Journal of Clinical Immunology, Vol. 11, No. 6, 1991

Treatment of Septic Thrombocytopenia with Immune Globulin

EDWARD R. BURNS,[1,2,4] VERNA LEE,[1] and ARYE RUBINSTEIN[3]

Accepted: June 25, 1991

Thrombocytopenia frequently complicates systemic infection and results from multiple possible mechanisms. We and others have demonstrated that platelet-associated IgG (PAIgG) levels are elevated in the majority of patients with septic thrombocytopenia. Corticosteroids may be undesirable as a treatment for thrombocytopenia for patients with severe infection because of their potential for suppressing the immune response. We hypothesized that septic thrombocytopenia is, in most cases, an immune disorder analogous to idiopathic thrombocytopenic purpura (ITP) which might respond to intravenous gamma-globulin as a treatment for increasing the platelet count in this disorder. Intravenous immune globulin (IVIG), 400 mg/kg daily for 3 days, was administered in a randomized double-blind placebo-controlled trial. Twenty-nine patients who developed thrombocytopenia during a documented, septic episode were studied. Patients with disseminated intravascular coagulation (DIC), hypersplenism, or drugs known to cause thrombocytopenia were excluded. Elevated PAIgG levels were documented in 52% of evaluable patients. Mean platelet counts in the IVIG group rose from 43K at study entry to 178K (411% rise) by Day 9. In the placebo group platelets rose from 51K to 125K (261% rise; $P = 0.02$). Seventy-seven percent of the IVIG group had a minimum peak rise of 35K, vs 56% of the placebo group. Three patients in the placebo group had a serious bleeding episode, vs one in the IVIG group. The use of IVIG to treat septic thrombocytopenia not associated with DIC leads to a more rapid, more sustained, and greater increase in platelet count than placebo. Its use is recommended in the septic patient who is bleeding or is likely to need invasive or surgical procedures.

KEY WORDS: Thrombocytopenia; sepsis; immune globulin; platelets.

[1]Department of Laboratory Medicine, Albert Einstein College of Medicine, Bronx, New York 10461.
[2]Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461.
[3]Department of Pediatrics, Albert Einstein College of Medicine, Bronx, New York 10461.
[4]To whom correspondence should be addressed at Department of Laboratory Medicine, Albert Einstein College of Medicine, 1400 Pelham Parkway South-6N22, Bronx, New York 10461.

INTRODUCTION

Thrombocytopenia is a frequent complication of infection in adults and neonates. Estimates of frequency of clinically significant thrombocytopenia range from 56% (1) to 77% (2) in large clinical studies, with up to one-third of patients with septicemia developing platelet counts below 50,000/mm^3 (3). There are numerous mechanisms to account for the thrombocytopenia including bone marrow suppression, disseminated intravascular coagulation (DIC) (4), direct damage to platelets by viruses and bacteria (5,6), and increased platelet clearance by the mononuclear phagocyte system.

One mechanism which has not received major attention but may be clinically important is immune-mediated thrombocytopenia. In one major study in adult patients (7) 73% of 44 patients with gram-negative or gram-positive sepsis and thrombocytopenia had elevated levels of platelet-associated IgG (PAIgG). PAIgG was rarely increased in nonthrombocytopenic patients with sepsis, thereby demonstrating the specificity of this finding for immune thrombocytopenia. In newborns, 82% of patients with viral or bacterial sepsis and thrombocytopenia had increased levels of PAIgG (8).

The major complication of thrombocytopenia, bleeding, is especially problematic in the extremely ill patient, who may already be prone to bleeding from stress ulcers and who may be in need of invasive diagnostic or therapeutic procedures such as catheterization or surgery. Specific hemostatic therapy for the thrombocytopenia of sepsis is cur-

0271-9142/91/1100-0363$06.50/0

Table I. Inclusion Criteria for Entry into Study

1. Platelet count of less than 75,000/mm^3
2. Suspected infection documented by one or more of the following:
   (a) Fever
   (b) Leukocytosis
   (c) Elevated band neutrophil count
   (d) Infiltrate on X-ray of chest consistent with pneumonia
   (e) Toxic granulations or Dohle bodies on peripheral smear
   (f) Positive gram stain of body fluid or exudate
3. Documented systemic infection by positive culture

Table II. Exclusion Criteria from Study

1. Patient thrombocytopenic before becoming infected
2. Patient has suppression of bone marrow megakaryocytopoiesis as defined by one or both of the following:
   (a) Bone marrow aspirate showing decreased megakaryocytes
   (b) Thrombocytopenia with an MPV that has fallen from normal
3. Thrombocytopenia due to bone marrow replacement by fibrosis or tumor
4. Presence of disseminated intravascular coagulation
5. Presence of hypersplenism
6. Patient on immunosuppressive therapy or corticosteroids
7. Patient had contraindication to receiving IVGG (e.g., IgA deficiency)

rently limited to the infusion of platelet concentrates. Since the mechanism for the thrombocytopenia of sepsis is often increased platelet clearance, it is not surprising that this approach is not uniformly successful.

We surmised that the immune thrombocytopenia of sepsis might respond to intravenous immune globulin (IVIG). This would be due to two properties of IVIG, namely, its ability to induce Fc receptor blockade (9, 10) and its ability to act as a source of antiidiotype antibody, which would suppress or block the autoantibodies directed against platelets (11, 12). The latter effect would be similar to that observed with the use of i.v. gamma-globulin in the treatment of spontaneous anti-Factor VIII antibodies (13) or idiopathic thrombocytopenic purpura (ITP) (14).

METHODS

Patients

The study was approved by the institution's Committee on Clinical Investigations and informed consent was obtained from all patients. To be included in the study, patients had to have documented sepsis and be acutely thrombocytopenic. Furthermore, the thrombocytopenia had to be related directly to the infection rather than be secondary to drug effects or disseminated intravascular coagulation. The specific inclusion and exclusion criteria used are presented in Tables I and II.

All patients entered into the study were evaluated with an initial complete blood count (CBC) including platelet count and mean platelet volume (MPV), a leukocyte differential, and a coagulation profile including prothrombin time, fibrinogen level, and fibrinogen degradation products assay. In addition, a serum chemistry profile was obtained to rule out patients with severe liver disease who might have hypersplenism. The majority of patients entered were cared for in the medical or surgical intensive care units. Many patients with septic thrombocytopenia were excluded for the purposes of this study because of the criteria in Table II.

Study Design and Treatment Plan

The study was designed as a randomized, double-blinded placebo-controlled trial. Hospital inpatients meeting the inclusion criteria were randomized into one of two arms and received either IVIG (Sandoglobulin; provided by Sandoz Pharmaceutical Corp.) or an albumin placebo product. Randomization numbers were provided at the onset of the study. Patients received either IVIG at a dose of 400 mg/kg per day for 3 days or a similar volume of placebo.

Data Collection

History, physical examination, and diagnosis were extracted from the patients' medical records. Daily temperatures were recorded.

Laboratory Tests. (i) Complete blood counts including platelet count and mean platelet volume were obtained daily. Leukocyte differentials were obtained at entry into the study and every 3 days thereafter. (ii) Bacterial cultures of blood, sputum, urine, and cerebrospinal fluid were obtained as necessary.

Specialized Studies. Specialized laboratory tests were performed on each patient at the time of inclusion into the study and approximately 1 week later.

Platelet-Associated IgG (PAIgG)

An EIA method developed by one of the investigators (ERB) was used as previously described (15).

In brief, Triton X-100 extracts of washed platelets were incubated with staphylococcal protein A-coated nylon beads. The beads were washed and incubated with alkaline phosphatase-conjugated $F(ab)_2$ fragments of anti-human IgG. A color reaction was developed with *p*-nitrophenyl phosphate. The reference range for 100 normal subjects was 0 to 2.0 ng/10^6 platelets. Patients with ITP had levels between 4.0 and 12 ng/10^6 platelets.

Monocyte Phagocytosis

Peripheral blood mononuclear cells were isolated from heparinized blood by Ficoll–Hypaque density separation. The cells were plated onto sterile culture dishes and incubated for 1 hr. Nonadherent cells were washed away and the remaining cells were coincubated with fresh autologous platelets that were previously tagged with fluorescein isothiocyanate. Wright-stained preparations of the adherent cells confirmed a purity of greater than 90% monocytes. After a 1-hr incubation at 37°C the dish was washed free of platelets and the adherent monocytes scraped off with a rubber policeman. A suspension of the cells was examined with a fluorescent microscope and the percentage of monocytes containing internalized platelets was calculated.

Lymphocyte Subsets

Helper and suppressor lymphocytes were quantitated using monoclonal antibody tags (CYTOSTAT T_4-RD1/T_8-FITC, Coulter Immunology, Hialeah, FL) and analyzed on a Coulter EPICS C analyzer.

Circulating Immune Complex (CIC) Levels

CICs were assayed in serum using a commercial micro-ELISA procedure (Diamedix, Miami, FL) that measures C1q binding. Normal values were <20 μg Eq/ml.

RESULTS

Thirty-eight patients met all the criteria for the study and were entered. Seven patients (three from the placebo group and 4 from the IVIG group) died of cardiogenic shock within the first 4 days of observation, and two patients (one from each group) withdrew from the study for personal reasons and could not be evaluated. Twenty-nine patients completed the full 9 days of follow-up and form the basis of the evaluation. In 25 of these patients microorganisms were cultured from blood (21), bile (1), and sputum (3). In the remaining four patients, sepsis was diagnosed on the combined basis of clinical presentation, fever, leukocytosis, and left-shifted leukocyte differential.

Table III. Characteristics of Study Patients at Entry

Characteristic	IVGG	Placebo	*P* -value
Age	61.5	59.8	0.83
WBC/mm^3	12.7	11.9	0.80
Temperature (°C)	37.7	37.8	0.82
Platelets/mm^3	43.2	51.4	0.28
Prothrombin time (sec)	13.5	16.1	0.20
Fibrinogen (mg/dl)	477.0	497.3	0.84
PAIgG (ng/10^6 plts)	2.7	3.3	0.59
CIC (μg/ml)	8.8	8.9	0.98
Monocyte phagocytosis (%)	9.5	17.2	0.34
T_4/T_8	1.8	3.1	0.12

The prestudy characteristics of the patients are listed in Table III. There were no statistical differences between groups.

Fifteen patients (52%) had elevated levels of PAIgG, but only 7 (24%) had increased levels of circulating immune complexes. Five patients (17%) had increased levels of monocyte phagocytosis. The mean platelet volume (MPV) was increased in 12 patients (41%). An abnormally low T_4/T_8 ratio was detected in only two patients (7%).

The response rate, defined as achieving a rise in platelet count of at least 35,000/mm^3 by day 9 of the study, was 77% in the IVIG group, versus 56% in the placebo group. Figure 1 presents the mean rise over baseline of the platelet count for each of the days of the study in the two groups. The mean counts over the course of the study were 77 ± 21K for the IVIG group and 63 ± 16K for the placebo group ($P = 0.032$ by Student's *t* test and $P = 0.033$ by Wilcoxon signed-rank test). The mean percentage rise in platelet count over the course of the study was 178 ± 51 for the IVIG group vs 125 ± 31 for the placebo group ($P = 0.02$) (Table IV). From day 4 onward, the mean percentage rise in platelets was considerably greater in the IVIG group (263 ± 117) than the placebo group (178 ± 61; $P = 0.008$). The mean platelet count was also greater in the IVIG group (125, 255 ± 42, 859) than in the placebo group (98,668 ± 28,482) on every day beginning with day 5 ($P = 0.001$).

The response rate was most pronounced in the IVIG group, whose initial platelet counts were less

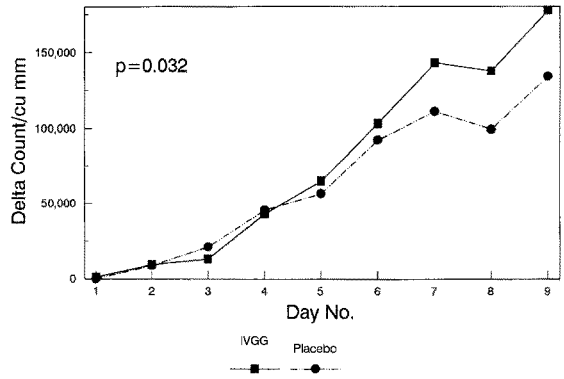

Fig. 1. Mean rise over baseline of the platelet count for the entire study period. The mean rises were 77 ± 21K for the IVIG group and 63 ± 16K for the placebo group ($P = 0.032$ by Student's t test and $P = 0.033$ by Wilcoxon signed-rank test). The difference is more apparent from day 4 onward, with the mean percentage rise in platelets being considerably greater in the IVIG group (263 ± 117) than the placebo group (178 ± 61; $P = 0.008$). The mean platelet count was also greater in the IVIG group (125,255 ± 42,859) than the placebo group (98,668 ± 23,482) on every day beginning with day 5 ($P = 0.001$).

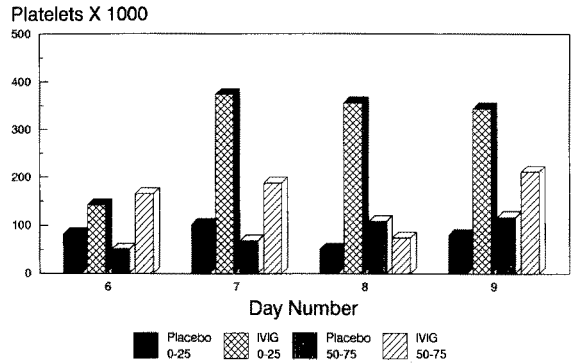

Fig. 2. Mean rises of platelet counts on days 6–9 of the placebo and intravenous immune globulin (IVIG) patients stratified as to initial platelet counts, i.e., 0–25,000 or 50,000–75,000/mm^3. P value for the 0–25,000 group is 0.01, while that of the 50,000–75,000 group is 0.07 (N.S.). The 25,000–50,000 group (data not shown) was also not significantly different.

than 25,000 or between 50,000 and 75,000/mm^3 (Fig. 2). Over the 9-day period, the mean platelet rise in the low-platelet IVIG group was 303 ± 85K, vs a rise of 72 ± 32K in the placebo group ($P = 0.01$). Those with counts between 25,000 and 50,000 responded no better than controls.

Patients were more likely to respond to IVIG if they had increased levels of CIC (75% response to IVIG, versus 33% for placebo) and increased monocyte phagocytosis (100% response to IVIG versus, 0% for placebo). The finding of an elevated level of PAIgG predicted a response to IVIG. Seventy-one percent (5/7) of patients with an elevated PAIgG responded to the drug, versus 44% (4/9) of PAIgG-positive patients in the placebo group. Patients whose PAIgG level fell during the study were two times more likely to respond to IVIG than placebo. Continued positivity of blood cultures had no influence at all on the response of the platelet counts to IVIG. Due to the small numbers of patients in each group, statistical significance could not be ascertained.

Five patients had clinically significant bleeding defined as gross hematuria, gastrointestinal bleeding, or hemorrhage from surgical wounds, during the study period, and all were severely thrombocytopenic. Four of the bleeding patients (80%) were in the placebo group. Prothrombin times were 15 sec or less (normal range, 10–13 sec) in these patients. In contrast, prothrombin times of greater than 18 sec were observed in nonbleeding patients.

Table IV. Mean Platelet Rises over Baseline over Course of Study

	i.v. gamma-globulin		Placebo	
Day	Delta/mm^3	% rise	Delta/mm^3	% rise
1	1,058	2.4	0	0
2	9,475	21.9	8,969	17.4
3	13,078	30.3	21,238	41.4
4	43,142	99.9	45,733	89.0
5	64,870	150.2	56,506	130.8
6	103,055	238.6	92,173	179.3
7	143,078	331.2	111,069	216.1
8	137,711	318.8	99,273	193.1
9	177,560	411.0	134,319	261.3
Mean ± SE	77,003 ± 21,905	178 ± 51	63,253 ± 16,045*	125 ± 31**

*$P = 0.032$.
**$P = 0.017$.

DISCUSSION

Thrombocytopenia is a major complication of severe systemic infection. While the lowered platelet counts most commonly seen usually do not cause clinical bleeding, they may preclude the ability safely to employ invasive diagnostic modalities and/or surgery.

The rationale for using IVIG in septic thrombocytopenia derives from this condition's similarity to ITP (16). If splenic phagocytosis of antibody-coated platelets is a primary mechanism causing the thrombocytopenia, then reticuloendothelial blockade with IVIG should be effective. The present study shows that some 40% have an elevated MPV; all had elevated levels of PAIgG, as did another 10% of patients with normal MPV values. This suggests that about one-half of patients had normal thrombopoiesis.

Our assay for PAIgG as well as others reported to measure total platelet IgG is not specific for antiplatelet antibodies. It appears to be a measure of platelet alpha granule IgG, which is increased in disorders of platelet destruction accompanied by active marrow megakaryocytopoiesis (17).

We chose a platelet count of 75,000/mm^3 or less as the criterion for entry into the study, since that value is accepted as the minimum level at which general surgical procedures may be performed safely. In a pilot study performed prior to the current protocol we found that the majority of patients presenting with the thrombocytopenia of sepsis had platelet counts ranging between 35,000 and 45,000/mm^3. Therefore, for the present study we arbitrarily chose 35,000 as the minimum criterion for response since that degree of platelet rise would bring most patients to a platelet count that would allow surgery without hemostatic compromise.

The data show that treatment with IVIG causes an increase in platelet count which is statistically greater than placebo over a 9-day follow-up period, the differences beginning at day 4 and becoming most pronounced at day 6. Since the course of therapy administered lasted 3 days, one would expect such an outcome if the drug is responsible. In terms of clinical benefit, treatment with IVIG appears to raise the platelet count to a level considered safe for surgery (i.e., >100,000/mm^3) a full day sooner than no treatment. The effect is most pronounced in the most thrombocytopenic patients with platelet counts below 25,000/mm^3.

The mechanism by which IVIG achieved its effect is unclear. The finding that PAIgG elevations were associated with a greater response rate in the drug-treated group gives some credence to RE system blockade as a mechanism for the drug's effect.

Recent studies have demonstrated that IVIG preparations have antiidiotypic antibodies directed against idiotypes on the GPIIb/IIIa autoantibodies of ITP patients (14). Thus, if autoantibodies are involved in the pathogenesis of septic thrombocytopenia, this property of IVIG may be operative. Some patients' platelet counts improved with IVIG despite continued ongoing sepsis (data not shown). This suggests a specific platelet promoting activity of the IVIG in the absence of generalized clinical improvement.

In summary, IVIG has been showed to cause a more rapid rise than placebo in the platelet count of patients with septic thrombocytopenia unrelated to DIC. The mechanism of the effect remains unknown but may involve RE receptor blockade, antiidiotypic antibody activity, or a generally beneficial effect of IVIG on sepsis.

Recent experimental data using newborn (18) and suckling (19) rat models of Group B streptococcal and *H. influenzae* (20) disease indicate that high-dose (2 g/kg) IVIG given concomitantly with antibiotics may impair bacterial clearance and worsen outcome. IVIG at a dose of 400 mg/kg enhanced survival in these models. The issue remains unresolved, but we saw no adverse effects of IVIG in our study.

Recent studies have shown that in ITP, a 2-day course of IVIG at a dose of 1 g/kg is as effective as the older standard dose of 400 mg/kg for 5 days (21, 22). To that end, we recommend this dose for septic thrombocytopenia and suspect that this dose may be more efficacious.

Since the natural course of septic thrombocytopenia is for eventual spontaneous improvement, we believe that random use of IVIG in the patient with low-grade thrombocytopenia and sepsis is unnecessary. IVIG would be efficacious in septic thrombocytopenia primarily in the absence of DIC, hypersplenism, and bone marrow failure. Because of the considerable expense of IVIG, we would limit our recommendations to two clinical conditions. In patients whose platelet count has dropped below 35,000 use of IVIG seems prudent inasmuch as it may stave off further decrements to levels associated with spontaneous hemorrhage. Further-

more, in patients whose sepsis is secondary to clinical entities known to require invasive intervention, such as patients with severe burns or intraabdominal infection, the more rapid normalization of the platelet count may allow such procedures to proceed more safely.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Sandoz Research Institute. The extraordinary help of the BMHC hospital pharmacy staff is gratefully acknowledged.

REFERENCES

1. Kreger BE, Craven DE, McCabe WR: Gram negative bacteremia IV. Reevaluation of clinical features and treatment of 612 patients. Am J Med 68:344–355, 1980
2. Riedler GF, Straub PW, Frick PG: Thrombocytopenia in septicemia. A clinical study for the evaluation of its incidence and diagnostic value. Helv Med Acta 36:23–38, 1971
3. Oppenheimer L, Hryniuk WM, Bishop AJ: Thrombocytopenia in severe bacterail infections. J Surg Res 20:211–214, 1976
4. Neame PB, Kelton JG, Walker IR, Stewart IO, Nossel HL, Hirsh J: Thrombocytopenia in septicemia: The role of disseminated intravascular coagulation. Blood 56:88–92, 1964
5. Hawiger J, Steckley S, Hammond D, *et al.*: Staphlococcal induced human platelet injury mediated by protein A and immunoglobulin G Fc fragment receptor. J Clin Invest 64:931–937, 1979
6. Ausprunk DH, Das J: Endotoxin induced changes in human platelet membranes: Morpphologic evidence. Blood 51:487–495, 1978
7. Kelton JG, Neame PB, Gauldie J, Hirsh J: Elevated platelet associated IgG in the thrombocytopenia of septicemia. N Engl J Med 300:760–764, 1979
8. Tate DY, Carlton GT, Johnson D, Sorenson RL, *et al.*: Immune thrombocytopenia in severe neonatal infections. J Pediat 98:449–453, 1981
9. Fehr J, Hoffman V, Kappeler U: Transient reversal of thrombocytopenia in idiopathic thrombocytopenic purpura by high dose intravenous gamma globulin. N Engl J Med 306:1254–1258, 1982
10. Morfini M, Vannucchi AM, Grossi A, *et al.*: Direct evidence that high dose intravenous gammaglobulin blocks splenic and hepatic sequestration of ^{51}Cr-labeled platelets in ITP. Thromb Haemostas 54:554, 1985
11. Dammacco F, Iodice G, Campobasso N: Treatment of adult patients with idiopathic thrombocytopenic purpura with intravenous immunoglobulin: Effects on circulating T cell subsets and PWM-induced antibody synthesis in vitro. Br J Haematol 62:125–135, 1986
12. Wangel AG, Lorenzetti M, Pettersson T: Polyclonal B-cell activation after intravenous immunoglobulin therapy in idiopathic thrombocytopenic purpura—an anti-idiotype response? Eur J Hematol 39:209–213, 1986
13. Sultan Y, Katzatchkine MD, Maisonneuve P, Nydegger UE: Anti-idiotypic suppression of autoantibodies to Factor VIII (antihaemophilic factor) by high dose intravenous gamma globulin. Lancet 2:765–768, 1984
14. Berchtold P, Dale GL, Tani P, McMillan R: Inhibition of autoantibody binding to platelet glycoprotein IIB/IIIa by anti-idiotypic antibodies in intravenous gamma globulin. Blood 74:2414–2417, 1989
15. Ellaurie M, Burns ER, Bernstein LJ, Shah K, Rubinstein A: Thrombocytopenia and human immunodeficiency virus in children. Pediatrics 82:905–908, 1988
16. Wilson JJ, Neame PB, Kelton JG: Infection induced thrombocytopenia. Semin Thromb Hemostas 8:217–233, 1982
17. George JN: Platelet immunoglobulin G: Its significance for the evaluation of thrombocytopenia and for understanding the origin of alpha granule proteins. Blood 76:859–870, 1990
18. Kim KS: High dose intravenous immune globulin impairs antibacterial activity of antibiotics. J Allerg Clin Immunol 84:579–588, 1989
19. Weissman LE, Lorenzetti M: High intravenous doses of human immune globulin suppress neonatal group B streptococcal immunity in rats. J Pediat 115:445–450, 1989
20. Schreiber J, Basker C, Priehs C, Sieber G: Deleterious effect of immune globulin in the treatment of H. influenzae B infection in infant rats. Pediat Res 21:334a, 1987
21. Baumann MA, Menitove JE, Aster RH, Anderson T: Urgent treatment of idiopathic thrombocytopenic purpura with single-dose gamma globulin infusion followed by platelet transfusion. Ann Intern Med 104:808, 1986
22. Berchtold P, McMillan R: Therapy of chronic idiopathic thrombocytopenia in adults. Blood 74:2309–2317, 1989