TACSM Abstract

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

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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 immunosupression can be detected as a function of phenotype

shift in granulocytes. More research is needed to identify factors that can prevent post-exercise immunosupression.