SPACE LIFE SCIENCES SYMPOSIUM (A1)
Applications of Space Medicine to Earth-Related Health Issues (3)

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CRYOPRESERVATION PROTOCOLS TO MONITOR ADHESION MOLECULE EXPRESSION ON POLYMORPHO-NUCLEAR LEUKOCYTES: A USEFUL TOOL FOR RESEARCH IN SPACE AND EARTHBOUND ANALOGUES?

Abstract

Background: In many experimental and clinical studies, surface adhesion molecules like ß2-integrine (CD11b) and L-selectin (CD62L) on polymorphonuclear leukocytes (PMN) are shown to interact with other immune cells and the vascular endothelium thereby influencing crucially the host defence. Because the assessment of the degee of expression of these adhesion molecules alters as a function of time between blood collection and measurement, they cannot be easily determined under field-conditions. Goals: 1.) To test five different protocols to cryo-preserve the native expression of CD11b and CD62L on human PMN and, 2.) to define the adequate condition to preserve activation-dependent regulation of 2-integrin-and of L-selectin expression. Methods: The baseline expression of CD11b and CD62L was quantified from whole blood (10l/assay) after incubation with monoclonal anti-CD11b/CD62L antibodies. Expression of adhesion molecules were determined by flow cytometry either immediately thereafter or 1 month of co-incubation (-80C) with dimethylsulfoxide (DMSO; 5-10%), polyethylenglycol (PEG; 10%) alone or in combination with DMSO. Accordingly, fresh whole blood was incubated with the chemoattractant N-formyl-met-leu-phe (fMLP 5*10-9M), and the adhesion molecule expression was assessed pre- and post-freezing. Results: Among all protocols tested, cryopreservation with PEG 10% was shown to be most suitable and describe in detail here: i.) control (native) values of CD11b showed stable values after 1 month at -80C ["postfreeze"] (relative fluorescence CD11b control=17.48plusminus2.0; CD11b postfreeze=22.8plusminus2.6; MVplusminusSEM, paired T-test, n.s.). When the cells were stimulated by fMLP, expression of CD11b increased expectedly as a marker of activation which could be cryopreserved as well (CD11b fMLP=106.3plusminus14.0; CD11b fMLP-postfreeze=103.7plusminus11.6; Mann-Whitney-U, n.s.). ii) In contrast, expression of CD62L significantly decreased after cryopreservation (CD62Lcontrol=23.20.97; CD62Lpostfreeze=15.9plusminus0.82, paired T-test; p¡0.001); fMLP resulted in an activation-dependent decrease ("shedding") which was further pronounced after freezing (CD62L fMLP=15.6plusminus1.5; CD62L fMLP-postfreeze=10.6plusminus1.0; Mann-Whitney-U, p¡0.02). However, fMLP activation-dependent relative changes could still be correctly mirrored after cryopreservation. Conclusion: The expression of both adhesion molecules, CD11b and CD62L, on PMN can be cryopreserved with PEG 10% for at least one month at -80C. CD11b is very stable. In contrast, CD62L appears to be more susceptible to alteration due to freezing-thawing, however, the relative changes of activation can still be reflected adequately. In summary, our cryopreservation protocol is easy to perform, requires only minute amounts of blood, and in this way it is suitable for application in space and earth bound analogues,

e.g. Antarctic Concordia base, when immediate measurement cannot be guaranteed. Acknowledgements: The authors are grateful to the support from to the German National Space Program supported by the German Space Agency DLR(50WB0719)