Circulating immune complexes do not affect microparticle flow cytometry analysis in acute coronary syndrome

Analysis of shed-membrane microparticles (MPs) remains a challenging issue. György et al recently reported in Blood that measuring MP by flow cytometry was flawed because of the overlapping biophysical properties of immune complexes, causing false-positive signals during analysis of synovial fluid from patients with rheumatoid arthritis. They suggested that similar problems might occur during analysis of circulating MP from patients with acute coronary syndrome, where the presence of circulating immune complexes has been reported, therefore raising a question on the validity of previous reports.

In view of György et al’s findings, we investigated the potential interaction of immune complexes with flow cytometry analysis of circulating MPs in patients with coronary artery diseases, using MP sensitivity to a brief exposure to Triton detergent. Ten patients with acute coronary syndromes (ACS) were included according to their clinical characteristics. Platelet-free plasma samples prepared as previously described were analyzed on a Millipore GUAVA EasyCyte 8HT flow cytometer. Fluorescent Megamix beads calibrated from 0.5 to 3 μm (Bicocyte) were used to define an analysis window (gate) consistent with the size of MPs (∼1 μm). AnnexinV–FITC (Roche), anti–CD41-PC7 (Beckman-Coulter), anti–CD235a-APC (BD-Bioscience), anti–CD144-PE (eBioscience) antibodies, their respective isotypes and Flowcount beads (Beckman-Coulter) were used to measure levels of annexinV+ MPs.}

Figure 1. Effect of Triton on MP flow cytometry analysis. (A) Representative experiment using plasma from a patient with ACS: PMP, RBC-MPs, and EMP levels were assessed using specific markers, either in absence (top panels; baseline) or presence (bottom panels) of Triton. Labeling with their respective isotype is not shown. MP number dramatically decreases after Triton lysis. (B) Plasma MP levels in ACS patients (n = 10) before and after Triton lysis. Data are expressed as mean ± SEM. Annexin V+ indicates annexinV+ MPs (*P < .01; Wilcoxon test).
MPs, platelet (PMPs), erythrocyte-derived (RBC-MPs), and endothelial MPs (EMPs), respectively.

Each plasma sample was evaluated immediately after addition of Triton 0.05% or vehicle, as described by György. Preliminary analysis with in vitro generated EMPs and circulating MPs from healthy subjects demonstrated that Triton (0.01 to 0.05%) dose-dependently decreased annexinV+ MP concentration to reach up to 99% inhibition, without affecting the biophysical properties of fluorescent Flowcount beads (not shown). Brief exposure to Triton significantly decreased all plasma MP subpopulations in ACS patients (Figure 1). A comparable effect of Triton on plasma MP analysis was also observed in n = 10 subjects with cardiovascular risk factors but no coronary disease, and in n = 10 patients with symptomatic stable coronary artery disease (data not shown).

These results demonstrate the absence of significant artifacts resulting from the interference of protein complexes with circulating MP detection in a population of ACS patients where circulating immune complexes have been reported. The discrepancy of the present findings with the work from György et al might result from a lower concentration of immune complexes in our population plasma when compared with inflammatory synovial fluid, or in differences in biophysical properties between these 2 biologic samples. Nevertheless, the results of György et al together with the present data point out the need for systematic use of Triton lysis as an additional control when establishing MP labeling using flow cytometry.

Response

Systematic use of Triton lysis as a control for microvesicle labeling

In their letter to the editor, Amabile and coworkers analyzed if immune complexes falsified flow cytometric microvesicle enumeration in acute coronary syndrome (ACS). Using Triton lysis, the authors found no significant artifacts resulting from interference with protein aggregates in the blood plasma of ACS patients. With their study they contributed to the correct assessment of microvesicles in ACS.

As the authors suggest, this observation may have resulted from the relatively low concentration of immune complexes in the tested plasma samples. Similarly to their findings, we found that rheumatoid arthritis (RA) and osteoarthritis (OA) plasma samples showed only minor positivity for immune complexes, making it unlikely that the antigen-antibody complexes affected microvesicle counting in RA or OA blood plasma samples. This was in contrast with synovial fluid samples from the site of inflammation (affected joints), where local production of autoantibodies resulted in very high concentration of immune complexes.

However, we found that certain blood plasma samples from patients with systemic lupus erythematosus showed high amounts of microvesicle-mimicking, Triton-resistant, immunoglobulin positive signals by flow cytometry (M.P., B.G., T. Szabó, L. Turiáik, A. Kittel, A. Polgár, E. Kiss, G. Nagy, K. Vékey, S. Gay, A. Falus, E.I.B., Differential detergent lysis differentiates immune complexes and microvesicles [cell-derived microparticles] in the blood plasma of patients with systemic lupus erythematosus, February 2012, manuscript in preparation). Thus, differentiation between protein aggregates and microvesicles might be necessary in blood plasma samples from diseases other than ACS.

Importantly, not only endogenously generated protein aggregates, but also factors during analysis may yield in severely confounding signals, which can only be differentiated from microvesicle-related events by Triton lysis. These include avidin-biotin complexes and primary-secondary antibody complexes formed during indirect immunofluorescent staining.

Strikingly, we found that self-aggregation of antibodies leads to microvesicle-mimicking signals. While using 2 different BD Biosciences antibodies we also found evidence for confounding signals. Agitation resulted in self-aggregation of an anti–CD14-PE antibody, and we have found recently that even without any agitation, anti–CD68-FITC antibody formed fluorescent aggregates, complement C4 null alleles, and myocardial infarction before age 45 years. Arterioscler Thromb Vasc Biol. 1995;15(5):665-668.


References

Circulating immune complexes do not affect microparticle flow cytometry analysis in acute coronary syndrome

Nicolas Amabile, Jean-Marie Renard, Christophe Caussin and Chantal M. Boulanger