USE OF RECOMBINANT BOVINE CYTOKINES IN PIGS VACCINATED AND CHALLENGED WITH STREPTOCOCCUS SUIS

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Summary

An experiment was conducted to determine the adjuvanticity of recombinant bovine interleukin-1ß (rBoIL-1ß) and recombinant bovine interleukin-2 (rBoIL-2) administered in conjunction with a single *S. suis* vaccination in pigs. Sixty, 4-wk-old pigs were allotted to 8 groups: 1) nonvaccinated controls; 2) vaccinated controls; 3) rBoIL-1ß, 100 ng/kg; 4) rBoIL-1ß, 1000 ng/kg; 5) rBoIL-1ß, 10,000 ng/kg; 6) rBoIL-2, 2.5 μg/kg; 7) rBoIL-2, 25 μg/kg; and 8) rBoIL-2, 250 μg/kg. All pigs (except group 1) were vaccinated on d 0 with a commercial *S. suis* vaccine (serotypes 1 and 2). At vaccination, pigs were injected intramuscularly with their respective cytokine treatments. Pigs received additional cytokine injections for 2 consecutive days. On d 21, all pigs were injected intravenously with 3.5×10^9 CFU of a log phase culture of *S. suis* (serotype 2). The highest dose of rBoIL-1ß exceeded the maximum tolerable dose for the cytokine; however, this dose of rBoIL-1ß protected pigs from the *S. suis* challenge. In pigs receiving rBoIL-1ß at 10,000 ng/kg, pathological lesions caused by *S. suis* were lowest when compared to other treatment groups. No mortality from *S. suis* challenge was observed in pigs that received the highest dose of rBoIL-1ß. These data clearly show that rBoIL-1ß (10,000 ng/kg), administered intramuscularly for 3 consecutive days at vaccination, is more effective than the *S. suis* vaccine alone in protecting pigs against a *S. suis* challenge.

(Key Words: Cytokine, Adjuvant, Pig, Vaccine.)

Introduction

Cytokines, particularly interferon gamma, interleukin-1 (IL-1), and IL-2, have been used successfully as adjuvants in several species. In pigs, recombinant porcine interferon gamma has been used in an effort to reverse dexamethasone-induced immunosuppression. Although recombinant porcine IL-1α and IL-2 have been cloned and expressed, they are not available for in vivo use. Human recombinant IL-2 has been evaluated both as a nonspecific immunomodulator and as an adjuvant in pigs. We have shown that recombinant bovine IL-1ß (rBoIL-1ß) and rBoIL-2 can be effective adjuvants to bovine herpesvirus-1 vaccination in cattle. It is likely that they also will be effective adjuvants in pigs.

Infection in pigs caused by *Streptococcus suis* is a widespread problem of the swine industry in the major swine-producing countries of the world. In the United States, awareness of the severity of *S. suis* infection has been relatively slow. However, in recent years there has been an increase in reports of *S. suis* infection in all ages of pigs, frequently causing

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[3]We thank American Cyanamid Co., Princeton, NJ, for providing the cytokines and partial financial support for this project.

meningitis, septicemia, pneumonia, and arthritis.

The widespread prevalence of *S. suis* infections has necessitated extensive research efforts on prevention and control measures. Bacterins have been used in the United States for the prevention of *S. suis* infection with some success. However, the rising incidence of *S. suis*, and the economic impact that this agent imposes on the swine industry makes the development of suitable vaccination programs imperative to control the disease. Therefore, the objective of this study was to determine if rBoIL-1ß and rBoIL-2 used in conjunction with a single *S. suis* vaccination increase immunity and resistance to a homologous *S. suis* challenge.

Procedures

Sixty, 4-wk-old pigs from a herd with no known history of *S. suis* were used. Eight pigs (except Group 1) were allotted by weight and gender to one of the following 8 groups: Group 1: nonvaccinated controls (4 pigs); Group 2: vaccinated controls; Group 3: vaccinated + rBoIL-1ß at 100 ng/kg; Group 4: vaccinated + rBoIL-1ß at 1,000 ng/kg; Group 5: vaccinated + rBoIL-1ß at 10,000 ng/kg; Group 6: vaccinated + rBoIL-2 at 2.5 μg/kg; Group 7: vaccinated + rBoIL-2 at 25 μg/kg; Group 8: vaccinated + rBoIL-2 at 250 μg/kg. At the start of the experiment (d 0), pigs were vaccinated intramuscularly with a commercial *S. suis* vaccine (Oxford Laboratories, types 1 and 2). At vaccination, pigs were injected intramuscularly with their respective cytokine treatment. Pigs received additional cytokine injections for 2 consecutive days. On d 21, all pigs were injected intravenously with 3.5×10^9 colony forming units of a log phase culture of *S. suis* type 2. Pigs were weighed weekly and body weights recorded. Pigs were observed daily following challenge (early morning), and the following clinical signs were recorded: dyspnea, nasal discharge, depression, lameness, and CNS disorders. Rectal temperatures were recorded daily from d 21 through 28. All pigs were euthanized by electrocution on d 28, and gross lesions, including meningitis, pleuritis, pericarditis, peritonitis, synovitis, and pneumonia (lung weight/body weight), were scored and recorded.

Results and Discussion

Depending on the dosage, in vivo use of rBoIL-1ß and rBoIL-2 caused dramatic effects on the physiology and immunology of 4-wk-old pigs. Pigs injected with rBoIL-1ß at 10,000 ng/kg displayed profound physiological effects in response to the cytokine treatment. Within 3 hours of injection, pigs showed behavior such as vomiting and lethargy. Continued injections of 10,000 ng/kg rBoIL-1ß caused some pigs to display CNS disturbances (paddling). The adverse effect of the highest dose of rBoIL-1ß was reflected in the poor growth performance in these pigs during the first 2 wk of the study (Table 1). However, as will be discussed later, even though these pigs were very severely affected by the rBoIL-1ß injections, they responded best to the *S. suis* challenge. Their enhanced resistance to *S. suis* is perhaps best shown by their positive average daily gain during the week of infection, when pigs in all other treatment groups were losing weight (Table 1). Pigs that were administered rBoIL-2 did not respond differently than control animals.

Similar to the growth performance data, Table 2 shows data indicating that pigs treated with the highest dose of rBoIL-1ß were least affected by the challenge with *S. suis*. The day after challenge with *S. suis*, pigs in all treatment groups showed similar clinical signs of disease. However, on d 2 postchallenge, pigs treated with rBoIL-1ß at 10,000 ng/kg were less affected clinically compared to control pigs. The trend for pigs from the highest dose rBoIL-1ß treatment group to have lower clinical signs of disease continued throughout the experiment. Because 3 out of 8 control pigs

died by d 3, the difference in clinical signs between the control pigs and the highest dose rBoIL-1ß pigs (no deaths) is certainly biased in favor of no treatment effect. Pigs treated with the highest dose of rBoIL-1ß did not die when challenged with *S. suis* (Table 3). Pathological lesions caused by *S. suis* were lowest in pigs that received rBoIL-1ß as a vaccine adjuvant when compared to values from control pigs (Table 3).

These data clearly show that rBoIL-1ß (10,000 ng/kg), administered intramuscularly for 3 consecutive days at vaccination, is more effective than the *S. suis* vaccine alone in protecting pigs against a *S. suis* challenge. Pigs treated with the highest dose of rBoIL-1ß had less severe clinical signs of the disease after challenge, better growth performance during the infection, and less severe pathological lesions caused by the bacteria. Also, no pigs in this treatment group died from the bacterial challenge. However, 10,000 ng/kg of rBoIL-1ß cannot be administered to pigs because of the adverse reaction to the cytokine at the time of administration. Clearly, it would be beneficial to find a dosage of rBoIL-1ß between 1,000 and 10,000 ng/kg that produced the same positive results as the highest dose of the cytokine but without adverse effects at the time of administration. Considering the encouraging results of this study, these possibilities should be explored.

Table 1. Average Daily Gain (lb) of Pigs Vaccinated and Challenged with *S. suis* and Administered rBoIL-1ß or rBoIL-2 as Adjuvants at Vaccination

	Treatment								
		rBoIL-1ß (ng/kg)			rBoIL-2 (µg/kg)				
Period (day)	Control	100	1,000	10,000	2.5	25	250	SE	Prob.
0-7	.64^{a}	.59^{a}	.68^{a}	.26^{b}	.64^{a}	.51^{a}	.57^{a}	.03	.001
0-14	.84^{ab}	.77^{a}	.84^{a}	.66^{b}	.84^{a}	.68^{ab}	.79^{ab}	.03	.06
0-21	.92^{ab}	.90^{ab}	.97^{a}	.79^{ab}	.95^{ab}	.77^{b}	.86^{ab}	.03	.05
21-28	-.33^{a}	-.18^{ab}	-.09^{ab}	.29^{b}	-.04^{ab}	-.31^{a}	-.15^{a}	.11	.07
0-28	.66^{ab}	.66^{ab}	.73^{a}	.66^{ab}	.68^{ab}	.51^{b}	.57^{ab}	.03	.03

Pigs were vaccinated on d 0 and administered cytokines on d 0, 1, and 2. All pigs were challenged with *S. suis* 21 d after vaccination. Values are least squares means, n=8. ^{ab}Means within rows not sharing common superscripts differ.

Table 2. Pooled Clinical Signs of Pigs Vaccinated and Challenged with *S. suis* and Administered rBoIL-1ß or rBoIL-2 as Adjuvants at Vaccination

		Treatment							
			rBoIL-1ß (ng/kg)			rBoIL-2 (µg/kg)			
Day	Nonvaccinates	Control	100	1,000	10,000	2.5	25	250	SE
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	8.0	7.3	9.3	8.1	7.5	7.4	9.9	7.5	.9
2	7.0	8.3[a]	5.7[ab]	7.3[ab]	5.1[b]	7.6[ab]	7.5[ab]	7.4[ab]	1.1
3	5.5	6.2[ab]	5.9[ab]	5.3[ab]	3.1[a]	5.3[ab]	8.7[b]	6.5[ab]	1.2
4	6.3	6.2[ab]	4.4[ab]	3.4[ab]	2.8[a]	4.8[ab]	7.2[b]	5.0[ab]	1.2
5	6.3	2.8	4.3	3.6	1.3	3.5	4.8	1.8	1.3
6	3.0	2.0[ab]	3.0[ab]	2.4[ab]	1.4[a]	1.8[ab]	4.7[b]	1.2[ab]	1.1
7	2.0	2.0	1.7	3.4	1.8	1.0	3.2	.83	.9

All pigs were challenged with *S. suis* 21 d (d 0) after vaccination. Scoring = 0 to 3 (normal to severe) for dyspnea, nasal discharge, depression, and CNS disorders; 0 to 4 (normal to down) for lameness; and 0 to 5 (normal to > 107°F) for rectal temperature. Values are least squares means. [ab]Means within rows not sharing common superscripts differ (P<.05).

Table 3. Mortality and Necropsy Findings of Pigs Vaccinated and Challenged with *S. suis* and Administered rBoIL-1ß or rBoIL-2 as Adjuvants at Vaccination

		Treatment							
			rBoIL-1ß (ng/kg)			rBoIL-2 (µg/kg)			
Item	Nonvaccinates	Control	100	1,000	10,000	2.5	25	250	SE
Mortality (%)	25.0	37.5	25.0	37.5	0.0	25.0	25.0	25.0	--
Necropsy Score	7.55	7.88[a]	5.14[b]	5.12[b]	5.00[b]	6.14[a]	7.14[a]	7.00[a]	.92
Lung Weight/ Body Weight (%)	1.59	1.65	1.57	1.72	1.27[c]	1.46	1.42	1.48	.16

All pigs were challenged 21 d after vaccination with *S. suis* and necropsied at death or 7 d after challenge. Necropsy scoring = 0 to 2 (normal to severe) for pleuritis, pericarditis, meningitis, and peritonitis and 0 to 4 (normal to severe) for synovitis. Values are least squares means. [ab]Means within rows not sharing common superscripts differ (P<.05). [c]Control vs. 10,000 ng/kg rBoIL-1ß, P=.10.