P1257
Poster Session V
Immunology, vaccination and host defences

THE EFFICACY OF TOLL-LIKE RECEPTOR4/MD-2COMPLEX AGONISTIC ANTIBODY UT12 FOR CHRONIC LOWER RESPIRATORY TRACT INFECTION CAUSED BY PSEUDOMONAS AERUGINOSA IN MICE

S. Nakamura[1], N. Iwanaga[1], K. Oshima[1], T. Kajihara[1], T. Takazono[1], Y. Imamura[1], K. Izumikawa[1], K. Yanagihara[2], S. Kohno[1]

[1]Second Department of Internal Medicine, Nagasaki University Hospital, Nagasaki, Japan ; [2]Department of Laboratory Medicine, Nagasaki University Hospital, Nagasaki, Japan

Objectives: The chronic lower respiratory tract infection (cLRTI) induced by *Pseudomonas aeruginosa* is the one of the intractable diseases. It is difficult to eradicate the *P.aeruginosa* from the LRT by only antibiotics-based treatment and repeated use of antimicrobial agents leads to occurrence of drug-resistant bacteria. The activation of host immunity is one of the alternative or adjunctive antibacterial therapy. Recently, we reported the UT12, toll-like receptor4/MD-2complex agonistic antibody, -mediated promotion of innate immunity for preventing the post-influenza pneumococcal pneumonia by promoting in mice (Tanaka A, et al. Clin vaccine Immunol 2013). In this study, we investigated the efficacy of UT12 against cLRTI caused by *P. aeruginosa* in mice.

Methods: We replaced the sterile plastic tube pre-coated by *P.aeruginosa* (clinical isolate in Nagasaki University Hospital) in the bronchus of mice according to the previous report (Yanagihara K. et al. AJRCCM 1997). UT12 (1 ug/mouse) was administered intraperitoneally every a week beginning at 7 days after inoculation throughout the experiment. At 14 days, 21 days, and 28 days after inoculation, mice were sacrificed and the number of viable bacteria (lung), and inflammatory cells (BALF) were analyzed. The level of inflammatory cytokines and chemokines in the lung homogenates was determined by ELISA. In addition, we performed phagocytosis assay and opsonophagocytic (OPH) killing assay by using the UT12 treated murine peritoneal neutrophils by flow cytometry. Furthermore we pretreated neutrophils with diphenyleneiodonium (DPI), oxidative burst inhibitor, and AEBSF, serine protease inhibitor, to elucidate the mechanism of UT12 mediated-promotion of bactericidal activity.

Results: The number of viable bacteria in the lungs (control vs UT12 treated group) was significantly decreased through observational period in the UT12 treated group, at 14 days (4.87 ± 0.30 vs 3.66 ± 0.41: $P< 0.05$), 21 days (4.89 ± 0.23 vs 2.05 ± 1.03: $P< 0.05$), and 28 days (5.04 ± 0.62 vs 1.51 ± 0.75: $P<0.01$) respectively. The number of neutrophil in BALF and the level of MIP-2 in the lungs were significantly elevated in the UT12 treated group. The viable bacterial counts estimated by OPH assay were significantly decreased in the UT12 pretreated neutrophils (4.11 ± 0.11vs 3.73 ± 0.07: $P< 0.05$), whereas not by those of $TLR4^{-/-}$ mice (3.95 ± 0.03, 3.93 ± 0.03), indicating the bactericidal activity of neutrophils was promoted by UT12. Furthermore the number of bacterial counts in the neutrophils pretreated with AEBSF, not DPI, was diminished significantly indicating that non-oxidative pathway might be important for UT12 mediated activation of bactericidal effect.

Conclusions: Our results suggest that toll-like receptor4/MD-2complex agonistic antibody UT12 has the potency to improve the cLRTI induced by *P. aeruginosa.* The immunostimulant therapy might be a candidate for the alternative or adjunctive therapy for refractory infectious diseases.