Platelets from patients with myocardial infarction can activate T cells

Elena E. Solomou,[1] Katerina Katsanaki,[1] Elena Kalyvioti,[1] Vasilios Gizas,[1] Angelos Perperis,[1] Dora Babali,[1] Evgenia Verigou,[1] Haralambos Gogos,[1] George Hahalis,[1] Periklis Davlouros[1] and Dimitrios Alexopoulos[2]

[1]Department of Internal Medicine, University of Patras Medical School, Rion, Patras and [2]B' Department of Cardiology, University of Athens Medical School, Athens, Greece

Correspondence: ELENA E. SOLOMOU - elenasolomou@hotmail.com

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Supplemental Data
Methods

Materials and Methods

1. **Patients and controls:** After written informed consent was obtained, we examined 33 patients (29 men, 4 women, mean age 62.5 years) with myocardial infarction with ST elevation (STEMI) and 10 aged-matched healthy volunteers. None of the patients with STEMI was receiving low dose aspirin before the event. Thirty one out of the 33 patients examined underwent angioplasty after the event. Only two patients of all patients included in the study did not go into angioplasty since coronary artery bypass graft surgery was suggested. All patients after angioplasty received dual anti-platelet therapy with aspirin along with clopidogrel or ticaglerol. The subjects in the healthy control group were not taking any medications and had similar characteristics with that of the patients examined. Another 5 patients with unstable angina served as the disease-control group. None of the patients was on any immunosuppressive treatment and none was diagnosed with an autoimmune disease. Three additional patients with STEMI received aspirin or clopidogrel before admission, and these patients were also analyzed as an internal control group. This study was submitted and approved by the Ethics Committee of the Patras University Hospital.
2. **Time points:** We analyzed the patients at the following time points; Day 0 was the day that the patient presented at the hospital and was just diagnosed with STEMI, before receiving any treatment. Day 5, was 5 days after the admission and after the diagnosis. Whenever patients didn't have any complications, on the fifth day after admission they were discharged. One month later the patients were also re-examined and blood samples were collected for analysis. In all time points described

above, heparinized peripheral blood was obtained and was used immediately after venipuncture, for the isolation of peripheral blood mononuclear cells (PBMCs) by density gradient centrifugation with lymphocyte separation medium (Organon, Durham, NC) as previously described (14). The same sample was used for the isolation of platelet rich plasma and plasma alone and these were used immediately in short term cell cultures as described below.

3. **Short term cultures.** In short term cultures we incubated isolated T cells from healthy volunteers with: a. Platelet-rich plasma from patients with STEMI (obtained as soon as their admission before receiving any treatment) or from healthy volunteers, b. Plasma alone from patients with STEMI or healthy volunteers, and c. With RPMI only and culture medium. In all these different culture settings we examined the percentages of activated CD4 T cells using the CD69 monoclonal antibody as an activation marker, by flow cytometry. Also, platelet rich plasma was used in three different cultures from patients with STEMI who received aspirin or clopidogrel immediately before their admission to examine possible differences between those patients on anti-platelet treatment or not.
4. **Tregs.** Based on our previous experience (14), we analyzed the percentages of Tregs in patients with STEMI using flow cytometry, at all three time points as described above (Day 0, day 5, and at one month of follow-up after the initial admission). We analyzed the percentages of $CD4^{+}CD25^{high+}FOXP3^{+}$ cells representing the Tregs. All experiments were performed using patients' samples and healthy subjects samples in parallel to eliminate any potential discrepancies between experiments.

5. **Expression of miR155.** The miR155 levels were evaluated using real-time PCR in samples from patients and healthy individuals. In STEMI patients the expression of miR155 was evaluated at admission and when the patients were discharged (Day 0 and day 5, respectively). The correlation of the expression of miR155 and Tregs was analyzed as described in the manuscript.