

Immediate hypersensitivity to platelet concentrate: allergic or not?

The frequency of hypersensitivity reactions following transfusion of labile blood products has been estimated as 5.4 cases per 10 000 units delivered in France and apheresis platelet concentrates (APC) account for 61% of the reactions (France annual haemovigilance report 2009, ANSM 2012). The mechanisms (allergic or non-allergic) as well as the specific agent involved remain unknown. We report two patients where allergic hypersensitivity to platelet concentrate (PC) was clearly demonstrated using specific mediator (histamine and tryptase) measurements and flow cytometry basophil activation tests. Plasma histamine concentrations were measured by a radioimmunoassay (Beckman Coulter, Immunotech, Marseille, France) and total mast cell tryptase by an automated fluoroimmunoassay (ThermoFisher scientific, Phadia SAS, Uppsala, Sweden). The basophil activation test was performed by challenging the patient's whole blood with the suspected agent and measuring marker CD63, a specific marker of basophil activation (Flow cytometry test, Flow2 Cast, Bülmann).

A male patient, 72 years old, experienced haemorrhage after femoral stent. He was transfused three red blood cell concentrates, one platelet concentrate mix (PCM), three methylene blue-inactivated fresh frozen plasmas within 4 h, and one APC 1 h later. Fifteen minutes after the beginning of the APC transfusion, he presented hypotension (systolic blood pressure < 50 mmHg), peripheral oxygen desaturation (72%), bronchoconstriction and urticaria. He was treated with adrenaline 0.025 mg intravenously, followed by a continuous infusion at a $0.04 \mu\text{g kg}^{-1} \text{ min}^{-1}$ rate. He recovered. Plasma histamine concentration was increased (66 nmol L^{-1} , 55 min after the reaction; normal values < 6 nmol L^{-1}), as well as was tryptase ($18 \mu\text{g L}^{-1}$, basal value 2 days later: $6.4 \mu\text{g L}^{-1}$; normal values < $12.5 \mu\text{g L}^{-1}$). The left-over APC and the APC supernatant obtained after centrifugation within 4 h of reaction at $1000 \times g$, and 15°C , were kept frozen at -20°C . A basophil activation test was performed five weeks after the reaction, with the APC and the APC supernatant, at four different concentrations, and showed positivity for both at dilutions 1:4, 1:20 and 1:100 (CD63 expression: 36%, 29% and 10%, respectively; normal values < 5%) (data not shown).

A female patient, 84 years old, presented with severe haemorrhage after cervical arthrodesis. She received six red

blood cell concentrates, six methylene blue-inactivated fresh frozen plasmas, followed by one PCM 80 min later. Twenty-five minutes after the beginning of the PCM transfusion, she had hypotension (systolic blood pressure: 40 mmHg), peripheral oxygen desaturation (92%), and erythema. She was treated with ephedrine 24 mg, adrenaline 0.7 mg intravenously, followed by a continuous infusion (1 mg h^{-1}). She recovered. Plasma histamine concentration was increased (537 nmol L^{-1} , 15 min after the reaction), as well as tryptase ($69 \mu\text{g L}^{-1}$ at 90 min, basal value nine days later: $16 \mu\text{g L}^{-1}$). Samples of the PCM, PCM supernatant and fresh frozen plasma were kept frozen as previously described. The basophil activation test, performed two months later showed positive for the four dilutions of the PCM and PCM supernatant tested (peak: 33% and 43%, respectively). The test was negative with the fresh frozen plasma (Fig. 1a), and with two methylene blue solutions (data not shown). A control subject tested in the same conditions appeared negative for the same PCM (Fig. 1b).

For the two cases, the cultures of the labile blood products were sterile, no antibodies against red blood cells or against the HLA system were detected, and there was no IgA deficiency. Latex allergy was ruled out by the absence of latex-specific immunoglobulin E.

Hypersensitivity reactions following transfusion are frequent, but their mechanisms are rarely investigated. Recently, a diagnostic procedure for elucidating the mechanisms of reactions to methylene blue-inactivated fresh frozen plasma has been proposed by the allergy committee of the French medicines and healthcare products regulatory agency (AFSSAPS/ANSM) (Mertes *et al.* 2011) and proved useful (Mertes *et al.* 2012). For the two cases reported here, the clinical signs favoured an immediate hypersensitivity mechanism, and the increased concentrations of histamine and especially tryptase (Fisher and Baldo 1998) confirmed that mast cell had been triggered, proving an allergic mechanism. The responsibility of the transfused PC was demonstrated by the basophil activation test. Even if the molecular antigen had not been identified, it was present in the platelet supernatant, but was not *per se* an offending agent, as shown by the negativity of the challenge with a control subject's blood. PC supernatants rarely elicit *in vitro* basophil degranulation in control subjects (Matsuyama *et al.* 2009), but testing of reactive patients had not been described yet. The possible causes of reaction include recipient-related (Savage *et al.* 2011), donor-related (Abe *et al.* 2011; Goldman and O'Brien 2011) and blood product-related mechanisms (Savage *et al.* 2012), as discussed elsewhere (Mertes *et al.* 2011). For these two patients, the evidence of the responsibility of the PC supernatant provided a rationale to prescribe washed PC as an alternative.

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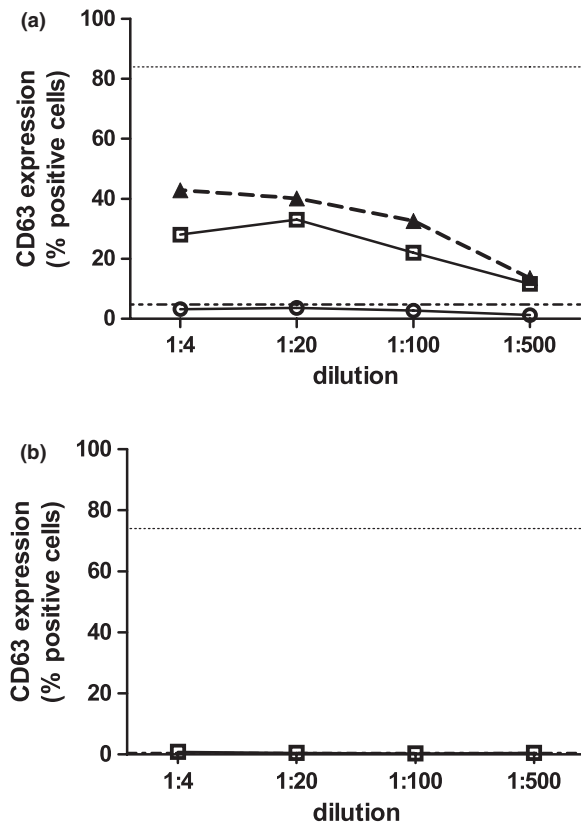


Fig. 1. Basophil activation tests. (a) Membrane expression of degranulation marker CD63 for case 2 as detected by flow cytometry: the patient's blood was incubated at 37 °C during 30 min with serial dilutions of the PC (□), PC supernatant (△) or methylene blue-inactivated fresh frozen plasma (○), administered before the reaction. A negative control (patient's blood + buffer) (.....) and two positive controls [patient's blood + anti-immunoglobulin E receptor antibody (.....) or fMLP (not shown)] were included. (b) Basophil activation test for a control subject, using the same platelet concentrate as in (a).

These results suggest that patients with hypersensitivity reactions following platelet transfusion can benefit from allergy mediator measurements during the reaction and basophil activation test

thereafter, as this diagnostic procedure allows personalized advices for subsequent transfusions. These investigations offer a new perspective for the understanding of transfusion-related hypersensitivity reactions and for the safety of patients.

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CONFLICT OF INTEREST

The authors have no competing interests.

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