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The York Platelet Syndrome: A third case

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Abstract

Our present study has described a third patient with the York Platelet Syndrome (YPS). The condition consists of a mitochondrial myopathy associated with unique platelet pathology. Their mitochondrial myopathy has not been completely delineated and will be the subject of further study. Platelet pathology in the new patient is essentially identical to that described in the first two patients. Thin sections of her thrombocytes reveal a normal complement of α and δ granules (dense bodies) in some, a decreased number in others and complete absence in a few. The unique pathological feature is the presence of giant organelles, including an intensely electron dense, huge body, the opaque organelle (OO) and a multilayered large body, the target organelle. In addition platelets from the new patient contain large masses and coils of smooth endoplasmic reticulum present infrequently in platelets of the first two patients. The giant opaque and target organelles appear to develop in rough and smooth endoplasmic reticulum of the parent megakaryocyte and mature in the dense tubular system of circulating platelets. The relationship of the unique platelet pathology and mitochondrial myopathy has not been defined.

Keywords

Giant granules; endoplasmic reticulum

Introduction

A series of reports in 2003 described the ultrastructural pathology, cytochemistry and analytical electron microscopy of megakaryocytes and platelets from a mother and child with life-long thrombocytopenia [1-4]. Their platelets were slightly enlarged, but functioned normally in aggregation studies and the laboratory revealed no signs of immune thrombocytopenia. Evaluation of their megakaryocytes [1] and platelets [2] by the whole mount technique and in thin sections revealed the presence of organelles never seen before in human cells. There were two different types of giant organelles in megakaryocytes and in platelets. No relationship could be identified between them and the organelles regularly present including alpha granules, dense bodies, mitochondria and lysosomes. One of the larger aberrant organelles was intensely electron opaque, readily apparent in unstained whole mount preparations as well as thin sections. The second appeared to be made up of

multiple layers resembling a target. In addition the mother and son have a mitochondrial myopathy that has not been fully characterized. The new patient with similar findings is unrelated to the original two cases. She has a similar myopathy and her platelets contain identical giant opaque bodies and target organelles. In addition, she has platelet structural changes observed in the initial family, but these are more extensive in her cells.

Materials and methods

Case report

Case histories of the woman and her male child, the first two patients with the York Platelet Syndrome (YPS), were described in earlier reports [1-4]. The mother and father are unrelated and their second child, a female, is unaffected. Parents of the third case are unrelated to each other or to the families of the first two cases. The 4-year-old patient presented here has had thrombocytopenia since birth and physical signs of the mitochondrial myopathic disorder found in the first two patients. Aside from the difficulty rising from the prone position and walking up stairs, she is a healthy 4-year-old female. Blood studies have shown no signs of immune thrombocytopenia, and other blood cells appear normal in all respects. Our blood studies were carried out on samples sent to us from the state where the family resides.

Preparation of platelet-rich plasma (PRP)

Blood samples from the child, her mother and father and from normal controls were mixed immediately with citrate citric acid, pH 6.5 (93 mmol/L sodium citrate, 70 mmol/L citric acid, and 140 mmol/L dextrose) in ratio of nine parts blood to one part anticoagulant [5]. Platelet-rich plasma (C-PRP) was separated from whole blood by centrifugation at 100 g for 20 minutes at 23 °C and was maintained at 37 °C until prepared for study.

Preparations of washed platelet suspensions (WPS)

Samples of C-PRP were combined with an equal volume of the citrate anticoagulant and centrifuged to pellets at 400 g for 15 minutes and 20 °C [6]. Supernatant was discarded and replaced with Hank's balanced salt solution (HBSS) containing either 0.1 mol/L EDTA or adenosine (5 mmol/L) and theophylline (3 mmol/L). After 30 minutes at 37 °C, the pellets were resuspended in the wash solution. The procedure was repeated twice and the platelets resuspended in HBSS with or without 0.1 mol/L EDTA at a final concentration of 5×10^5 /mL. The washed suspensions were maintained in the 37 °C water bath until used in specific experiments.

Preparation of platelet whole mounts

Small drops of PRP or washed platelets were placed on formvar-coated, carbon-stabilized grids, rinsed within 10–15 s with drops of distilled water, dried from the edge with pieces of filter paper and waved in the air to remove residual moisture [7]. The grids were inserted into the electron microscope without fixation or staining.

Preparation of thin sections

Fixation of control and patient platelets was accomplished by combining the samples with an equal volume of 0.1% glutaraldehyde in White's saline. After 15 minutes the samples were centrifuged to pellets and the supernatant fixative was removed and replaced with 3% glutaraldehyde in the same buffer. The samples resuspended in the second aldehyde fixative were maintained at 4 °C for 30 minutes, then sedimented to pellets. Supernatant was removed and replaced with either 1% osmic acid in distilled water containing 1.5% potassium ferrocyanide for 1 hour at 4 °C. All samples were dehydrated in a graded series of

alcohol and embedded in Epon 812. Thin sections cut from the plastic blocks on an ultramicrotome were examined unstained or after staining with uranyl acetate and lead citrate to enhance contrast [8]. Examination was carried out in a (F.E.I. Co. Hillsboro, OR, USA) 301 electron microscope.

Results

General

Platelet counts in blood samples from the new patient were decreased and the mean platelet volume slightly elevated. Thin sections of her platelets revealed the irregular increase in size suggested by the mean platelet volume. Many of her thrombocytes contained the usual number of alpha granules (α Gr), mitochondria and dense bodies (Figure 1). Others contained few or were devoid of these organelles (Figure 2).

Pathology

Giant organelles: The giant, electron opaque organ-elles (DO) and the huge target organelles (TO) found in megakaryocytes and platelets from the first two individuals with the York Platelets Syndrome (YPS) [1-4] were present in thin sections of thrombocytes from the new patient. Their frequency was variable, as observed in the first two individuals. Some platelets contained only one type of the two giant organelle (Figure 3), but the presence of both opaque organelles (OO) and TO in the same platelet thin section was not uncommon (Figure 4). Giant OO were often grouped together (Figure 5), and appeared to grow by fusion (Figures 6-8). Their final size probably arose in this manner (Figures 9 and 10). Both types of giant organelle were visible in whole mount platelet preparations (Figures 11 and 12), but the large OO were more easily identified because of their opacity. The large TO appeared to develop one layer at a time resulting in their unique, target-like appearance (Figure 13). The first stage was a ring of dense material just inside the membrane of an element of the dense tubular system (DTS) of channels (Figure 14). The core of the TO could be opaque (Figures 15-20), or less dense than adjacent rings. TO appeared to become more dense with time and difficult to distinguish from the OO (Figures 19 and 20).

The presence of flat masses and scrolls of smooth endoplasmic reticulum (SER) from themegakaryocytes in the platelets from the third YPS patient were observed much more often than in cells from the first two individuals (Figures 21-28). The resemblance to channels of the DTS was very strong, and suggested that the fundamental pathology of the YPS developed in rough (RER) and smooth endoplasmic reticulum (SER) of their megakaryocytes, and the dense tubular system of circulating platelets [10, 11]. Most of the proteins synthesized in their megakaryocytes were delivered to lamellae of the Golgi complexes to be concentrated into vesicles that fused to form the α granules, δ granules (dense bodies) and lysosomes [12, 13]. In YPS megakaryocytes, however, substantial quantities of the newly synthesized proteins were not moved from RER and SER to Golgi complexes. This may have been an early or late defect in the transportation mechanism because most platelets had some or a normal complement of and granules, and lysosomes. Coils of SER are prominent in platelets of Figures 21-28, but their precise relationship to giant organelles is not clear. A relationship to the DTS shown in Figures 14-16, 18, and 20 is clear, suggesting that the giant organelles had evolved in SER and matured in the DTS.

Discussion

Discovery of a third patient with the same disorder as the mother and son previously described [1-4] has elevated the status of this rare condition from a case report to a syndrome named after the initial family. The new patient with the York Platelet Syndrome

(YPS) has features of the mitochondrial myopathy found in the first two patients. She has difficulty rising from a prone position and walking up stairs. The child has not yet had a muscle biopsy to determine if her mitochondria are devoid of the same enzymes found deficient in the York family mother and son. This will be done in the near future, as well as biochemical studies of her platelet mitochondria.

The platelet pathology in the third patient with the YPS is identical in most respects to findings reported in the first patients. Giant electron opaque organelles were a dominant feature. Examination of her platelets prepared by the whole mount technique revealed the huge, opaque organelles easily distinguished from the normal-sized dense bodies. Stages in their development from electron dense chains and clusters could also be identified in thin sections. We have shown previously that the chains and clusters are present in platelets from patients with the Hermansky-Pudlak Syndrome (HPS) whose platelets are devoid of dense bodies [9]. Thus they are not involved in formation of the serotonin, adenine nucleotide rich delta organelles missing in HPS.

Stages in development of the giant opaque organelles can be observed more clearly in thin sections of platelets from the new patient. The presence of many small and enlarged dense clusters, examples of them fusing together to form larger masses and their fusion with giant opaque organelles suggest this as the pathological process for the huge opaque body formation.

Platelets from the new patient also contain the giant target organelles present in megakaryocytes and thrombocytes of the York family members. Their origin was more difficult to determine than that of the giant opaque organelles. However, reacting their platelets for peroxidase activity with the diaminobenzidine procedure revealed staining of internal contents and outer membranes enclosing the giant, target-like organelles [4]. The findings support the concept that the giant opaque and target organelles in platelets from patients with the YPS derive from the rough RER and smooth SER of the megakaryocytes and channels of DTS in their platelets.

Platelets from the new patient with the YPS contained many more examples of flat masses and scrolls formed by closely associated elements of the SER and DTS than were observed in thrombocytes from the original two cases. The channels were pressed together in flat masses or in large scrolls resembling myelin figures. However, myelin figures consist of single membrane sheets, while the flat masses and scrolls in YPS platelets are clearly individual channels of SER and DTS pressed closely together. Similar structures have not been observed in normal platelets or in other platelet disorders. Their presence is a further indication that the basic pathology of the YPS involves the endoplasmic reticulum in the megakaryocytes and the dense tubular system in platelets.

In summary the present study has presented the third case of the York Platelet Syndrome. The disorder is characterized by the association of a mitochondrial myopathy with unique platelet pathology. Megakaryocytes and circulating platelets contain giant opaque organelles and massive, multi-layered organelles resembling targets in thin section. Our studies have shown that the huge organelle contain calcium, acid hydrolases and platelet peroxidase. Normally these products are synthesized or taken up by the RER and SER, and delivered to the Golgi complex [12, 13]. There the protein products and elements are passed from the cis- to the trans-face where they are sorted into separate vesicles. Fusion of vesicles forms the α , δ and lysosomal organelles filling the platelet cytoplasm. Clearly the YPS platelets can form quantities of normal sized organelles. Formation of giant organelles containing the products that usually go to separate organelles suggests that a significant amount of these products are not delivered to the Golgi complex at an early stage when normal organelles are formed, or

are retained after the normal process has been completed. Further studies will be required to define the nature of the defects in transfer of synthesized proteins to the Golgi apparatus in YPS, megakaryocytes, as well as the relationship between the mitochondrial myopathy and unique platelet pathology.

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Figure 1.

Thin section of a platelet from the new patient with the York Platelet Syndrome (YPS). The cell is entirely comparable in morphology to normal platelets. Its discoid form is supported by a circumferential coil of microtubules (MT). The cytoplasm is filled with organelles, including numerous alpha granules (Gr) and occasional dense bodies (DB). Mag $\times 33\,000$.

Figure 2.

Thin section of another of the new patient's platelets. This example is devoid of all the structures present in the cell shown in Figure 1. A few small clumps of dense tubular system elements and dilated channels of the open canalicular system (OCS) are the only structures present. Mag $\times 33\,000$.

Figure 3. Thin section of two of the new patient's platelets containing organelles characteristic of the YPS. The opaque organelles (OO) in the platelet on the left is larger than any of the four target organelles (TO) in the platelet on the right. Mag $\times 29\,400$.

Figure 4. Platelet from the new YPS patient containing a small opaque organelle (OO) and two giant target organelles (TO) revealing the several alternating rings of light and dark material which suggested the name chosen for these structures. Max $\times$ 29 400.

Figure 5.
Patient platelet containing five opaque organelles (OO) in close proximity in the same cell.
Max $\times 72\,000$.

Figure 6. Patient platelet containing one, fully developed opaque organelle (OO-1), and a second (OO-2) appearing to be developing by fusion. Elements of the dense tubular system (DTS) are closely associated. Mag $\times 65\ 000$.

Figure 7.
Patient platelet with two opaque organelles (OO) in the process of fusion. Mag $\times 57\,600$.

Figure 8. Giant opaque organelle (OO) in a patient platelet closely associated with elements of the dense tubular system (DTS). Small dense particles in the cell may be precursor particles (PP) becoming opaque organelles by fusion. Mag $\times 70\,000$.

Figure 9. Patient platelet with two giant opaque organelles (OO). In this example masses of smooth endoplasmic reticulum (SER) are also present. Mag $\times 25\ 200$.

Figure 10. Giant opaque organelles (OO) in close association with elements of the dense tubular system. Mag $\times 80\,000$.

Figure 11. Whole mount of a patient platelet. Giant opaque organelles (OO) are similar in opacity to dense bodies (DB) but are much larger. Mag $\times 20\,800$.

Figure 12. Whole mount of a patient platelet containing dense bodies (DB), opaque organelles (OO) and target organelles (TO). The target organelles are less electron dense than opaque organelles and dense bodies. Mag $\times 20\,800$.

Figure 13. Thin section of target organelles (TO) and opaque organelles (OO) in a patient platelet. The organelles appear to be in different stages of development. All are in close association with channels of the dense tubular system. Mag $\times 57\,600$.

Figure 14.

An early stage in development of target organelles. The development of electron dense staining at the peripheral margins of elements of the dense tubular system (DTS) in patient platelets appeared to be the earliest sign of target organelle (TO) development. Mitochondria (Mi) in this section appear larger than normal. Mag $\times 80\,000$

Figure 15. Platelet from the patient showing developmental stages in formation of target organelles (TO) and their association with the dense tubular system (DTS). Mag $\times 38\,500$.

Figure 16.
YPS platelet containing four fully formed target organelles (TO). Mag $\times 46\,000$.

Figure 17. Platelet from the new YPS patient with two target organelles (TO), one in a stage of formation, and opaque organelles (OO). Mag $\times 38\,500$.

Figure 18.
Two target organelles (TO) at different stages of development. Mag $\times 44\,000$.

Figure 19.
Huge target organelle (TO) that may be transforming into an opaque organelle. Mag $\times 38$ 500.

Figure 20. YPS platelet with two target organelles (TO) at different stages of development closely associated with elements of the DTS. Nobs (Nb) are present at the periphery of the larger, more mature, target organelle. Mag $\times 48\,000$.

Figure 21.
YPS platelet containing developing target organelles (TO) and two coils of endoplasmic reticulum (ERC). Mag $\times 29\,400$.

Figure 22.
Patient platelet containing four endoplasmic reticulum coils (ERC). Mag $\times 29\,400$.

Figure 23. Patient platelet containing three endoplasmic reticulum coils (ERC). Elements of the dense tubular system (DTS) are present within them. Mag $\times 29\,400$.

Figure 24.
YPS patient platelet with five coils of endoplasmic reticulum (ERC), and a developing target organelle (TO). Mag $\times 38\,500$.

Figure 25. Patient platelet containing multiple endoplasmic reticulum coils (ERC) and (DTS). Mag $\times$ 38 500.

Figure 26. Patient platelet containing multiple coils of smooth endoplasmic reticulum (ERC), and a mature target organelle (TO). Mag $\times 38\,500$.

Figure 27.
Patient platelet with multiple coils of endoplasmic reticulum (ERC). Mag $\times 40\,500$.

Figure 28. Patient platelet with opaque organelles (OO), target organelles (TO), and several coils of smooth endoplasmic reticulum (ERC). Mag $\times 38\,500$.