Depletion Of Neutrophils From Induced Sputum Using A Novel Bead And Sieve-Separation System

O. Holz¹, F. Schaumann¹, O. Janssen¹, B. Lavae-Mokhtari¹, L. Witte¹, N. Krug¹, J.M. Heinrich², J.M. Hohlfeld¹
¹Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany; ²PluriSelect GmbH, Leipzig, Germany

Introduction

To obtain macrophages/monocytes from induced sputum of well characterized patient groups e.g. for preclinical in-vitro tests of novel drug compounds, low stress cell isolation methods are required.

In this study we tested if the novel Pluribead® (PluriSelect) methodology can be used for the depletion of neutrophilic granulocytes from induced sputum.

Methods

Sputum of 12 healthy volunteers (non-smokers, mean (SD) age: 38±11, FEV₁: 104.2±7.3 % pred.) was used in this study. The volunteers inhaled 2 µg lipopolysaccharide (LPS, Clinical Center Reference Endotoxin, NIH Bethesda, USA), which was nebulized during standardized and flow controlled inhalations (Aeroneb solo, Inspiration Medical). Sputum was induced 6 h after LPS provocation.

• NaCl Inhalation (6x5 Min, 3%, 4%, 5%)
• Sputum plug selection
• Homogenization (DTT)
• Filtration, Washing, Centrifugation
• Sputum was mixed with CD3 and CD15 coupled catcher-beads
• 30 min incubation under soft rotation.
• Depletion of bead coupled cell populations by placing cells on a 30 µm sieve

Results

The median (IQR) percentage of neutrophils before separation was 69 (60; 74) %. After depletion with Pluribeads, neutrophils were reduced to 8 (1; 16) %. The relationship between amount of antibody and the cell number is important. In our hands a maximum of 2 Mio total cells with 200 µL of CD15 Pluribeads resulted in optimal depletion of sputum neutrophils.

Fig. 2: Depletion of Pluribead bound cells by sieving

Fig. 5: Changes in the percentage of sputum macrophages, lymphocytes and non-squamous epithelia cells after depletion with CD15 and CD3 Pluribeads

The proportion of lymphocytes was not reduced. Using flow cytometry we observed that CD3 positive cells could not be detected in homogenized sputum, which is compatible with the observed lack in binding of these catcher beads.

Conclusion

• CD 15 Pluribeads can be used to deplete neutrophils from sputum samples.
• The depletion procedure is fast and simple
• Enriched sputum macrophages are viable and their TNF or IL8 release can still be modulated by LPS and/or Dexe.
• CD 3 Pluribeads cannot be used for the depletion of lymphocytes from DTT homogenized sputum samples.

Acknowledgements

The initial experiments and the optimisation of the method was supported by PluriSelect, Leipzig, Germany

Contact

Dr. Olaf Holz
Fraunhofer ITEM, Hannover, Germany
olaf.holz@item.fraunhofer.de