fairly large and were lined by endothelial cells. Lymphoid tissue was abundant but not hyperplastic. No germinal centers or marginal zones were seen. A moderate number of megakaryocytes were identified. Blood vessels showed thickening and hyalinization of the wall.

RESULTS

The histiocytes observed in the three cases of ITP as seen in sections stained with hematoxylin and eosin (figs. 1–4) appeared as clear cells measuring 15
to 18 μ in diameter with a faint, slightly bluish to purplish granular pattern in the cytoplasm and a round to oval, usually centrally located, vesicular nucleus of about 3 μ in diameter. A nucleolus was identified in some cells but was never prominent. The cellular outline was not too well defined and occasionally cells were seen to fuse into each other. These cells occurred in clusters or isolated in the red pulp outside the lumen of the sinusoids. Somehow similar cells were identified in one spleen removed from a patient with thalassemia (fig. 6). These showed a denser cytoplasm with a more prominent granular and reticular pattern and measured 12 to 15 μ in diameter. The reactions of the cytoplasmic granules in the histiocytes of ITP and thalassemia with various histochemical dyes are summarized (table 1).

DISCUSSION

The morphologic appearance of the foam cells in ITP suggests that they belong to the reticuloendothelial system and can best be defined as histiocytes.9 The nonlipid nature of these cells has been proven by the negative fat stains in Case No. 1. The reaction of the intracellular granules in the histiocytes with PAS, alcian blue, aniline blue, methenamine silver and the orthochromasia with toluidine blue demonstrates the presence of a mucopolysaccharide, most probably of neutral nature.10,11 Resistance to digestion with testicular hyaluronidase and ribonuclease illustrate the absence of hyaluronic acid, chondroitin sulfates A, C and ribonucleic acid.10 These cells bear no resemblance to
Fig. 5.—Collections of typical lipid globules in spleen of patient No. 3. This is a common finding in normal spleens. x 420 (enlarged 5 times) hematoxylin-eosin.

the lipid globules which are commonly seen in the spleen,12 and which were encountered in Case No. 3 (fig. 5) and in 15 of the total 115 spleens examined. They are also entirely different from the cells seen in Gaucher's disease which are much larger in size and contain typical striations in the cytoplasm. The reticuloendothelial cells in Niemann-Pick, Hand-Schuller Christian and hypercholesterolemia are characterized by a high lipid content, a feature which is not present here.

Foamy nonlipid histiocytes containing mucopolysaccharide granules have never been described in reviews dealing with the histopathology of the spleen in ITP.13,14 Carpenter et al. briefly mentions one case with infiltration of "non-lipid containing foamy macrophages" among 85 spleens removed for ITP. These authors subsequently excluded this case from their series because of the presence of these macrophages which was considered unusual for ITP. A review of the literature reveals two additional cases in which somewhat similar cells were found in the spleen, bone marrow and lymph nodes of patients with splenomegaly and normal platelet counts.4

The histiocytes in thalassemia are smaller in size, present staining reactions which are similar to the ones in ITP but are more intense. The other difference is the metachromasia with toluidine blue in contrast to the orthochromasia in ITP. These observations confirm the presence of an acid mucopolysaccharide in the foam cells of thalassemia as indicated by Sen Gupta et al.3

Lipid macrophages have been reported in splenomegaly and thrombocyto-
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Fig. 6.—Arrows point to a group of histiocytes in a spleen from patient with thalassemia. x 420 (enlarged 5 times) hematoxylin-eosin.

penia\textsuperscript{15} with three types of granules: coarsely pigmented, unpigmented, and finely unpigmented. The latter did not stain for lipid. Malinin\textsuperscript{16} described cells similar to the coarsely granular macrophages. These stained positive for lipid only after boiling in acetate buffer for one minute. The cells reported in our three cases were all finely granular and remained negative for lipid after similar boiling.

The origin of the mucopolysaccharide in the foam cells in ITP is unknown. In the case of thalassemia, Sen Gupta et al.\textsuperscript{3} suggested that erythroid cells could represent the source of the mucopolysaccharide granules. Other possible sources may also be considered. Nondigestible PAS positive granules have also been demonstrated in eosinophils, lymphocytes, monocytes, basophils\textsuperscript{17} and in megakaryocytes.\textsuperscript{18} Moreover, this material could originate in a variety of other tissues, since mucopolysaccharides are among the most widely distributed substances in the body. Morales\textsuperscript{19} reported nonlipid, PAS positive histiocytes in the spleen of a child with congenital rhabdomyoma and tuberous sclerosis and suggested that the intracellular material represented storage material from the tumor of the heart and the abnormal neural cells. Whether foam cells occurred in organs other than the spleen in these three cases of ITP is not known since only surgically removed spleens were studied.

The clinical and hematologic findings in these three patients were similar to those in most cases of ITP. The failure of splenectomy to produce a rise in platelet count in the third case may be related to the presence of platelet ag-
Table 1.—Histochernical Staining Reactions of Foam Cells in Spleens from Patients with ITP and Thalassemia

<table>
<thead>
<tr>
<th>Stains</th>
<th>ITP</th>
<th>Thalassemia</th>
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<tbody>
<tr>
<td>Sudan IV</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oil Red O</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Nile Blue Sulfate</td>
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<td>0</td>
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<tr>
<td>Osmic Acid</td>
<td>0</td>
<td>9</td>
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<td>Gomori’s iron reaction</td>
<td>0</td>
<td>0</td>
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<tr>
<td>PAS</td>
<td>+</td>
<td>+++</td>
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<tr>
<td>PAS with diastase</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Alcian blue</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Alcian blue with hyaluronidase</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Toluidine blue</td>
<td>+</td>
<td>Orthochromasia +</td>
</tr>
<tr>
<td>Toluidine blue with ribonuclease</td>
<td></td>
<td>Orthochromasia +</td>
</tr>
<tr>
<td>Mallory’s aniline blue</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Gomori’s methenamine silver</td>
<td>+</td>
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<td>Gomori’s methenamine silver</td>
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<td>+++</td>
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<tr>
<td>with diastase</td>
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</table>

(The reactions are graded from 0 to 3 by intensity).

glutinins. All of these patients had received steroids but since this therapy is widely used in this disease, it would not seem related to the presence of the foam cells. Foamy histiocytes were not demonstrated in the spleens of the other cases of ITP who also received steroids and have never been reported in spleens of individuals receiving steroids for other disorders.

The significance of these cells in ITP is unknown. Since they were present in only a few cases, they may indicate a variant of this disorder. It is also possible that these patients had a histiocytosis manifested by a thrombocytopenia. Additional studies will be necessary to establish a definite clinicopathologic correlation.

SUMMARY

1. Foamy histiocytes with mucopolysaccharide granules are reported in 3 of 47 spleens removed from patients with the clinical picture of chronic idiopathic thrombocytopenic purpura (ITP).
2. The absence of lipid in these histiocytes is demonstrated in one case.
3. These cells resemble the foam cells of thalassemia by their mucopolysaccharide content and absence of lipid.
4. The possibility of a histiocytosis manifesting as ITP is suggested.

SUMARIO IN INTERLINGUA

1. Histiocytos spumose con granulos de mucopolysaccharida esseva trovate in tres ex 47 splenes ablationate ab patientes con le tableau clinic de chronic purpura thrombocytopenic idiopathic.
2. Le absentia de lipido in iste histiocytes esseva demonstrate in un del casos.
3. Iste cellulas resimila le cellulas spumose de thalassemia per lor contento de mucopolysaccharido e le absentia de lipido.
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4. Es suggerite le possibilitate que histiocytosis se manifesta como idiopathic purpura thrombocytopenic.

ADDENDUM

In a recent article in Blood, 18:73, 1961, Saltzstein reported similar cells in the spleens of patients with ITP. A variable amount of stainable lipid was found. Whether the absence of lipid in some cases and its presence in others is significant must await further evaluation.

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REFERENCES

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