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Role of Toll-like receptor 13 in pneumococcal recognition by macrophages

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Background: Streptococcus (S.) pneumoniae is a major cause of pneumonia, sepsis and meningitis, resulting in high morbidity and mortality rates. Pneumococcal infection leads to powerful inflammatory reaction which contributes substantially to damage. Recently, Toll-like receptors (TLR) have been implicated as central triggers of this inflammatory host response, mostly TLR 2, 4 and 9. Yet, as MyD88-deficient mice show a more severely impaired immune response than TLR 2/4/9-triple knockouts, we hypothesized that other TLRs might be involved.

Material/methods: Bone-marrow derived macrophages (BMDM) of wild-type (WT), MyD88/TRIF-, TLR 2-, TLR 4-, TLR 13-, TLR 2/4-double, TLR 2/13-double and TLR 2/4/13-triple knockout mice were infected with antibiotic-treated S. pneumoniae serotypes 2 (D39), 3, 4, 7F, 14, 19A a as well as pneumolysin-deficient serotype 2 strain and examined for cytokine production and cell death. To identify a possible reason for their different killing potencies, serotypes were characterized on their level of released pneumolysin by immunoblot.

Results: After infection with D39, BMDM showed elevated levels of interleukin (IL)-1beta, IL-6 and increased cell death. Both, cell death and IL-1beta secretion was an effect of pneumolysin as the pneumolysin-deficient D39 (D39ΔPLY) was not able to induce either, but only IL-6. BMDM lacking MyD88/TRIF which is associated with a complete loss of TLR signaling showed almost no IL-1beta and IL-6 release after S. pneumoniae challenge. Single deficiency of TLR 13 – but not of TLR 2 or 4 – diminished cytokine secretion significantly, but only combined deficiency of TLR 2 and 13 decreased IL-1beta and IL-6 levels as strongly as deficiency of MyD88/TRIF. Next, we tested different S. pneumoniae serotypes to generalize our findings and identify a general pattern of TLRs responsible

for pneumococcal recognition. The ability to induce an immune reaction and to kill host macrophages varied greatly between the different serotypes that were tested. All serotypes increased IL-6 generation and all except serotype 3 stimulated IL-1beta secretion. The different serotypes showed varying patterns of TLRs mediating pathogen recognition, but all serotypes were at least partially recognized by TLR 13 – a receptor for bacterial RNA. All serotypes except serotype 3 led – in varying degrees – to increased macrophage death. In immunoblot, we detected different levels of released pneumolysin by the different serotypes which, however, did not correlate with the extent of their cytotoxicity. Deficiency of MyD88/TRIF, TLR 2, 4 or 13 did not lead to significant differences in macrophage death following S. pneumoniae infection.

Conclusions: Different serotypes of S. pneumoniae activate macrophages to a different extent. Activation is mediated primarily by the concerted action of TLR 2 and 13, with TLR 13 playing the major part. We conclude that pneumococcal RNA plays an important role in the recognition of S. pneumoniae by macrophages.