Online supplementary material for:

A Decoy Receptor 3 Analogue Reduces Localized Defects in Phagocyte Function in Pneumococcal Pneumonia

Helen M. Marriott, Marc Daigneault, Alfred A. R. Thompson, Sarah R. Walmsley, Sharonjit K. Gill, Derrick R. Witcher, Victor J. Wroblewski, Paul G. Hellewell, Moira K. B. Whyte and David H. Dockrell

Description of murine models used in study

These models utilized different strains of pneumococci with varying degrees of virulence. The dose and time of study were varied to interrogate the roles of specific elements of the host response including those of recruited neutrophils, resident alveolar macrophages and lung T-cells. Serotype 1 *S. pneumoniae* infection results in established pneumonia of moderate severity, serotype 2 *S. pneumoniae* results in higher bacterial colony counts in the lung and blood and greater mortality, while serotype 4 *S. pneumoniae* posses an additional virulence factor pili, which limits the effectiveness of host defences and accelerates disease progression and death.[1,2] Supplementary Figure 1 illustrates the aspects of the host response investigated in each model.

Supplementary Methods

Pneumococcal infection model

For pulmonary infection mice were anaesthetized with ketamine and acepromazine, a midline incision made, the trachea exposed and a 24 gauge catheter inserted. Pneumococci were delivered to the lungs in 20µl volume using gel loading tips, the incision closed and the mice allowed to recover in a warmed cage with free access to food and water. Mice received 1 $x10^7$ colony forming units (cfu) of each strain to create a model of established pneumonia of varying severity and were studied at set time periods in the evolution of pneumonia as outlined in supplementary Figure 1. We performed some experiments with 1 $x10^4$ cfu of serotype 1 *S. pneumoniae* since clearance in this model is dependent on alveolar macrophages without a requirement for additional recruited immune cells.[1] We also performed some experiments using 5 $x10^5$ cfu of serotype 4 *S. pneumoniae* as this dose is consistently above the tipping-point at which resident alveolar macrophages protection fails and pneumonia is established. This model also probes key events in the transition to pneumonia and highlights a role for T-cells in the host response at an early stage of pneumonia evolution, when there are still comparatively low numbers of recruited neutrophils in bronchoalveolar lavage (BAL)

fluid. Mice were killed by exsanguination following overdose of sodium pentabarbitone and bronchial alveolar lavage (BAL), blood and lungs collected as described previously.[1]

Neutrophil depletion

C57BL/6, *gld* and *lpr* mice were treated intraperitonealy with 100µg/mouse anti-mouse Ly6G (Gr-1) antibody, clone RB6-8C5 (eBioscience, San Diego, CA) or isotype control 24hr before instillation with $1x10^7$ colony forming units (CFU) serotype 2 S. pneumoniae. Gr-1 leads to lysis of neutrophils by complement dependent and complement independent mechanisms and results in rapid removal of neutrophils from the circulation within 1 minute of administration.[3] As shown in supplementary Figure 2 the antibody effects did not include significant increases in neutrophil apoptosis.

Decoy receptor treatment

C57BL/6, *gld* and *lpr* mice were treated subcutaneously with 400µg/mouse DcR3-a every 12h, starting immediately before instillation of *S. pneumoniae*. Control mice were similarly treated with 400µg/mouse BSA.[4] Mice were instilled with $5x10^5$, or in experiments measuring T-cell activation $1x10^7$, CFU serotype 4 S. pneumoniae.

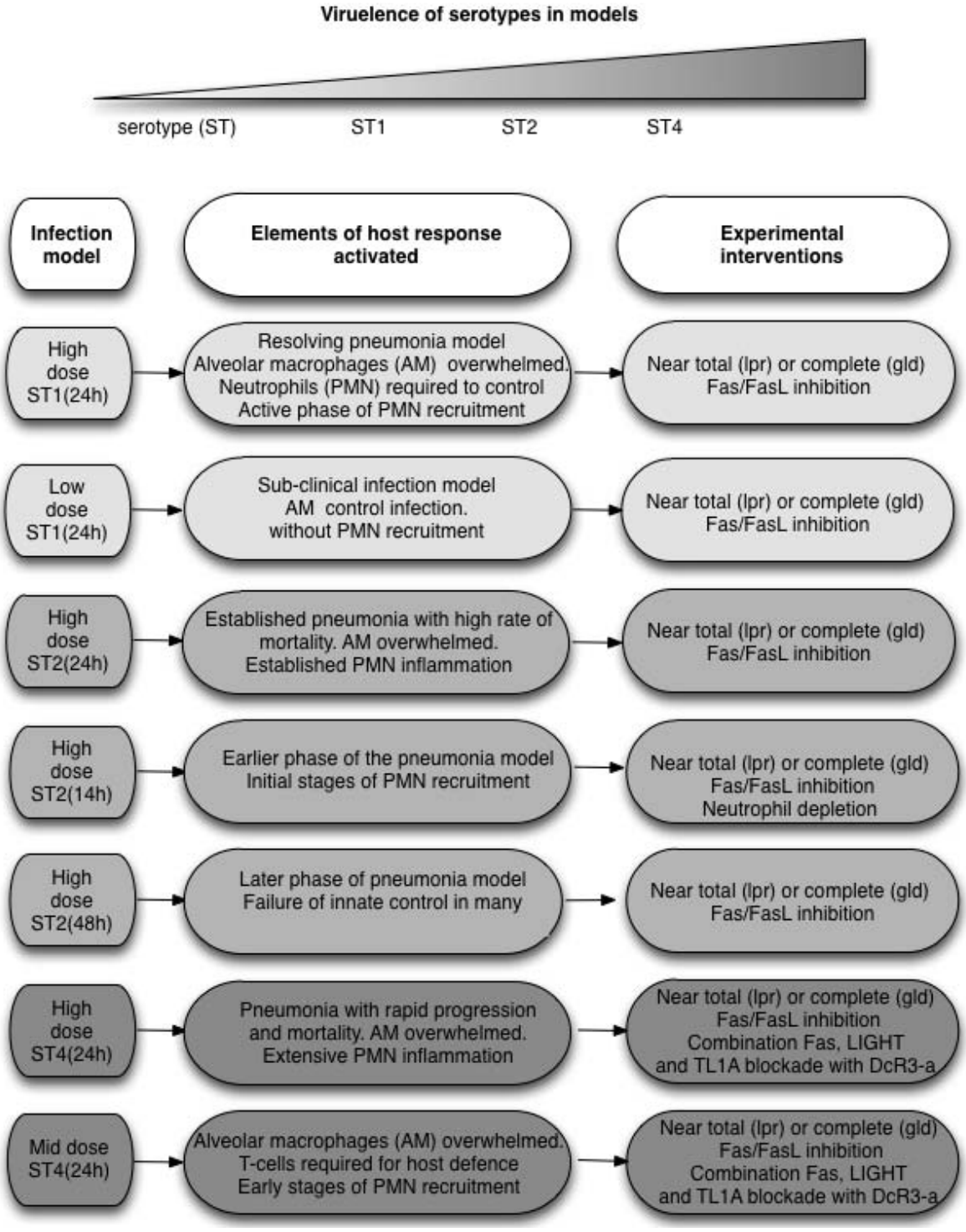
Supplementary References

1. Dockrell DH, Marriott HM, Prince LR, et al. Alveolar macrophage apoptosis contributes to pneumococcal clearance in a resolving model of pulmonary infection. J Immunol 2003;**171**(10):5380-8 2003/11/11].

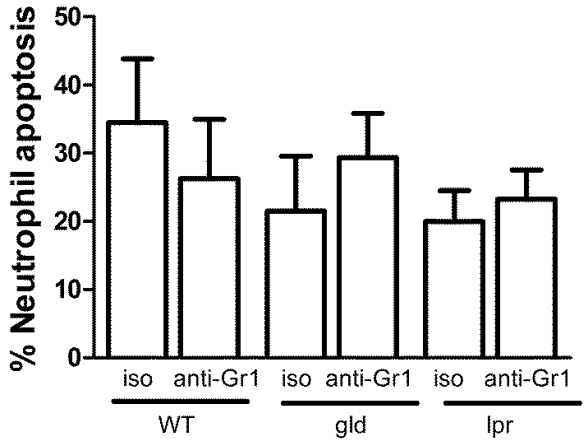
2. Barocchi MA, Ries J, Zogaj X, et al. A pneumococcal pilus influences virulence and host inflammatory responses. Proceedings of the National Academy of Sciences of the United States of America 2006;**103**(8):2857-62 doi: 0511017103 [pii] 10.1073/pnas.0511017103 published Online First: 2006/02/17].

3. Abbitt KB, Cotter MJ, Ridger VC, Crossman DC, Hellewell PG, Norman KE. Antibody ligation of murine Ly-6G induces neutropenia, blood flow cessation, and death via complement-dependent and independent mechanisms. Journal of leukocyte biology 2009;**85**(1):55-63 doi: 10.1189/jlb.0507305 published Online First: 2008/10/18].

4. Matute-Bello G, Liles WC, Frevert CW, et al. Blockade of the Fas/FasL system improves pneumococcal clearance from the lungs without preventing dissemination of bacteria to the spleen. J Infect Dis 2005;**191**(4):596-606 doi: JID32394 [pii] 10.1086/427261 published Online First: 2005/01/19].

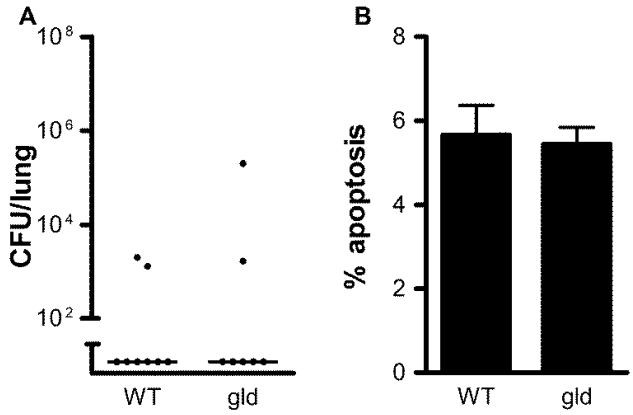


Supplementary Figure 1: Flow diagram showing the aspects of the host response investigated in each infection model and the experimental interventions applied to each



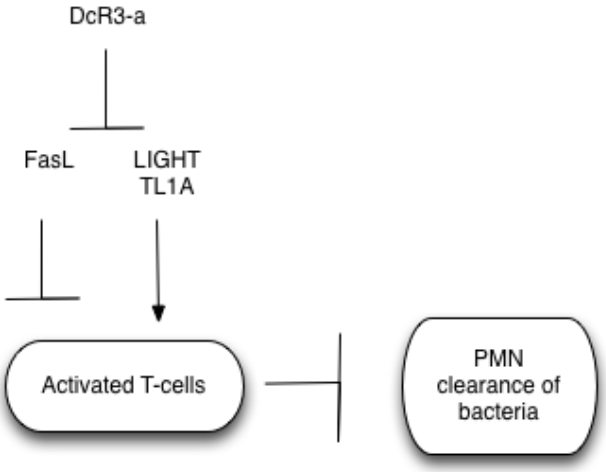
Supplementary Figure 2: Treatment with anti-Gr-1 antibody does not result in neutrophil apoptosis

Peripheral blood neutrophils from wild type control mice (WT) and mice deficient in Fas ligand (*gld*) or Fas (*lpr*) were isolated by negative magnetic selection. Neutrophils were treated with 5 µg/ml anti-Gr1 antibody (anti-Gr1) or isotype control (iso). Rates of apoptosis were assessed by morphology on cytospins prepared after 9h of culture, n=2.



Supplementary Figure 3: Mice lacking FasL had no reduction in alveolar macrophage-dependent bacterial clearance

A) Colony forming units (CFU) of bacteria in lung homogenates from wild type control mice (WT, n=8) and mice deficient in Fas ligand (*gld*, n=7) 24h after intratracheal instillation of $1x10^4$ CFU serotype 1 pneumococci, line at median. B) The percentage of apoptosis in bronchial alveolar lavage cells in the same experiments as A), mean+SEM (average % macrophages WT=99.2%, gld 99.6%), p=not significant by t-test.



Supplementary Figure 4: Proposed model illustrating how FasL and other DcR3 ligands interact in modulating key host responses against *S. pneumoniae* in the lung.