TACSM Abstract

Using image-based flow cytometry to monitor exercise-induced changes in granulocyte function

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Category: Masters

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ABSTRACT

Introduction. Strenuous aerobic exercise is known to disrupt innate immune function for up to 24-h, resulting in a period of time where individuals are more likely to get sick; granulocytes play a key role in this innate immune response. Recently our laboratory validated a new, image-based method of simultaneously assessing granulocyte phagocytosis and oxidative burst to divide granulocytes into high-, moderate-, and low-active phenotypes. The purpose of the present study was to use image-based flow cytometry method to assess the effect of strenuous aerobic exercise on the change in granulocyte activation phenotype. Methods. After providing their informed consent, subjects were asked to complete 60-min of treadmill exercise (4, 15-min intervals: 7-min fast walking, 8-min jogging). Blood was collected prior to exercise, immediately after, 2-h, and 4-h post exercise. Heparinized blood (100 µL) was mixed with S. aureus bioparticles-pHrodo (Life Technologies; 100 µL) and Dihydroethidium (DHE; Sigma-Aldrich; 2.5 µg/mL) in 4 identical tubes and each incubated for 10, 20, or 40-min in a 37°C water bath. An additional tube kept on ice and used as a negative control. All subsequent processing steps were completed on ice in the dark to minimize additional activation of cells. Following the water bath incubation, N-ethylmaleimide (10 mM) was added to halt phagocytosis, preventing the uptake of additional bioparticles. Suspensions were labeled with CD66b-APC (eBioscience; DF:50) and CD45-APCeFluor780 (eBioscience; DF:50) for 30-min. RBCs were then lysed and WBCs fixed using a commercial Fix/Lyse solution (eBioscience). Prior to acquisition, 7AAD (EMD Millipore; DF:20) was added to stain nuclear DNA. All samples were collected on a Millipore-Amnis FlowSight calibrated each day with manufacturer-provided calibration beads. A minimum of 10,000 granulocyte (CD66b+/CD45-) events were acquired using the FlowSight equipped with blue (488 nm; 60 mW) and red (642 nm; 100 mW) lasers. Side Scatter (SSC) was detected using a dedicated SSC laser (785 nm; 10 mW). Samples were compensated and analyzed post-acquisition using Amnis IDEAS software (v.6.0.208.0). Results. As a function of incubation time, exercise resulted in a progression suppression of the presence of the “High” activity phenotype. To a lesser extent exercise also affected those granulocytes that were “Moderate” and “Low” active. As recovery progressed, there was a gradual recovery in the “High” active phenotype. Conclusion. To our knowledge the present study is the first to demonstrate that exercise-induced immunosuppression can be detected as a function of phenotype.
shift in granulocytes. More research is needed to identify factors that can prevent post-exercise immunosuppression.