SHORT COMMUNICATIONS

Immunization of Mice Against *Streptococcus suis* Serotype 2 Infections using a Live Avirulent Strain

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ABSTRACT

In this study, the IgG response of mice injected with two virulent strains and one avirulent *Streptococcus suis* capsular type 2 strain was compared by Western blotting. The serum from mice immunized against the avirulent strain could recognize most proteins of the various strains tested and similar results were obtained with serum from mice injected with virulent strains. The live avirulent strain was injected twice (days 0 and 10) to groups of five mice, and four virulent strains from different geographical origins were used to challenge the animals. All mice, except one in one group, survived the challenge. These results suggest that a live avirulent strain could be used for immunization of swine, the natural host.

RÉSUMÉ

Dans cette étude, la technique d'immunobuvardage a été utilisée pour comparer la réponse en IgG de souris auxquelles on a administré une souche avirulente de *Streptococcus suis* serotype 2 à celle de souris auxquelles on a administré deux souches virulentes du même sérotype. Le sérum des souris immunisées avec la souche avirulente a reconnu la plupart des protéines qui étaient reconnues par le sérum des souris immunisées avec les souches virulentes. La souche avirulente a été injectée aux jours 0 et 10 à des groupes de cinq souris. Les souris furent ensuite infectées en utilisant, pour les différents groupes, une des quatres souches virulentes provenant de différentes régions géographiques. Toutes les souris, sauf une dans un groupe, furent protégées contre l'infection. Ces résultats suggèrent que l'utilisation de cette souche avirulente devrait être considérée pour d'éventuels essais de protection chez l'hôte naturel, le porc, en utilisant la bactérie vivante.

Streptococcus suis capsular type 2 is an important swine pathogen, causing mainly meningitis, septicemia and arthritis (1). Attempts to control diseases with antibiotics and/or vaccination have often been disappointing, even if autogenous inactivated whole cell vaccines have shown promise (1). Successful passive immunization of mice using antisera directed against different *S. suis* proteins have been reported (2,3) but active immunization against *S. suis* cellular proteins of a given strain failed to protect mice against heterologous strains (4). Live *S. suis* strains have previously been used to protect pigs against the disease but several injections were necessary for good protection (5).

In a previous study, it was noted that the electrophoretic protein profile of an avirulent strain was similar to those of virulent isolates (6). The failure of some avirulent strains to cause disease may be related, at least in part, to their inability to increase capsule production *in vivo* (6). One aim of this study was to compare the IgG response of mice immunized with an avirulent strain and virulent strains of *S. suis*. Another objective was to evaluate the protective potential of this avirulent isolate using a murine experimental model of infection (7). This model has been used in pathological studies (8) and in attempts to predict virulence for the natural host (9).

Five *S. suis* capsular type 2 strains were used; the reference strain (735), isolated in Denmark, was provided by Dr. J. Henrichsen, Statens Seruminstitut, Copenhagen. One isolate from the United States, AAH4, was provided by Dr. Brad Fenwick, Kansas State University. One Mexican isolate, J590, was provided by Dr. Jose Luis Monter Flores, University of Toluca. Two isolates, 1591 and 1330, were from our collection. Strain 1330 was avirulent whereas the other four strains were virulent for mice and pigs (7).

We evaluated, by Western blotting, the ability of IgG produced against various strains to recognize proteins from homologous and heterologous strains. Sera of mice injected with 10^8 cells (formalin-killed) of strains 1330, 735 and 1591 were used. For Western blots, cells were cultured overnight in Todd-Hewitt broth at 37°C, harvested by centrifugation, washed and resuspended in 3 mL of K_2HPO_4 (0.1M, pH 7.0). Cells were then processed three times in a French press cell, treated with lysozyme (5 mg/mL), and the supernatants, containing cytoplasmic and membrane proteins, were recovered after centrifugation (12,000 × *g*, 20 min). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (7.5% acrylamide) was then performed (10) in order to separate cellular proteins. Following SDS-PAGE, material was transferred from the slab gel to the nitrocellulose

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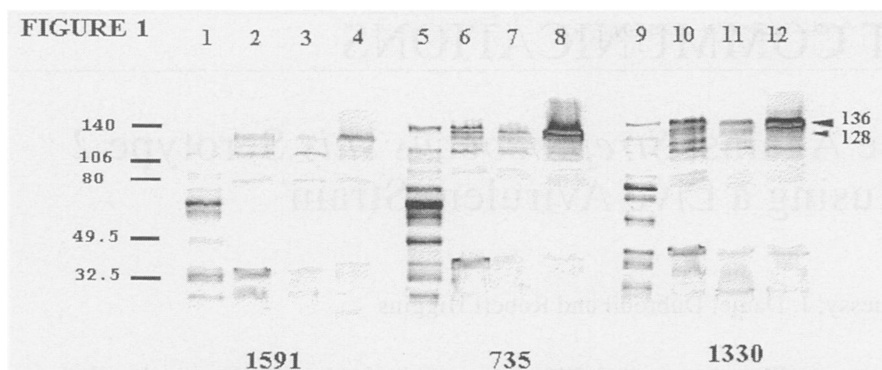

Fig. 1. Western blots of cellular proteins of *Streptococcus suis* capsular type 2 strains (7.5% acrylamide-SDS-PAGE). Protein profiles were revealed using mice antisera against strain 1330 (lanes 2, 6,10), strain 1591 (lanes 3, 7, 11) and strain 735 (4, 8, 12). Molecular weight markers in kDa. Below, strain identification numbers. Lanes 1, 5 and 9 were Coomassie blue stained proteins on acrylamide gels before transfer to nitrocellulose.

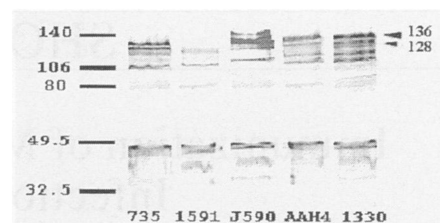

Fig. 2. Comparison, by Western blots, of cellular proteins of various *Streptococcus suis* capsular type 2 strains (7.5% acrylamide-SDS-PAGE) using mice antisera against strain 1330. Molecular weight markers in kDa. Below, strain identification numbers.

membrane by the methanol-Tris-glycine system (11) and the protein profiles were revealed using the various antisera and a peroxidase-labeled goat antiserum raised against murine IgG (Sigma Chemicals, St.Louis, Missouri) and 4-chloro-1-naphthol in cold methanol mixed with H_2O_2.

For immunization assays, four groups of five mice were injected intraperitoneally with 10^7 live cells of strain 1330 on days 0 and 14. Four other control groups were injected with PBS only. All groups of mice were challenged on day 21 by intraperitoneal injection of 10^8 cells of one of the four virulent strains. Western blots were repeated three times, the experiments with animals were repeated twice and the guidelines of the Guide to the Care and Use of Experimental Animals from the Canadian Council on Animal Care were followed.

The antisera raised against the various strains recognized most proteins of these strains and from two other virulent strains. However, some differences were detected in each strain using the different antisera. Mouse antisera against the avirulent strain recognized most proteins also recognized by antisera against the virulent strains (Fig. 1). This indicated that the mouse IgG response to the avirulent strain could recognize many proteins of virulent strains. In particular, the antiserum obtained by injecting mice with the avirulent strain recognized a protein of about 136 kDa in all virulent strains except one; this protein was not dectected in strain 1591 as previously noted (4,9), but was present in strain 1330 (Fig. 2). This 136 kDa protein was shown to be the most immunogenic *S. suis* cellular protein (4), and was recognized by a monoclonal antibody raised against the muraminidase released protein (MRP) (9,12).

Since the avirulent strain led to the production of IgG recognizing many proteins of virulent isolates and since antibodies directed against some proteinaceous epitopes had been shown to protect against the disease (4), it was suggested that antibodies directed against the avirulent strain proteins could induce protection against the virulent strains. Indeed, all mice from three groups were protected against mortality while four of five mice were protected in the fourth group (J590) (Table I). All mice, except one, died in the control groups.

TABLE I. Active immunization of mice against virulent strains of *Streptococcus suis* serotype 2 using a live avirulent strain

Mice challenged[a] with *S. suis* strain	No. of sick mice[b]/ No. of injected mice (Control groups[c])	No. of dead mice/ No. of injected mice (Control groups)
735	0/5 (5/5)	0/5 (4/5)
J 590	1/5 (5/5)	1/5 (5/5)
1591	1/5 (5/5)	0/5 (5/5)
AAH4	0/5 (5/5)	0/5 (5/5)
1330	(0/5)	(0/5)

[a] Mice were injected intraperitoneally with 10^8 CFU of each strain one week following the second injection with strain 1330
[b] Mean numbers of mice which showed nervous signs and/or prostration during the week following the experiment. Results are the mean of two separate experiments
[c] Control groups were injected twice with PBS before the challenge

Considerable genetic diversity has been found among *S. suis* isolates (13), but the avirulent strain succeeded in inducing a protection in mice against virulent strains from various geographical origins. Since a 110 kDa extracellular factor, previously reported as a virulence marker (12), was not detected in this strain it could indicate that this factor is not essential for protection.

In a previous study, the 136 kDa cellular protein was recognized by antibodies produced against a 128 kDa cellular protein, present in all strains (Fig. 1)(9). Since the sera of mice immunized with strain 1591, that does not possess the 136 kDa protein, recognized a 136 kDa in all other tested strains, it could also indicate that this strain possesses a protein, probably the 128 kDa protein, with epitopes shared by the 136 kDa protein.

Other structures, not considered in this study, such as capsular polysaccharides, may be involved in the immunity against *S. suis* but *S. suis* polysaccharides are poorly immunogenic (14). The IgG response of mice and pigs to *S. suis* capsular type 2 cellular proteins was shown to be similar (4). Thus, this study suggests that the avirulent strain 1330 would be a good candidate for vaccination of swine with live bacteria.

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