Platelet associated immunoglobulins in primary biliary cirrhosis: a cause of thrombocytopenia?

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SUMMARY Thrombocytopenia in cirrhotic patients is usually attributed to splenic pooling whereas in idiopathic thrombocytopenic purpura it is related to platelet bound immunoglobulin (PA-IgG). Since primary biliary cirrhosis (PBC) is an autoimmune disorder we have undertaken a prospective study to assess the frequency and possible relationship of PA-IgG to thrombocytopenia in this condition. Sixty two primary biliary cirrhosis patients (28 precirrhotic; 34 cirrhotic) were studied. Twenty five patients (40%) had raised PA-IgG of whom 18 had cirrhosis. There was a significant inverse correlation between platelet count and PA-IgG (p<0·001) and between platelet count and spleen size (p<0·001). Thrombocytopenia (platelets $< 100 \times 10^9/l$) was found in nine patients (15%); all nine had raised PA-IgG and eight were cirrhotic with an enlarged spleen. Two cirrhotic patients with persistent thrombocytopenia and bleeding episodes were treated with prednisolone and showed a useful therapeutic response. These results suggest that immune mediated platelet destruction and splenic pooling of platelets may both play a part in the thrombocytopenia observed in primary biliary cirrhosis.

Thrombocytopenia in patients with primary biliary cirrhosis has mainly been attributed to splenic pooling in an enlarged spleen, secondary to portal hypertension,[1] although other factors, such as intrinsic platelet abnormalities leading to reduced survival have also been implicated.[2] On the other hand thrombocytopenia in patients with idiopathic thrombocytopenic purpura is now recognised as being autoimmune in nature; there is strong evidence that an IgG autoantibody directed against platelet antigens causes increased platelet destruction.[3–5] Progress in this field has been hampered by the difficulty of studying the binding of antibody to platelets but in recent years several techniques which measure platelet associated immunoglobulin (PA-IgG) have been developed.[6–9] The more widespread application of this investigation has resulted in the demonstration of PA-IgG in other conditions such as systemic lupus erythematosis,[10] neoplastic disorders,[11] chronic active hepatitis[12] and alcoholic cirrhosis;[13] these findings add to the suggestion that immune mechanisms may mediate thrombocyto-

penia in many more disorders than was previously thought likely.

Primary biliary cirrhosis ('chronic non-suppurative destructive cholangitis') is an autoimmune disease characterised by many immunological abnormalities including the presence of autoantibodies in the serum[14] and hypergammaglobinaemia,[15] and associated with other autoimmune disorders such as thyroid disease.[16] Raised PA-IgG has been reported in one patient with primary biliary cirrhosis who had thrombocytopenia,[13] suggesting that immune thrombocytopenia may also develop in this condition. In view of the potential therapeutic implications of this finding, we have carried out a prospective study to assess the frequency of PA-IgG in primary biliary cirrhosis and its possible relationship to thrombocytopenia in this condition.

Methods

PATIENTS
Sixty two patients (57 women, five men) with primary biliary cirrhosis were studied; the diagnosis being established on standard biochemical, immunological and histological criteria.[16] Thirty four

patients were cirrhotic and 28 precirrhotic on liver biopsy. An estimate of splenomegaly was made (grade 0–3) by an independent observer (JDC) on a standard [99]technetium liver and spleen scan. Two cirrhotic primary biliary cirrhosis patients with persistent thrombocytopenia, epistaxis and gastrointestinal bleeding in whom an immune mediated destruction of platelets was suspected were treated with prednisolone therapy and their clinical course carefully monitored.

Full blood counts and platelet counts in all patients were carried out on a Coulter Counter, Model S plus III. Platelet associated immunoglobulins were estimated using a modification of a method previously described using ^{125}I-labelled Staphylococcal Protein A (SPA).[17–19]

Platelets from patients with primary biliary cirrhosis were harvested by differential centrifugation, washed in phosphate buffered saline pH 7·2 (PBS) and stored at 4°C in phosphate buffered saline containing 15·4 mmol/l sodium azide. Normal platelets from at least 10 group O blood donors were similarly harvested and pooled ('pooled normal platelets'). Staphylococcal Protein A (Pharmacia Ltd – Uppsala) was radio-iodinated by the chloramine T method with Na[125]I (Amersham International) and stored in 20 μl aliquots at −40°C. This stock radiolabelled SPA was diluted appropriately before use. Duplicate aliquots of either test or pooled normal platelets containing 10^8 platelets were resuspended in and incubated with 100 μl of working dilution of SPA ^{125}I at 4°C for 60 minutes. Unbound SPA ^{125}I was removed by washing×3 in phosphate buffered saline. The platelet button was resuspended in phosphate buffered saline and bound radioactivity counted. The PA-IgG was reported as the ratio of SPA[125]I bound to test platelets, compared with that bound to 'pooled normal platelets'. The normal range of PA-IgG for a cohort of 50 normal people was up to 1·65 (mean 0·89±2 SD (SD=0·38)).

The Spearman rank correlation coefficient was used to test the correlation of platelet count with PA-IgG and with spleen size. The χ^2 test was applied to analyse the association of thrombocytopenia (platelet count $<100\times10^9$/l; normal range 150–400×10^9/l) with raised PA-IgG ratio and with an enlarged spleen.

Results

The PA-IgG results in all patients tested are shown in Fig. 1. There were raised levels of PA-IgG in 25 patients with primary biliary cirrhosis (40%), the range of values was 0·8 to 16 (mean 1·8). Raised PA-IgG was more commonly found in cirrhotic than
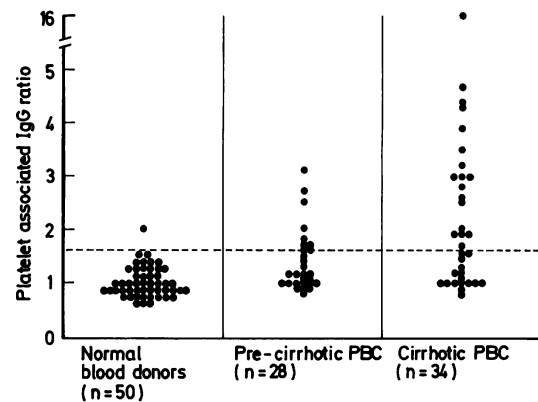

Fig. 1 *Platelet-associated IgG ratio in patients with primary biliary cirrhosis and normal controls. Line represents upper limit of normal range (mean±2 S.D.).*

pre-cirrhotic patients (53% *vs* 25%).

In the 62 primary biliary cirrhosis patients there was a significant inverse correlation between platelet count and PA-IgG ratio (r=−0·51 p<0·001) (Fig. 2) and between platelet count and spleen size (r=−0·63 p<0·001). Nine primary biliary cirrhosis patients (eight with cirrhosis) had platelet counts below 100×10^9/l for at least three months. This thrombocytopenia was significantly associated with both raised PA-IgG (9/9, p<0·0005) and an enlarged spleen (8/9, p<0·005). (Figs 3a and b).

The clinical course of the two primary biliary cirrhosis patients with persistent thrombocytopenia, epistaxis and gastrointestinal bleeding who were treated with prednisolone is shown in Fig. 4. Patient
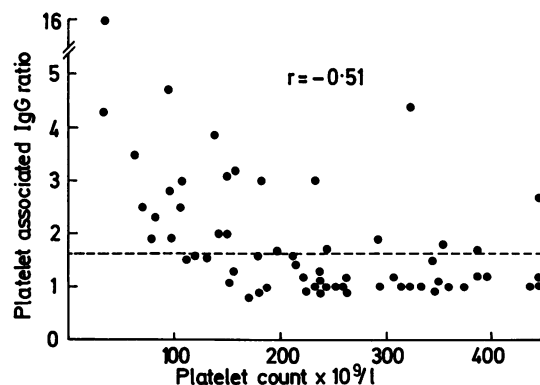

Fig. 2 *Relationship between PA-IgG and platelet count in primary biliary cirrhosis patients. Line represents upper limit of normal range (mean±2 S.D.).*
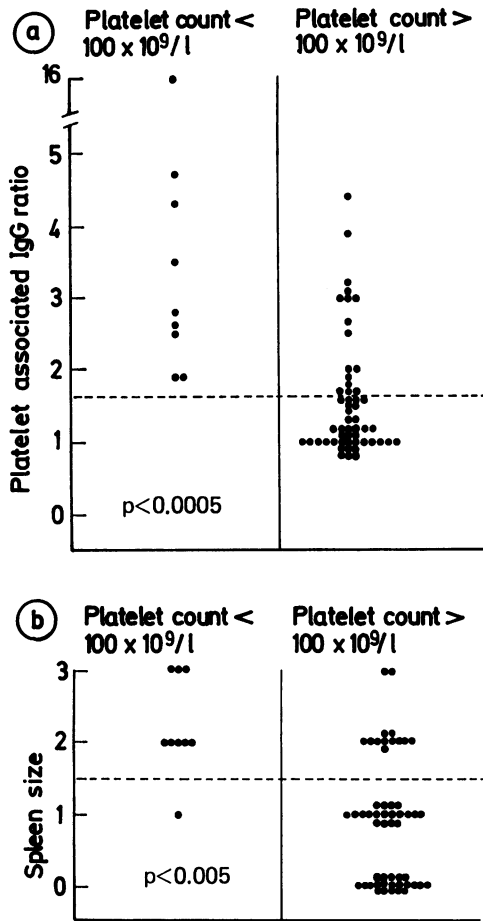
Fig. 3 *(A) Relationship between PA-IgG and thrombocytopenia (platelets <100×10⁹/l) in primary biliary cirrhosis patients. Line represents upper limit of normal range (mean±2 S. D.).*
(b) Relationship between spleen size and thrombocytopenia (platelets <100×10⁹/l) in primary biliary cirrhosis patients. Line represents division between considerable splenomegaly (spleen size 2 & 3) normal or slight splenomegaly (spleen size 0 & 1).

1 (Fig. 4a) showed a dramatic therapeutic response with a marked improvement in her platelet count and cessation of bleeding. When her immunosuppressive therapy was stopped her platelet count slowly fell towards pretreatment levels, associated with a raised PA-IgG of 4·3. Patient 2 (Fig. 4b) followed a similar though less remarkable clinical course. When prednisolone was stopped her platelet count also fell, associated with a raised PA-IgG of 16 and epistaxis recurred. Restarting prednisolone

therapy resulted in a partial clinical response with an increase in her platelet count associated with a decrease in PA-IgG to 2·8. No reactive thrombocytosis had been observed in either patient following previous bleeding episodes.

Discussion

The clinical value of PA-IgG estimations has been shown for a variety of disorders. A large number of radioisotopic methods have been devised for the quantitation of PA-IgG. The majority rely on a labelled anti-human IgG antibody for its detection. We have used ^{125}I labelled staphylococcal protein A. This detects mainly IgG 1, 2 and 4, although IgG 3 and IgM are detected to some extent.[20] Evidence suggests that the predominant subclass of IgG found on platelets in patients with immune thrombocytopenia is IgG 1.[21] [22] One recent study though has suggested a hitherto unrecognised frequent involvement of PA-IgM in ITP[23] so it is possible the method used may underestimate total platelet-associated immunoglobulin.

Our study has shown that raised PA-IgG occurs in some patients with primary biliary cirrhosis and that they are more commonly associated with cirrhotic than non-cirrhotic patients. The pathogenic role of this platelet bound immunoglobulin is still far from clear. In ITP some studies have shown an inverse correlation between PA-IgG and platelet count[6] [7] [9] but another study has been unable to confirm this.[23] Similarly a significant inverse correlation between PA-IgG and platelet survival has been shown in idiopathic thrombocytopenic purpura,[5] but has not been found in other thrombocytopenic patients.[24] The application of PA-IgG measurements to these other patient groups has resulted in modification of the original assumption that raised PA-IgG levels indicate specific antiplatelet antibody.[6] [7] It is now suggested that possibly only 10% of PA-IgG is directly related to a shortened platelet life span[25] and that the remaining 60–90% of PA-IgG is either non-specifically associated with platelets or is directed towards easily solubilised platelet antigens.[26] Some of the immunoglobulin bound to platelets in primary biliary cirrhosis could conceivably be related to changes in receptor availability and function,[27] secondary to the altered lipid composition of platelets that occurs in chronic liver disease.[28] Despite the immunological nature of primary biliary cirrhosis the incidence of PA-IgG in the cirrhotic patients (53%) is lower than that previously reported in alcoholic cirrhosis.[13] This is consistent with the concept that some of the PA-IgG detected in primary biliary cirrhosis is formed by a mechanism common to other forms of chronic liver
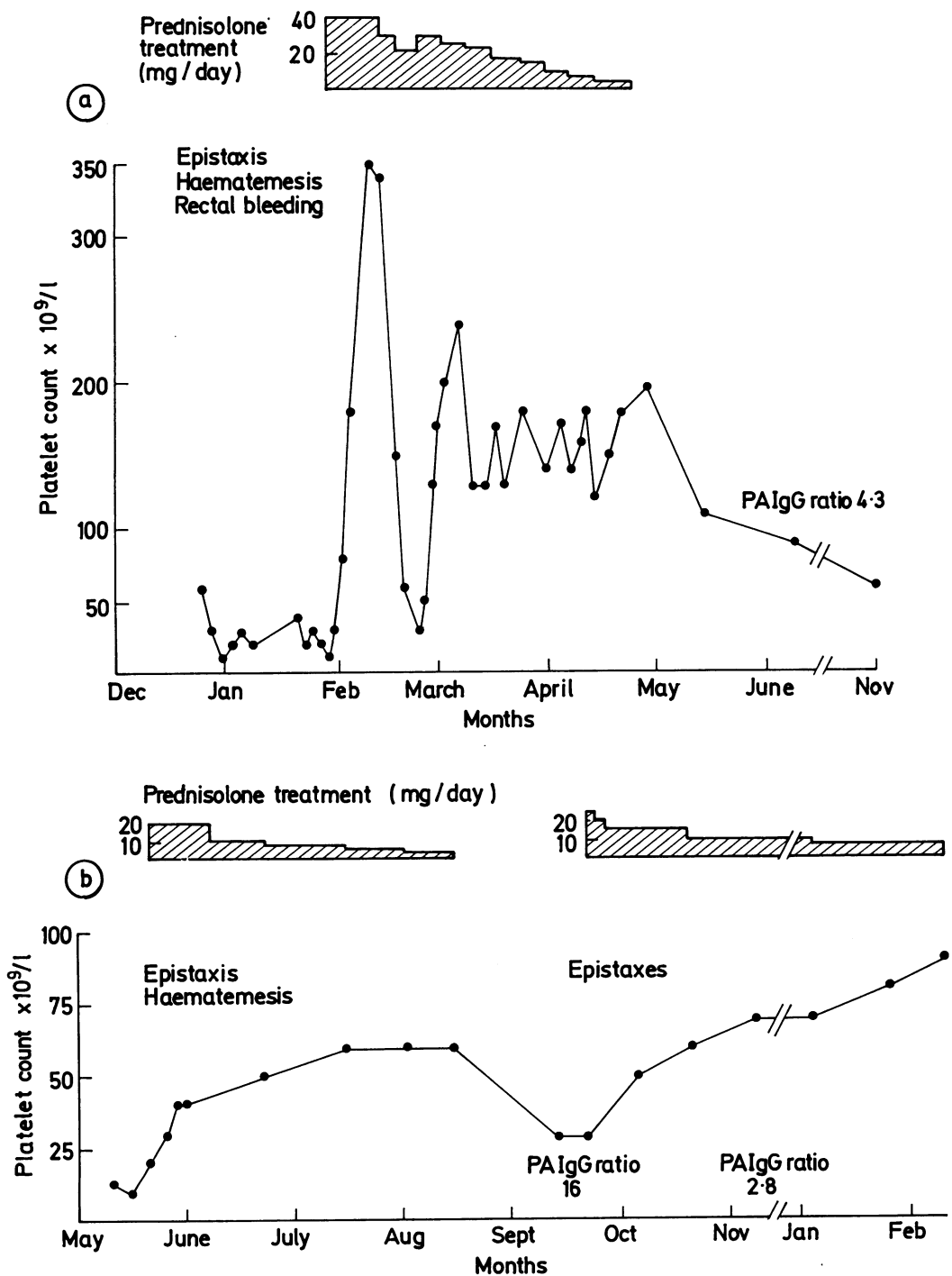
Fig. 4 *Response of platelet count to steroid treatment in two primary biliary cirrhosis patients.*

disease, and may not be specifically 'autoimmune' in aetiology.

Nonetheless studies in other forms of chronic liver disease have not shown the inverse correlation between platelet count and PA-IgG,[12] [13] which has been found in this study. This inverse correlation is similar to idiopathic thrombocytopenic purpura[6] [7] [9] and suggests that immune mediated platelet destruction is contributing to the thrombocytopenia found in some primary biliary cirrhosis patients. Our finding of raised PA- IgG in all nine primary biliary cirrhosis patients with persistent thrombocytopenia also supports this hypothesis, and suggests that an immune mechanism may be as important a factor in thrombocytopenia as splenic pooling in an enlarged spleen. The possible immunological nature of platelet destruction is further supported by the two patients reported here who exhibited a useful therapeutic response to steroids. The increase in platelet count during immunosuppressive treatment and the raised PA-IgG when taken off therapy are both typical of classical idiopathic thrombocytopenic purpura, and suggest an immune pathogenesis for thrombocytopenia common to both diseases.

The significant inverse correlation of platelet count with spleen size also found in this study is consistent with previous data indicating that pooling of platelets in an enlarged spleen is a cause of thrombocytopenia.[1] Our study, however, would suggest that this is an over simplification of the mechanisms involved and that immune mediated platelet destruction in the spleen plays an additive role.

A better understanding of the complex pathophysiology of thrombocytopenia in primary biliary cirrhosis and other forms of chronic liver disease may be reached by measurement of PA-IgG in conjunction with studies of platelet survival and sites of destruction. The finding of raised PA-IgG in thrombocytopenic patients can both raise the suspicion that immune mediated platelet destruction is occurring and suggest that successful management may include measures directed towards modification of the immune process.

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