Bull Yamaguchi Med School 32 : 1-9, 1985

Ultrastructural Study on the Spleens of 28 Patients with ITP: Platelet Phagocytosis of Cordal Macrophages

*Tokuhiro Ishihara, Yoshimi Yamashita, Toshikazu Gondo, Tadaaki Yokota, Toshiaki Kamei, Mutsuo Takahashi, Fumiya Uchino and Noboru Matsumoto**

The First Department of Pathology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan

*The School of Allied Health Sciences, Yamaguchi University, Ube, Yamaguchi 755, Japan

(Received August 14, 1985)

Abstract Spleens of 28 patients with idiopathic thrombocytopenic purpura have been observed histologically, immunohistologically and electron microscopically. Spleens of fifteen patients had the foamy cells in the red pulp. Some foamy cells had positive materials for anti-human platelet antibody, using the unlabelled immunoperoxidase method. Electron microscopically, many platelets in varying stages [of intracellular digestion from intact-appearing platelets to myelinlike materials were disclosed in the foamy cells.

We suggest that there are two types of the macrophage in the spleen from patients with ITP. One does not reveal foamy cytoplasm, because phagocytized platelets are immediately digested by the lysosomal enzyme within cytoplasm of macrophages. The other has foamy cytoplasm, because phagocytized platelets are incompletely digested and remain as myelinlike materials within cytoplasm of macrophages.

Key Words: ITP, Ultrastructure, Spleen, Platelet, Cordal macrophage

Introduction

There are morphologically two types of the macrophage in spleens from patients with ITP. One has the foamy cytoplasm, and the other has not. The frequency of occurrence of foamy cells is 20 to 50 % of spleens from patients with ITP. Since Landing et al.[1] and Saltzstein[2] first reported the occurrence of lipid-containing histiocytes in spleens from patients with ITP, there have been many reports about the foamy cells in ITP. The foamy cells are not noted in normal spleen. However, platelets normally survive for 7 days in circulating blood and then are removed by macrophages in the spleen[3]. Many foamy cells develop in the spleen during accelerated removal of platelets resulting

in deposition of platelets in splenic macrophages where they can be seen in various stages of degeneration. However, definitive evidence has been lacking. In addition, although a few investigators also reported about the ITP spleen which did not have the foamy cells, it is unclear why the foamy cells are detected on some spleens.

In this paper, we present the histology, immunohistology using unlabelled antibody peroxidase-anti-peroxidase (PAP) method, and the ultrastructure of the spleens from 28 patients with ITP.

Materials and Methods

The pertinent clinical data of 28 patients who were diagnosed as ITP and were underwent splenectomy are summerized in Table 1. The patients, three males and twenty five females, ranged in age from 2 to 63 years (mean 30) at the time of the splenectomy.

For histological examination, tissue fragments of the spleens were fixed in 10% buffered formalin. Sections from paraffin blocks were stained with hematoxylin and eosin. PAP stain with Sternberger's method[4] was employed for the demonstration of human platelet antigen (Dako Immunoglobulins, Copenhagen).

For transmission electron microscopy, small pieces of the spleen were obtained from several different sites immediately after the operation. They were fixed in 2.1% glutaraldehyde at 4°C and postfixed in 1% osmium tertoxide. They were dehydrated in a graded series ethanol and embedded in Epon 812. Semithin sections (0.5 μ thick) stained with alkaline toluidine blue were examined in a light microscopy to select the appropriate areas for electron microscopic study. The ultrathin sections cut with an LKB or an Ivan Sorvall ultramicrotome were stained with uranyl acetate and lead citrate and examined in a Hitachi HS-8 and/or a H-300 electron microscope. Small pieces of the spleen were fixed in cold 2.1% glutaraldehyde in cacodylate buffer and incubated in the solution containing lead nitrate and beta-glycerophosphate for the demonstration of acid phosphatase activity.

Table 1 Morphological findings in spleens from 28 patients with ITP

Case	Age (Yr) at splenectomy	Sex	Weight of spleen (Gr)	Number of foamy cells
1	2	F	90	−
2	5	F	150	###
3	6	F	60	−
4	14	F	140	++
5	16	F	280	###
6	16	F	190	###
7	20	F	190	+
8	23	F	180	−
9	24	F	440	++
10	27	F	200	++
11	27	F	190	−
12	27	F	160	++
13	28	F	270	−
14	28	F	160	−
15	30	F	250	++
16	31	M	270	###
17	31	F	170	++
18	32	F	60	−
19	32	M	180	−
20	33	F	320	++
21	34	F	160	−
22	44	F	170	###
23	44	F	120	−
24	48	F	320	++
25	51	F	110	++
26	52	F	210	−
27	52	F	200	−
28	63	M	150	−

F: Female, M: Male, −: no foamy cell +a small number, ++a moderate number, ###a large number

Results

All spleens were slightly enlarged as shown in table 1. Macroscopically, there were no characteristic changes.

Histological findings; There are no characteristic findings in thirteen out of 28 spleens from ITP patients. However, germinal centers in white pulp were enlarged in some cases, and eosinophils infiltrated in the red pulp in

a few cases. Other fifteen spleens contained variable number of foamy cells in their medullary cords. These cells had pale cytoplasm with irregular granular stippling and poorly defined, foamy vacuolation. These cells were scattered throughout the cords, but occasionally formed small nests (Fig. 1). Some macrophages and foamy cells in all spleens from 15 patients more or less contained variable amounts of immunoreactive materials for anti-human platelet antibody (Fig. 2). Some macrophages in spleens from other 13 patients also contain variable amounts of immunoreactive materials.

Electron microscopic findings;

Several macrophages were scattered in the red pulp. Cordal macrophages had moderately developed Golgi apparatus and rough endoplasmic reticulum, a few mitochondria, and some lysosomal dense bodies. Some macrophages contained various kinds of cellular debris recognizable as degraded platelets. Several intact-appearing platelets were scattered in the cordal spaces (Fig. 3). Some platelets were engulfed by the cytoplasmic processes of macrophages (Fig. 4). Recently engulfed but still intact-appearing platelets were surrounding by a single membrane within the cytoplasm of the macrophages. The membrane surrounding the engulfed platelets became denser and these platelets revealed focal areas of cytoplasmic degradation (Fig. 5), such as some darking of the cellular matrix and condensation of their specific granules. As the intracellular digestion progressed further, the phagocytized platelets were condensed into coarsely granular or mottled inclusions with loss of integrity of platelet organelles (Fig. 6). These findings were noted in all spleens. In addition, variable number of macrophages in 15 spleens in which foamy cells were noted light microsc-

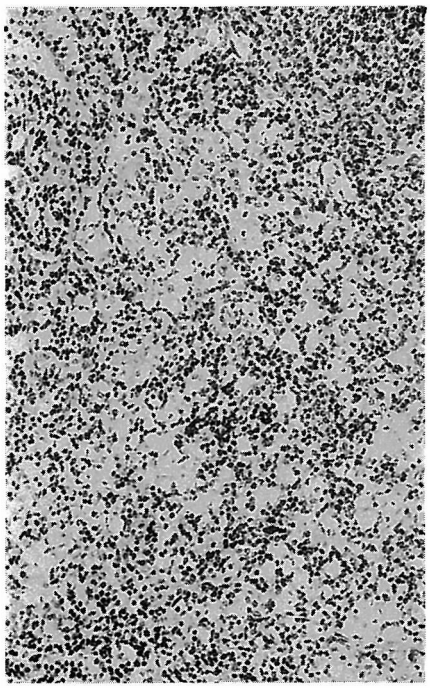

Fig. 1 Many clusters of foamy cells in medullary cords of the red pulp. H.E.

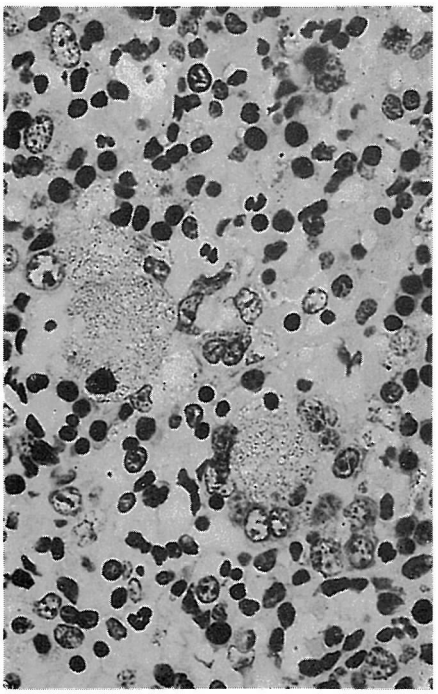

Fig. 2 Three cells contains diffuse granular dense materials in the cytoplasm. PAP stain for anti-human platelet antibody

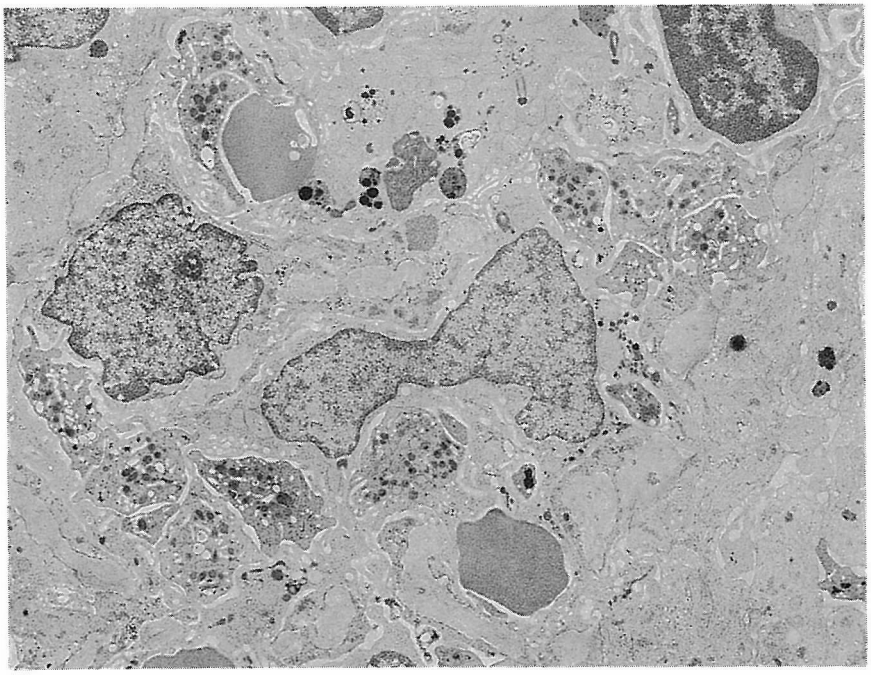

Fig. 3 Many intact platelets are noted in the cordal space. ×4, 600

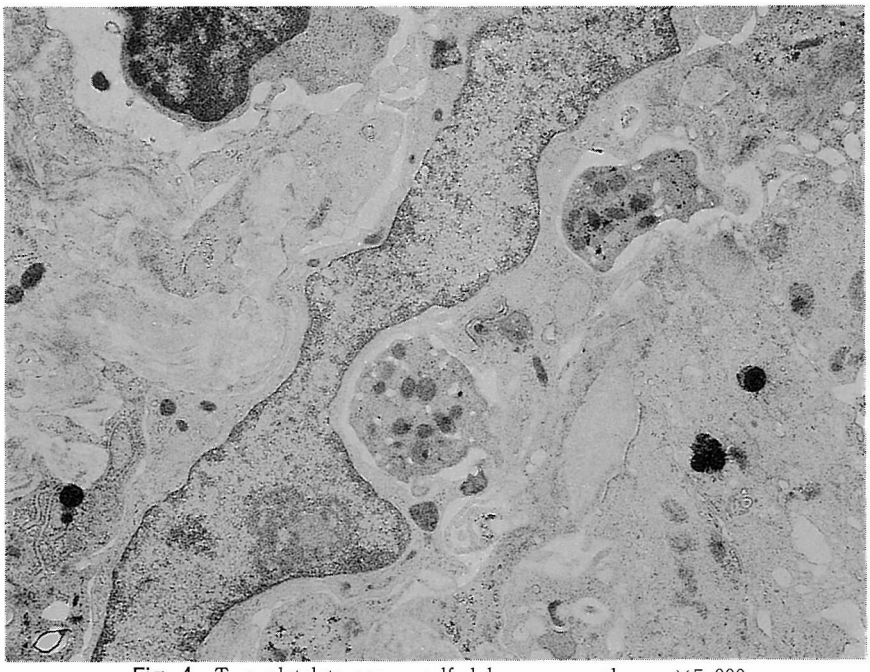

Fig. 4 Two platelets are engulfed by a macrophage. ×5, 000

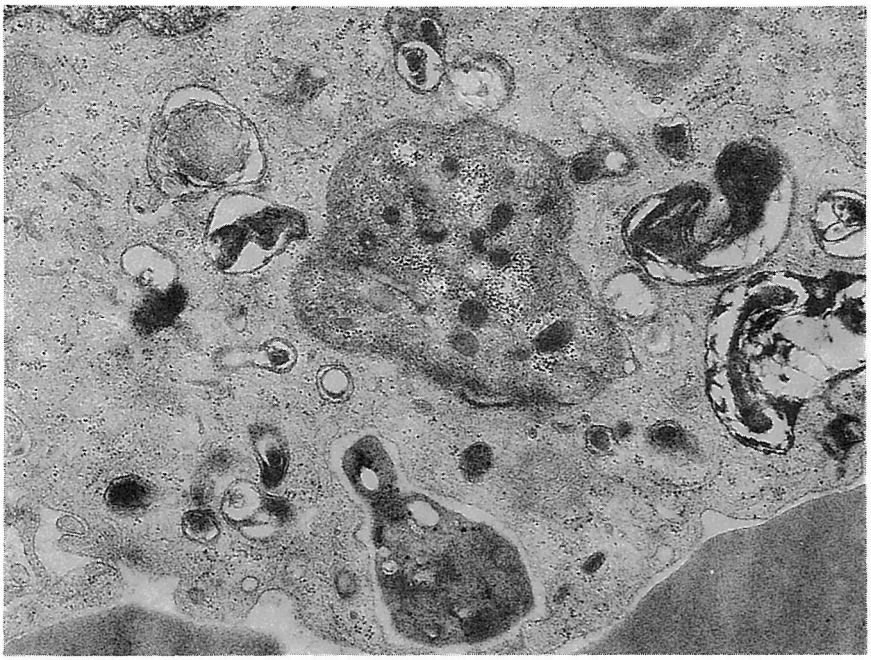

Fig. 5 Various stage of digested platelets are shown. ×10, 000

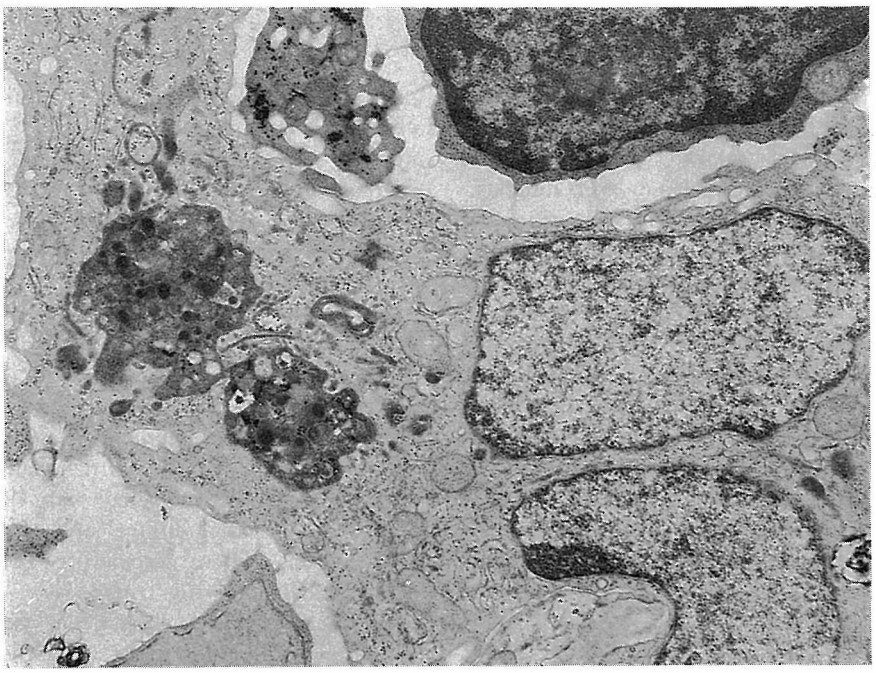

Fig. 6 Two engulfed platelets reveal condensation of platelet granules. ×8, 000

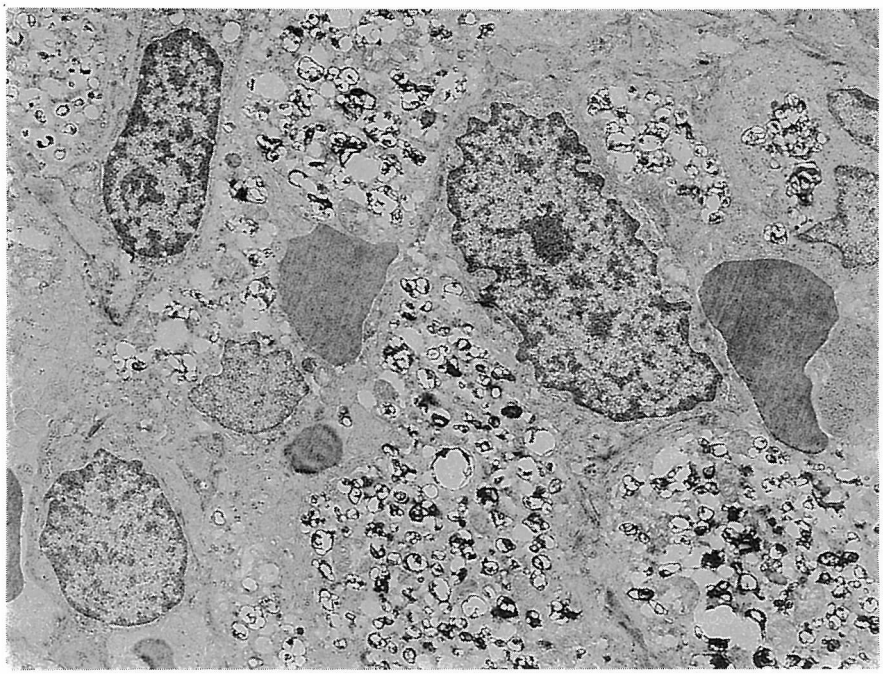

Fig. 7 Myelinlike structures as end-products of platelets destruction are contained in several macrophages. ×4, 000

opically contained numerous myelinlike structures (Fig. 7). Based on the light microscopic study of semithin section stained with toluidine blue, the foamy cells demonstrable in the light microscopy were estimated to be identical to these macrophages containing numerous myelinlike structures. There were a few lysosomes in the cytoplasm of most of foamy cells. Acid phosphatase activity was not demonstrated around or within the recently engulfed platelets, but at the advanced stage of intracellular digestion of platelet, this enzyme activity was noted around and within phagocytized platelets (Fig. 8). However, there was no demonstrable activity in most of myelinlike structures (Fig. 9).

Discussion

Branehög reported that before splenectomy platelet mean life span of ITP patients was very short and platelet production 2.8 times normal[3]. That is, platelets are normally or increaseingly produced in the bone marrow from ITP patients, but platelet count of peripheral blood decreases. It means that the destruction of platelets occurs elsewhere. Najean and Ardaillou[5] reported that a good correlative was found between the site of sequestration and the short- and long-term results of splenectomy: success in more than 90% of cases with splenic sequestration but complete failure in 70% with hepatic sequestration. Aster and Keene[6] suggested that in ITP, as in other clinical and experimental situations where circulating cells were sequestered following their reaction with antibodies, lightly sensitized cells were removed from the circulation at a relatively slow rate by the spleen, while more heavily sen-

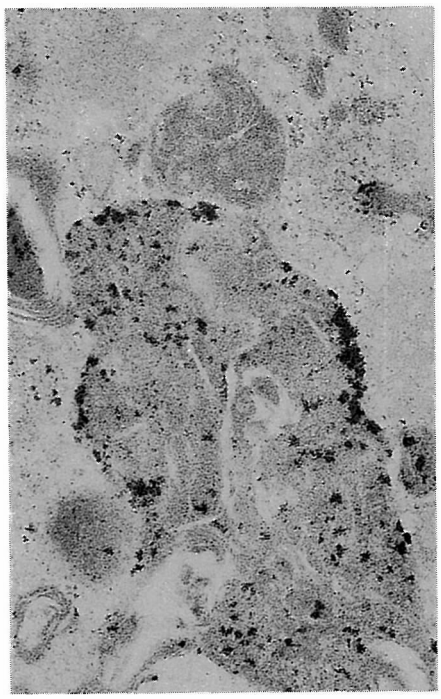

Fig. 8 Acid phosphatase activity is demonstrable in the phagocytized platelet. ×26, 000

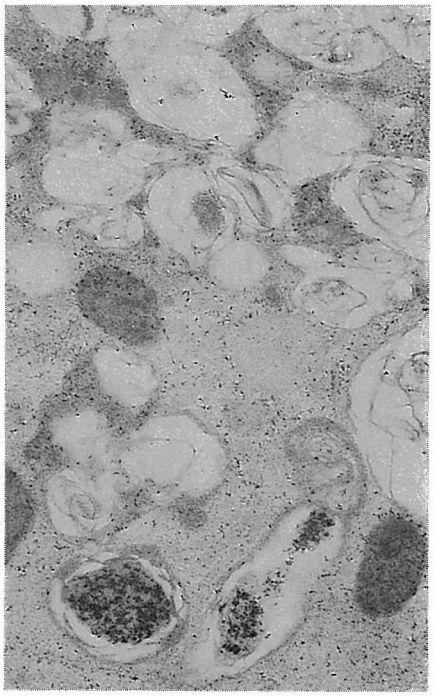

Fig. 9 A few positive materials for acid phosphatase are noted in the dense materials, and no activity within myelinlike structures. ×20, 000

sitized cells were liable to rapid destruction in the liver. Splenectomy is often effective in the treatment of ITP patients, and it has been presumed that the success of this operation is due to removal of a major site of platelet destruction. However, the effect of splenectomy does not always related to the morphological changes of the spleen[7]. Although there were no foamy cells in the spleen of 13 cases, there were several cases in which the splenectomy was effective. In their cases, many platelets were stored in the cordal spaces. The spleen normally stored a large number of circulating platelets, permitting them to come into contact with the antibody in an enviroment where a high concentration of antibody was present. Stasis of many sensitized platelets has occured owing to some immunological mechanism. Nobody has been proved that the stasis of sensitized platelets occured in the spleen from patients with ITP. Tavassoli and McMillan[8] stated that extracellular platelets might show focal mass of cytoplasmic degradation. Luk et al[9]. have never been observed fragmentation of platelets prior to phagocytosis. We did not find the degenerated platelet extracellularly in the spleen from ITP patients. We suggest that extracellular platelets are intact at least morphologically. Next, many platelets are phagocytized by the cordal macrophages. In addition, many macrophages with dense bodies are demonstrated in the spleens. However, macrophages containing myelinlike structures are not seen. These macrophages have a large amount of reactive materials for acid phosphatase. The findings show that many platelets are phagocytized by the macrophages in which the phagocytized plate-

lets were immediately digested. The foamy cells are relatively characteristic in the spleen from ITP patients, but not specific. Dollberg et al.[10] summerized that the finding of lipid-laden histiocytes could not be related to any clinical or laboratory data, and that in all cases of ITP not satisfactorily responding to surgery, the spleen contained the characteristic histiocytes. In addition, they described that the large histiocytes could be recognized easily in paraffin hematoxylin and eosin sections only when they were very numerous, but when occuring in moderate numbers or only a few, it was difficult or impossible to recognize the large histiocytes, even when their existence had been proved. We observed very carefully on light microscopy, therefore, we found the foamy cells in 15 out of 28 spleens from ITP patients. Firkin et al.[11] suggested that the platelet was destroyed within the phagocytic cell by a combination of the histiocytes' own enzyme systems as well as the catalytic enzymes of the platelet itself. Luk et al.[9] reported that the degradation of platelets in the phagolysosomes of macrophages was incomplete and resulted in the formation of myelinlike structures which accumulate in large numbers of the macrophages. In addition, they suspected that the incomplete platelet breakdown might be due to a deficiency of specific lysosomal enzymes. Recently we[12,13] proved that the macrophages in the subcutaneous granuloma induced by the injection of platelets easily trans formed into lipid-laden macrophages with foamy appearance (foamy cell). We suggested the mechanism for formation of foamy cells as the following. Under the state of accelerated phagocytosis of platelets by the macrophages, such as ITP, the amount of injected platelet membranes was beyond capacity of lysosomal digestion. Thus, the incompletely degraded membrane constituents, especially membrane-derived phospholipids, remain in the macrophages, and they are most responsible for the foamy appearance of these macrophages. The data in this study indicate that the various kinds of cellular debris and myelinlike structures contained within the macrophages is derived from platelets through the process of cytoplasmic degradation.

Our consideration about the mechanism of the sequestration and digestion of platelets in the spleen from patients with ITP is shown as follows. First, the sensitized platelets showing morphologically intact, were located in the cordal spaces. After then, most of them were engulfed by the cytoplasmic processes of macrophages. The engulfed platelets were gradually degraded within the cytoplasm of macrophage. As progression of intracellular degradation of platelets within the macrophages, macrophage transformed into two different types. One has several dense bodies in the cytoplasm. The other has many myelinlike structures showing foamy appearance on the light microscopic observation. We suggest that the former has enough lysosomal enzyme to digest the engulfed platelets, but the latter has a little. In other words, if the macrophages immediately digest the engulfed platelets, the macrophages change the former. If the macrophages can not digest the membrane or granules of platelets which contain much phospholipids, the macrophages transform into the foamy cells. When the macrophages very slowly digest the engulfed platelets, the macrophages temporarily transform into the foamy cells, and then change slowly to the macrophages.

The authors appreciate the members of the Third Department of Internal Medicine, First and Second Department of Surgery, Yamaguchi University School of Medicine.

References

1) Landing, B.H., Strauss, L., Crocker, A.C., et al.: Thrombocytopenic purpura with histiocytosis of the spleen. *N. Engl. J. Med.*, 265 : 572 1961.

2) Saltzstein, S.L.: Phospholipid accumulation in histiocytes of splenic pulp associated with throbmocytopenic purpura. *Blood*, 18 : 73, 1961.
3) Branehög, I.: Platelet kinetics in idiopathic thrombocytopenic purpura (ITP) before and at different times after splenectomy. *Br. J. Haematol.*, 29 : 413, 1975.
4) Sternberger, L.A., Hardy, P.H. Jr., Cuculis, J.J., et al.: The unlabelled antibody enzyme method of immunohistochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. *J. Histochem. Cytochem.*, 18 : 315, 1970.
5) Najean, Y. and Ardaillou, N.: The sequestration site of platlets in idiopathic thrombocytopenic purpura: its correlation with the results of splenectomy. *Br. J. Haematol.*, 21 : 153, 1971.
6) Aster, R. and Keene, W.: Sites of platelet destruction in idiopathic thrombocytopenic purpura. *Br. J. Haematol.*, 16 : 61, 1969.
7) Ishihara, T., Matsumoto, N. and Uchino, F.: Foamy histiocytes in the spleen associated with idiopathic thrombocytopenic purpura. *Acta Pathol. Jpn.*, 24 : 273, 1974.
8) Tavassoli, M. and McMillan, R.: Structure of the spleen in idiopathic thrombocytopenic purpura. *Am. J. Clin. Pathol.*, 64 : 180, 1975.
9) Luk, S.C., Musclow, E. and Simon, G.T.: Platelet phagocytosis in the spleen of patients with idiopathic thrombocytopenic purpura (ITP). *Histopathology*, 4 : 127, 1980.
10) Dollberg, L., Casper, J., Djaldetti, M., et al.: Lipid-laden histiocytes in the spleen in thrombocytopenic purpura. *Am. J. Clin. Pathol.*, 43 : 16, 1965.
11) Firkin, B.G., Wright, R., Miller, S., et al.: Splenic macrophages in thrombocytopenia. *Blood*, 33 : 240, 1969.
12) Ishihara, T., Akizuki, S., Yamanami, S., et al.: Foamy cells in ITP spleens and in granulomas induced by murine platelets, commercialized phospholipids, and erythrocyte membrane. Histological and ultrastructural studies. *Acta Pathol. Jpn.*, 33 : 943, 1983.
13) Ishihara, T., Akizuki, S., Yokota, T., et al. Foamy cells associated with platelet phagocytosis. *Am. J. Pathol.* 114 : 104, 1984.