**Immunogenicity of an Autogenous Streptococcus suis Bacterin in Preparturient Sows and Their Piglets in Relation to Protection after Weaning**

Christoph Georg Baums,¹* Christian Brüggemann,¹ Christoph Kock,¹ Andreas Beineke,² Karl-Heinz Waldmann,³ and Peter Valentin-Weigand¹

Institut für Mikrobiologie, Zentrum für Infektionsmedizin, Stiftung Tierärztliche Hochschule Hannover, D-30173 Hannover, Germany¹; Institut für Pathologie, Stiftung Tierärztliche Hochschule Hannover, D-30173 Hannover, Germany²; and Klinik für kleine Klauentiere und forensische Medizin und Ambulatorische Klinik, Stiftung Tierärztliche Hochschule Hannover, D-30173 Hannover, Germany³

Received 20 April 2010/Returned for modification 24 May 2010/Accepted 17 August 2010

*S. suis* is an important porcine pathogen causing meningitis and other invasive diseases in piglets of different ages. Application of *S. suis* serotype 2 bacterins to specific-pathogen-free (SPF) weaning piglets has been demonstrated to protect against the homologous serotype. However, autogenous *S. suis* bacterins are also applied to sows and suckling piglets in the field. Therefore, comparative evaluation of different bacterin immunization regimes, including sow vaccination, was performed in this study. The main objectives were to determine the immunogenicity of an *S. suis* bacterin in sows prepartum and its influence on active immunization of piglets. Experimental infection of 6- and 8-week-old weaning piglets was performed to elucidate protective efficacies. Humoral immune responses were investigated by an enzyme-linked immunosorbent assay (ELISA) measuring muramidase-released protein (MRP)-specific IgG titers and by opsonophagocytosis assays. Bacterin application elicited high MRP-specific IgG titers in the serum and colostrum of sows, as well as opsonizing antibodies. Piglets from vaccinated sows had significantly higher MRP-specific titers than respective piglets from nonvaccinated sows until 6 weeks postpartum. Vaccination of suckling piglets did not result in high MRP-specific titers nor in induction of opsonizing antibodies. Furthermore, neither vaccination of suckling nor of weaning piglets from immunized sows was associated with a prominent active immune response and protection at 8 weeks postpartum. However, protection was observed in respective 6-week-old weaning piglets, most likely because of protective maternal immunity. In conclusion, this study provides the first results suggesting protective passive maternal immunity for *S. suis* serotype 2 after bacterin vaccination of sows and a strong inhibitory effect on active immunization of suckling and weaning piglets, leading to highly susceptible growers.

*S. suis* causes various pathologies, such as meningitis, arthritis, septic shock, and endocarditis (11). Furthermore, *S. suis* serotype 2 is also an important zoonotic agent (9). *S. suis* is characterized by a high diversity, and different serotypes might be involved in invasive diseases in pigs (6, 24). However, most of the experimental studies have been performed with serotype 2. Based on comparative evaluation of virulence of wild-type strains in intranasal infection experiments, serotype 2 isolates expressing the 136-kDa muramidase-released protein (MRP) and the 110-kDa extracellular factor (EF) are regarded as more virulent than serotype 2 strains which lack these factors or express MRP and a large factor (EF) are regarded as more virulent than serotype 2 strains. MRP+ EF+ serotype 2 strains (mrp+ eff+ cps2) have frequently been isolated from diseased piglets and have also been detected in some human cases in Europe (2, 20, 22). Furthermore, this genotype is associated with wild boars in Germany and has caused severe meningitis in a hunter (5).

MRP is an immunogenic surface-associated protein (4). Sera from convalescent and bacterin-vaccinated piglets have generally high MRP-specific antibody titers (3). Immunization of piglets with a subunit vaccine, including MRP and EF, elicited partial protection against the homologous, highly virulent MRP+ EF+ serotype 2 strain (25). However, vaccination with purified MRP alone failed to induce protection, and high MRP-specific-antibody titers were not associated with protection (3, 25).

*S. suis* immune prophylaxis is hampered by the lack of a vaccine protecting piglets against more than one serotype (4). In the field, autogenous vaccines are commonly used in herds with *S. suis* problems. Serotype 2 bacterins elicited protection against serotype 2 but not serotype 9 strains in specific-pathogen-free (SPF) weaning piglets (3, 25). Importantly, induction of opsonizing antibodies by bacterin immunization correlated with protection (3).

*S. suis* problems might occur at different ages, including in suckling and weaning piglets as well as growers. For prophylaxis, autogenous bacterins are applied to preparturient sows, piglets, or both in porcine practice (10). The protective efficacies of the different *S. suis* vaccination regimes are unknown.
since comparative evaluations have not been described. Maternal antibodies might exhibit positive or negative effects of various degrees on vaccine-induced immune responses in pregnancy, as has been shown for different pathogens (17, 18). The working hypothesis of this study was that S. suis immunization of preparturient sows might elicit protective passive maternal immunity but might also influence active immunization of piglets. The results of this study showed that vaccination of preparturient sows with an autogenous bacterin elicited a prominent humoral immune response associated with induction of opsonizing antibodies. In contrast, bacterin application did not elicit opsonizing antibodies in their suckling and weaning piglets. Accordingly, these piglets were unprotected at 8 weeks.

MATERIALS AND METHODS

Pig herd. All piglets investigated in this study were from a single closed farrow-to-finish farm with 105 sows with a history of S. suis problems in weaning piglets and growers. Immunization of pigs against S. suis started with this study and was performed only with the S. suis bacterin described below. All sows received porcine circovirus 2 vaccination (Circovac; Merial, Germany) 5 weeks antepartum and Enteritidis coli and Clostridium perfringens type C immunization (Entersisol Coli-Clost; Boehringer Ingelheim, Germany) 3 weeks antepartum. Seven days postpartum, sows were immunized against parvovirus and Escherichia coli rhusiopathiae (Parvovacur, Merial, Germany). Fourteen days postpartum, sows and suckling piglets were vaccinated against porcine respiratory and reproductive syndrome virus (Ingelvac PRRS MLV; Boehringer Ingelheim, Germany). Furthermore, a Mycoplasma hyopneumoniae vaccine (Stellamune Mycoplasma; Pfizer, Germany) was applied to suckling piglets at ages 5 and 26 days.

Weaning was performed in the 4th week postpartum. Cross-fostering was not practiced with the piglets included in this study. Five days before challenge, respective piglets were transported to the institute for experimental infection under safety level 2 laboratory conditions.

Bacterial strains and growth conditions. S. suis strain Br3/6 is an mrrp+/epf+/sly+/cps2 strain which was isolated from the brain of a piglet of this particular herd with severe fibrinosuppurative meningitis. Strain 10 is an mrrp+ epf+ sly+ cps2 reference strain which has been shown to be highly virulent in experimental infections of piglets. Strain 10 was used and was characterized by the two antisera in the present study. S. suis strain of sequence type 99 which was originally isolated from a pig with meningitis (6).

Preparation of the bacterin. The bacterin was generated with S. suis strain Br3/6 grown overnight in Bacto Todd-Hewitt broth (Becton Dickinson Diagnostics, Heidelberg, Germany) at 37°C and inactivated in 0.1% formaldehyde (no centrifugation of the culture). Emulsigen was added as an adjuvant (20% [vol/vol]). Each immunization dose contained approximately 109 CFU. The mixtures were stirred for 8 h, screened for sterility, and stored at 8°C.

Immunoassays protocols. The first two experiments, sows in the same gestation stage (5 weeks antepartum) were randomly divided into an S. suis immunization group and a control group. The two groups were housed in the same unit. The only difference in treatment between the two groups was application of the S. suis bacterin in the 5th and 3rd weeks antepartum. After the 2nd experiment, all piglets of this particular herd were immunized with the S. suis bacterin in the 5th and 3rd weeks antepartum. The sows (and piglets) investigated in this herd had not been vaccinated against S. suis before inclusion of these animals in this study. Piglets were vaccinated either in the 2nd and 4th weeks postpartum (1st and 2nd experiments) or in the 4th and 6th weeks postpartum (3rd experiment).

In the 1st experiment, all piglets used for immunization and subsequent challenge were from different sows (either immunized or nonimmunized). In the 2nd and 3rd experiments, all piglets of a sow were divided randomly into two groups, one used for S. suis immunization and the other as a control. The two groups were not separated physically. For challenge, two piglets, one vaccinated and one nonvaccinated, from each sow were used (randomly selected among healthy and well-developed piglets).

Challenge experiments. Sixty piglets from a single herd described above were infected experimentally and cared for in accordance with the principles outlined in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Treaty Series, no. 123: http://conventions.coe.int/Treaty/EN/Treaties/html/123.htm ANX: permit no. 33.9-42502-04-07/1395 and 09/1684). The piglets were challenged intravenously with 1 × 104 CFU of strain Br3/6 either at an age of 6 weeks (1st experiment) or at an age of 8 weeks (2nd and 3rd experiments). The health status of the animals was monitored every 6 to 10 h after challenge. In the case of high fever (≥40.5°C), apathy as well as anorexia, and in all cases of clinical signs of acute polyarthritis or severe menin gitis, animals were euthanized for reasons of animal welfare. All surviving piglets were sacrificed 9 days postinfection.

Serum and colostrum samples. Serum samples were collected from all sows 5 weeks and 1 week antepartum (pre- and postvaccination, respectively). Colostrum was obtained within 4 and 6 h after the start of birth and frozen immediately at −20°C.

In the 1st experiment, serum was collected from piglets in the 2nd, 4th, and 6th weeks postpartum (pre-1st, pre-2nd, and postvaccination, respectively). In the 2nd and 3rd experiments, an additional serum sample was collected in the 8th week postpartum (all samples prior to experimental infection).

Histopathological and bacteriological screening. The histological screenings were carried out and scored with blinded experiments as described previously (2). All tissues screened histologically were also investigated bacteriologically through culture- and PCR-based detection of the challenge strain (20).

Detection of MRP-specific antibodies. MRP-specific IgG (IgG1 and IgG2) was determined by ELISA as described previously (3, 13). Based on the information of the manufacturer of the mouse anti-porcine IgG1 and IgG2 antisera (Serotec; Kidlington, Oxford, United Kingdom), the definition of subclasses was based upon electrophoretic mobility, with IgG1 migrating faster than IgG2 as described by Metzger and Fougereau (16). However, electrophoresis does not allow differentiation of the six porcine IgG subclasses described more recently on the basis of genomics (7, 12). Since there are currently no purified standards for the different IgG variants, it is impossible to determine the subclasses or allotypic variants of subclasses of the two antisera recognize (J. E. Butler, personal communication).

Briefly, the anti-MRP ELISA was performed with Maxisorb plates (Nunc, Rochester, NY) coated with 5 μg recombinant MRP (rMRP) or bovine serum albumin (BSA) (background measurement) in carbonate buffer. Mouse anti-porcine IgG1 and IgG2 antisera (Serotec) and peroxidase-conjugated goat anti-mouse IgG antiserum (Dianova, Hamburg, Germany) were used as primary and secondary antibodies, respectively. The plates were developed with 2,2-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) (Boehringer, Mannheim, Germany) and 0.002% H2O2 as a substrate. Absorbance was measured at 405 nm. Every serum sample and the controls were measured in a duplicate series of 4 (reference serum, 7) 2-fold dilutions in phosphate-buffered saline–Tween 20 (PBST) (starting with 1:200). A convalescent-phase serum obtained from a piglet 20 days after experimental infection with the MRP+ EPF+ serotype 2 reference strain 10 was used as a standard and defined to have 100 ELISA units. Optical densities were converted to antibody concentrations through log linear regression analysis after background subtraction. The ELISA units of each sample were defined as the mean of the calculated units for each of the four dilutions of the two series. Data were considered only if they met the following criteria: deviation of duplicates of <15%, slope of the linear portion of the reference standard curve.
between 0.8 and 1.2 for IgG1 and between 1 and 1.4 for IgG2, correlation coefficient between 0.9 and 1.0, and controls within established ranges.

**Purification of serotype 2 capsular polysaccharides (CPS2) and detection of CPS2-specific antibodies.** Purification of CPS2 and detection of CPS2-specific IgG antibodies in an ELISA were performed as described previously (3). In this ELISA, a peroxidase-conjugated goat anti-swine IgG (heavy plus light chains) antiserum (Dianova, Hamburg, Germany) was used.

**Opsonophagocytosis assay.** Opsonophagocytic killing in the presence of 20% (vol/vol) porcine serum was assayed as described previously (3). The same batch of frozen glycerol stocks of an *S. suis* strain Br3/6 culture (logarithmic growth phase) was used during the whole experiment (13, 19). *S. suis* strain Br3/6 was incubated with porcine neutrophils at a starting multiplicity of infection of 0.35 (1.75 x 10⁶ CFU incubated with 5 x 10⁶ neutrophils). The survival factor represented the ratio of CFU at 1 h to CFU at time zero. Results of an opsonophagocytosis assay were accepted only if survival factors of strain Br3/6 were below 0.2 in the positive control (porcine hyperimmune serum against BSA-conjugated serotype 2 capsular polysaccharides) and above 1.5 in the negative control (serum from gnotobiotic piglets). The serotype 9 strain A3286/94 was included as a control to confirm specificity of killing mediated by the positive-control serum (survival factor above 1.2).

**Statistical analysis.** Statistical analysis with the Mann-Whitney test was performed to analyze differences between the different groups of piglets used in each experiment. The Wilcoxon test was used for comparison of different time point values within the same group. The data in the Kaplan-Meyer survival diagrams were analyzed with the log rank test. Probabilities lower than 0.05 were considered significant.

**RESULTS**

**Characterization of *S. suis* bacterin immunogenicities in sows.** A main objective of this work was to evaluate immunogenicity of a bacterin applied 5 and 3 weeks antepartum in dams, since this had not been investigated previously. In all three experiments, vaccination of sows with the autogenous *S. suis* bacterin elicited a significant IgG serum response against the surface-associated protein MRP, which was used as an antigen in respective ELISAs (IgG1 and IgG2; see Materials and Methods). Serum IgG anti-MRP titers were significantly higher in vaccinated sows than in nonvaccinated sows of the first two experiments (Fig. 2A; see also Fig. S1A in the supplemental material). Accordingly, colostrum from immunized sows contained significantly higher titers of anti-MRP IgG than colostrum from nonvaccinated animals (Fig. 2B; see also Fig. S1B). The MRP-specific IgG1 and IgG2 titers were about twice as high in colostrum than in sera 1 week antepartum (e.g., for vaccinated sows of the 1st experiment: median ELISA units for IgG1 and IgG2 of 839 and 2098 for colostrum and 508 and 946 for serum, respectively; Fig. 2A and B).

Application of bacterin to weaning piglets did not elicit a prominent immune response against serotype 2 capsular polysaccharides in our previous study (3). Since immunogenicity of capsular polysaccharides might be greater in older animals, the sera of the sows of the 1st experiment were also screened with respect to CPS2-specific antibodies. As shown in Fig. 2C, bacterin application elicited a significant total IgG response against CPS2 in serum from preparturient sows (*P* = 0.028). This resulted in significantly higher anti-CPS2 titers for vaccinated sows 1 week antepartum than for unvaccinated sows (*P* = 0.049).

In our previous study, we demonstrated correlation of induction of opsonizing antibodies with protection in *S. suis* immunization (3). Therefore, titers of opsonizing antibodies were also determined in this study. As shown in Fig. 3, immunization of sows induced opsonizing antibodies antepartum, leading to sufficient killing of the challenge strain by porcine neutrophils in the presence of post- but not prevaccination serum (*P* = 0.0001). In accordance, titers of opsonizing antibodies in sera from vaccinated sows against the homologous serotype 2 *S. suis* strain were significantly higher than respec-
tive titers for unvaccinated sows (1 week antepartum; \( P = 0.00004 \)) (Fig. 3).

In conclusion, quantitative (anti-MRP and anti-CPS2 titers) and functional (opsonization) analysis of humoral immunity against \( S. \) suis serotype 2 in sows indicated induction of a strong and efficient immune response after bacterin vaccination 5 and 3 weeks antepartum. Analysis of humoral immunity in suckling and weaning piglets and challenge experiments were performed to investigate if the induced maternal immunity interfered with active immunization of suckling (1st and 2nd experiments) and weaning (3rd experiment) piglets and if the maternal immunity provided protection in 6-week-old (1st experiment) and 8-week-old (2nd experiment) piglets.

Protective efficacies of sow and suckling piglet vaccination in weaning piglets. In the 1st experiment, vaccination of suckling piglets with an autogenous \( S. \) suis bacterin was performed alone or in combination with sow immunization (Fig. 1). Application of the bacterin to suckling piglets only did not result in significant protection against morbidity after \( S. \) suis challenge 2 weeks after weaning (\( P = 0.2 \)) (Fig. 4A). In contrast, weaning piglets from vaccinated sows, which were immunized as suckling piglets, showed less morbidity after homologous challenge than respective control piglets (\( P = 0.009 \)) (Fig. 4A and Table 1). Accordingly, pathohistological lesions were also less severe in these piglets (Table 2). Furthermore, the challenge strain was detected in only 1 tissue sample from each of 3 piglets weaned from vaccinated sows, in contrast to detection of the challenge strain in a total of 11 tissue samples collected from 5 different control piglets (Table 3).

MRP-specific IgG titers were significantly higher for piglets from vaccinated sows in the 2nd, 4th, and 6th weeks postpartum than for piglets of unvaccinated sows (IgG1 and IgG2; Fig. 5A). A significant increase in mean MRP-specific IgG titers was not observed in any of the three groups of the 1st experiment (Fig. 1 shows the experimental design, and Fig. 5A shows...
results). Twelve days postvaccination, there was a significant difference in titers of MRP-specific IgG1 but not IgG2 between the group of vaccinated piglets raised by unvaccinated sows and the control group ($P = 0.042$) (Fig. 5A). However, 5 of these 6 vaccinated piglets had IgG1 titers below 40 ELISA units, which were based on controls of the ELISA not considered to be clearly positive.

Titers of opsonizing antibodies determined in neutrophil killing assays were significantly higher in the 2nd week postpartum for piglets from vaccinated sows than for piglets from nonvaccinated sows (Fig. 5B) but not comparable to the high titers detected in the sera of the vaccinated sows (Fig. 3). At 6 weeks postpartum, titers of opsonizing antibodies had declined significantly in suckling piglets from vaccinated sows, resulting in comparable titers of opsonizing antibodies for all three groups.

In conclusion, autogenous bacterin vaccination of suckling piglets failed to elicit a significant MRP-specific serum IgG response or an increase in titers of opsonizing antibodies. A significant protection of respective 6-week-old piglets against nonvaccinated sows (Fig. 5B) but not comparable to the high titers detected in the sera of the vaccinated sows (Fig. 3). At 6 weeks postpartum, titers of opsonizing antibodies had declined significantly in suckling piglets from vaccinated sows, resulting in comparable titers of opsonizing antibodies for all three groups.

In conclusion, autogenous bacterin vaccination of suckling piglets failed to elicit a significant MRP-specific serum IgG response or an increase in titers of opsonizing antibodies. A significant protection of respective 6-week-old piglets against nonvaccinated sows (Fig. 5B) but not comparable to the high titers detected in the sera of the vaccinated sows (Fig. 3). At 6 weeks postpartum, titers of opsonizing antibodies had declined significantly in suckling piglets from vaccinated sows, resulting in comparable titers of opsonizing antibodies for all three groups.

### Table 1. Evaluation of protection induced by vaccination of sows and/or piglets with an autogenous bacterin of *S. suis* strain Br3/6 ($mrp^+/epf^+shy^+cps2$) in a homologous intravenous challenge experiment with weaning piglets and growers

| Expt | Vaccination group                  | Challenged piglets | No. of piglets with clinical symptoms | No. of piglets with max. body temp (°C) |
|------|-----------------------------------|--------------------|--------------------------------------|----------------------------------------|
|      |                                   | Morbidity$^a$      |                                      |                                        |
|      |                                   | Mortality$^b$      |                                      |                                        |
|      |                                   | CNS | Lameness | Unspecific | <40.5 | ≥40.5 |
| 1st  | Sows and suckling piglets         | 6   | 6       | 2/6      | 2/6   | 0/6  | 1/6  | 1/6  | 4/6  | 2/6  |
|      | Suckling piglets only             | 6   | 6       | 5/6      | 2/6   | 1/6  | 1/6  | 1/6  | 1/6  | 5/6  |
|      | Control                           | 6   | 6       | 6/6      | 6/6   | 0/6  | 4/6  | 2/6  | 0/6  | 6/6  |
| 2nd  | Sows and suckling piglets         | 8   | 6       | 6/6      | 3/6   | 1/6  | 5/6  | 1/6  | 1/6  | 5/6  |
|      | Suckling piglets only             | 8   | 6       | 6/6      | 4/6   | 0/6  | 4/6  | 1/6  | 0/6  | 6/6  |
|      | Sows only                         | 8   | 6       | 6/6      | 4/6   | 1/6  | 3/6  | 1/6  | 1/6  | 5/6  |
|      | Control                           | 8   | 6       | 6/6      | 4/6   | 1/6  | 3/6  | 1/6  | 1/6  | 5/6  |
| 3rd  | Sows and weaning piglets          | 8   | 9       | 6/9      | 5/9   | 2/9  | 6/9  | 0/9  | 2/9  | 3/9  |
|      | Sows only                         | 8   | 9       | 8/9      | 7/9   | 0/9  | 8/9  | 1/9  | 2/9  | 7/9  |

$^a$ Expressed as no. of diseased piglets/no. tested.

$^b$ Expressed as no. of dead piglets/no. tested.

$^c$ Expressed as no. of piglets with symptoms/no. tested.

$^d$ Max., maximum.

### Table 2. Scoring of fibrinosuppurative lesions of weaning piglets and growers intravenously infected with *S. suis* strain Br3/6 ($mrp^+/epf^+shy^+cps2$) after immunization of sows and/or piglets with a bacterin of *S. suis* strain Br3/6

| Expt | Vaccination group                  | Challenged piglets | Meningitis, pleocystis, or choroiditis | Pleuritis, peritonitis or pericarditis | Synovialitis | Splenitis$^b$ or hepatitis | Pneumonia | Score $\sigma^c$ |
|------|-----------------------------------|--------------------|--------------------------------------|--------------------------------------|--------------|---------------------------|-----------|
|      |                                   | Age (wk) | No. in group | 5 | 3 | 1 | 4 | 2 | 1 | 4 | 2 | 1 |                   |
| 1st  | Sows and suckling piglets         | 6   | 6       | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 1.7          |           |
|      | Suckling piglets only             | 6   | 6       | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 (1)$^d$ | 0 (1)$^d$ | 0  | 0  | 1.7 (2.7)$^d$ |
|      | Control                           | 6   | 6       | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1.7          |           |
| 2nd  | Sows and suckling piglets         | 8   | 6       | 1 | 0 | 2 | 1 | 0 | 3 | 0 | 1 | 2 | 1 | 1 | 1 | 0  | 3.8  |
|      | Suckling piglets only             | 8   | 6       | 1 | 1 | 0 | 1 | 0 | 3 | 0 | 0 | 1 | 3 | 1 | 0 | 0  | 2.7  |
|      | Sows only                         | 8   | 6       | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 1 | 2 | 0 | 0  | 3.3  |
|      | Control                           | 8   | 6       | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 3 | 1 | 1 | 0 | 0  | 3.2  |
| 3rd  | Sows and weaning piglets          | 8   | 9       | 2 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 2 | 2 | 4 | 0 | 0  | 2.8$^e$ |
|      | Sows only                         | 8   | 9       | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 3 | 4 | 0 | 0  | 2.8$^e$ |

$^a$ Scoring of 4 and 5 indicates moderate to severe diffuse or multifocal fibrinosuppurative inflammations. Scoring of 2 and 3 indicates mild focal fibrinosuppurative inflammation. Individual single perivascular neutrophils received a score of 1.

$^b$ Neutrophilic accumulation of the splenic red pulp.

$^c$ $\sigma = \Sigma \text{score}_{\max} / n_{\min}$, (2).

$^d$ Fibrinosuppurative lesions were not detected in two immunized piglets which developed body temperatures above 40.5°C after experimental infection. However, one of these piglets had a moderate granulomatous pneumonia, and the other had a mild multifocal lymphohistiocytic hepatitis. The challenge strain was detected in both lesions. The pathological scores provided in the parentheses take these two lesions into account.

$^e$ Severe fibrinosuppurative endophthalmitis associated with the challenge strain was diagnosed in one piglet (receiving the score 4).

$^f$ Moderate diffuse, lymphoplasmacytic and suppurative cystitis associated with the challenge strain was diagnosed in one piglet (receiving the score 4).
specific IgG2 but not IgG1 was observed from the 4th to 8th weeks postpartum, a slight but significant increase of MRP-specific IgG1 and IgG2 calculated from the decline period (no significant differences). The theoretical half lives of MRP-specific IgG1 and IgG2 calculated from the declines observed from the 2nd to the 4th week postpartum of the unvaccinated suckling piglets born from vaccinated sows were 6.9 days (standard deviation [SD] = 1.1) and 6.7 days (SD = 1.2), respectively. In the group of piglets derived from nonvaccinated sows with bacterin application in the 2nd and 4th weeks postpartum, a slight but significant increase of MRP-specific IgG2 but not IgG1 was observed from the 4th to 8th week postpartum (P = 0.046) (see Fig. S2). At an age of 8 weeks, the mean MRP-specific IgG1 and IgG2 titers for these piglets, which were vaccinated during the suckling period, were 45 and 92 ELISA units and thus only slightly higher than those for the unvaccinated control group, with mean values of 16 and 52 ELISA units, respectively (P = 0.016 and 0.1, respectively). None of the vaccination regimes tested in the 2nd experiment induced opsonizing antibodies in piglets at ages of 6 and also 8 weeks (mean survival factors of S. suis strain Br3/6 were between 1.3 and 1.5 in the opsonophagocytosis assay with no significant differences).

In conclusion, neither application of S. suis bacterin to preparturient sows nor that to suckling piglets or both elicited protection in 8-week-old piglets. The detected positive MRP-specific IgG2 titers were not associated with opsonizing activity. The lack of opsonizing antibodies most likely explains the lack of protection.

**Protective efficacies of sow and weaning piglet vaccination in growers.** The objective of the 3rd experiment was to assess if active immunization of piglets weaned from bacterin-vaccinated sow can elicit an active immune response and protection (using the same S. suis bacterin). In contrast to the previous experiments, the bacterin was applied in the 4th and 6th weeks instead of the 2nd and 4th weeks postpartum (Fig. 1). As demonstrated in Fig. S3A in the supplemental material, MRP-specific IgG1 and IgG2 titers declined from the 2nd to the 4th and the 4th to the 8th week postpartum for nonvaccinated piglets and for vaccinated piglets (with the exception of one vaccinated piglet). However, MRP-specific IgG1 but not IgG2 titers were significantly higher for vaccinated piglets than for nonvaccinated piglets at an age of 8 weeks (P = 0.015 and 0.83, respectively). Application of the bacterin to weaning piglets did not elicit significant titers of opsonizing antibodies against the homologous serotype 2 strain (P = 0.51 for comparison of pre- and post-vaccination sera and P = 0.085 for comparison of sera from vaccinated and nonvaccinated 8-week-old pigs) (see Fig. S3B). In agreement, morbidity and mortality were above 50% in vaccinated and nonvaccinated piglets challenged with this strain, with no significant differences between the two groups (Table 1 and Fig. 4C). Differences between the two groups also were not recorded with respect to pathohistological alterations and detection of the challenge strain in tissue samples (Tables 2 and 3). In conclusion, quantitative and qualitative serological investigations of bacterin-vaccinated piglets weaned from bacterin-vaccinated sows indicated a lack of a prominent active immune response in these weaned piglets, in

### TABLE 3. Reisolation of the challenge strain from piglets which had been intravenously infected with S. suis strain Br3/6 (mrp<sup>e</sup> epf<sup>e</sup> sly<sup>e</sup> cps2) after vaccination of sows and/or piglets with a homologous bacterin of S. suis

| Expt | Vaccination group | Age (wk) | No. in group | Tonsils | Lung<sup>b</sup> | Serosa<sup>c</sup> | Spleen | Liver | Brain or CSE<sup>d</sup> | Joint fluid<sup>ad</sup> |
|------|-------------------|----------|--------------|---------|--------------|----------------|--------|-------|----------------|------------------|
| 1st  | Sows and suckling piglets | 6 | 6 | 0/6 | 0/6 | 0/6 | 1/6 | 0/6 | 0/6 | 2/6 |
|      | Suckling piglets only | 6 | 6 | 0/6 | 0/6 | 0/6 | 1/6 | 1/6 | 2/6 | 1/6 |
|      | Control | 6 | 6 | 0/6 | 0/6 | 0/6 | 1/6 | 2/6 | 1/6 | 4/6 |
| 2nd  | Sows and suckling piglets | 8 | 6 | 0/6 | 1/6 | 0/6 | 1/6 | 1/6 | 3/6 | 2/6 |
|      | Suckling piglets only | 8 | 6 | 0/6 | 0/6 | 0/6 | 1/6 | 0/6 | 0/6 | 2/6 |
|      | Sows only | 8 | 6 | 0/6 | 2/6 | 0/6 | 1/6 | 2/6 | 1/6 | 4/6 |
|      | Control | 8 | 6 | 0/6 | 0/6 | 1/6 | 1/6 | 1/6 | 2/6 | 4/6 |
| 3rd  | Sows and weaning piglets | 8 | 9 | 1/9 | 3/9 | 0/9 | 4/9 | 2/9 | 5/9 | 4/9 |
|      | Sows only | 8 | 9 | 0/9 | 2/9 | 1/9 | 6/9 | 3/9 | 5/9 | 7/9 |

*<sup>a</sup> Challenge strains were identified through PCR as described in Materials and Methods. Values are expressed as no. of pigs for isolation from site/no. tested.
*<sup>b</sup> One cranial lobe was investigated.
*<sup>c</sup> Pleural, peritoneal or pericardial cavity.
*<sup>d</sup> Cerebrospinal fluid.
agreement with the lack of protection observed in the challenge experiment.

DISCUSSION

In this study, we performed for the first time experiments to evaluate S. suis bacterin vaccination regimes with sow and suckling piglet immunization. This is of significance since S. suis might cause diseases at very different ages, including in suckling and weaning piglets and in growers. Therefore, there is a very frequent demand for vaccination of sows and suckling piglets in porcine practice (10). However, vaccination trials have been described only for weaning piglets, with one exception (1).
This study demonstrates that bacterin vaccination of sows 5 and 3 weeks ante partum elicited a prominent IgG serum response against the immunogenic MRP antigen. Importantly, vaccination of sows was associated with induction of opsonizing antibodies and significantly higher titers of MRP-specific antibodies in colostrum. It is noteworthy that the sows in this study were not seronegative prior to vaccination. The immune responses of naïve sows, which are rarely found in the field, might be different. Importantly, previous exposure to S. suis was associated with positive IgG1 and IgG2 titers against MRP but not with titers of opsonizing antibodies sufficient to induce killing of the homologous serotype 2 strain under the experimental conditions. This is in agreement with findings of our previous study demonstrating that positive anti-MRP IgG titers are not associated with opsonization (3). Interestingly, bacterin application elicited antibodies against capsular polysaccharides in sows of the 1st experiment, which might have substantially contributed to the opsonizing activity. This suggests differences in immunogenicities of an S. suis serotype 2 bacterin in sows and piglets, since vaccination of weaning piglets with a similar bacterin formulation did not induce anti-CPS2 titers contributing to opsonization (3).

Higher titers of opsonizing antibodies for vaccinated sows than for nonvaccinated sows are an important result of this study, since opsonizing antibodies might be crucial for protection (3). Accordingly, suckling piglets from vaccinated sows had significantly higher titers of opsonizing antibodies than piglets from nonvaccinated sows 2 weeks postpartum. This finding suggests that preparturient sow vaccination is sufficient for providing opsonizing antibody titers in 2-week-old piglets, most likely associated with protection. Accordingly, Amass et al. (1) observed partial protection in 2-week-old piglets after preparturient sow vaccination with an S. suis serotype 14 bacterin. At age 6 weeks, no significant differences were observed between titers of opsonizing antibodies among the different groups of the 1st experiment. Thus, opsonizing antibodies might not explain the difference observed in morbidity and mortality. On the other hand, we cannot rule out that these sera lost some opsonizing activity, since all sera of this study were frozen and thawed twice prior to conducting the neutrophil killing assay. Nevertheless, the lack of detectable opsonizing antibodies in sera from all other piglets of this study is in agreement with the high morbidity and mortality observed after challenge of these piglets.

Li et al. observed induction of opsonizing antibodies and partial protection in an S. suis vaccination trial with piglets after vaccination with surface antigen one (SAO) and the adjuvant QuilA (14). In contrast, no protection and induction of opsonizing antibodies were recorded in their previous study using the same antigen but a lower antigen dose and the adjuvant Emulsigen (15). The authors discuss these differences with respect to a Th1 response induced by QuilA but not Emulsigen, since high IgG2 titers were found only with QuilA (14). We used the same monoclonal antibodies differentiating IgG1 and IgG2 in our studies but did not detect significant differences between the ratios of these two IgGs among groups with significant differences in protection and titers of opsonizing antibodies, either in this (results not shown) or in a previous study (3). Furthermore, high anti-MRP and anti-SA0 IgG2 titers were observed for animals lacking protection, as well as titers of opsonizing antibodies sufficient to induce killing of the challenge strains *in vitro* in this and our previous study (6), respectively.

In the course of the manuscript revision process, we learned from the manufacturer of the monoclonal antibodies (Serotec) that they differentiated IgG1 and IgG2 subclasses by electrophoretical mobility as described by Metzger and Fougerau (16). This is not in accordance with a more recent classification of porcine IgG isotypes based on genomics (3, 14, 15). Since there are no purified standards for the 11 different porcine IgG variants, it cannot be excluded that the monoclonal antibodies provided by Serotec recognize groups of subclasses rather than a single one (J. E. Butler, personal communication). Importantly, there is no evidence at this point that differentiation of porcine IgG1 and IgG2 responses allows conclusions on a Th1 versus Th2 response.

Furthermore, induction of the IgG3 isotype might be important for protection against *S. suis*, since sequence analysis suggests that this isotype is likely activating complement and binding FcγRs and might thus be important for opsonization (7). It remains a task of future S. suis studies to identify correlations between specific porcine IgG isotypes and titers of antibodies opsonizing *S. suis*.

In agreement with the serological results with the sows, suckling piglets from vaccinated sows had significantly higher MRP-specific IgG1 and IgG2 titers than piglets from unvaccinated sows in the 2nd week postpartum. As expected, these maternal antibodies declined in the following weeks. At age 6 but not at 8 weeks, significant differences in MRP-specific IgG titers were still observed between piglets from vaccinated sows and piglets from nonvaccinated sows (1st and 2nd experiments). Serum half-life for IgG in suckling piglets ranges from 8 to 10 days (8). The half-lives of MRP-specific IgG1 and IgG2 calculated in this study from the data of unvaccinated suckling piglets from vaccinated sows of the 2nd experiment are in line with the published data (6.9 days with an SD of 1.1 and 6.7 days with an SD of 1.2). Importantly, the decline of specific antibodies was very similar in suckling piglets from immunized sows vaccinated during the suckling period (no significant difference), indicating that the high titers for these piglets detected in the 2nd, 4th, and 6th weeks are related to maternal antibodies. On the other hand, it cannot be completely ruled out that an active MRP-specific humoral immune response after bacterin application in piglets which had sucked colostrum from vaccinated sows was not recorded because of the high titers of maternal antibodies.

The missing or weak immune responses observed with regard to suckling piglet vaccination in this study raise the question of whether this was due to inhibition by maternal antibodies (or other colostrum components) or immature adaptive immunity in suckling piglets (8). Since this study did not include colostrum-deprived piglets, this question remains open. It is noteworthy that other studies have observed a good immune response in suckling piglets with vaccines against other pathogens and have demonstrated that maternal antibodies reduced the impact of vaccination in swine (17).

Two previous studies demonstrated induction of protective immunity in 8-week-old weaning piglets through application of an S. suis serotype 2 bacterin at ages of 3 to 4 and 6 weeks postpartum (3, 25). In the 3rd experiment of this study, pro-
tection was not observed, though a similar bacterin was applied at the same age. Though all three studies were performed with serotype 2, only the strain used in this study carries an eps gene encoding a large variant of EF. Therefore, differences in protective efficacies of bacterin application to weaning piglets between the three studies might be related to the differences between these S. suis serotype 2 strains. It is noteworthy that differences in EF phenotypes of serotype 2 strains are known to be associated with differences in epidemiology and virulence, as outlined in the introduction. On the other hand, the piglets included in the two cited studies were SPF animals, whereas the piglets of the 3rd experiment of this study were weaned from vaccinated sows. Therefore, a likely explanation for the differences in protective efficacies is that passive maternal immunity reduced the impact of vaccination and in particular prevented induction of opsonizing antibodies and protection.

In summary, this study demonstrates for the first time that immunization of preparturient sows with an autogenous S. suis bacterin can induce humoral immune responses, including induction of opsonizing antibodies. Their piglets showed significant differences with respect to quantitative and qualitative humoral immunity. Protection in the 6th but not the 8th week postpartum might be explained by passive maternal immunity. How an active protective immune response might be elicited in such piglets with passive maternal antibodies remains an important challenge of future studies.

ACKNOWLEDGMENTS

We thank Hilde Smith (DLO-Institute for Animal Science and Health, Netherlands) for providing strain 10 and the nonencapsulated isogenic mutant 10epsAEF. M. Beyerbach (Stiftung Tierärztliche Hochschule Hannover, Hannover, Germany) kindly provided support in statistical analysis. Andrea Düngelhoef, Sophie Gschaider, Jana Seele, Nadine Büttner, and Christoph Hirche are acknowledged for their excellent work during their practicals. Hans-Joachim Schubert (Stiftung Tierärztliche Hochschule Hannover) is acknowledged for helpful discussions of the concept of this study.

This study was financially supported by the Deutsche Forschungsgemeinschaft (DFG), Bonn, Germany (SFB587), and IDT Biologika GmbH Dessau-Tornau.

REFERENCES

1. Amass, S. F., G. W. Stevenson, B. D. Vyverberg, T. W. Hufsk, K. E. Knox, and L. A. Grote. 2000. Administration of a homologous bacterin to sows prefarrowing provided partial protection against streptococcosis in their weaned pigs. Swine Health Prod. 8:217–219.

2. Baums, C. G., U. Kaim, M. Fulde, G. Ramachandran, R. Goethe, and P. Valentijn-Weigand. 2006. Identification of a novel virulence determinant with serum opacification activity in Streptococcus suis. Infect. Immun. 74:6154–6162.

3. Baums, C. G., C. Kock, A. Beineke, K. Bennecke, R. Goethe, C. Schröder, K. H. Waldmann, and P. Valentijn-Weigand. 2009. Streptococcus suis bacterin and subunit vaccine immunogenetics and protective efficacies against serotypes 2 and 9. Clin. Vaccine Immunol. 16:200–208.

4. Baums, C. G., C. Kock, A. Beineke, K. Bennecke, R. Goethe, C. Schröder, K. H. Waldmann, and P. Valentijn-Weigand. 2009. Streptococcus suis bacterin and subunit vaccine immunogenetics and protective efficacies against serotypes 2 and 9. Clin. Vaccine Immunol. 16:200–208.

5. Baums, C. G., C. J. Verkühlen, T. Rehm, L. M. Silva, M. Beyerbach, K. Pohlmeier, and P. Valentijn-Weigand. 2007. Prevalence of Streptococcus suis genotypes in wild boars of Northwestern Germany. Appl. Environ. Microbiol. 73:711–717.

6. Beineke, A., K. Bennecke, C. Neis, C. Schröder, K. H. Waldmann, W. Baumgartner, P. Valentijn-Weigand, and C. G. Baums. 2008. Comparative evaluation of virulence and pathology of Streptococcus suis serotypes 2 and 9 in experimentally infected growers. Vet. Microbiol. 128:423–430.

7. Butler, J. E., N. Wertz, N. Deschatz, and L. Kacskovics. 2009. Porcine IgG: structure, genetics, and evolution. Immunogenetics 61:209–230.

8. Butler, J. E., Y. Zhao, M. Sinkora, N. Wertz, and L. Kacskovics. 2009. Immunoglobulins, antibody repertoire and B cell development. Dev. Comp. Immunol. 33:321–333.

9. Gottschalk, M., M. Segura, and J. Xu. 2007. Streptococcus suis infections in humans: the Chinese experience and the situation in North America. Anim. Health Res. Rev. 8:29–45.

10. Hasebroek, F., P. Passman, K. Chiers, D. Maes, R. Ducatelle, and A. Decoutere. 2004. Efficacy of vaccines against bacterial diseases in swine: what can we expect? Vet. Microbiol. 100:255–268.

11. Higgins, R., and M. Gottschalk. 2005. Streptococcal diseases, p. 769–783. In B. Straw, S. D. Allaire, W. Mengeling, and D. Taylor (ed.), Diseases of swine. Iowa State University, Ames, IA.

12. Kacskovics, L., J. Sun, and J. E. Butler. 1994. Five putative subclasses of swine IgG identified from the cDNA sequences of a single animal. Immunol. 153:3565–3573.

13. Kock, C., A. Beineke, M. Seitz, M. Ganter, K. H. Waldmann, P. Valentijn-Weigand, and C. G. Baums. 2009. Intranasal immunization with a live Streptococcus suis isogenic eps mutant elicited slysin-neutralization titers but failed to induce opsonizing antibodies and protection. Vet. Immunol. Immunopathol. 132:235–244.

14. Li, Y., M. Gottschalk, M. Exgle, L. Lacouture, J. D. Dubreuil, P. Willson, and J. Harel. 2007. Immunization with recombinant Sfo protein confers protection against Streptococcus suis infection. Clin. Vaccine Immunol. 14:708–714.

15. Li, Y., G. Martinez, M. Gottschalk, L. Lacouture, P. Willson, J. D. Dubreuil, M. Jacques, and J. Harel. 2006. Identification of a surface protein of Streptococcus suis and evaluation of its immunogenic and protective capacity in pigs. Infect. Immun. 74:491–497.

16. Metsger, J. J., and M. Fougerauf. 1967. Characterization of two subclasses of gamma G immunoglobulin in swine. C. R. Acad. Sci. Seances Acad. Sci. D 265:724–727.

17. Nguyen, T. V., L. Yuan, M. S. Azevedo, K. I. Jeong, A. M. Gonzalez, C. Iosef, K. Lovgren-Bengtsson, B. Morein, P. Lewis, and J. L. Saif. 2006. High titers of circulating maternal antibodies suppress effector and memory B-cell responses induced by an attenuated rotavirus priming and rotavirus-like particle-immunostimulating complex boosting vaccine regimen. Clin. Vaccine Immunol. 13:745–749.

18. Nguyen, T. V., L. Yuan, M. S. Azevedo, K. I. Jeong, A. M. Gonzalez, C. Iosef, K. Lovgren-Bengtsson, B. Morein, P. Lewis, and J. L. Saif. 2006. Low titer maternal antibodies can both enhance and suppress B cell responses to a combined live attenuated human rotavirus and VLP-ISM vaccine. Vaccine 24:2302–2316.

19. Romero-Steiner, S., D. Libutti, L. B. Pajs, J. Dykes, P. Anderson, J. C. Whitin, H. L. Keyserling, and G. M. Carbone. 1997. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against Streptococcus pneumoniae using differentiated HL-60 cells. Clin. Diagn. Lab. Immunol. 4:415–422.

20. Silva, L. M., C. G. Baums, T. Rehm, J. H. Wisselink, R. Goethe, and P. Valentijn-Weigand. 2006. Virulence-associated gene profiling of Streptococcus suis isolates by PCR. Vet. Microbiol. 115:117–127.

21. Smith, H. E., M. Damman, J. van der Velde, F. Wagenaar, J. H. Wisselink, N. Stockhofe-Zurwieden, and M. A. Smits. 1999. Identification and characterization of the eps locus of Streptococcus suis serotype 2: the capsule protects against phagocytosis and is an important virulence factor. Infect. Immun. 67:1750–1756.

22. Smith, H. E., F. H. Reek, U. Vecht, A. L. J. Gielkens, and M. A. Smits. 1993. Repeats in an extracellular protein of weakly pathogenic strains of Streptococcus suis type 2 are absent in pathogenic strains. Infect. Immun. 61:3318–3326.

23. Vecht, U., J. H. Wisselink, J. E. van Dijk, and H. E. Smith. 1992. Virulence of Streptococcus suis type 2 strains in newborn germfree pigs depends on phenotype. Infect. Immun. 60:550–556.

24. Wisselink, H. J., H. E. Smith, N. Stockhofe-Zurwieden, K. Peperkamp, and U. Vecht. 2000. Distribution of capsular types and production of muramidase-released protein (MRP) and extracellular factor (EF) of Streptococcus suis strains isolated from diseased pigs in seven European countries. Vet. Microbiol. 74:237–248.

25. Wisselink, H. J., U. Vecht, N. Stockhofe-Zurwieden, and H. E. Smith. 2001. Protection of pigs against challenge with virulent Streptococcus suis serotype 2 strains by a muramidase-released protein and extracellular factor vaccine. Vet. Rec. 148:473–477.