Platelet-Associated Immunoglobulin, Platelet Size, and the Effect of Splenectomy in the Wiskott-Aldrich Syndrome

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Wiskott-Aldrich syndrome (WAS) thrombocytopenia is a X-linked immunodeficiency disorder characterized by the triad of eczema, hemorrhage, and recurrent infections. The hemorrhagic diathesis is due to profound thrombocytopenia with the distinctive observation of severely decreased mean platelet volume (MPV). The etiology of the thrombocytopenia remains an enigma, although several authors have reported data to suggest that it is due to production of an intrinsically abnormal platelet.

Management of WAS-associated thrombocytopenia is a difficult clinical problem, since the long-range efficacy of platelet transfusion is limited. Although bone marrow transplantation of compatible bone marrow has been successful in some patients, suitable donors are not always available. We previously reported our experience in the management of WAS thrombocytopenia by splenectomy and noted increased platelet counts and improved clinical status with good long-term results when prophylactic antibiotic coverage was instituted. During the course of that study, we observed that the decreased MPV had returned to normal in two post-splenectomy subjects and that autologous platelet survival normalized in one patient. We also noted that platelet-associated immunoglobulin (PAIgG) was elevated in eight of ten presplenectomy WAS patients. In light of the presence of adequate megakaryocytes in WAS bone marrow and the normalization of autologous platelet survival, mean platelet volume, and platelet count after splenectomy, we decided to further investigate the effect of splenectomy on platelet volume distribution and PAIgG in WAS.

MATERIALS AND METHODS

Patient Selection

Patients who had been referred to the Metabolism Branch of the National Cancer Institute for evaluation of suspected WAS were studied. The diagnosis of WAS was based on the clinical triad of severe thrombocytopenia, recurrent infections, and eczema. Many of the patients had a family history of the disorder. Other criteria used to establish the diagnosis included skin test anergy to all antigens tested—including tetanus toxoid, diphtheria toxoid, candida—poor lymphocyte blast transformation to specific antigens, poor mixed lymphocyte reactions, failure to make specific antibodies to immunizations with pneumococcal polysaccharide types I or II, low or absent isohemagglutinins, accelerated catabolism of serum immunoglobulins, and depressed monocyte-mediated antibody-dependent cellular cytotoxicity.

Laboratory Studies

Platelet counts were performed in the routine hematology laboratory by either electrical impedance or phase contrast microscopy, depending on the absolute level. Blood was collected in 13 mmol/L sodium citrate and 1 mmol/L EDTA as previously described. MPV was measured on purified platelets isolated on arabinogalactan gradients. This technique was necessary due to profound thrombocytopenia and decreased platelet size in WAS. Previous attempts to size WAS platelets in whole blood samples were unsuccessful. Other studies in this laboratory have demonstrated that the use of arabinogalactan system, total platelet populations can be isolated with greater than 95% average recovery. Cell sizing was performed using an electrical impedance system equipped with a logarithmic amplifier. Platelet volume was determined using the median from probability plot analyses of the volume distributions. Fifty normal subjects were evaluated to establish the normal MPV size range. Thrombocytopenic control subjects with severely reduced platelet counts were evaluated.
Platelet volume distribution in seven WAS subjects before splenectomy. All subjects were severely thrombocytopenic. Shaded area represents normal platelet volume distribution. Ordinate represents frequency; abscissa is volume in femtoliters.

Paired studies of platelet size in seven WAS subjects pre- and post-splenectomy demonstrated a substantial increase in platelet size after splenectomy for each subject. Serial measurement of MPV before splenectomy in several WAS patients demonstrated consistent reductions in both platelet size and platelet count. Subjects studied serially postsplenectomy exhibited a consistent increase in MPV, and the platelet volume distribution was completely normal.

Platelet-Associated Immunoglobulin

PAIgG (femtograms per platelet) was significantly elevated (>14.8 fg per platelet, 3 SD above normal) in 13 of 14 WAS subjects (x = 78.9 ± 23.3, SD) tested prior to splenectomy. None of the thrombocytopenic subjects with platelet hypoproduction demonstrated elevated PAIgG levels, while 11 of 11 thrombocytopenic patients with a clinical and/or laboratory diagnosis of immune platelet destruction had significantly elevated PAIgG (x = 66.1 ± 33.5, SD). Seven WAS subjects studied postsplenectomy with normal or improved platelet counts had normal PAIgG. Five of these were evaluated in a paired study pre- and post-splenectomy, and each demonstrated consistent normalization of PAIgG postsplenectomy.

Correlation of Platelet Size and PAIgG With Subsequent Clinical Course

Two patients had complicated clinical courses with recurrent thrombocytopenia after an initially successful splenectomy. One patient had a prolonged postoperative course complicated by hepatitis and opportunistic infections. During the course, severe thrombocytopenia recurred associated with an increase in PAIgG, but MPV and volume distribution remained in the normal range. A second subject also developed recurrent thrombocytopenia and elevated PAIgG, but platelet size also remained entirely normal.

DISCUSSION

Markedly decreased platelet size associated with thrombocytopenia is a consistent observation that is...
well described in eusplenic WAS patients and is unique to this disorder. The etiology of the thrombocytopenia and the reduced platelet volume have previously been attributed to either rapid elimination of a defective platelet or ineffective thrombopoiesis. The quantity and quality of megakaryocytes in WAS bone marrow appear to be normal by light microscopy, and ultrastructural studies have been inconclusive as to a consistent abnormality. Autologous platelet survival in this syndrome is generally reduced, although not always to a degree sufficient to account for the level of thrombocytopenia, while allo- geneic platelet survival is reported to be comparable to that of patients with bone marrow failure. Studies by two groups have failed to show conclusive evidence for immune-mediated autologous platelet destruction.

The present study is unique in that it examines a large group of WAS patients before and after splenectomy. Our previous observations that splenectomy improves WAS thrombocytopenia as well as platelet function suggested that other factors besides ineffective thrombopoiesis contribute to the platelet abnormality. Platelet volume distribution analysis in eusplenic patients clearly indicates that some normal-sized platelets are produced by WAS megakaryocytes. Moreover, postsplenectomy, the platelet volume distribution returns to normal as the platelet count increases. This clearly suggests that splenic function is contributory to the production of the small WAS platelet. This observation is interesting, since thrombocytopenia syndromes with shortened platelet survival are usually associated with increased MPV. This difference could be due to either an intrinsic abnormality of the WAS platelet or to some form of unique splenic interaction. Ochs and co-workers described a single postsplenectomy patient (A.S.) whose MPV was 5.05 μM. Although this is below the normal range for the patients of these authors, it is larger than the MPV for their eusplenic patients, and thus suggestive of a postsplenectomy effect in their patient as well.

The significance of elevated PAIgG in the eusplenic WAS patients is unclear and may be due to artifact, although the parallel postsplenectomy changes in platelet count and platelet volume suggest that the observation is valid and possibly related to the production of thrombocytopenia. The recurrence of elevated PAIgG with relapse of thrombocytopenia also suggests that the two events are related. Knutsen et al. have reported a single WAS patient who initially responded to splenectomy; however, this patient subsequently relapsed with recurrent thrombocytopenia and was also found to have elevated PAIgG.

A number of workers have questioned the specificity of elevated PAIgG in autoimmune thrombocytopenia. Kelton and Steevens suggested that the elevated platelet-associated IgG in immune thrombocytopenic purpura (ITP) may not be specific for immune-mediated platelet destruction and could be deposited as the result of increased platelet surface area or a generalized altered affinity for all plasma proteins. However, the recent observations by Lo Buglio et al. tend to support the validity of elevated PAIgG in autoimmune thrombocytopenia. We have measured platelet-associated IgM in these WAS patients and have not found elevated levels indicative of nonspecific adsorption, although this could be due to low serum IgM levels in WAS. Our large control group with nonimmune thrombocytopenia did not exhibit elevated PAIgG. Thus, it does not seem likely that severe thrombocytopenia alone could account for false PAIgG elevations. Inaccurate platelet counts or platelet fragments could contribute to falsely elevated PAIgG values, but our washing and platelet counting procedures should prevent these discrepancies.

It is also conceivable that the elevated PAIgG in WAS is due to nonspecific deposition of immune complexes on the platelet surface. The observations by others that allogeneic platelet survivals in WAS patients are equivalent to those for hypoplastic thrombocytopenia suggests that immune complexes are not a major factor, since both allogeneic and autologous platelets should be affected by immune complexes. We have, however, observed one patient who received no increment from allogeneic platelet support at the initiation of platelet transfusions, and other patients have been observed to undergo transient episodes of refractoriness to platelet transfusion both before and after splenectomy even with HLA-matched platelets. Unfortunately, we have no PAIgG or size data for our patients during these periods, and thus it is possible that immune complexes may be a source of elevated PAIgG.

Two of our patients redeveloped thrombocytopenia after an initially successful response to splenectomy. In both cases, the recurrence of thrombocytopenia was associated with transiently elevated PAIgG, but without a change in platelet size. Repeat volume distribution analysis did not reveal a recurrent subpopulation of small platelets, nor did it reveal large platelets seen in typical autoimmune thrombocytopenia. Both subjects ultimately recovered with either prednisone or cytotoxic therapy. This suggests that an intact spleen is critical to the creation of the small WAS platelet, and while other tissues may become the site of platelet destruction, they do not affect platelet size. The failure of MPV to increase during relapse may indicate that WAS thrombopoiesis is defective in some fashion.

Parkman and co-workers have described the
absence of a 115,000-dalton surface glycoprotein from WAS lymphocytes and a reduction in glycoproteins Ia and Ib in WAS platelets. This observation may indicate that an intrinsic abnormality of the WAS platelet and Ib in WAS platelets. This observation may indicate an intrinsic abnormality of the WAS platelet and Ib in WAS platelets. This process might occur only in the spleen under conditions that would favor attachment of a low-affinity antibody and subsequent partial loss of platelet membrane. It is also possible that the spleen could serve as a principle site of antibody synthesis, antibody attachment, or specialized platelet–macrophage interaction. Recent preliminary studies by Slichter et al15 suggest that platelet-associated anti-bodies can also cause ineffective thrombocytopenia. Thus, it is also possible that the WAS spleen could exert an effect on bone marrow megakaryocytes. Definitive proof for immune-mediated platelet destruction in WAS will require identification of the specific antigen or antibody and could provide an interesting model for the interaction of target cells and macrophages.

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