

# Quantifying Lung Macrophages to Understand Increased Susceptibility to Bacterial Pneumonia with Age

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Pneumonia affects 450 million people worldwide. It is believed that elderly individuals, 65 years or older, are more susceptible to acquiring the disease, due to changes in the aging immune system. In the absence of infection, the concentrations of inflammatory cytokines, including tumor necrosis factor (TNF), increase in the blood and tissues as humans age, initiating an immune response. This age-associated inflammation triggers an increase in the number of macrophages; however, their antimicrobial function decreases with age. This novel investigation aimed to determine if age-associated inflammation and exposure to TNF alter macrophage presence in the lungs with age. Lungs from wildtype (WT) and TNF knockout (KO) mice, aged three months (young) and 22-24 months (old) in each experimental group, were stained with markers specific to macrophages, actively dividing cells, and T cells. Areas of positive staining were quantified and normalized to total lung tissue area using imaging software and an ImageJ Immunohistochemistry module. The trend of macrophages present in the lungs of old WT and old TNF KO mice was found to be increased compared to the controls; however, differences were not statistically significant due to the variability within old mice ( $p=0.0840$  one way ANOVA). Furthermore, young TNF KO mice appeared to have fewer macrophages than their older counterparts; however, when old groups of WT and TNF KO mice were compared, no such difference was noted. These results suggest that TNF may alter the number of lung macrophages in young mice but not in old mice. The findings will assist in the improved design of therapeutic interventions to modulate the levels of macrophages and their antibacterial function in the lungs to better protect the elderly during pneumonia.