Supplemental information

Detection of nasal lavage *M. pneumoniae*-specific and total Immunoglobulin levels

Maxisorp 96-wells plates (Corning Costar, Corning, NY, USA) were coated overnight with *M. pneumoniae* M129 (ATCC 29342) cell lysate for the detection of *M. pneumoniae*-specific antibodies and coated with an anti-human universal immunoglobulin (SouthernBiotech, Birmingham, AL, USA) for the detection of total antibodies levels. After blocking with PBS/BSA, nasal lavage samples diluted in 0,1% BSA/PBS were added and incubated overnight. Standards for *M. pneumoniae*-specific antibodies were created by making a twofold dilution series of *M. pneumoniae*-IgA, IgG and IgM positive controls (Virion\Serion GmbH, Wurzburg, Germany). Undiluted positive controls were set to 100 Arbitrary Units. To create standards for total IgA, IgG and IgM we used purified IgA (InvivoGen, San Diego, CA, USA), purified IgG and purified IgM (Sigma-Aldrich, St. Louis, MO, USA). For the detection of specific isotypes we used goat anti-human IgA-Alkaline Phosphatase, goat anti-human IgM Horse radish peroxidase and goat anti-human IgG-Alkaline phosphatase (Sigma). 3,3',5,5'-Tetramethylbenzidine (TMB) or para-Nitrophenylphosphate (Sigma-Aldrich) was used as a substrate. Optical density was measured at 450 nm or 405 nm respectively using a microplate reader (SpectraMax iD3, Molecular Devices, San José, USA). Antibody levels were normalized to nasal lavage total protein to account for sampling variation. Total protein levels were measured using CBQCA Protein Quantitation Kit (Thermo Fisher Scientific, Waltham, MA, USA).

Functional antibody assay

To assess a potential inhibitory effect of nasal lavage antibodies on *M. pneumoniae* epithelial adhesion, A549 respiratory epithelial cells were cultured in 24-wells plates until confluent (Greiner Bio-one International, GmbH, Kremsmünster, Austria). Medium was replaced with nasal lavage which was

diluted 10-fold in RPMI 1640 containing L-glutamine (Gibco, Carlsbad, USA) and subsequently, 10^9 Colony Forming Units (CFUs) per well of *M. pneumoniae* strain M129 were added. After 4h incubation, unbound bacteria were washed off and epithelial cells were then lysed by passing through a 25G needle in deionized water leaving adhering bacteria intact. The resulting suspension was plated out on PPLO agar plates and CFUs were counted.

Additional information on statistical analysis

Nasal lavage *M. pneumoniae*-specific and total antibody levels were measured in quadruplicate, whereas other measurements were performed in duplicate. Technical duplicates were averaged before data analysis. *M. pneumoniae*-specific and total antibody levels were assumed to be log-normally distributed and log(10)-transformed before statistical hypothesis testing. *M. pneumoniae* bacterial load, number of Antibody Secreting Cells and number of adhering bacteria in our adhesion assay were not assumed to be (log-)normally distributed and analysed using non-parametric tests.