To the editor:

Tissue factor expression on platelets is a *dynamic* event

The so-called controversy about the presence of tissue factor (TF) in platelets is a reality that our research group has been dealing with since 2003, when we published our first data on this topic.

We have read with interest the letter by Bouchard et al,[1] and we agree with the authors that discrepancies in published data on this topic may be due to differences in methods, reagents, and experimental conditions used by different investigators. We believe that the opportunity to use reagents and methods already validated in other laboratories might help in reproducing data and solving such important issues as the present one.

Results reported by Bouchard et al indicate that human platelets stimulated with protease-activated receptor (PAR)–1 and PAR-4 agonist peptides do not express TF at 2 hours.[1] This evidence and our experience on this topic prompted us to carry out experiments in the same conditions described by the authors. Our results confirm that the amount of TF present on the platelet surface after 2 hours stimulation with PAR-1 agonist peptide and thrombin receptor-activating peptide (TRAP)–6 is negligible (1.5 ± 0.2, 1.7 ± 0.1, 1.5 ± 0.2 MFI for control, PAR-1 and TRAP-6 stimulation, respectively; Figure 1). After a 15-minute stimulation, however, PAR-1 and TRAP-6 significantly increase platelet TF expression (2.7 ± 0.2 and 2.6 ± 0.3 MFI, respectively; $P < .01$) compared with resting platelets (1.8 ± 0.2 MFI). This indicates that TF expression by platelets in response to certain agonists is an early event, confirming data already published by our group.[2]

Specifically, in 2003 we showed that platelet activation by adenosine diphosphate (ADP), TRAP, epinephrine, thromboxane analog U46619, and calcium ionophore A23187 resulted in a concentration-dependent expression of TF on the cell surface.[2] The kinetics of this event was very rapid, because TF was detected on the platelet surface as early as 3 minutes after stimulation; a constant increase was observed up to 30-60 minutes, then a return to basal levels within 2-4 hours. Interestingly, at 2 hours, platelet-associated TF did not significantly differ from that observed in resting conditions. To ensure the identity of the platelet-associated TF, flow cytometric analyses were performed using 3 different monoclonal antibodies against TF (2 of them were commercially available, and one was kindly provided by Prof Yale Nemerson, Mt Sinai School of Medicine, New York, NY [deceased, 2009]) and a fluorescein isothiocyanate–labeled recombinant factor VIIa. Moreover, the exposed TF was confirmed to be functionally active because it was able to generate factor Xa as demonstrated by chromogenic assay. More recently, we also demonstrated that acute coronary syndrome patients had a significantly higher amount of TF-positive platelets than stable angina patients or controls and that the functional activity of platelet-associated TF was significantly higher in acute coronary syndrome patients than in stable angina patients and controls,[3] thus suggesting that TF exposed on the platelet surface might have a pathophysiologic relevance in arterial thrombosis.

In conclusion, we agree with Bouchard et al that platelets do not express detectable TF after 2 hours' stimulation. Nevertheless, our data support the evidence that platelets do express TF as a result of a rapid and dynamic process. Thus, its detection may considerably vary if observed at different time points after in vitro cell stimulation.

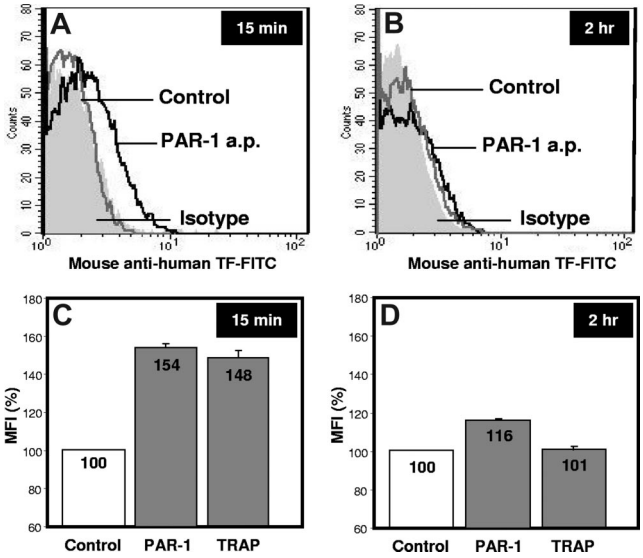

Figure 1. Platelet-associated TF expression. Washed platelets from healthy subjects were left untreated (Control) or stimulated with PAR-1 agonist peptide (PAR-1 a.p.) or TRAP-6 for 15 minutes or 2 hours at 37°C. Samples were then incubated for 15 minutes with saturating concentration of mouse anti–human TF–fluorescein isothiocyanate (American Diagnostica, catalog no 4507CJ) and mouse anti–human CD41-phycoerythrin monoclonal antibodies (Instrumentation Laboratories, catalog no A07781).[2] Fluorescein isothiocyanate– and phycoerythrin-conjugated isotype controls were used in all the experiments to quantify the background labeling. TF-positive platelets were determined in 10 000 CD41-positive events per sample. Mean fluorescence intensities (MFI) were calculated from fluorescence histograms for the gated population, and data were analyzed with CellQuest Pro 5.2.1 software (Becton Dickinson). (A-B) Representative fluorescence histograms of 3 independent experiments performed in triplicate. (C-D) The mean value of the results, reported as %MFI ± SD.

Marina Camera
Department of Pharmacological Sciences, Università degli Studi di Milano and Centro Cardiologico Monzino IRCCS, Milan, Italy

Marta Brambilla
Centro Cardiologico Monzino IRCCS, Milan, Italy

Vincenzo Toschi
Division of Haematology and Blood Transfusion and Thrombosis Centre, AO Ospedale San Carlo Borromeo, Milan, Italy

Elena Tremoli
Department of Pharmacological Sciences, Università degli Studi di Milano and Centro Cardiologico Monzino IRCCS, Milan, Italy

Contribution: M.C. conceived of research, analyzed data, and wrote the paper; M.B. conceived of research, designed experiments, analyzed data, and reviewed the manuscript; and V.T. and E.T. conceived of research and reviewed the manuscript.

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Correspondence: Marina Camera, PhD, Department of Pharmacological Sciences, Università degli Studi di Milano, Via Balzaretti, 9; 20133 Milan, Italy; e-mail: marina.camera@unimi.it or marina.camera@ccfm.it.

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To the editor:

Transmission of leukemic donor cells by allogeneic stem cell transplantation in a context of familial CLL: should we screen donors for MBL?

A family history of chronic lymphocytic leukemia (CLL) is one of the best characterized risk factors for the development of CLL. First-degree relatives of individuals with CLL have a 3- to 8-fold increased risk for CLL.[1,2] CLL often has an indolent behavior, but some patients show an aggressive course, are resistant to purine analogs and therefore are eligible for allogeneic stem cell transplantation (ASCT).[3]

We report here the case of a 45-year-old man who was referred in 1997 for CLL with lymphocytosis (30×10^9/L) and lymphadenopathies. Immunophenotyping studies on blood cells revealed monoclonal B lymphocytes (Matutes Score 5), and a κ immunoglobulin light chain restriction. Because of a quick relapse after fludarabine treatment and the existence of a HLA matched brother, the patient underwent a peripheral blood stem cell transplantation with myeloablative conditioning in March 2001. A limited chronic graft-versus-host disease (GVHD) was observed and the patient received cyclosporine for 2 years. Finally, complete remission and full donor chimerism were obtained.

In March 2006, the 63-year-old donor brother was referred for lymphocytosis (35×10^9/L) evolving over a period of 2 years. Immunophenotyping of blood cells revealed a monoclonal B-cell population expressing λ immunoglobulin light chain restriction (Matutes Score 5). In October 2006, the recipient brother presented with lymphadenopathies and a discrete lymphocytosis (5.5×10^9/L). Flow cytometric analysis identified a monoclonal B-cell population expressing λ immunoglobulin light chain.

Both brothers exhibited normal karyotypes, but FISH analysis showed a monoallelic deletion at 13q14. PCR amplification of IGHV-D-J rearrangements with FR1 primers demonstrated a monoallelic, unmutated VH1 (IGHV 4-69*01) rearrangement in the initial B-CLL clone in the recipient before PMSCT.[4] After allograft, this patient showed a new unmutated, biallelic VDJ rearrangement (VH1 4-69*01 + VH4-34*01). An identical biallelic rearrangement was detected in the donor CLL (Figure 1). Further PCR amplification and sequencing retrospectively confirmed the presence of this biallelic IGHV-D-J rearrangement in the donor blood before ASCT, while WBC was normal (Figure 1).

Monoclonal B-cell lymphocytosis (MBL) is an asymptomatic hematologic condition characterized by lymphocytosis $< 5 \times 10^9$/L and a CLL-like phenotype.[5] Here we report the second case of transmission of CLL by ASCT, linked to the presence of MBL in the graft. In our observation, CLL developed as an aggressive form in both brothers, whereas Perz described indolent CLL in the recipient and persistence of MBL in the donor. Of note, the latter case showed a somatically mutated VH4-34 rearrangement whereas in the present cases, VH genes were unmutated.[6] Taken together, this suggests that genetic microenvironment and immune system do not influence CLL course.

These observations raise the question of identifying occult malignancies in transplant donors, specifically for CLL.[7] Indeed, MBL is more frequent in sibling transplant donors to CLL patients (15.4%) than in the general population (3%), and MBL prevalence increases with age in first-degree relatives of patients with sporadic CLL.[8,9]

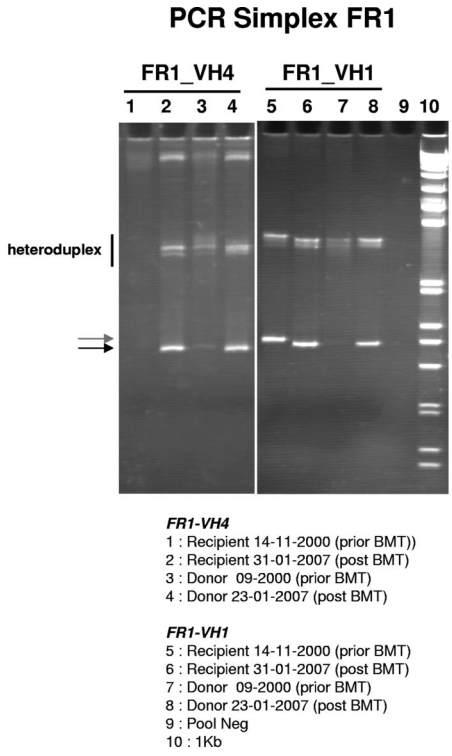

Figure 1. PCR analysis of IGHV-D-J rearrangements using FR1 Biomed-2 primers. Samples as indicated. Arrows indicate clonal rearrangements. Vertical black bar indicates the position of heteroduplexes.

In conclusion, we propose the use of sensitive methods for the systematic screening of blood donors for the presence of MBL, particularly when a history of CLL is known in the family.[10,11]

Pascale Flandrin-Gresta
Laboratoire Hématologie, CHU Saint-Etienne, Saint-Etienne, France

Mary Callanan
Laboratoire de Génétique Onco-Hématologique, CHU, Grenoble; Inserm U823, Institut Albert Bonniot, Grenoble; and Université Joseph Fourier-Grenoble I, Institut Albert Bonniot, UMR-S823, Grenoble, France

Nathalie Nadal
Laboratoire Hématologie, CHU Saint-Etienne, Saint-Etienne, France

Jerome Jaubert
Service Hématologie Clinique, ICL, Saint-Priest-en-Jarez, France