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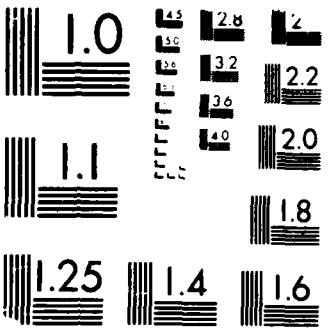

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Abstract

A commercial Bordetella bronchiseptica bacterin that does not contain adjuvant was evaluated in strain 13/N guinea pigs for efficacy against an airborne challenge of virulent B. bronchiseptica. Vaccinated animals developed humoral antibody titers that ranged from 128 to 1024, as measured by ELISA. When challenged with 325 median lethal doses (LD_{50}) of B. bronchiseptica in a small particle aerosol, the vaccinated guinea pigs were fully protected from lethal effects. Only minimal acute tracheitis with mild multifocal lymphatic hyperplasia occurred in the vaccinated, challenged animals. However, the induced immune response did not completely eliminate the challenge organisms within the 30-day observation period. Sham-vaccinated guinea pigs, on the other hand, died of a fulminant bronchopneumonia within 6 days following aerosol challenge. The commercial bacterin, therefore, provided protection against a massive airborne challenge, and prevented the inducement of significant pathological alterations.

Efficacy of a Commercial Bacterin in Protecting Strain 13 Guinea Pigs Against *Bordetella bronchiseptica* Pneumonia

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Bordetella bronchiseptica Bacterin in Guinea Pigs

The animals described in this report were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

Abstract

A commercial Bordetella bronchiseptica bacterin that does not contain adjuvant was evaluated in strain 13/N guinea pigs for efficacy against an airborne challenge of virulent B. bronchiseptica. Vaccinated animals developed humoral antibody titers that ranged from 128 to 1024, as measured by ELISA. When challenged with 325 median lethal doses (LD_{50}) of B. bronchiseptica in a small particle aerosol, the vaccinated guinea pigs were fully protected from lethal effects. Only minimal acute tracheitis with mild multifocal lymphatic hyperplasia occurred in the vaccinated, challenged animals. However, the induced immune response did not completely eliminate the challenge organisms within the 30-day observation period. Sham-vaccinated guinea pigs, on the other hand, died of a fulminant bronchopneumonia within 6 days following aerosol challenge. The commercial bacterin, therefore, provided protection against a massive airborne challenge, and prevented the inducement of significant pathological alterations.

Enzootic pneumonia in the guinea pig (Cavia porcellus), first described in 1913, was shown to be caused by Bordetella bronchiseptica (1). Fatal epizootics among guinea pig breeding colonies have continued to occur (2-4). In addition to bronchopneumonia, it also induces abortion, still births, infertility, and otitis media (5-7). Daily prophylactic administration of antibiotics will prevent B. bronchiseptica outbreaks, but does not totally eliminate the bacteria, and any lapse in drug therapy results in recrudesence of the disease (2). Because of the management problems and absence of bactericidal action associated with daily antibiotic therapy, vaccination is the preferred means of preventing B. bronchiseptica outbreaks in guinea pig colonies (2-4,8,9).

Losses from periodic epizootics of acute, fatal B. bronchiseptica pneumonia have been encountered in the strain 13/N guinea pig conventional production colony at our Institute. These outbreaks were brought under control by following a program of routine vaccination of weanling guinea pigs, as previously recommended (2). The bacterin used was obtained from the Comparative Pathology Section, Veterinary Resources Branch, National Institutes of Health (Bethesda, MD). However, the bacterin became unavailable, and a suitable, commercially-produced bacterin was sought as a replacement. Aluminum hydroxide, which is an adjuvant used in a majority of commercial B. bronchiseptica bacterins, causes an extreme local tissue reaction in the guinea pig (10), thereby making it necessary to identify a bacterin devoid of tissue damaging adjuvant. One such commercial product[1] has been shown to be efficacious in protecting vaccinated dogs against aerosol challenges of virulent B. bronchiseptica (11).

The purpose of this study was to evaluate the efficacy of the non-adjuvanted bacterin[1] in protecting strain 13/N guinea pigs against a lethal

challenge of virulent B. bronchiseptica presented in a small particle aerosol.

Materials and Methods

Animals: Inbred strain 13/N, female, 6-week-old guinea pigs (390-450 g) were obtained from the Institute's conventional production colony. Selected animals did not exhibit humoral antibodies to B. bronchiseptica, as determined by three separate sera evaluations, conducted 25, 15, and 3 days prior to the start of the vaccination regimen. Sera for antibody assay were obtained from 2 ml blood samples drawn from the anterior vena cava (12). Blood withdrawal procedures were accomplished after ketamine[2] (30 mg/kg) and xylazine[3] (6 mg/kg) administration. Each animal was ear-tagged for identification, individually housed in polycarbonate cages bedded with hardwood chip bedding[4], and maintained within ventilated racks[5]. Control animals were housed within the same room, but in racks separate from exposed animals. Open formula guinea pig ration[6] and water were provided ad libitum throughout the study.

Vaccination: Animals were vaccinated, intramuscularly, twice with 0.2 ml of either phosphate buffered saline (PBS) or a commercial non-adjuvanted bacterin[1] on days 0 and 21. The bacterin[1] is a cellular extract of antigenic material from a highly immunogenic strain of B. bronchiseptica that is recommended for prevention of canine bordetellosis, and as an aid in controlling canine infectious tracheobronchitis (13).

Bacterium: A frozen (-70°C) stock culture of a guinea pig isolate of B. bronchiseptica, strain SHGP-1, was revived, aerobically incubated, concentrated, and resuspended to a concentration of 1.7×10^9 colony forming units (CFU)/ml, as previously described (14).

Respiratory challenge: Animal exposures were performed using whole-body dynamic aerosol equipment as described previously (15). The Henderson-type aerosol transit tube was modified by incorporation of an animal exposure box. Each animal was exposed for 10 min to either tryptose phosphate broth

(TPB) or a concentration of B. bronchiseptica. Respiratory minute volumes were estimated in accordance with Guyton's formula (16). Total inhaled dose was calculated using the minute volumes and the aerosol concentration of bacteria delivered. The inhaled dose of virulent bacteria received by the challenged animals was adjusted to 325 median lethal doses (LD_{50}), 4.3×10^5 CFU (14).

Bacterial assay: Colony assay procedures were used to quantitate B. bronchiseptica in aerosol samples and tissue swab specimens. Assays for aerosol samples were performed by inoculating 5% sheep blood agar with 0.2 ml amounts of serially diluted test samples. Culture plates were incubated at 37°C for 24 hr, then concentration (CFU/ml) was determined. Samples from the larynx, trachea, and lungs were obtained by aseptic swabbing of tissues. Swabs were streaked onto blood agar, MacConkey agar, and urea slants for identification and quantification of tissue levels of B. bronchiseptica. The identity of isolated colonies as B. bronchiseptica was accomplished using the Gram stain and accepted biochemical tests (17).

Serology: Humoral antibody was quantified with an enzyme-linked immunosorbent assay (ELISA), as described previously (14). Titers, expressed as the reciprocal of serum dilution (Table 1), were considered positive at 32. This was a twofold dilution higher than the highest positive reaction, due to non-specific binding of titrated antibody, noted in blank control wells (i.e., wells without antigen).

Pathology: Tissues for histopathological examination, which included all major organs and vaccination site musculature, were fixed in a solution of 10% formaldehyde in PBS (pH 7.4), embedded in paraffin, sectioned at 6 µm, and stained with hematoxylin and eosin.

Experimental design: Five groups of guinea pigs were formed by random

allocation (Table 1). Animals in groups I and III received 0.2 ml of undiluted bacterin[1] on days 0 and 21. Guinea pigs in group IV received placebo injections of PBS. The remaining two groups of guinea pigs (II and V) were not injected. Seven days before the initial bacterin injection, daily monitoring of body weight and rectal temperature of all animals was initiated and continued throughout the study. On day 51 (30 days after the second bacterin injection), guinea pigs in groups III and IV were challenged with an inhaled dose of 4.3×10^5 of virulent B. bronchiseptica, which represents 325 median lethal doses (LD_{50}). Groups I and II were exposed to an aerosol of sterile TPB. The guinea pigs in group V represented unvaccinated, unchallenged controls. All animals were clinically evaluated twice daily after exposure. Serum was obtained from each animal two days before virulent challenge to determine the concentration of humoral antibodies to B. bronchiseptica. Surviving animals were similarly re-evaluated at 27 days after aerosol challenge. Moribund animals were necropsied and select tissues were examined for bacterial content and for development of pathological alterations. Four animals from each group exhibiting minimal ($\leq$ 2) death losses were similarly evaluated 8 days after aerosol exposure. Control animals, and all animals that survived, were evaluated 30 days after exposure.

Results

Clinical observations: Weight gains and rectal temperatures of all groups of guinea pigs were within normal limits prior to aerosol challenge. Neither bacterin[1] nor PBS administration affected either of these observations. Groups I and II, challenged with sterile diluent (TPB), exhibited weight gains and normal body temperatures similar to those observed in the control animals (Group V). The PBS-vaccinated animals that were challenged with virulent B. bronchiseptica (Group IV) ceased gaining weight within two days after aerosol exposure. Elevated temperatures and weight losses were noted by day three after exposure for this group. All animals in Group IV were moribund within 6 days of exposure, and had evidenced a 15-20% loss in body weight. Elevated rectal temperatures, sustained above 39.5°C, began after exposure and continued until death. Group III bacterin[1]-vaccinated animals, similarly challenged with a virulent aerosol, demonstrated a slight elevation in body temperature (0.5°C) within 3 days after challenge, but the temperatures returned to normal by day 7. Weight gains for animals in Group III became static within 2 days after challenge. Weight losses of up to 8% were noted in some animals in this group. At approximately 14 days after challenge, each of the animals in Group III reversed their static weight, or weight loss trends. Normal weight gains were subsequently maintained until termination of the study at 30 days. No animal in Group III died.

Serology: Animals vaccinated with PBS, as well as control animals, (Groups II, IV, and V) did not demonstrate significant ($\geq$32) antibody titers (Table 1). Animals in Group I and Group III, vaccinated with bacterin[1], exhibited humoral antibody titers that ranged from 128 to 1024.

Gross pathology: All animals from Group IV had lesions that were similar in distribution and severity. Upper respiratory tract, pulmonary, and

thoracic pathological alterations were as previously observed (14). No gross pathological alterations were noted in animals from the remaining groups.

Histopathology: Guinea pigs from Group IV had a suppurative, necrotizing bronchopneumonia and a necrotizing tracheitis. Tissues from animals of Groups I, II, and V were essentially normal. Bacterin[1]-vaccinated animals that were challenged with virulent B. bronchiseptica (Group III) exhibited minimal acute diffuse tracheitis with a mild multifocal lymphocytic hyperplasia of the lung parenchyma. Normal tissue histology was noted at the injection sites for all vaccinated animals.

Bacteriology: Guinea pigs that were sham-vaccinated and exposed to lethal doses of B. bronchiseptica (Group IV) yielded high numbers of organisms from the larynx, trachea, and lung swabs (Table 2). Animals vaccinated with the bacterin[1] and similarly challenged (Group III) had moderate numbers of B. bronchiseptica in the larynx and trachea only. The lung tissue swabs from Group III were negative for B. bronchiseptica. All of the guinea pigs in the remaining groups of animals (Groups I, II, and V) yielded negative cultures for swabs taken from larynx, trachea, and lungs.

Discussion:

Endemic B. bronchiseptica causes severe problems when recrudescence occurs in susceptible, unprotected guinea pig colonies. Survivors of B. bronchiseptica epizootics are not acceptable for use in respiratory tract research studies, and would be of marginal value in other biomedical investigations. Prevention of disease outbreaks in a conventional colony can best be accomplished by implementing a bacterin-based prophylaxis program. This enhances the quality of animal model which, in turn, yields accurate and reproducible data.

Bronchicine®, the commercial bacterin evaluated, is a non-adjuvanted whole cell extract of B. bronchiseptica. The immunogens present in the bacterin[1] induced significant humoral antibody titers (128 to 1024) when administered to guinea pigs by intramuscular injections on days 0 and 21. Antibody titers reflected relative protection against airborne challenge of virulent organisms. None of the vaccinated animals developed clinical signs or histopathological changes indicative of bordetellosis following a massive challenge of virulent B. bronchiseptica in a small particle aerosol. The commercial bacterin provoked this protective immune response in the absence of added adjuvant, thereby eliminating adjuvant-induced abscesses at the injection site.

An unexpected observation was the persistence of low numbers of B. bronchiseptica in the larynx and trachea of vaccinated animals. Obviously, the mucosal immune response was insufficient to totally eradicate the challenge bacteria. Yet, when combined, the elicited humoral and cell mediated immunity afforded protection against pathological events. Such a virus of virulent bacteria would provide an excellent source of pathogens for airborne transmission to naive animals. This is the most probable principal

reservoir of B. bronchiseptica in guinea pig colonies which suffer from endemic bordetellosis. Additional studies should delineate the duration of persistence of organisms in immune guinea pigs, the ease of transmission to susceptible guinea pigs, and the duration of induced immunity.

Based on these data, a vaccination program was implemented for the strain 13/N guinea pig colony in our Institute. Animals are given the two-dose regimen with a booster injection every six months. The six month booster injection interval was arbitrarily selected until definitive dose response curves can be established. There has been no evidence of B. bronchiseptica-induced disease within the colony since the vaccination program began. The quality of animals available for the Institute's research programs has been improved, with a resultant enhancement of investigative work that utilize the strain 13/N guinea pigs.

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Table 1

Survival and humoral antibody response in vaccinated and unvaccinated strain 13 guinea pigs against aerosol challenge with Bordetella bronchiseptica, SHGP-1

Group	Antigen	Inhaled Challenge Dose[a] (CFU)	Dead/Total (%)	Antibody Titer[b] (ELISA)
I	Vaccine[c]	Diluent[d]	0/8 (0%)	235
II	None	Diluent	0/8 (0%)	0
III	Vaccine	4.3×10^5	0/12 (0%)	513
IV	PBS	4.3×10^5	12/12 (100%)	0
V	None	None	0/8 (0%)	0

[a] Bordetella bronchiseptica, SHGP-1; Challenged 30 days after the last vaccination.

[b] Geometric mean of the reciprocal of the ELISA humoral antibody titer 2 days prior to challenge.

[c] Bronchicine®, Dellen Labs; 0.2 ml on days 0 and 21.

[d] Tryptose phosphate broth.

Table 2

Bordetella bronchiseptica levels in selected tissues

Group	Number Cultured	Antigen	Aerosol Exposure	Day After Exposure[a]	Culture Results[b]		
					Larynx	Trachea	Lungs
I	4	Vaccine[c]	Diluent[d]	30	0	0	0
II	4	None	Diluent	30	0	0	0
III	8	Vaccine	325 LD_{50}	30	2.0[e]	2.0	0
IV	12	PBS	325 LD_{50}	6	3.8	3.8	3.5
V	4	None	None	30	0	0	0

[a]Day after exposure when tissue specimens were obtained for culture.

[b]Grading system for *B. bronchiseptica* isolates:

1+ = Urea slant negative; < 5 colonies on blood or MacConkey agar.

2+ = Urea slant negative; 5-25 colonies on blood or MacConkey agar.

3+ = Urea slant positive at $\leq$ 48 hr; 25-100 colonies on blood or MacConkey agar.

4+ = Urea slant positive at $\leq$ 24 hr; > 100 colonies on blood or MacConkey agar.

[c]Bronchicine®, Dellen Labs.

[d]Tryptose phosphate broth.

[e]Geometric mean for the tissues examined.

Footnotes

[1]Bronchicine®, Dellen Labs, Inc., Omaha, NE

[2]Vetalar®, Parke Davis, Morris Plains, NJ

[3]Rompun®, Haver-Lockhard, Shawnee, KS

[4]Beta Chip®, Northeastern Products Corp., Warrensburg, NY

[5]Ventilated Animal Rack®, Lab Products, Inc., Rochelle Park, NJ

[6]NIH Production Guinea Pig Chow, Agway, Inc., St. Marys, OH

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