MPs, platelet (PMPs), erythrocyte-derived (RBC-MPs), and endothelial MPs (EMPs), respectively.[5]

Each plasma sample was evaluated immediately after addition of Triton 0.05% or vehicle, as described by György.[3] 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 of 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.

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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).[1] 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. Turiák, Á. Kittel, A. Polgár, E. Kiss, G. Nagy, K. Vékey, S. Gay, A. Falus, E.I.B., Differential detergent lysis differentiates immune cmplexes 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 the amount of which increased with increasing protein concentration of the sample (B.G., T. Szabó, L. Turiák, M. Wright, P. Herczeg, Z. Lédeczi, Á. Kittel, A. Polgár, K. Tóth, B. Dérfalvi, G. Zelenák, I. Böröcz, B. Carr, G. Nagy, K. Vékey, S. Gay, A. Falus, E.I.B., Improved flow cytometric assessment reveals distinct microvesicle [cell-derived microparticle] signatures in joint diseases, February 2012, manuscript in preparation).

Given the variable protein concentrations among individual biologic samples, which may lead to different rates of protein aggregation, in accordance with Amabile et al,[1] we recommend the

use of the simple and inexpensive Triton lysis to prove the vesicular nature of events in all flow cytometric microvesicle assessments.

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To the editor:

Fingolimod blocks immunosurveillance of myeloma and B-cell lymphoma resulting in cancer development in mice

Fingolimod (FTY720, 2-amino-2-propane-1,3-diol hydrochloride) is a remarkably efficient immunosuppressive drug that was recently approved as the first oral treatment for multiple sclerosis. Fingolimod prevents lymphocyte egress from lymph nodes by targeting 4 of 5 sphingosine-1-phosphate receptors.[1] Two phase 3 trials showed that fingolimod significantly reduced multiple sclerosis disease progression compared with placebo or standard treatment.[2,3] However, both trials concluded that longer studies were required to assess possible long-term risks.[2,3] Reports from studies in mice and humans strongly support a crucial role of lymphocytes and adaptive immunity in both preventing cancer and fighting established tumors in a process called cancer immunosurveillance.[4,5] In transplant recipients, lifelong immunosuppressive treatment is associated with an increased risk of cancer.[6]

We investigated the effect of fingolimod on cancer immunosurveillance mediated by tumor-specific $CD4^+$ T cells in mouse models for myeloma and B-cell lymphoma. We used severe combined immunodeficient (SCID) mice made transgenic for a T cell receptor (TCR) that recognizes a tumor-specific idiotypic (Id) antigen that is secreted by MOPC315 myeloma and F9 B-cell lymphoma.[7,8] The

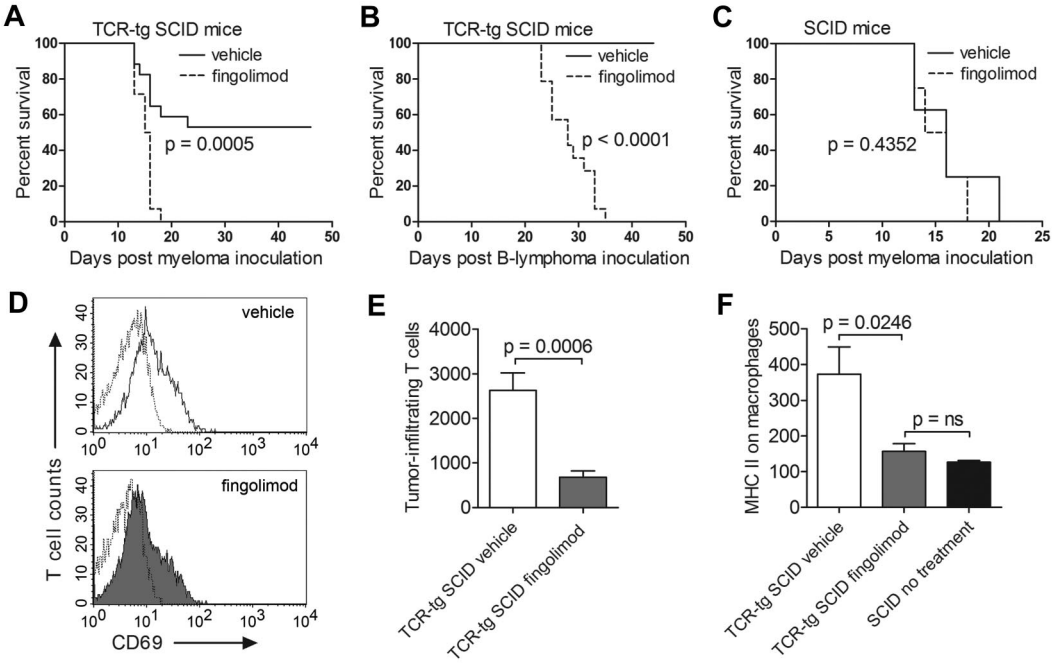

Figure 1. Fingolimod blocks antitumor immunity and prevents rejection of myeloma and B-cell lymphoma in TCR-transgenic SCID mice. (A) Id-specific TCR-transgenic (TCR-tg) SCID mice were inoculated subcutaneously with 1.6×10^5 MOPC315 myeloma cells and treated daily with either fingolimod (FTY720, Selleck Chemicals; 2 μg/g bodyweight) or with vehicle only (0.8% DMSO; Sigma-Aldrich) delivered intraperitoneally. Tumor growth was followed by palpation. Mice were euthanized when the tumor reached 10 mm in diameter (n = 14-17). (B) Id-specific TCR-transgenic SCID mice were inoculated subcutaneously with 1.6×10^5 F9 B-lymphoma cells and treated daily with fingolimod or with vehicle only. F9 cells are A20 B-lymphoma cells transfected with Id-containing L-chain from MOPC315[7] (n = 14-16). (C) Nontransgenic SCID mice were inoculated subcutaneously with 1.6×10^5 MOPC315 cells and treated daily with fingolimod or with vehicle only (n = 8). (D-F) Id-specific TCR-transgenic and nontransgenic SCID mice were inoculated subcutaneously with 10^5 MOPC315 myeloma cells embedded in Matrigel.[9] TCR-transgenic SCID mice were treated daily with fingolimod (1 μg/g bodyweight) or with vehicle only (n = 8-10). SCID mice were left untreated (n = 2). At day +8, draining lymph nodes and Matrigel plugs were dissected and analyzed by flow cytometry.[9] (D) CD69 expression on gated Id-specific $CD4^+$ T cells in draining lymph nodes of representative TCR-transgenic SCID mice treated with vehicle only (top) or fingolimod (bottom). Dotted lines indicate an isotype-matched control antibody. (E) Number of Id-specific T cells per Matrigel plug (mean ± SEM). (F) Expression of the activation marker MHC class II on Matrigel-infiltrating $CD11b^+$ macrophages (geometric mean ± SEM). *P* values were calculated with the log-rank test (A-C) and the *t* test (E-F). ns indicates not significant.