Identification of Cystic Fibrosis Newborn Neutrophils through Flow Cytometry

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Cystic fibrosis is often characterized by severe airway disease due to a cycle of inflammation and infection caused by neutrophil dysfunction, which becomes increasingly severe. Due to the importance of understanding disease progression at an early stage, the purpose of our study was to develop a surface stain to identify neutrophils within fixed and frozen CF newborns by first studying adult CF samples. In this study, we costained for a number of markers including CD63, CD66b, CD16, and HNE, and we used flow cytometry protocol to compare surface expression on whole blood and airway neutrophils of adult CF samples. Our results showed that CD63 expression in live neutrophils was significantly higher in airway compared to whole blood samples. In addition, CD16 and CD63 were expressed within fixed/frozen neutrophils, but with only a four-fold increase from blood to airway, which suggests that CD16 is not a definitive marker. Permeabilization of fixed and frozen neutrophils showed high levels of human neutrophil elastase (HNE) expression, but HNE was marginally higher in airway neutrophils with permeabilization compared to unpermeabilized samples. Since markers such as HNE were present on neutrophils without permeabilization, our results suggest that the samples were being permeabilized by the method of fixation, which could sufficiently alter the cellular structure in order to reduce expression, and formation of microcrystals of ice could lead to leakage. This suggests that surface staining alone is insufficient to conclusively identify neutrophils within fixed/ frozen samples and further study with image cytometry is necessary to target neutrophils.