Host-pathogen interactions during (myco)bacterial respiratory tract infections
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Specific ICAM-3 grabbing nonintegrin-related 1 (SIGNR1) expressed by marginal zone macrophages is essential for the defense against pulmonary *Streptococcus pneumoniae* infection

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Abstract

The dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) homolog, SIGN-related 1 (SIGNR1) is a pathogen receptor expressed by splenic marginal zone and peritoneal macrophages and is essential for clearance of Streptococcus pneumoniae by phagocytosis after intraperitoneal infection. Here, we identified an important in vivo function for SIGNR1 in S. pneumoniae infection induced via its natural entrance route. Upon intranasal infection with S. pneumoniae, SIGNR1-deficient mice did not clear bacteria from lung and blood, and displayed severely enhanced inflammatory parameters compared to the wild-type mice. However, SIGNR1 is not expressed by alveolar macrophages, suggesting that another mechanism than a decrease in phagocytosis is responsible for this difference. Natural anti-phosphorylcholine IgM produced by marginal zone B cells is essential for protection against infection with S. pneumoniae. Strikingly, during infection, SIGNR1-deficient mice failed to produce a rapid anti-phosphorylcholine IgM response. Marginal zone macrophages have been suggested to capture antigens for presentation to marginal zone B cells. We demonstrated that marginal zone macrophages from SIGNR1-deficient mice in contrast to wild-type mice are not able to capture pneumococci from blood, suggesting that SIGNR1 on marginal zone macrophages captures S. pneumoniae for antigen presentation to and activation of marginal zone B cells resulting in an anti-phosphorylcholine IgM response.
Introduction

The first line of defense against invading *Streptococcus pneumoniae* is provided by the innate immune system. There is strong evidence that the complement system is important in pneumococcal infection (1), and the classical pathway, partially mediated by the binding of natural IgM antibodies to bacteria, is the most important pathway for activation of this system during innate immunity to *S. pneumoniae* (2).

Rapid antibody responses to pneumococci are also essential for effective elimination of the pathogen and, in this context, the spleen, with its highly specialized lymphoid compartment, plays a central role in clearing blood-borne pathogens. Pneumococcal polysaccharides are T-cell independent type 2 (Ti-2) antigens and splenic marginal zone B cells play an important role in such responses (3, 4). Splenic marginal zone B cells join B1 B cells to generate the initial humoral IgM response in the initial 3 days of a primary immune response to particulate bacterial antigens (5).

A role in the defense against pneumococcal infection may also exist for marginal zone macrophages that have been shown to capture and concentrate both Ti-2 and particulate antigens circulating in the blood (6, 7). Intravenous injected encapsulated *S. pneumoniae* was rapidly captured by marginal zone macrophages (7). The dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) homolog, SIGN-related 1 is a C-type lectin involved in the capture of Ti-2 antigens by marginal zone macrophages, since the rapid capture of pathogen polysaccharides such as mannan and dextran by marginal zone macrophages upon intravenous injection is inhibited by antibodies against SIGNR1 (8, 9). Moreover, using blocking antibodies Kang et al. demonstrated that SIGNR1 is involved in the capture of different serotypes of capsular polysaccharides from *S. pneumoniae* (10). Recently, Lanoue et al. demonstrated that that SIGNR1 plays a crucial role in the immune response against intraperitoneal infection of *S. pneumoniae* serotype 2 and 14 (11).

The authors show that SIGNR1 expressed by peritoneal macrophages plays a crucial role in the binding and concomitant phagocytosis of the bacteria. However, the natural infection pathway of *S. pneumoniae* is through inhalation via the upper airways and SIGNR1 is not expressed by the residing alveolar macrophages. Therefore, we have investigated the role of SIGNR1 in the defense against pulmonary infection with *S. pneumoniae* using mice with a genetic deletion of SIGNR1 (SIGNR11/1) (11). We demonstrate that SIGNR11/1 mice are more susceptible to pulmonary *S. pneumoniae* infection than WT mice, and that SIGNR11/1 mice fail to induce an early IgM response against *S. pneumoniae*. Furthermore, our data suggest that SIGNR1 expressed by marginal zone macrophages is pivotal for the capture of *S. pneumoniae* from blood and is involved in the induction of the early
IgM response against *S. pneumoniae* which might be necessary for protection against these bacteria.

**Materials and Methods**

**Mice and bacteria**
SIGNR1<sup>−/−</sup> mice were a generous gift from A. McKenzie (Cambridge, UK). C57Bl/6x129 WT and SIGNR1<sup>−/−</sup> mice were bred in the animal facility of the VU University Medical Center under specific pathogen-free conditions, and were kept in the animal facilities of the VU University Medical Center and the Academic Medical Center in Amsterdam, The Netherlands. Age and sex-matched mice were used in all experiments. The experiments have been approved by the Animal Care and Use Committee of the University of Amsterdam and VU University Medical Center. *S. pneumoniae* serotype 3 (ATCC 6303, Rockville, MD) were used in mid-logarithmic phase and concentrations were determined by plating dilutions on sheep-blood agar plates.

**Immunofluorescence analysis**
8 μm cryosections were fixed in dehydrated acetone for 10 minutes, rehydrated with PBS and stained for 90 minutes at 37°C with primary antibodies ED31 (anti-MARCO), SER-4 (anti-Sialoadhesin) or ER-TR9 (anti-SIGNR1). The appropriate secondary antibodies were used for detection. Heat killed *S. pneumoniae* serotype 3 was detected by a pneumococcal polysaccharide serotype 3-specific rabbit polyclonal (Statens Serum Institute, Copenhagen, Denmark).

**In vivo antigen capture by marginal zone macrophages**
Naïve mice were injected intravenously into the tail veins with either 100 μl FITC-dextran (0.5 mg/mL, 500-kDa dextran-FITC; Molecular Probes, Eugene, OR), or 200 μl heat killed *S. pneumoniae* serotype 3 (1.3x10<sup>6</sup> CFU/ml). After 45 minutes mice were sacrificed and spleens were isolated and antigens were detected by immunofluorescence analysis.

**Binding Assay**
Raji-1 cells stably expressing SIGNR1 or freshly isolated peritoneal macrophages were preincubated in adhesion buffer (20 mM Tris, pH 8.0, 150 mM NaCl, 1 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>, 0.5% BSA) with either 2 mg/ml mannan (SIGMA, St. Louis, MO), 50 mM EGTA, 2.6x10<sup>7</sup> CFU/ml heat killed *S. pneumoniae* serotype 3 (ATCC 6303, Rockville, MD), 1x10<sup>9</sup> CFU/ml *Escherichia coli* (strain AMC B12G1) or 2x10<sup>9</sup> CFU/ml heat killed non-typeable *Haemophilus influenzae* (strain 12 kindly donated by S.J. Barenkamp, St. Louis, MO) for 15 minutes at room temperature. FITC-dextran
(50 μg/mL, 500-kDa dextran-FITC; Molecular Probes, Eugene, OR) was added and after 45 minutes at 37°C analyzed by flow cytometry.

Induction of pneumonia
Pneumonia was induced as described previously (12). Mice were inoculated with bacteria in a 50μl volume (5x10⁴ CFU) intranasally, and 24 or 48 hours after infection, mice were sacrificed. CFUs were determined from tissue homogenates. Cytokines and chemokines were measured using specific ELISAs (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. Lungs for histology were harvested at 24 and 48 hours after infection, fixed in 10% buffered formalin in PBS for 24 hours and embedded in paraffin. 4 μm thick lung sections were stained with H&E, and scored by a pathologist who was blinded for groups (13). Lung inflammation and damage were scored by analyzing the entire lung surface with respect to the following parameters: interstitial inflammation, edema, endothelialitis, bronchitis and pleuritis. Each parameter was graded on a scale of 0 to 5; from 0: absent to 5: severe. The total “lung inflammation score” was expressed as the sum of the scores for each parameter, the maximum being 25. Plasma samples were analyzed for the presence of anti-phosphorylcholine (PC) IgM antibodies by ELISA on PC-BSA coated wells. Specificity was determined by subtracting the coating buffer background levels from the PC coated values. All data are expressed as mean ± SEM unless indicated otherwise. Comparisons between groups were performed with Mann-Whitney U tests using GraphPad Prism version 3.00, GraphPad Software (San Diego, CA). Values of P<0.05 were considered statistically significant.

Immunization
Naive animals were injected intraperitoneally with 0.1 mg/mL TNP-KLH in 0.5 ml PBS. Sera were collected before and weekly thereafter for assessment of TNP-KLH specific IgG antibodies by ELISA.

Results
SIGNR1 expression and function
The C-type lectin SIGNR1 is expressed by splenic marginal zone macrophages and peritoneal macrophages (8, 14), but not by alveolar macrophages in the lung (data not shown). First the lack of SIGNR1 expression by marginal zone and peritoneal macrophages of SIGNR1−/− mice was confirmed (fig. 1A and B). SIGNR1 deficiency in marginal zone macrophages from SIGNR1−/− mice did not alter the architecture of the macrophage subsets present in the marginal zone (fig. 1A), since the localization of both the MARCO+ marginal zone macrophages and the sialoadhesin+...
marginal zone metallophilic macrophages were comparable to WT (fig. 1A). I.v. injection of the TI-2 antigen dextran-FITC results in a rapid capture by marginal zone macrophages through SIGNR1 (B). As expected, the marginal zone macrophages from SIGNR1<sup>−/−</sup> mice failed to capture dextran-FITC after i.v. administration (fig. 1A). Similarly, SIGNR1<sup>−/−</sup> peritoneal macrophages efficiently bound dextran-FITC, whereas peritoneal macrophages from SIGNR1<sup>−/−</sup> mice did not (fig. 1C). These data support an essential role for SIGNR1 in the capture of TI-2 antigens by both marginal zone and peritoneal macrophages.

![Figure 1](image)

**Figure 1:** SIGNR1 captures blood-borne TI-2 antigens in vivo. A) The splenic marginal zone from SIGNR1<sup>−/−</sup> mice contains both marginal zone macrophage subsets (marginal zone macrophages (MZM) and marginal metallophilic macrophages (MMM) respectively), but does not capture TI-2 antigens. Spleens from naïve WT (left) and SIGNR1<sup>−/−</sup> (right) mice were stained for MARCO<sup>−</sup> M2M in red and SER-4<sup>−</sup> M1M in green (upper panel), for SIGNR1 with ER-TR9 (middle panel) and for capture of Dextran-FITC 45 minutes after i.v. administration (lower panel). These data are representative for three animals of both genotypes. B and C) Peritoneal macrophages from SIGNR1<sup>−/−</sup> mice do not capture TI-2 antigens. Peritoneal macrophages were analyzed for SIGNR1 expression using ER-TR9 (B), and binding to dextran-FITC was analyzed in the presence and absence of the blocking reagents mannan and EGTA (C). These data are representative for three independent experiments. For color figure, please see page 256 of this thesis.

**SIGNR1 interacts with *S. pneumoniae* serotype 3**

In order to investigate the role of SIGNR1 in the infection with the virulent *S. pneumoniae* serotype 3, the SIGNR1 interaction with this pneumococcus was studied using a dextran-blocking assay. Cell-lines expressing SIGNR1 efficiently capture dextran-FITC and this interaction was blocked by pre-incubation of the transfectants with *S. pneumoniae* serotype 3 (fig. 2A). In contrast, pre-incubation with other bacteria such as *Escherichia coli* and non-typeable *Haemophilus influenzae* did not inhibit binding of SIGNR1 transfectants to dextran-FITC (fig. 2A). Thus, SIGNR1 binds whole *S. pneumoniae* serotype 3, whereas it does not interact with other bacteria such as *E. coli* and *H. influenzae* (fig. 2A).
SIGNR1<sup>−/−</sup> mice are more susceptible to pulmonary infection with *S. pneumoniae* serotype 3

Next, WT and SIGNR1<sup>−/−</sup> mice were challenged by intranasal administration of 5x10<sup>4</sup> CFU *S. pneumoniae* serotype 3. To investigate the role of SIGNR1 during infection of *S. pneumoniae*, we determined the bacterial load in the lungs 24 and 48 hours after infection. Between 24 and 48 hours after infection, the bacterial load in SIGNR1<sup>−/−</sup> mice increased, whereas the WT mice started clearing the bacteria (fig. 2B). Thus, SIGNR1<sup>−/−</sup> mice are unable to clear the pneumococci, suggesting that SIGNR1 is involved in the early defense against *S. pneumoniae* infection. Clearance of bacteria from the respiratory tract during pneumococcal pneumonia strongly depends on the efficacy in mounting a local inflammatory response. To investigate the role of SIGNR1 in the inflammatory response, we evaluated the inflammation of the lung during infection with *S. pneumoniae* in vivo after 24 and 48 hours. Relative lung wet weights were calculated and lung histology sections were scored as described in Materials and Methods. Lungs of SIGNR1<sup>−/−</sup>
mice were enlarged and SIGNR1/− mice displayed a significantly increased inflammation of the lungs when compared to WT (fig. 2C and D). In accordance, lungs from SIGNR1/− mice contained more neutrophils than those from WT and substantial more edema, as demonstrated by H&E staining and increased wet lung weight (fig. 2E and C). Thus, S. pneumoniae induced a strong inflammatory response in SIGNR1/− mice, which was associated with an increased influx of neutrophils into the lungs. Next, we investigated whether SIGNR1/− mice have different pulmonary cytokine responses to respiratory tract infection with pneumococci. Therefore, we determined the concentrations of the pro-inflammatory cytokines IL-1β, TNFα and the chemokine KC 24 and 48 hours after infection. SIGNR1/− mice did not exhibit an increased immune response early after infection, since cytokine levels did not differ between SIGNR1/− and WT mice after 24 hours of infection (fig. 3A, B and C). However, at 48 hours of infection, the cytokine levels in the lungs from SIGNR1/− mice were strongly elevated due to massive inflammation (fig. 3A, B and C). Furthermore, five of eight SIGNR1/− mice developed bacteremia after 24 hours and seven of eight after 48 hours, compared to three of eight WT mice after both 24 and 48 hours. Dissemination of bacteria to spleen and subsequent growth was strongly increased in SIGNR1/− mice, since they had 100-fold more CFUs in spleen after 48 hours than did WT mice (fig. 3D). These data demonstrate that, although SIGNR1/− mice are able to mount a neutrophil-attracting inflammatory response upon infection with S. pneumoniae, the pneumococci are not cleared from the lung. Moreover, SIGNR1/− mice are not able to clear the bacteria from blood, suggesting that SIGNR1 is necessary for clearance of blood-borne pathogens.

![Figure 3: Upon infection with S. pneumoniae, SIGNR1/− mice display similar early pulmonary cytokine responses compared to WT mice. Cytokine production in the lung (A, B and C) was measured 24 and 48 hours after infection. Bacterial outgrowth from the spleen (D) was measured 48 hours after infection. *P <0.05, **P<0.001](image-url)
SIGNR1 is essential for the capture of blood-born S. pneumoniae serotype 3

Although SIGNR1 is not expressed by resting alveolar macrophages in the lung, we investigated whether SIGNR1 is upregulated after infection with S. pneumoniae. Immunofluorescence analysis of lung tissues demonstrated that SIGNR1 expression was not induced upon S. pneumoniae infection (data not shown), indicating that the failure of the SIGNR1+/- mice to clear the S. pneumoniae is not due to an altered function of alveolar macrophages. This was supported by a similar cytokine profile compared to WT after 24 hours (fig. 3A). To further elucidate the role of SIGNR1 in the clearance of S. pneumoniae, we studied the capture of S. pneumoniae by marginal zone macrophages. After intravenous injection, S. pneumoniae was captured by the marginal zone in the WT whereas hardly any S. pneumoniae was detected in the marginal zone of SIGNR1+/- mice (fig. 4A and B). Moreover, S. pneumoniae particles that were observed in the spleen of the SIGNR1+/- mice were primarily localized in the red pulp (fig. 4C). Thus, SIGNR1 is

SIGNR1 in pneumonia

Figure 4: SIGNR1 is pivotal for the in vivo capture of blood-borne S. pneumoniae serotype 3 by the marginal zone. A) S. pneumoniae is not captured in vivo by the splenic marginal zone in SIGNR1+/- mice. The capture of i.v. injected S. pneumoniae was determined in 5 representative visual fields of spleen sections of WT (upper) and SIGNR1+/- (lower panel) mice after 45 minutes. Spleens were stained for MARCO+ marginal zone macrophages (M2M) in red and S. pneumoniae in green. Inset shows pneumococci colocalizing and in close contact with WT MARCO+ M2M. Inset shows the absence of pneumococci in the marginal zone of SIGNR1+/- mice. B) S. pneumoniae is captured by the marginal zone of the WT spleen whereas the pneumococci are hardly detected in the marginal zone of the SIGNR1+/- spleen. The amount of S. pneumoniae particles residing in the marginal zone of either WT or SIGNR1+/- mice was counted and compared. The capture of i.v. injected S. pneumoniae was determined in 5 representative visual fields of spleen sections of WT and SIGNR1+/- mice after 45 minutes. C) The S. pneumoniae particles present in the SIGNR1+/- spleen reside mostly in the red pulp area of the spleen in contrast to the S. pneumoniae particles present in the WT spleen which target to the marginal zone. These data are representative for three animals of both genotypes. For color figure, please see page 256 of this thesis.
essential for the capture of whole \textit{S. pneumoniae} by the marginal zone of the spleen.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{SIGNR1\textsuperscript{-/-} mice fail to raise a rapid anti-phosphorylcholine IgM antibody response in vivo during infection with \textit{S. pneumoniae}. The anti-phosphorylcholine (PC) IgM levels were measured in plasma before (A) and 24 (B) or 48 hours (C) after infection with 5x10\textsuperscript{4} CFU \textit{S. pneumoniae}. Horizontal bars represent mean anti-PC IgM levels. ***P<0.001}
\end{figure}

\textit{SIGNR1} is pivotal for the induction of a natural IgM response against \textit{S. pneumoniae}

Splenic marginal zone B cells are essential for the rapid antibody response against TI-2 polysaccharides, such as the capsular polysaccharides of \textit{S. pneumoniae} (15). In the early phase of pneumococcal infection, IgM antibodies, in particular natural antibodies against the \textit{S. pneumoniae}-epitope phosphorylcholine (PC), play an important role in the protection against pneumococci (16, 17). As SIGNR1 is able to capture \textit{S. pneumoniae} from blood, resulting in \textit{S. pneumoniae} accumulation in the marginal zone, close to marginal zone B cells, we investigated whether SIGNR1 is involved in the rapid increase of natural antibody production by marginal zone B cells. Therefore, we determined the anti-PC IgM response during the infection. Strikingly, whereas in WT mice natural anti-PC IgM levels increased upon infection, no increase in natural anti-PC IgM production was observed during infection in SIGNR1\textsuperscript{-/-} mice (fig. 5). These data suggest that SIGNR1-mediated capture of blood-borne \textit{S. pneumoniae} is necessary for an efficient anti-PC IgM response by marginal zone B cells. However, upon administration of an irrelevant antigen (TNP-KLH), no differences in IgG response were observed between WT and SIGNR1\textsuperscript{-/-} mice (fig. 6). Hence, SIGNR1\textsuperscript{-/-} mice do display a normal follicular B cell response.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{SIGNR1\textsuperscript{-/-} mice display a normal follicular B cell-mediated immune response. The anti-TNP-KLH IgG levels were measured in serum at day 0, 7 and 14 days after intraperitoneal administration of 0.1 mg/mL TNP-KLH.}
\end{figure}
Discussion

In this study we have identified an important in vivo function for the murine homolog of DC-SIGN in S. pneumoniae infection induced via its natural entrance route. We demonstrate that SIGNR1−/− mice are more susceptible to intranasally administrated S. pneumoniae than WT mice. Strikingly, in contrast to infected WT mice, infected SIGNR1−/− mice fail to induce natural anti-PC IgM antibodies, known to be pivotal for protection against S. pneumoniae (16-19). Our data suggest that the SIGNR1−/− marginal zone macrophages are essential for the natural anti-PC IgM response by marginal zone B cells.

The marginal zone of the spleen is a unique compartment that facilitates the capture of blood-borne antigens for eliciting rapid antibody responses against bacteria (20). Marginal zone macrophages are strategically situated in the marginal zone to capture antigens from blood. Recently, we have demonstrated that SIGNR1, a murine homolog of DC-SIGN, is specifically expressed by marginal zone macrophages and mediates the capture of blood-borne TI-2 antigens (8, 9). Similar to DC-SIGN, SIGNR1 interacts with different pathogens, including viruses and bacteria (10, 14), suggesting that SIGNR1 might be an important pathogen receptor on these macrophages. Marginal zone macrophages efficiently capture particulate S. pneumoniae present in blood (6) and our data demonstrate that SIGNR1 mediates capture of these particulate bacteria, since marginal zone macrophages from SIGNR1−/− mice do not capture i.v. injected S. pneumoniae serotype 3 (Figure 4). The failure of the SIGNR1−/− mice to clear the bacteria from blood after infection and the 100-fold increase in splenic bacterial load compared to WT mice supports the importance of SIGNR1 as a pathogen receptor on marginal zone macrophages. Recently, Lanoue et al. demonstrated that SIGNR1 is involved in the capture of S. pneumoniae (11). In a peritonitis model using S. pneumoniae serotype 2 and 14, Lanoue et al. demonstrated that SIGNR1 expressed by peritoneal macrophages is important in the binding and subsequent phagocytosis of the pneumococci as peritoneal macrophages derived from SIGNR1−/− mice fail to clear injected S. pneumoniae, which causes severe infection (11). Thus, SIGNR1 is an important receptor for the defense against S. pneumoniae and is involved in the clearance of bacteria from the peritoneal cavity. However, the common route of infection by S. pneumoniae is through the upper airways. Strikingly, alveolar macrophages do not express SIGNR1, suggesting that SIGNR1 might not be involved in the defense against S. pneumoniae upon infection via the natural route. Therefore, we have mimicked the natural route of S. pneumoniae infection. In our experiments the SIGNR1−/− mice displayed a significantly increased inflammation of the lungs when compared to WT mice characterized by extensive infiltration with neutrophils and substantial more edema (Figure 2). Alveolar macrophages do not express SIGNR1, and we did not observe SIGNR1 expression after infection (data not
shown), indicating that SIGNR1 is not directly involved as a pathogen receptor on alveolar macrophages in the clearance of the infection. The increased influx of neutrophils in the SIGNR1−/− mice suggested that the neutrophils were unable to clear the bacteria even though the SIGNR1−/− mice were able to mount an inflammatory response.

Interestingly, in pneumococcal infection, natural IgM antibodies against PC play an important role (16-19). Recently Lanoue et al. demonstrated that the levels of anti-PC antibodies do not differ between naïve WT and SIGNR1−/− mice (11). In this study we show that although the SIGNR1−/− mice display a normal follicular B cell-mediated immune response, intranasal infection of S. pneumoniae serotype 3 results in an elevation of anti-PC IgM antibodies during infection only in the WT mice. The SIGNR1−/− mice did not mount an efficient anti-PC IgM response as no increase in anti-PC IgM was observed in the blood from SIGNR1−/− mice during infection. These data suggest that the inability of the SIGNR1−/− mice to clear the infection is due to the lack of early IgM antibodies against S. pneumoniae. The marginal zone B cells are essential in the induction of a rapid IgM response against encapsulated bacteria, such as S. pneumoniae (15, 21). Although marginal zone macrophages are ideally localized to capture blood-borne antigens, their role in the early innate immune response is unclear. Early experiments suggested that marginal zone macrophages, due to their localization and ability to capture blood-borne antigens, are critical components of the TI-2 antibody responses (3, 22). Recently, Kang et al. demonstrated that SIGNR1 on marginal zone macrophages efficiently captures pneumococcal polysaccharides (10). Marginal zone macrophages might capture polysaccharides for processing and presentation of the TI-2 antigens to the marginal zone B cells. However, in vivo depletion of marginal zone macrophages did not lead to a decreased TI-2 response against soluble TI-2 antigens (20, 23, 24). Our data suggest that the capture of whole encapsulated bacteria by SIGNR1+ marginal zone macrophages is essential to induce an early immune response in vivo, which is pivotal for the protection against S. pneumoniae. It is possible that whole encapsulated bacteria are not efficiently recognized by marginal zone B cells during infection in vivo, and that the marginal zone macrophages are necessary for capture and processing of the bacteria followed by the presentation of bacterial fragments to marginal zone B cells. The close localization of the marginal zone macrophages and B cells suggests that either whole bacteria or antigens from the processed bacteria might be presented by marginal zone macrophages to marginal zone B cells, reminiscent of the role of the human homolog of SIGNR1 in HIV-1 transmission; HIV-1 is captured by dendritic cells through DC-SIGN and transmitted to T cells (25), suggesting that these C-type lectins are able to capture pathogens to present either whole particulate antigens or components to adjacent leukocytes.
Moreover, marginal zone macrophage-produced signals following the capture of bacteria might be necessary in the activation of marginal zone B cells, since TI-2 antigens alone are not sufficient to induce proliferation of marginal zone B cells, and co-signals from mitogens present on bacteria as well as from accessory cells such as macrophages are needed to activate the marginal zone B cells (26). Thus, SIGNR1" marginal zone macrophages may be involved in natural antibody production against \textit{S. pneumoniae}, which is pivotal for defense against \textit{S. pneumoniae}. Further studies are needed to demonstrate whether the lack of anti-PC IgM antibodies is the only factor involved in the increased susceptibility of the SIGNR1" mice and how SIGNR1 expressed by marginal zone macrophages is involved in the early humoral immune response against \textit{S. pneumoniae}, either through activation of marginal zone B cells or by presentation of captured antigens to marginal zone B cells.

\textbf{Acknowledgments}

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\textbf{References}


