Pneumolysin binds to the mannose receptor C type 1 (MRC-1) leading to anti-inflammatory responses and enhanced pneumococcal survival

Karthik Subramanian, Daniel R. Neill, Hesham A. Malak, Laura Spelmink, Shadia Khandaker, Giorgia Dalla Libera Marchiori, Emma Dearing, Alun Kirby, Marie Yang, Adnane Achour, Per-Åke Nygren, Marie Yang, Laura Plant, Aras Kadioglu and Birgitta Henriques-Normark

Streptococcus pneumoniae (the pneumococcus) is a major cause of mortality and morbidity globally, and the leading cause of death in children under 5 years old. The pneumococcal cytolysin pneumolysin (PLY) is a major virulence determinant known to induce pore-dependent pro-inflammatory responses. These inflammatory responses are driven by PLY-host cell membrane cholesterol interactions, but binding to a host cell receptor has not been previously demonstrated. Here, we discovered a receptor for PLY, whereby pro-inflammatory cytokine responses and Toll-like receptor signalling are inhibited following PLY binding to the mannose receptor C type 1 (MRC-1) in human dendritic cells and mouse alveolar macrophages. The cytokine suppressor SOCS1 is also upregulated. Moreover, PLY–MRC-1 interactions mediate pneumococcal internalization into non-lysosomal compartments and polarize naïve T cells into an interferon-γ low, interleukin-4 high and FoxP3+ phenotype. In mice, PLY-expressing pneumococci colocalize with MRC-1 in alveolar macrophages, induce lower pro-inflammatory cytokine responses and reduce neutrophil infiltration compared with a PLY mutant. In vivo, reduced bacterial loads occur in the airways of MRC-1-deficient mice and in mice in which MRC-1 is inhibited using blocking antibodies. In conclusion, we show that pneumococci use PLY–MRC-1 interactions to downregulate inflammation and enhance bacterial survival in the airways. These findings have important implications for future vaccine design.

S. pneumoniae is a common colonizer of the upper respiratory tract of healthy children. However, it is also a major cause of life-threatening diseases such as pneumonia, septicaemia and meningitis, resulting in the death of over 800,000 children annually. The cholesterol-binding pore-forming toxin PLY is expressed by most resident immune cells in alveoli and mediate protection from pathogens. The mannose receptor MRC-1 (also known as CD206) is an M2 phenotype marker and a phagocytic receptor that is mostly expressed by tissue macrophages, including alveolar macrophages. MRC-1 binds to endogenous and microbial antigens such as capsular polysaccharides. Furthermore, studies have demonstrated that MRC-1 influences pneumococcal uptake by Schwann cells and olfactory cells, but they did not show colocalization. It is not clear which macrophage receptors recognize pneumococci in the nasopharynx and lungs and what bacterial properties interact with the receptors mediating pneumococcal uptake. Here, we discovered a role for PLY in driving anti-inflammatory responses and lysosomal escape in macrophages and DCs by directly binding to MRC-1, thereby promoting pneumococcal internalization and survival in the host.

We first compared the cytokine response induced by PLY by infecting different immune cells—primary human monocyte-derived DCs, neutrophils and THP-1 monocyte-derived macrophages—with a low dose (multiplicity of infection (MOI) of 1) of the pneumococcal strain T4R (which expresses PLY) or its isogenic PLY mutant T4RΔply. The non-encapsulated strain T4R (an isogenic capsular mutant of the encapsulated serotype 4 strain T4) was used for the in vitro experiments to increase bacterial uptake since the capsule impedes bacterial adhesion to host cells. We found lower secretion of the pro-inflammatory cytokines tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-12 from DCs challenged with PLY-proficient T4R compared with the mutant T4RΔply, which was in contrast to THP-1-derived macrophages and neutrophils (Fig. 1a; Supplementary Fig. 1a,b). This PLY-dependent inhibition of cytokine responses was also observed...
using the encapsulated strains T4 and T4Δply (Fig. 1b). The cytokine inhibition was independent of cell death, as determined by measuring lactate dehydrogenase release (Supplementary Fig. 1c), but dependent on bacterial uptake since secretion of TNF-α was reduced after phagocytosis was blocked using cytochalasin D and wortmannin (Supplementary Fig. 1d). Treatment with cytochalasin D, an inhibitor of actin polymerization, inhibited cytokine production by DCs and THP-1 macrophages in a PLY-independent manner. Pretreatment with purified endotoxin-free PLY at 100 ng ml⁻¹ inhibited IL-12 production by ~50% from DCs infected with T4Δply in a dose-dependent manner, independent of cell death (Supplementary Fig. 1e). We then further studied the effect of using different pneumococcal strains and challenge doses. We infected DCs, THP-1 macrophages, neutrophils and bone-marrow-derived macrophages (BMDMs) with the pneumococcal strain D39 of serotype 2 or its isogenic PLY mutant T4RΔply, at different MOIs and measured IL-1β release and cell death (Supplementary Fig. 1f–i). We observed that at lower infection doses (MOI of 0.1 or 1), the mutant D39Δply induced higher levels of IL-1β release in DCs and BMDMs (but not in neutrophils or THP-1 macrophages) independent of cell death. However, at a MOI of 10, the pattern was reversed, and wild-type D39 induced higher IL-1β release, but this was also accompanied by an approximately twofold higher rate of cell death.

We then performed a Toll-like receptor (TLR) signalling quantitative PCR (qPCR) array using RNA from DCs infected for 9 h with T4R or T4Δply. Expression of all genes, except IFNB1 (encoding IFN-β1), was upregulated following infection with T4Δply compared with T4R-infected cells (Supplementary Fig. 1j). This result indicated that PLY expression has a general inhibitory effect on cytokine induction and inflammatory signalling in DCs.

To explore the mechanisms behind this inhibitory effect of PLY on DCs, we measured the expression of the negative regulators of nuclear factor-κB (NF-κB), AP-1 (activator protein 1), and STAT1 (signal transducer and activator of transcription 1) pro-inflammatory signalling pathways. We identified that suppressor of cytokine signalling 1 (SOCS1) mRNA was upregulated in DCs infected with T4R, but not with T4Δply (Fig. 1c). A kinetic analysis revealed that SOCS1 mRNA increased 6 h post infection and peaked at 9 h (Supplementary Fig. 1k). Concurrent with the mRNA result, the protein levels of SOCS1 were higher in DCs at 9 h post infection with T4R compared with T4Δply (Fig. 1d). However, SOCS1 expression remained unaffected in THP-1 macrophages (Supplementary Fig. 1l), confirming the cell-type-specific effect.

Since SOCS1 is a known inhibitor of STAT signalling, we measured the levels of phosphorylated STAT1 and found that phosphorylation in T4R-infected DCs was delayed compared with T4Δply (Fig. 1c). Pretreatment with the STAT inhibitor stattic inhibited the secretion of TNF-α, IL-1β and IL-12 (Supplementary Fig. 1m–o). In addition to STAT1, we found lower levels of NF-κB in T4R-infected DCs compared with T4Δply (Fig. 1f). Together, our data suggest that PLY expression inhibits pro-inflammatory signalling via STAT1 and NF-κB in DCs, possibly via the induction of the cytokine suppressor SOCS1.

**Fig. 1 | PLY inhibits cytokine responses and inflammatory signalling in DCs by upregulating SOCS1.** a, TNF-α secretion from human DCs (n = 6), THP-1 macrophages (n = 4) and primary neutrophils (n = 4) following infection with the wild-type strain T4R or its isogenic PLY mutant T4Δply. b, TNF-α secretion from DCs infected with encapsulated strains, T4 or T4Δply (n = 3 donors). c, SOCS1 mRNA levels in T4R- or T4Δply-infected DCs at 9 h post infection (n = 3 donors). d, Flow cytometry histogram plot showing SOCS1 protein levels in T4R- or T4Δply-infected DCs at 9 h post infection. Percentage of SOCS1-positive cells is indicated in parentheses. e, STAT1 phosphorylation in T4R- or T4Δply-infected DCs at 3–5 h post infection. f, Western blot showing the levels of nuclear NF-κB (p65) in T4R- or T4Δply-infected DCs at 4 h post infection. Histone H2A served as loading controls. Blots are representative of data from two independent experiments. Data in a–c are the mean ± s.e.m. *P < 0.05 by two-tailed Wilcoxon matched-pairs signed-rank test (a, b) or paired two-tailed t-test (c). Data in d and e are representative of three independent experiments.
To identify the host receptor interacting with PLY, we performed a pull-down assay using purified PLY. Thirty-two proteins were identified exclusively in DC lysates, and three of these proteins were surface proteins: integrin alpha M, MRC-1 and galectin 1 (Supplementary Table 1). We further investigated the lectin receptor MRC-1, since it has previously been reported to have immunosuppressive properties\(^{11}\). To confirm the interaction between MRC-1 and PLY, we performed immunoprecipitation of MRC-1 from native DC lysates using anti-PLY coupled beads (Supplementary Fig. 2a).

To assess whether the binding of MRC-1 to PLY was mediated via glycan recognition, we performed enzymatic deglycosylation of PLY to remove bound glycans, which would be evidenced by the slightly higher electrophoretic gel mobility (Supplementary Fig. 2b). PLY to remove bound glycans, which would be evidenced by the slightly higher electrophoretic gel mobility (Supplementary Fig. 2b).

Importantly, MRC-1 co-immunoprecipitated with both native and deglycosylated PLY from native DC lysates (Supplementary Fig. 2c). We found that MRC-1 was selectively expressed by DCs and macrophage colony stimulating factor (M-CSF)-derived macrophages (M2 polarized), but not by THP-1 macrophages, neutrophils or granulocyte-macrophage colony-stimulating factor (GM-CSF)-derived macrophages (M1 polarized) (Supplementary Fig. 2d–e). Interestingly, DCs exhibited an upregulated expression of MRC-1 following infection with T4R compared with T4RΔply (Supplementary Fig. 2f).

In addition, the non-pore-forming PLY mutant (PdB) showed reduced binding compared with cytolytic PLY (Fig. 2b), indicating that domain 4 of PLY is key to the MRC-1–PLY-interaction, as purified PLY colocalized with MRC-1 and the early hydrate recognition domains of MRC-1 (CTLD4-7-Fc). We found that domain 4 of PLY is key to the MRC-1–PLY-interaction, as purified PLY colocalized with MRC-1 and the early hydrate recognition domains of MRC-1 (CTLD4-7-Fc).

Since DCs are professional antigen-presenting cells, we investigated the role of MRC-1 in DC-primed CD4\(^+\) T helper cell cytokine responses after pneumococcal challenge. In contrast to T4R-PLY-infected cells, we found that DCs depleted of MRC1 using siRNA and infected with T4R elicited higher IFN-γ (a T helper type 1 cytokine) and lower IL-4 (T helper type 2 cytokine) levels from naive T helper cells in co-culture compared with DCs treated with control siRNA (Fig. 3c,d). A similar trend was observed in DCs stimulated with purified PLY. To further characterize the phenotype of T cells co-cultured with DCs, we measured FoxP3, a regulatory T cell marker 24, and found that DCs infected with T4R (but not with T4RΔply) and those treated with purified PLY induced higher FoxP3 expression in naive T helper cells after co-culture (Fig. 3c; Supplementary Fig. 3c). FoxP3 upregulation in T cells was abolished when co-cultured with DCs treated with MRC1 siRNA. Similar to human DCs, mouse BMDMs from wild-type mice that were infected with D39 (in contrast to D39Δply) and co-cultured with CD4\(^+\) mouse T cells resulted in higher regulatory (expressing FoxP3 and IL-10) T cells and lower T helper type 1 cells (expressing T-bet and IFN-γ) compared to BMDMs from MRC1−/− mice (Fig. 3e; Supplementary Fig. 3d–f).

To verify our findings in vivo, we challenged wild-type C57BL/6J mice intranasally with 1×10\(^{5}\) colony-forming units (CFU) of wild-type T4 or the mutant T4Δply. At 6 h post infection, bronchoalveolar lavage fluid (BALF) was collected and lung alveolar macrophages isolated. We observed intracellular colocalization of strain T4 with MRC-1, but not with lysosomes (Fig. 4a). In contrast, intracellular T4Δply did not colocalize with MRC-1, but co-stained with lysosomes (Fig. 4a, Supplementary Fig. 4a). Ex vivo, mouse alveolar macrophages secreted lower levels of pro-inflammatory cytokines following infection with T4R compared with T4RΔply. This difference was reduced by pretreatment with an anti-MRC-1 antibody (Supplementary Fig. 4b,c). In agreement with these results, T4Δply-infected mice had higher levels of pro-inflammatory cytokines,
Fig. 2 | MRC-1 colocalizes with PLY and intracellular pneumococci in DCs. **a**, Representative sensorgram of three independent surface plasmon resonance experiments showing the dose-dependent binding profile of recombinant PLY (12.5–200 nM) over immobilized MRC-1. RU, response units. **b**, ELISA showing the binding of immobilized MRC-1 constructs, CTLD4–7-Fc or CR-FNII-CTLD1-Fc (1.25–10 µg ml−1) with full-length (FL) PLY, toxoid PdB, PLY domains 1–3 and domain 4. Mannan (Man) was used as a specific ligand for CTLD4–7 to block interactions with PLY, and galactose (Gal) was used as a negative control for the blocking assay. Bovine serum albumin (BSA) was used as a negative control. Bound PLY was detected using anti-PLY antibodies. Data are the mean ± s.e.m. of two independent experiments, each containing three replicates per condition. **c**, Wild type (WT) DCs or MRC1 siRNA-treated DCs were incubated with purified active PLY or mutant PLY (PdB) (200 ng ml−1) for 45 min. Immunofluorescence staining shows that active PLY colocalizes with MRC-1 and EEA-1 (early endosomes) in contrast to the non-pore-forming mutant PLY (PdB). **d**, DCs were infected with T4R or T4RΔply for 90 min. Immunofluorescence staining shows that intracellular T4R colocalizes with MRC-1, while T4RΔply does not colocalize with MRC-1 but with lysosomes (Lysotracker) (white arrows). All scale bars, 5 µm. Data in **c** and **d** are representative of three independent experiments.
TNF-α, IL-12 and IL-1β, and lower levels of anti-inflammatory cytokines, IL-10 and transforming growth factor-β (TGF-β), in the BALF compared with mice infected with T4 (Fig. 4b; Supplementary Fig. 4d). In addition, T4Δply infected mice had higher numbers of neutrophils and monocytes in the BALF (Supplementary Fig. 4e). The enhanced inflammation and higher infiltration of phagocytes were concurrent with the higher clearance of T4Δply compared with T4 from the airways of infected mice (Fig. 4c). Mice pretreated with antibodies to block MRC-1 before infection with T4 had significantly higher levels of TNF-α and IL-12, and lower levels of IL-10 in BALF at 6h post infection compared with isotype antibody-treated controls (Fig. 4d; Supplementary Fig. 4f). Mice treated with an anti-MRC-1 antibody had 50% lower bacterial counts in the lower airways compared with controls (Fig. 4e), and intracellular bacteria did not colocalize with MRC-1 in alveolar macrophages (Supplementary Fig. 4g). Importantly, MRC1−/− mice also had significantly decreased bacterial numbers in the nasopharynx at 7 and 14 days post challenge compared with wild-type mice in a pneumococcal carriage model with strain D39 (Fig. 4f). In wild-type mice, MRC1+ macrophages were found to rapidly accumulate in the...
nasopharynx following pneumococcal colonization (Supplementary Fig. 5a,b). Similar to mice treated with an anti-MRC-1 antibody, \( \textit{MRC1}^{−/−} \) mice had higher levels of the pro-inflammatory cytokines TNF-α and IL-6, and lower levels of anti-inflammatory IL-10 and TGF-β in the nasopharynx compared with wild-type mice at 6 h and 1 day post infection (Supplementary Fig. 5c–f).

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**Fig. 4 | MRC-1 mediates PLY-induced suppression of early inflammatory responses in vivo.**

\( \textit{a} \). Primary alveolar macrophages were isolated from C57BL/6J mice infected with T4 or T4\( \Delta\)ply at 6 h post infection. Immunofluorescence staining showed that PLY-proficient pneumococci (T4) colocalize with MRC-1 unlike T4\( \Delta\)ply, which colocalizes with the lysosome marker (Lysotracker). Scale bars, 5 μm. Images are representative of data from five mice per group.

\( \textit{b,c} \), TNF-α levels (\( n=12 \)) and bacterial count (\( n=13 \)) in BALF from mice infected with either T4 or T4\( \Delta\)ply at 6 h post infection. \( \textit{d,e} \), Levels of TNF-α (\( n=8 \)) and bacterial count (\( n=9 \)) in BALF of mice pretreated with anti-MRC-1 (0.1 mg ml⁻¹) or isotype antibody (ab) and infected with strain T4 for 6 h. \( \textit{f} \), Bacterial count (CFU) per mg nasopharyngeal homogenates of wild-type or \( \textit{MRC1}^{−/−} \) mice infected with strain D39 over a 14-day carriage experiment. \( n=6 \) mice per data point per strain. \( \textit{g} \), Model suggested for PLY-mediated immunomodulation. PLY-proficient pneumococci induce internalization into alveolar macrophages (AMs) and DCs via interaction with MRC-1. PLY-expressing pneumococci colocalize with MRC-1 in non-lysosomal compartments and block inflammatory cytokine secretion by upregulating SOCS1, thereby promoting regulatory T (T\( \text{reg} \)) cell responses and bacterial survival in the airways. T\( \text{h}1 \), T helper type 1. For \( \textit{b–e} \), data are mean ± s.e.m. (of three independent experiments for \( \textit{b–e} \)); \(* * * P<0.001\), \(* * P<0.01\) by two-tailed Mann–Whitney U-test (\( \textit{b–e} \)) or two-way ANOVA with Tukey's post-test (\( \textit{f} \)).
MRC-1-mediated phagocytosis is of particular significance in the lungs, as MRC-1 is abundantly expressed by alveolar macrophages. A previous study highlighted the crucial role of the scavenger receptor MARCO in anti-pneumococcal immunity in the nasopharynx and suggested a minimal role for MRC-1. However, the authors in that study used a 100-fold higher infection dose (1 x 10^7 CFU) for colonization compared with our study, and we have previously shown that in contrast to high-density infection, low-density pneumococcal carriage induces immunoregulatory responses characterized by a sustained elevation of nasopharyngeal TGF-β1, regulatory T cells and MRC-1-expressing macrophages. In the current study, we demonstrate that the infection dose determines the nature of cytokine response to PLY, whereby lower infection doses lead to cytokine inhibition. Hence, our results suggest that the infection dose is critical when studying host responses to pneumococcal infections.

PLY is not a typical adhesin and has previously been considered to be cytosolic and released only following bacterial lysis. However, recent transmission electron microscopy data have shown that pneumolysin can be localized on the surface, suggesting that it can be available for interactions with host receptors. The above data support our discovery that PLY interacts with MRC-1, which is a finding that represents a conceptual change in our current understanding. Our results suggest that the MRC-1–PLY interaction is not mediated by glycan recognition, since MRC-1 also binds to deglycosylated PLY, and the interaction is specifically mediated by type lectin domains 4–7 of MRC-1 and domain 4 of PLY.

In conclusion, we discovered a significant role for PLY, whereby MRC-1 acts as a receptor for PLY, enabling pneumococci to invade MRC-1-proficient immune cells, including DCs and alveolar macrophages, in the airways, thereby dampening cytokine responses to establish intracellular residency of pneumococci. While MRC-1 has previously been demonstrated to bind pneumococcal capsular polysaccharides, we show here that it can also directly bind to PLY. This is a hitherto unknown survival mechanism for the pneumococcus and has important implications for future vaccine design against infection. The potential mechanisms involved are summarized in Fig. 4g.

**Methods**

**Pneumococcal strains used.** The encapsulated *S. pneumoniae* serotype 4 strain TIGR4 (T4; ATCC BAA-334) as well as its non-encapsulated isogenic mutant T4R and its isogenic PLY-deficient mutants T4Rαply and T4RAply were used in this study. Clinical isolates of serotype 1 pneumococci expressing non-haemolytic PLY (BHN31 of ST306) and a clonally related strain expressing haemolytic PLY (BHN32 of ST228) were also used. Bacteria were grown on blood agar plates at 37°C in 5% CO2 for 24 h of infection. Cytolysin D (0.5 mM), wortmannin (0.1 mM) (Sigma) or staurosporine (5 μM) (Tocris Biosciences) was added to cells 15 min before pneumococcal infection. In some experiments, DCs were incubated with endothelin-free PLY at 0.2 μg/ml diluted in R10 medium.

**Human monocytic leukemia THP-1 cells** (ATCC TIB-202) were cultured in RPMI 1640 medium. For differentiation into macrophages, THP-1 cells were treated for 48 h with 20 ng/ml of phorbol myristate acetate (Sigma).

**Neutrophils** were isolated from whole blood following lysis of red blood cells and enriched using an EasySep human neutrophil enrichment kit (Stem Cell Technologies) according to the manufacturer’s instructions. Purified neutrophils were verified to be ~99% CD66b+CD16+. Human naive T cells were purified from fresh peripheral blood mononuclear cells using a EasySep Human Naive CD4+ T Cell Isolation kit (Stem Cell Technologies) and were verified by flow cytometry to be >95% CD3+CD4+.

**Isolation of mouse BMDMs and culture.** Bone marrow cells were flushed from mouse femurs and tibia. Macrophages were grown from bone marrow cells in Dulbecco’s modified Eagle’s Medium (Sigma) 10% (v/v) fetal calf serum (FCS; Sigma), penicillin (100 U/ml), streptomycin (100 mg/ml) and L-glutamine (100 mM; Sigma) supplemented with M-CSF (20 ng/ml; R&D Systems). Cultures were maintained in a humidified atmosphere (5% CO2) at 37°C, and medium was replaced on days 3 and 6. On day 6, cells were plated for use in assays. A total of 6.25 x 10^6 BMDMs were cultured alone (untreated) or infected with D39, D39△ply, D39△cps or DKO (D39△cps△ply) (1 macrophage;10 bacteria) or stimulated with purified PLY (4 μg/ml). After 24 h of incubation, supernatants were collected and used to assess cytokine production by ELISA or to determine the density of infection by Miles and Misra dilution.

**BMDM–T cell co-culture.** Naive CD25−CD4+ T cells were purified from spleens of C57BL/6 or MRC1−/− mice by negative selection (Miltenyi Biotec). Non-CD4+ T cells and CD4+ memory T cells were labelled with biotinylated antibodies before the addition of anti-biotin microbeads and magnetic separation. CD4+ T cell purity was >90%. Purified T cells were added to 24 hr pneumococcal-stimulated BMDMs at a ratio of 15:1 for 5 days. Culture supernatants were collected for ELISA, and cells were stained for flow cytometry.

**Cell viability assays.** Cytotoxicity was determined in the culture supernatants by measuring the release of the enzyme lactate dehydrogenase compared with a 100% lysis control using a Cytotoxicity kit (Roche) according to the manufacturer’s instructions.

**Real time qPCR.** Total cellular RNA was extracted from cells using a RNeasy Kit (Qiagen). The concentration and purity of isolated RNA was determined spectrophotometrically using a Nanodrop ND 1000 spectrophotometer. Complementary DNA (cDNA) was synthesized from isolated RNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems). The qPCR was performed using Taq Universal SYBR Green Supermix (Bio-Rad) according to the manufacturer’s instructions. The following primers were used: Hs_SOCS1_1_SG, Hs_MRC1_1_SG and Hs_GAPDH_1_SG. Each primer pair was validated for specificity by performing a melt curve analysis of the PCR product to ensure the absence of primer dimers and nonspecific products. The mRNA expression level was normalized to the level of GAPDH, and relative expression was determined using the ΔΔCT method. The TLR Signaling qPCR array (Qiagen) was performed according to the manufacturer’s instructions and analysed using the GeneGlobe Data Analysis Center (Qiagen).

**Mouse experiments and isolation of alveolar macrophages.** All mice experiments were performed in accordance with the local ethics committee (Stockholms Norra djurförsöksätska nämnd). Male wild-type C57BL/6 mice (6–7 weeks old) were used. Experiments with MRC1−/− mice were performed at the University of Liverpool with the approval of the UK Home Office and the University of Liverpool’s ethics committee. MRC1−/− mice were generated on a mixed 129SvJ−/− background, and then backcrossed to the C57BL/6 strain for at least seven generations. Homozygous knockout mice were bred and maintained at the University of Nottingham and were a gift from L. Martinez-Pomares (University of Nottingham). Wild-type and MRC1−/− mice used for infection were sex and age matched and no more than 12 weeks of age at the start of the study. Wild-type and MRC1−/− mice were randomized independently to time points by technical staff with no role in the study’s design. Researchers were blinded to the experimental group until the data analysis stage. Sample size calculations were performed using the appropriate designs and the values stated below were used.
determined to give 90% power, alpha = 5%, assuming an expected mean CFU for the wild type of 1,000 CFU per ml and s of 5% (determined from previous studies). The experiments were powered to detect an expected difference between groups of 10%.

Pneumococcal nasopharyngeal carriage model. For induction of pneumococcal nasopharyngeal carriage, mice were lightly anaesthetized and 10 μl of PBS containing 1 x 10^5 CFU D39 was administered into the nostrils. The dose was confirmed by viable count following infection. At pre-chosen time intervals following infection, mice were killed and nasopharynx, draining cervical lymph nodes and lungs were collected, passed through a 30-μm cell strainer or homogenized using an Ultra-Turrax T8 homogenizer (IKA). Bacterial counts were determined from tissue homogenates by viable count on blood agar plates.

Invasive pneumococcal disease model. Mice were sedated by inhalation of 4% isofluorane, and 50 μl of PBS containing 1 x 10^6 CFU of wild-type T4 or the PLY mutant T4Apyl was administered into the nostrils. To block MRC-1, 20 μg of 0.1 mg/ml monoclonal anti-MRC-1 antibody (Abcam) or isotype matched control (Abcam) was administered intranasally 30 min before infection. Post sacrifice, the BALF was spun down at 400 g for 7 min at 4 °C, resuspended in R10 medium (RPMI 1640 containing 2 mM L-glutamine and 10% FBS) and plated on coverslips for 1 h to allow cells to attach. Unattached cells were removed by washes with PBS. Macrophages were verified phenotypically by flow cytometry (CD11c+ Siglecs F+). The percentage of neutrophils (CD11b^+Ly6G^+^-) and monocytes (CD11b^+Ly6C^-) in the BALF was quantified by flow cytometry following gating for viable cells stained using the fixable viable dye eFlour 780 (Thermo Fisher Scientific).

MRC-1 knockdown using siRNA. DGs (6 x 10^5) were electroporated with 5 μg siRNA from Life Technologies against MRC-1 (s53926, s39327, s33928) or scrambled control siRNA (4390843, 4390846) on day 4 of DC differentiation. The cells were electroporated using a Bio-Rad gene pulser (square wave, 500 V , 0.5 ms with a single impulse) and immediately resuspended in fresh R10 medium. The cells were used 48 h after siRNA electroporation. Treatment with siRNA reduced MRC-1 protein expression by ~80% as evaluated by western blotting (Supplementary Fig. 3A).

Flow cytometry. Cells were fixed with 4% paraformaldehyde and stained with a mouse anti-MRC-1 antibody (Abcam) and a goat-anti mouse Alexa Fluor 488 secondary antibody (Life Technologies). For intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with ice-cold methanol. Cells were stained with phos-STAT1 (Tyr 701) Alexa Fluor 488 conjugated rabbit antibody (Cell Signaling), rabbit anti-SOCS1 (ab135718) and assessed by flow cytometry following gating for viable cells stained using the fixable viable dye eFlour 780 (Thermo Fisher Scientific).

Quantification of cytokines. For cytokine measurements, cell-free culture supernatants were collected 18 h post infection and frozen at ~20 °C. The levels of human TNF-α, IL-12p70, IL-10, IFN-γ and IL-4 were measured using an OptEIA ELISA kit (BD Biosciences). The levels of mouse TNF-α, IL-12p70, IL-10, IFN-γ and IL-17 were measured using an Cytometric Bead Array Kit (BD Biosciences). Data for injections of PLY over MRC-1 were fitted to a Langmuir 1:1 interaction using Biacore 4.1 software and the dissociation equilibrium constant was calculated from average association and dissociation rate constants obtained from three separate dilution series analysed on two different sensor chips. Human serum albumin, bovine serum albumin and trastuzumab (Herceptin) were injected at the non-specific binding controls. The non-specific binding was subtracted before calculating MRC-1 binding was pre-incubated with 100 nM of MRC with 0.5 μM D-mannose or D-glucose (Sigma) for 1 h in running buffer before injection of the mixed samples over the PLY immobilized chip.

ELISA to measure MRC-1-PLY binding. Briefly, 96-well flat-bottomed plates (Sigma, UK) were coated overnight with 1.25–10 μg/ml of mouse receptor, full-length (2534-MR-050) or truncated constructs CTD4–7–Fc, CRFNI-CTD4–7–Fc, a gift from Luisa Martinez-Pomares (University of Nottingham), in the presence or absence of galactose or mannose (Sigma) in coating buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, pH 9.6). Wells were blocked with 200 μl of 20% (v/v) FBS in PBS for 2 h, and then washed three times with 250 μl PBS, 0.05% (v/v) Tween 20 (Sigma). A total of 10 ng/ml of PLY, PLY Δ NTerminal domain 1–3, or PLY domain 4 was added and incubated at 37 °C for 1 h. Wells were washed again with PBS, and bound proteins were detected using a PLY polyclonal antibody (Abcam ab71811) in blocking buffer. Plates were incubated with anti-rabbit IgG alkaline phosphatase (Abcam, ab6722) in blocking buffer. Bound antibodies were detected using the chromogenic substrate p-nitrophenylphosphate for 30 min. NaOH (1 M) was added to all wells and the absorbance was measured at 405 nm.

To study the specific interaction of MRC-1 with PLY versus capsular polysaccharides, immobilized MRC-1 was incubated with PLY (0–5 μg/ml) in the presence or absence of 2.5 μg/ml of purified serotype 2 or type 4 capsules (Statens Serum Institut). Bound PLY was detected using mouse anti-PLY antibody and mouse IgG-HRP. Binding of the purified capsule to MRC-1 was detected using rabbit anti-capsule and anti-rabbit IgG-HRP. Bound antibodies were detected using the chromogenic substrate tetramethylbenzidine. Phosphoric acid (1 M) was used as the stop solution and absorbance was measured at 450 nm.

Immunofluorescence microscopy. Briefly, cells were fixed with 4% paraformaldehyde buffered in PBS for 10 min. Subsequently, the cells were permeabized using PBS containing 0.5% Tween 20 for 15 min. To block nonspecific interactions, cells were incubated with 5% FBS in PBS for 1 h. Lysosomes were stained using Lysotracker deep red (Thermo Fisher Scientific) before fixation. Early endosomes were stained using Alexa 647 conjugated anti-EAA-1 (Abcam). PLY was stained using mouse anti-PLY (Abcam) and Alexa 488 goat anti-mouse secondary antibody (Thermo Fisher Scientific). MRC-1 was detected using rabbit anti-MRC-1 (Abcam) and Alexa 555 goat anti-rabbit secondary antibody (Thermo Fisher Scientific). Pneumococci were stained using rabbit anti-pneumococcal anti-serum (Eurogentec) labelled with Alexa 488 using a Zenon Rabbit IgG Labeling kit (Thermo Fisher Scientific). Type 1 clinical strains were stained using anti-serum Type 1 (Statens Serum Institut). Samples were washed twice with PBS between the antibody incubations and mounted on slides using ProLong Diamond antifade reagent containing 4.6-diamidino-2-phenylindole (DAPI; Thermo Fisher Scientific). Images were acquired using a Delta Vision Elite microscope under a ×100 objective (GE Healthcare).
Western blotting. Cells were lysed with RIPA buffer containing 1× protease inhibitors (Roche) on ice for 15 min. Cell debris and nuclear material were pelleted by centrifuging at 13,000× g for 10 min at 4°C. The supernatant was transferred to a fresh tube and the pellet was suspended in 5× SDS sample buffer and resolved on 4–12% Bis-Tris gel (Invitrogen). Proteins were transferred to a polyvinylidene fluoride membrane and blocked with 5% skim milk powder in PBS containing 0.1% Tween 20. Proteins were detected using the following antibodies: mouse anti-human MHC-I (Abcam), SOCS antibody, NF-κB (p65) antibody (Santa Cruz) and a phospho-IκBα antibody (Cell Signaling Technologies) was used as a loading control. Anti-rabbit IgG or anti-mouse IgG conjugated to HRP (GE Healthcare) were used as secondary antibodies. Blots were developed using an Amersham ECL Plus Western blotting detection system (GE Healthcare) and a ChemiDoc XR+ (Bio-Rad Laboratories).

Statistical analysis. Data were statistically analysed using GraphPad Prism v5.04. Data of immune cells prepared from human donor blood were analysed using Wilcoxon matched-pairs signed rank tests. Data from THP-1 macrophages were analysed using Mann–Whitney tests. Comparison between groups was done using one-way or two-way ANOVA followed by Bonferroni or Tukey’s post-tests as indicated. All numerical data were analysed using unpaired t-tests. Differences were considered significant at *P < 0.05; **P < 0.01; NS, denotes not significant.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary. The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Author contributions
K.S., D.R.N., L.S., A. Kadoglu and B.H.-N. designed the study. K.S., L.S., G.D.L.M., H.M., S.K., J.D., M.Y., D.R.N., A.A., J.N., P.-Å.N., A. Kirby and L.P. performed the experiments. K.S., L.S., D.R.N., A. Kadoglu and B.H.-N. wrote the manuscript, and the other authors contributed to the writing. All authors read and approved the final version of the manuscript.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to A.K. or R.H.-N.

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Experimental design

1. Sample size

Describe how sample size was determined.

Each experiment was usually performed thrice. The sample size was chosen in order to achieve statistical significance. The exact number of samples (donors/mice) for the individual experiments are mentioned in the figure legends.

2. Data exclusions

Describe any data exclusions.

Data were generally not excluded.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

Experiments were repeated using different human donors and mice to check for reproducibility. Attempts at replication were successful.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Mice were randomly assigned to the experimental groups.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

No blinding was done but the major findings were also independently verified to be consistent by our collaborators in this study.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a  Confirmed

☐  The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)

☐  A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐  A statement indicating how many times each experiment was replicated

☐  The statistical test(s) used and whether they are one- or two-sided

Only common tests should be described solely by name; describe more complex techniques in the Methods section.

☐  A description of any assumptions or corrections, such as an adjustment for multiple comparisons

☐  Test values indicating whether an effect is present

Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.

☐  A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)

☐  Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.
7. Software

Describe the software used to analyze the data in this study.

Data were statistically analysed using GraphPad Prism version 5.04 for manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

No restrictions

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

All antibodies used in this study were purchased from commercial sources. The source of the antibodies are mentioned in the methods section.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Primary human dendritic cells and neutrophils were freshly isolated from buffy coat of healthy donors provided by Karolinska University Hospital and Uppsala University Hospital. Human THP-1 cell line was purchased from ATCC [TIB-202].

b. Describe the method of cell line authentication used.

Cell-lines were authenticated by using different frozen batches of cell lines and experiments were repeated using different batches of cells.

c. Report whether the cell lines were tested for mycoplasma contamination.

The THP-1 cell line was tested for mycoplasma and found to be negative. The primary cells were freshly isolated from buffy coat and used directly for the experiments.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

N/A

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

All mice experiments were performed in accordance with the local ethical committee (Stockholms Norra djurförsöksetiska nämnd). Six- to seven- weeks old male wild-type C57BL/6J mice were purchased from Charles River Laboratories. Experiments with MRC-1-/- mice were done at the University of Liverpool with the approval of the UK Home Office and the University of Liverpool ethics committee. MRC-1-/- mice were generated on a mixed 129SvJ and C57BL/6 background, and then backcrossed to C57BL/6 strain for at least 7 generations. Homozygous knockout mice were bred and maintained at the University of Nottingham and were a generous gift of Dr. Luisa Martinez-Pomares (University of Nottingham).

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

N/A