Ann Hematol (1991) 62: 232–234

Annals of
Hematology

Case report

Post-transfusion purpura as the main manifestation of a trilineal transfusion reaction, responsive to steroids: flow-cytometric investigation of granulocyte and platelet antibodies

N. Puig[1], M. J. Sayas[1], J. A. Montoro[1], J. V. Villalba[1], and A. Pla[2]

[1] Center of Transfusion of Comunidad Valenciana, Valencia, and [2] Department of Hematology, Dr. Peset's Hospital, Valencia, Spain

Received December 7, 1990/Accepted March 1, 1991

Summary. We report a typical case of post-transfusion purpura (PTP) due to anti-Pl^{A1} in a 65-year-old woman. Serological studies were carried out using flow cytometry (FCM). The patient also developed red cell alloantibodies that produced a delayed hemolytic transfusion reaction (DHTR) and broad HLA antibodies. Treatment with high-dose intravenous IgG (HDIgG; a first-generation preparation) was ineffective, but a course of steroids resulted in a rapid increase in the the platelet count.

Key words: Post-transfusion purpura – Delayed hemolytic transfusion reaction

Introduction

Post-transfusion purpura (PTP) is a rare form of immune thrombocytopenia characterized by sudden, profound, and self-limited thrombocytopenia occurring 5–10 days after transfusion of products containing platelet material (for reviews see [6, 10, 11]). Generally, the patient has a previous history of sensitization by pregnancy and/or transfusion. Most cases reported involve women, and platelet-specific alloantibodies can be identified in the serum of practically all of them. The mechanism by which autologous platelets are destroyed remains unclear. At least five platelet-specific antigen systems have been described (HPA-1, HPA-2, HPA-3, HPA-4, HPA-5) [20], and any of them may potentially induce an alloimmunization. However, the majority of PTP cases are due to anti-HPA-1a (anti-Pl^{A1}) [10–12]. Many patients with PTP also develop multispecific HLA antibodies [10, 11]. In contrast, in only a few cases is a delayed hemolytic transfusion reaction (DHTR) presented in association with the PTP [3, 14]. We describe here the successful use of flow cytometry (FCM) for the simultaneous detection of platelet and leukocyte antibodies in a case of PTP caused by anti-Pl^{A1}. The particular clinical features of our patient were the association of PTP with a DHTR as well as an excellent response to low-dose steroid therapy and possible, but not proven, failure of high-dose IgG (HDIgG), the treatment that has recently been established as the therapy of choice for this syndrome [12].

Offprint requests to: N. Puig, Laboratorio de Inmunología Leucoplaquetar, Centro de Transfusión de la Comunidad Valenciana, Avenida del Cid, 65 E-46014 Valencia, Spain

Case report

A 65-year-old woman was admitted to the hospital because of upper gastrointestinal bleeding. Endoscopy showed multiple gastroduodenal erosions secondary to ingestion of nonsteroidal anti-inflammatory drugs. The blood count was Hb 8 g/dl, WBC 7×10^9/l, and the platelet count was 202×10^9/l. On admission, the patient received two units of packed red cells without showing any apparent reaction. Twenty years earlier she had been transfused because of another attack of gastric bleeding. She had had three healthy children and one abortion. A further transfusion was prescribed 8 days later but was interrupted when chills and shivers were observed. Twenty hours after the second transfusion (9 days after the first), she developed widespread petechiae and ecchymoses along with genitourinary and nasal bleeding. Her platelet count was 1.5×10^9/l, WBC 15×10^9/l, and Hb 8.5 g/dl. The clotting times were normal. The direct red cell test was positive. Anti-Jk^a reactivity was identified, both in the patient's serum and in the eluate. Bilirubin was 0.5 mg/dl, LDH 600 U/l, and haptoglobin 45 mg/dl. The bone marrow was not examined. A diagnosis of PTP associated with DHTR was made, and treatment with intravenous methylprednisolone (0.5 mg/kg/day) and HDIgG (Gamma-Venin 400 mg/kg/day for 5 days) was set up. The bleeding stopped and the platelet count steadily returned to normal values 10 days later. The steroid therapy was kept up for another 5 days and was then discontinued. A rapid drop in the platelet count to 15×10^9/l was observed and the methylprednisolone was reinstated, leading to a second complete response within a few days (Fig. 1). Methylprednisolone was administered for 1 month and gradually reduced without a new relapse of thrombocytopenia occurring. The patient needed no further transfusions.

The main serological findings are summarized in Table 1. Platelet and granulocyte studies were done with the immunofluorescence technique [19] and flow-cytometric analysis (FACScan system) [9, 18]. Chloroquine-treated platelets were used to get rid of the HLA reactivity while maintaining the platelet-specific reactivity. The direct platelet testing remained positive (polyspecific and anti-IgG

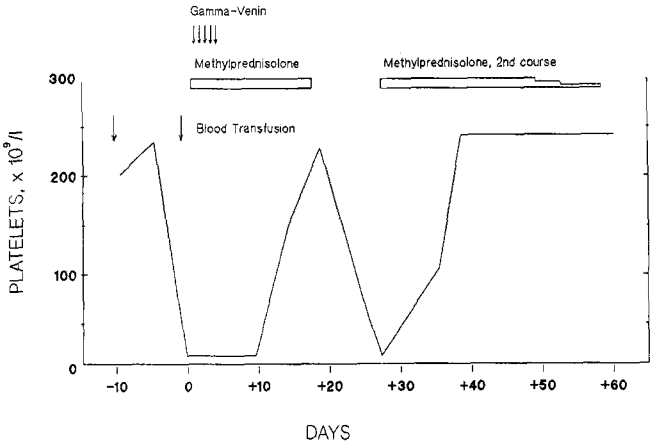

Fig. 1. Clinical course of patient in relation to treatment

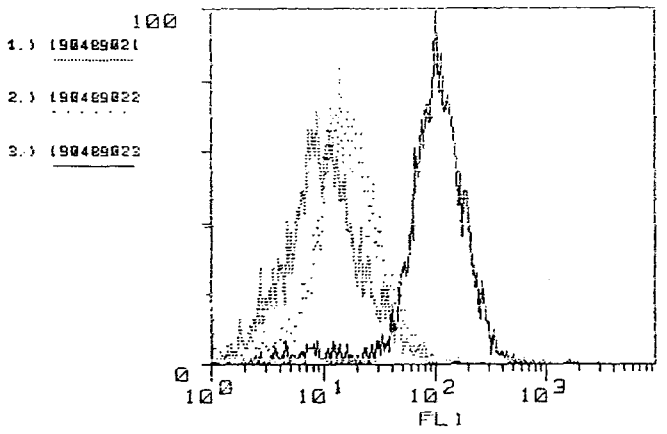

Fig. 2. Comparative flow-cytometric histograms showing the binding of the negative control sera and the patient's serum (*3*) to Pl^{A1}-positive chloroquine-treated platelets. AB serum (*1*) and multispecific anti-HLA serum (*2*) without platelet-specific antibodies were employed as negative controls

reagent) for more than 60 days. It was not possible to obtain an appropiate eluate from the patient's platelets. Strong, broad HLA antibodies were detected using both the lymphocytotoxicity technique [16] and FCM. No granulocyte-, monocyte-, and/or lymphocyte-specific antibodies were observed after exhaustive platelet absorption. The patient's serum reacted strongly with chloroquine-treated platelets with both polyspecific antiglobulin and anti-IgG. Figure 2 shows the reactivity of the patient's serum against Pl^{A1}-positive platelets. The anti-Pl^{A1} specificity was subsequently confirmed by Dr. Muñiz, using the MAIPA method [8], at the Institute for Clinical Immunology and Transfusion Medicine, Giessen, FRG. The patient was typed as Pl^{A1} negative, as were two (the first and the second) of her three children. The third child was typed as PL^{A1} positive. The patient's HLA-DR antigens were DRw6, DR4, Drw52, and DRw53. The typing of the patient's red cells was: O, DCe/dce, MNSs, Jk(a−b+), Fy(a+b+), kk, Le(a−b+), Pl−.

Discussion

We have described a typical case of PTP in a Pl^{A1}-negative woman who developed multiple blood cell antibodies after a blood transfusion. It is noteworthy that the direct platelet testing remained positive for more than 2 months in spite of a recovery from thrombocytopenia. Unfortunately, an eluate from the patient's platelets to clarify the specificity of the autoantibodies was not obtained. Three mechanisms have been proposed to explain the destruction of autologous platelets in this syndrome, but none are completely satisfactory: simultaneous development of cross-reactive autoantibodies [15], immune complex formation with innocent-bystander destruction of platelets, and absorption onto autologous platelets of transfused soluble antigens [7]. All of them could produce a positive direct antiglobulin test. In a recent report, Dieleman et al. [5] describe their observation and subsequent demonstration, using Western blotting, that Pl^{A1}-negative platelets may acquire Pl^{A1} antigen after incubation with plasma from Pl^{A1}-positive donors. This phenomenon supports the hypothesis that passive acquisition of specific platelet antigens is a potential pathogenic mechanism for inducing PTP. A unique case of atypical PTP due to passive transfer of anti-Pl^{A1} antibody by blood transfusion has also been reported [1].

Our patient had multispecific HLA antibodies. These antibodies are detected in many cases of PTP [10, 11], suggesting that there is a hyperimmune response in such patients. Nevertheless, unlike one case recently reported [3], no granulocyte-specific antibodies were observed after exhaustive platelet absorption of HLA antibodies by platelets. In contrast, the patient presented with a moderate DHTR due to anti-Jka. The development of red cell antibodies in association with PTP has been described in very few patients, and only two of them developed a clinical DHTR [3, 14].

The case reported here was DRw52 and DRw6. As is well known, there is a strong association between these two antigens and alloimmunization against certain platelet antigens [13, 4].

The treatment for PTP has recently been revised by Mueller-Eckhardt and Kiefel [12], who conducted a multicentric study of HDIgG for PTP and revised the cases reported in the literature. A total of 17 PTP patients treated with HDIgG were evaluated. There were 16 responses and only one failure. Five of the 16 responders relapsed,

Table 1. Results of the direct and indirect immunofluorescence tests (DIT, IIT) and the lymphocytotoxicity tests

	DIT	IIT	IIT following platelet absorption	IIT following chloroquine treatment
Platelets	+	+	−	+
Granulocytes	−	+	−	NT
Monocytes	−	+	−	NT
Lymphocytes	−	+	−	NT

Patient's HLA antibodies: + multispecific (PRA 100%)
Patient's HLA typing: A2,−; B12, B49; DR4, DRw6; DQw1, DQw3; DRw52, DRw53

NT, Not tested

but they attained complete remission after a second course of HDIgG. The authors conclude that in spite of the lack of a controlled prospective study, which most likely will never be possible, HDIgG is the best treatment for PTP. Other treatments such as plasma or blood exchange, procedures more cumbersome and associated with adverse reactions, have been effective in many patients, although failures and relapses have also been observed [10, 11]. The therapeutic benefit of the steroids in this syndrome is not well established. Almost all patients have received prednisone but its effectiveness has not been adequately evaluated. There are only two cases in the literature that show a clear steroid response [14, 21]. Both these patients received intravenous methylprednisolone at a dose of 2 mg/kg/day, in one of them an early relapse occurred after tapering off prednisone, followed by a second, complete response upon reinstatement [21].

The final effectiveness of the HDIgG treatment in our patient cannot be adequately evaluated for two reasons. First, when the relapse occurred a second course of HDIgG was not attempted, and it is possible that she would have attained a second remission after another course, as has been described for some patients in the study by Mueller-Eckhardt and Kiefel [12]. Second, the immunoglobulin employed (Gamma-Venin), is a first-generation, Fc-depleted preparation and it has been proven [2, 17] that these preparations are less effective, supporting the importance of intact Fc for efficacy. Practically all patients in the Mueller-Eckhardt/Kiefel study received IgG from 3rd and/or 2nd generations. In contrast, our patient's respone to steroid therapy is clearly demonstrated by the typical response-relapse-response pattern (Fig. 1).

We believe that it may be advisable to start a course of steroids simultaneously with HDIgG (3rd and/or 2nd generations) for the treatment of PTP. A sinergistic effect of low-to-moderate doses of prednisone could be useful and harmless and could protect the patient from a rare but not impossible failure with HDIgG.

Acknowledgements. We thank Mr. R. Rodriguez, Ms. M. Gómez, Mr. A. Llop, and Ms. M. Gimeno for their careful technical assistance in carrying out this study and Dr. A. Ribera and Dr. E. Muñiz for their valuable help in confirming the serological specificity.

References

1. Ballem PJ, Buskard NA, Decary F, Doubroff P (1987) Post-transfusion purpura secondary to passive transfer of anti-PLA^1 by blood transfusion. Br J Haematol 66: 113–114
2. Burdach EG, Evers KG, Geursen RG (1986) Treatment of acute idiopathic thrombocytopenic purpura of childhood with intravenous immunoglobulin G: comparative efficacy of 7S and 5S preprations. J Pediatr 109: 770–775
3. Chapman JF, Murphy MF, Berney SI, Ord J, Metcalfe P, Amess JAL, Waters AH (1987) Post-transfusion purpura associated with anti-Bak^a and anti-PLA^2 platelet antibodies and delayed haemolytic transfusion reaction. Vox Sang 52: 313–317
4. de Waal LP, van Dalen CM, Engelfriet CP, von dem Boene AE (1986) Alloimmunization against the platelet-specific Zw^a antigen, resulting in neonatal alloimmune thrombocytopenia or post-transfusion purpura, is associated with the supertypic DRw52 antigen including DR3 and DRw6. Hum Immun 17: 45–53
5. Dieleman LA, Brand A, Claas FHJ, van De Keur C, Wituliet M, Giphart MJ (1989) Acquired Zw^a antigen on Zw^a-negative platelets demonstrated by Western blotting. Br J Haematol 72: 539–542
6. Kalish RI, Jacobs B (1987) Post-transfusion purpura: initiation by leukocyte-poor red cells in a polytransfused woman. Vox Sang 53: 169–172
7. Kickler TS, Ness PM, Herman JH, William RB (1986) Studies on the pathophysiology of post-transfusion purpura. Blood 68: 347–350
8. Kiefel V, Santoso S, Weisheit M, Mueller-Eckhardt C (1987) Monoclonal antibody-specific immobilization of platelet antigens (MAIPA): a new tool for the identification of platelet-reactive antibodies. Blood 70: 1722–1726
9. Marti GE, Magruder L, Schuette WE, Gralnick HR (1988) Flow-cytometric analysis of platelet surface antigens. Cytometry 9: 448–455
10. McCarthy LJ, Menitove JE (1985) Immunologic aspects of platelet transfusion. American Association of Blood Banks. Arlington, VA
11. Mueller-Eckhardt C (1986) Post-transfusion purpura. Br J Haematol 64: 419–424
12. Mueller-Eckhardt C, Kiefel V (1988) High-dose IgG for post-transfusion purpura revisited. Blut 57: 163–167
13. Mueller-Eckhardt C, Kiefel V, Kroll H, Mueller-Eckhardt G (1989) HLA-DRw6, a new immune response marker for immunization against platelet alloantigen Br. Vox Sang 57: 90–91
14. Slichter SJ (1982) Post-transfusion purpura: response to steroids and association with red blood cell and lymphocytotoxic antibodies. Br J Haematol 50: 599–605
15. Stricker RB, Lewis BH, Corash L, Shuman RB (1987) Post-transfusion purpura associated with an autoantibody directed against a previously undefined platelet antigen. Blood 69: 1458–1463
16. Terasaki PI, McClelland J (1964) Microdroplet assay of human serum cytotoxins. Nature 204: 998–1000
17. Tovo PA, Miniero R, Fiandino G, Saracco P, Messina M (1984) Fc-depleted vs intact intravenous immunoglobulin in chronic ITP. J Pediatr 984: 676–677
18. Veys PA, Gutteridge CN, Macey M, Ord J, Newland AC (1989) Detection of granulocyte antibodies using flow-cytometric analysis of leucocyte immunofluorescence. Vox Sang 56: 42–47
19. von dem Borne AEGK, Verneugt FWA, Oosterhof F, von Piesz E, Brutel de la Rievière A, Engelfriet CP (1978) A simple immunofluorescence test for detection of platelet antibodies. Br J Haematol 39: 195–207
20. von dem Borne AEGK, Décary F (1990) ICSH/ISBT Working Party on Platelet Serology. Nomenclature of Platelet-Specific Antigens. Vox Sang 58: 176
21. Weisberg LJ (1984) Prednisone therapy of post-transfusion purpura. Ann Intern Med 10: 76–77