In vivo imaging of the pathophysiological changes and dynamics of immune cells in influenza virus-infected mouse lung

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Influenza virus is a respiratory pathogen that causes pandemics and seasonal epidemics. The pathophysiological changes and *in vivo* dynamics of immune cells in influenza virus-infected lungs are poorly understood. In this study, we established an *in vivo* imaging system that combines two-photon excitation microscopy and fluorescent influenza viruses of different pathogenicity (backbones of H5N1 A/Vietnam/1203/2004 or A/Puerto Rico/8/34 [H1N1; PR8]). This approach allowed us to monitor and correlate several parameters and pathophysiological changes including the spread of infection, pulmonary permeability, pulmonary perfusion speed, the number of pulmonary neutrophils, and neutrophil motion in the lungs of live mice. Several pathophysiological changes were larger and occurred earlier in mice infected with a highly pathogenic H5N1 influenza virus compared with those in mice infected with a PR8 strain. Time-lapse imaging analysis revealed that in influenza virus-infected lung, pulmonary neutrophil numbers temporally increased and neutrophil movement changed to longer periods of the slow motion after the climax of neutrophil recruitment. We also made real-time observations of cell-cell interactions, with morphological changes, between infected cells and alveolar macrophages infiltrating the alveoli of infected lungs. These findings demonstrate the potential of our *in vivo* imaging system to provide novel information about the pathophysiological consequences of virus infection and the immune response to influenza virus.