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An Important Role for Polymeric Ig Receptor-Mediated Transport of IgA in Protection against *Streptococcus pneumoniae* Nasopharyngeal Carriage

Keer Sun,* Finn-Eirik Johansen,‡ Lars Eckmann,‡ and Dennis W. Metzger*2*

The importance of IgA for protection at mucosal surfaces remains unclear, and in fact, it has been reported that IgA-deficient mice have fully functional vaccine-induced immunity against several bacterial and viral pathogens. The role of respiratory Ab in preventing colonization by *Streptococcus pneumoniae* has now been examined using polymeric IgR knockout (pIgR−/−) mice, which lack the ability to actively secrete IgA into the mucosal lumen. Intranasal vaccination with a protein conjugate vaccine elicited serotype-specific anti-capsular polysaccharide Ab locally and systemically, and pIgR−/− mice produced levels of total serum Ab after vaccination that were similar to wild-type mice. However, pIgR−/− mice had 5-fold more systemic IgA and 6-fold less nasal IgA Ab than wild-type mice due to defective transport into mucosal tissues. Wild-type, but not pIgR−/− mice were protected against infection with serotype 14 *S. pneumoniae*, which causes mucosal colonization but does not induce systemic inflammatory responses in mice. The relative importance of secretory IgA in host defense was further shown by the finding that intranasally vaccinated wild-type and pIgR−/− mice were not protected from colonization. Although secretory IgA was found to be important for protection against nasal carriage, it does not appear to have a crucial role in immunity to systemic pneumococcus infection, because both vaccinated wild-type and pIgR−/− mice were fully protected from lethal systemic infection by serotype 3 pneumococci. The results demonstrate the critical role of secretory IgA in protection against pneumococcal nasal colonization and suggest that directed targeting to mucosal tissues will be needed for effective vaccination in humans. *The Journal of Immunology*, 2004, 173: 4576–4581.

Colonization of the upper respiratory tract by *Streptococcus pneumoniae* is a major cause of community-acquired pneumococcal infection (1). Defense against pathogens in the respiratory tract relies upon immune mechanisms located in the airways (2) and the alveolar spaces (3, 4) and while the currently licensed 7-valent polysaccharide (PS) conjugate vaccine given s.c. can elicit effective defense against systemic *S. pneumoniae* infection, it provides only marginal protection against nasopharyngeal carriage, presumably due to an inability to induce effective immunity at respiratory sites (3). As Gram-positive, extracellular bacteria, encapsulated pneumococci are eliminated from the lung primarily through phagocytosis and intracellular killing by alveolar macrophages and neutrophils recruited to the site of infection (5). Pneumococcal-specific Ab efficiently facilitates this phagocytosis process. Nonetheless, the relative importance of mucosal vs serum Abs in controlling pneumococcal nasal colonization still remains poorly defined (6–8).

Dimeric IgA, with an incorporated J chain, is the predominant Ab isotype expressed by B cells in the mucosal lamina propria. It is actively transported by the epithelial polymeric Ig receptor (pIgR) and released into mucosal secretions as secretory IgA (SIgA), a complex of dimeric IgA with a bound secretory component (the extracellular domain of the pIgR; Ref. 4). Luminal SIgA is believed to interfere with pathogen adherence to mucosal epithelial cells, a process called immune exclusion (9). Human serum IgA has also been reported to induce complement-mediated killing of *S. pneumoniae* and to enhance phagocytosis (8). Mice with a disruption in the pIgR gene (pIgR−/− mice) cannot transport IgA or IgM into the mucosal lumen, and thus offer an opportunity to determine the importance of respiratory SIgA in host defense against pneumococcal colonization. Previous studies have shown that pIgR−/− mice have reduced protection against influenza virus infection in the upper respiratory tract, which cannot be substituted by serum IgG (2). Nevertheless, it has been reported that IgA is not necessary for vaccine-induced protection against bacterial and viral mucosal pathogens, including the influenza virus (10), rotavirus (11), *Shigella flexneri* (12), and *Helicobacter pylori* (13).

We have now evaluated protection by mucosal and systemic Abs in pIgR−/− and wild-type mice after intranasal (i.n.) and parenteral immunization with polysaccharide conjugate vaccine, using IL-12 as a mucosal adjuvant as previously described (14). The role of SIgA in the defense against pneumococcal respiratory infection was also investigated using IgA gene-deficient (IgA−/−) mice. The results show that while serum Ab can protect against systemic infection (15), secretory IgA is critical for control of nasal carriage.

**Materials and Methods**

**Mice**

pIgR−/− mice on a B6 × 129 background were originally developed as described (16). Due to the defective transport of IgA into the mucosal...
lumen, there are significantly higher levels of IgA in sera of these pIgR mice compared with wild-type mice. IgA gene-deficient (IgA−/−) mice (17) were obtained from Baylor College of Medicine (Houston, TX). Wild-type C57BL/6129 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and used as controls for both strains. All mice were bred and maintained at the Albany Medical College Animal Facility following Institutional Animal Care and Use Committee guidelines (Albany, NY).

Immunizations

To induce mucosal Ab responses, mice were immunized i.n. using IL-12 as an adjuvant essentially as described previously (14). In brief, mice were anesthetized and immunized i.n. on days 0 and 21 with 2.5 μg of serotype 14 PS conjugate vaccine (PS14-CRM197; kindly provided by Wyeth Vaccines, Pearl River, NY) mixed with 1 μg of murine IL-12 (Wyeth Vaccines), followed by i.n. administration of 1 μg of murine IL-12 alone on days 1, 2, and 3. The total volume of vaccine and IL-12 per inoculation was 25 μl in PBS containing 1% normal mouse serum (NMS). PS3-CRM197 serotype 3 vaccine (1 μg in 200 μl saline; Wyeth Vaccines) was given i.p on days 0 and 21 together with 2 mg/ml alum (Rehydrogel Low Viscosity Gel; Reheis, Berkeley Heights, NJ). Control animals received either no pretreatment before infection or were treated with IL-12 only. No immune responses were noted in the absence of IL-12 coadministration.

Sera and nasal washes

Serum samples were obtained by tail vein bleeding or by retro orbital puncture under anesthesia. In addition, 3 days after infection, anesthetized mice were bled at the Albany Medical College Animal Facility following Institutional Animal Care and Use Committee guidelines (Albany, NY).

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Mice were sacrificed 3 days after i.n. infection and the lungs were removed for histological analyses. Paraffin-embedded tissues were sectioned to a thickness of 5 μm and stained with H&E by standard methods.

**Statistical analysis**

Statistical comparisons of Ab levels were performed on log-transformed data by the Student’s t test. Mann-Whitney U test analysis was used for comparison of nasal carriage between experimental groups.

**Results**

**Serotype 14 PS-specific Ab responses in wild-type and pIgR−/− mice after i.n. vaccination and infection**

Serotype 14 PS conjugate vaccine was delivered i.n. to wild-type and pIgR−/− mice using IL-12 as an adjuvant to induce mucosal immunity. The immunized mice were then bled 4 wk later (7 days after secondary boosting) and serum samples were examined before bacterial challenge by ELISA to determine PS14-specific systemic Ab levels (Fig. 1A). Although there were no significant differences in total and IgG serum Ab levels between the two groups of mice, IgA was found to accumulate in the sera of pIgR−/− mice. Lung infection with serotype 14 S. pneumoniae induced similar increases in systemic Ag-specific Ab production in the two groups of mice (Fig. 1B). Naïve mice had no detectable PS14-specific Abs in sera, and i.n. infection of these mice induced a small but detectable serum Ab response (data not shown).

Ab levels in nasal washes of immunized mice were also examined. Nasal PS14-specific IgA Ab was detected in wild-type but not pIgR−/− mice (Fig. 1C). Because the most abundant Ab iso-type in the upper respiratory tract is IgA, there was also little total Ab found in nasal washes of pIgR−/− mice. Similarly, after infection, nasal Abs were only found in wild-type mice (Fig. 1D).

**Mucosally immunized pIgR−/− mice are not protected against nasal carriage by S. pneumoniae**

Ten days after boosting with vaccine, the mice were challenged i.n. with serotype 14 pneumococci and 72 h later, the number of pneumococci in the nose was quantified (Fig. 2A). The effect of immunization on protection against bacterial carriage was found to be significant only in wild-type mice (p < 0.05 compared with unimmunized mice treated only with IL-12). Immunization resulted in more than a 10-fold decrease in bacterial load in these mice but no significant decrease was observed in pIgR−/− mice. Nasal vaccination did not induce the same level of Ab responses in all mice (Fig. 1) and the numbers of nasal-associated bacteria tended to have a negative correlation with Ab levels in nasal washes, i.e., the five wild-type mice with total nasal PS14-specific Ab levels >0.1 OD were also the mice that had nearly complete clearance of pneumococci from their noses (Fig. 2B). There was also a slight negative correlation between the numbers of nasal-associated bacteria and serum levels of PS14-specific Ab. In contrast to the results in wild-type mice, immunization had no discernable effect on nasopharyngeal carriage in pIgR−/− mice (no significant difference from unimmunized mice). Although significant levels of serum (but not nasal) Abs were detected in these mice, there was no correlation between numbers of nasal-associated pneumococci and levels of serum Ab in individual pIgR−/− mice. These results indicate that SIgA is necessary for protection against pneumococcal colonization.

**The role of SIgA in mediating protection against bacterial carriage**

To further address the role of SIgA in protection against pneumococcal nasal carriage, studies were performed using IgA−/− mice. Vaccination i.n. with PS14 conjugate vaccine in the presence of
IL-12 tended to induce lower overall serum Ab responses in IgA−/− mice compared with wild-type mice but these differences were not statistically significant (Fig. 3A). However, the IgA−/− mice produced little respiratory Ab (Fig. 3B) and immunization of IgA−/− mice did not induce protection against bacterial colonization (Fig. 3C). There was no correlation between serum Ab titers and bacterial carriage in individual IgA−/− mice. As above, the vaccination protected wild-type mice from colonization, which was correlated with significant mucosal Ab titers.

**Passive protection by pIgR−/− serum**

The results indicated that mucosal Ab plays a necessary role in protection against nasal colonization, a role that cannot be replaced by Ab in serum. However, it was not clear whether the mucosal Abs possessed unique protective functions or if serum Abs could also mediate protection if they were able to reach the bacteria in the upper respiratory tract. To investigate the protective capability of serum PS14-specific Abs present in pIgR−/− mice, bacteria were opsonized in vitro with immune serum collected from pIgR−/− mice vaccinated i.n. with IL-12 as above and then used to challenge wild-type and IgA−/− mice. The observed passive protection provided by pIgR−/− immune serum in both strains (Fig. 4) indicates that the susceptibility of vaccinated pIgR−/− mice to pneumococcal carriage simply reflects the inability of Abs to be transported to the respiratory tract mucosal surface.

**Nasal colonization by serotype 14 pneumococci does not induce inflammatory responses**

Because nasal colonization of humans by *S. pneumoniae* occurs asymptptomatically and in the absence of inflammation, it was important to ensure the lack of lung inflammation in our mouse model of colonization. Mice were sacrificed 3 days after i.n. infection and lungs were prepared for histological analysis. No significant tissue alternations were observed after serotype 14 pneumococcal infection (Fig. 5), suggesting that nasal infection with this strain does not lead to lung inflammation, which in turn, could initiate systemic infection. Three days after i.n. challenge, bacterial burden in the upper respiratory tract, lung, and bloodstream were also examined by plating nasal washes, lung homogenates, and whole blood on blood agar plates. All mice were nasally colonized after i.n. infection (~1000 CFU per mouse); however, there were few bacteria in the lower respiratory tract (~10 CFU per mouse) in either pIgR−/− or wild-type mice. There were no detectable bacteria in the blood of infected pIgR−/− or wild-type mice. Thus, i.n. inoculation of serotype 14 bacteria did not lead to systemic infection.

**The role of secretory Ab in protection against systemic infection**

In a final set of experiments, the role of Abs in protection against systemic infection was determined. For this purpose, mice were immunized i.p. and then challenged i.p with pneumococcal serotype 3, a serotype that, unlike serotype 14, leads to invasive disease. It was found that immunization of both wild-type and pIgR−/− mice induced high levels of serum Abs and that pIgR−/− mice had much higher IgA Ab titers compared with wild-type mice (Fig. 6A). In addition, both groups of mice were fully protected from a lethal dose of bacteria (Fig. 6B). Thus, although pIgR−/− mice do not express secretory Abs and are not protected against nasal carriage, they are fully protected against systemic infection after parenteral vaccination.

**Discussion**

In the present work, we examined the biological activity of respiratory Ab in protecting mice against nasal colonization with *S. pneumoniae*. Mice that lack expression of the pIgR and that are, therefore, defective in epithelial transport of dimeric IgA, were not protected against nasal carriage of *S. pneumoniae* after mucosal immunization. IgA−/− mice were similarly not protected against nasal colonization. However, i.p. vaccination of these mice resulted in systemic immunity that was fully protective against an i.p. challenge of *S. pneumoniae*. The results indicate that parenteral vaccination to induce serum Abs is not sufficient to protect against nasal carriage and that mucosal-derived IgA Ab is necessary to clear this noninflammatory condition in which there is likely to be no leakage of serum Ab into the upper respiratory tract.

The importance of respiratory Abs in the host defense against pneumococcal colonization was established using pIgR−/− mice that lack the ability to actively secrete dimeric IgA and pentameric IgM into the mucosal lumen (2). In addition, we exploited an i.n. vaccination protocol that used IL-12 as a mucosal adjuvant and
FIGURE 6. PS3-specific serum Ab responses and protection against systemic infection with *S. pneumoniae.* A, Mice were immunized i.p. with PS3-CRM197 conjugate vaccine in alum and 7 days after boosting, levels of serum PS3-specific total and IgA Abs were determined. There was an accumulation of serum IgA in plgR<sup>−/−</sup> mice compared with wild-type mice as expected (*, *p* < 0.05). B, Four weeks after boosting, the mice were challenged i.p. with serotype 3 *S. pneumoniae.* Infected mice were monitored daily for survival. ○, nonimmunized wild-type mice; □, nonimmunized plgR<sup>−/−</sup> mice; ▲, immunized wild-type mice; ▼, immunized plgR<sup>−/−</sup> mice.

that was previously shown by our laboratory and others to yield high levels of protective Abs in the lungs of mice (18, 19). Vaccination i.n. with pneumococcal PS14 conjugate vaccine induced similar patterns of specific serum Ab isotype responses in wild-type and plgR<sup>−/−</sup> mice, and almost the same levels of serum Abs after infection. However, nasal IgA Ab was not detected in plgR<sup>−/−</sup> mice and protection against nasal carriage was not observed. Although mucosal leakiness in plgR<sup>−/−</sup> mice has been described (16), we did not observe leakage of serum IgA into the upper respiratory tracts of these mice. In individual wild-type mice, levels of nasal bacteria were negatively correlated with Ab levels in nasal washes; in contrast, there was no correlation between numbers of pneumococci in the nose and serum Ab levels in individual plgR<sup>−/−</sup> mice. There was also no defect in the ability of serum Abs from plgR<sup>−/−</sup> mice to opsonize pneumococci, but in the absence of plgR, the Abs could not be actively secreted into the mucosal lumen. Passive protection from nasal carriage that was provided by plgR<sup>−/−</sup> immune serum demonstrated the functional activity of these Abs and the necessity of secreting them into the mucosal lumen to decrease nasal colonization. It has been reported that IgA is not necessary for vaccine-induced protection against bacterial and viral mucosal pathogens, including the influenza virus (10), rotavirus (11), *S. flexneri* (12), and *H. pylori* (13). Nevertheless, our results agree with previous studies demonstrating the susceptibility of plgR<sup>−/−</sup> mice to respiratory virus infections (2).

Histological analysis of lungs from mice infected with the serotype 14 pneumococcus strain showed lack of inflammatory cell infiltration. Therefore, the mice cleared colonization through a noninflammatory mechanism rather than as an indirect effect of protection against an inflammatory systemic infection.

IgA<sup>−/−</sup> mice were previously shown by our laboratory to have a general defect in specific antiviral Ab production after i.n. immunization, a defect that could be overcome through the use of IL-12 as a vaccine adjuvant (19). In this study, we found that incorporating IL-12 into the vaccination protocol led to essentially comparable amounts of total serum Ab levels in wild-type and IgA<sup>−/−</sup> mice, but the latter mice lacked protection against nasal carriage. This finding clearly demonstrates that SIgA is the most important Ab isotype in mediating protection against pneumococcal carriage. We do not at this point know whether this induced SIgA may mediate protection through interaction with other immune factors induced by IL-12, although IL-12 given by itself before i.n. challenge conferred no protection (Fig. 2A). However, SIgA does not appear to play a crucial role in defense against systemic infection once the pathogen has breached the mucosal barrier.

The protective activity of IgA against *S. pneumoniae* and other pathogens has previously been studied (8, 20), but it has been difficult to distinguish the activity of serum IgA vs SIgA, especially when both are produced in the same host at the same time. The use of plgR<sup>−/−</sup> mice in this study allowed us to distinguish between the protective role of SIgA against nasal carriage, which may lead to epithelial penetration, and systemic immunity against blood-borne *S. pneumoniae.* In this regard, it is interesting to note that human plgR interacts directly with a surface protein of *S. pneumoniae,* SpsA (CbpA; Refs. 21–23), through Ig-like domains 3 and 4 of the receptor (24, 25). This interaction may support *S. pneumoniae* adherence and epithelial penetration (23, 25), but excess secretory component, either free or bound to SIgA in nasal secretions, will favor blocking of pneumococci. However, it should also be noted that SpsA (CbpA) does not bind to murine plgR (24) and plgR<sup>−/−</sup> mice are not differently susceptible to pneumococcal invasion compared with plgR<sup>+/+</sup> mice (Fig. 6).

A full understanding of SIgA function is not only of theoretical interest, but also of practical significance in designing appropriate vaccination strategies. Although nasal colonization of humans by *S. pneumoniae* occurs asymptomatically, it is likely that bacteria can be spread to susceptible populations or mutate into antibiotic-resistant strains. Investigations into the protective function of SIgA against nasal colonization with *S. pneumoniae* suggest that vaccination efforts against respiratory pathogens should focus primarily on inducing effective mucosal immune responses (26). The present work has not only confirmed some of the properties of IgA previously documented (27), but has also definitively demonstrated the crucial role for SIgA in local defense against pneumococcal colonization under noninflammatory situations.

References


