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Evaluation of the humoral and cellular immune response to different antigens of *Corynebacterium pseudotuberculosis* in Canindé goats and their potential protection against caseous lymphadenitis

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Abstract

Corynebacterium pseudotuberculosis is the etiologic agent of caseous lymphadenitis, a disease that affects goats and sheep, and can cause severe economic losses. In this study, four different antigenic extracts were obtained from the attenuated strain T1, which was isolated in the state of Bahia (Brazil). Forty-four Canindé breed goats were divided in five groups, each receiving a different antigen solution and saline buffer as a control. The humoral response was monitored through the identification of specific IgG by indirect ELISA and Western Blotting, and the production of IFN-γ was followed in order to observe the activation of cellular response. After twelve weeks of antigen inoculation, the animals were challenged with 2×10^5 CFU of a wild strain, also isolated in Bahia, and necropsy was performed on all animals twelve weeks afterwards. It was observed that the attenuated bacteria gave a protection of 33.3%, in addition to the weak humoral response elicited. Animals inoculated with secreted antigen associated with Freund's incomplete adjuvant and oligodeoxynucleotide containing unmethylated CpG dinucleotides (CpG ODN) showed a strong humoral response, but this inoculation could not prevent the spread of challenge bacteria in the majority of animals. These results demonstrate the immunogenic potential of the attenuated T1 strain in the development of a vaccine against caseous lymphadenitis in goats.

Keywords: Caseous lymphadenitis; Goats; *C. pseudotuberculosis*; CpG ODN; Vaccine

1. Introduction

Caseous lymphadenitis is an infectious disease that affects small ruminants, particularly goats and sheep. The bacteria *Corynebacterium pseudotuberculosis* is the causative agent of the pathology (Batey, 1986; Brown and Olander, 1987; Williamson, 2001). The major clinical feature of this disease is the development of granulomas in peripheral and internal lymph nodes, containing necrotic tissue. In goats, other organs can be

Abbreviations: TPP, three phase partitioning; FIA, Freund's incomplete adjuvant; BHI, brain and heart infusion; ODN, oligodeoxynucleotides.

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affected, such as lungs and liver. The disease exists worldwide (Sting et al., 1998; Pepin et al., 1999; Paule et al., 2003), and the bacteria can affect other species, like horses, bovines, but it rarely affects humans (Peel et al., 1997; Mills et al., 1997). *C. pseudotuberculosis* released from superficial abscesses persists in environment for a longtime and may become a source of contamination for the other animals in herd (Pepin et al., 1999). In the northeast region of Brazil, there is a high prevalence of the disease in goats, causing elevated economic losses, due to a reduction in weight gain and milk production, as well as problems in the commercialization of products derived from animal meat (Unanian et al., 1985; Brown and Olander, 1987; Brown et al., 1987).

Therapeutic treatment of the disease is not effective, as the pathogen has an intracellular location, and the distribution of drugs inside of the granuloma is poor. The puncture of the peripheral affected lymph nodes is the only viable treatment, but it can cause the spread of the bacteria in the environment, therefore elevating the risk of contamination (Nairn and Robertson, 1974). The internal granulomas are difficult to diagnose and may be a source of contamination for other animals (Ellis et al., 1987).

Several experimental trials have been developed in order to achieve a reliable vaccine to control the disease in sheep and goats. Different antigen preparations have been employed, such as formalin-killed bacterin, bacterial cellular wall and phospholipase D toxoid (Cameron et al., 1972; Brogden et al., 1984, 1996; Brown et al., 1986; Eggleton et al., 1991). An association of bacterin and formalin inactivated exotoxin was also tested, resulting in partial immunity characterized by fewer affected lymph nodes in each animal and fewer animals presenting with disease (Piontkowski and Shivvers, 1998).

In this study we used 44 Canindé breed goats from the state of Bahia in Brazil. Canindé is a naturalized Brazilian breed from northeastern Brazil and the experimental animals were from the same flock of purebreds. We tested the protective capacity and protection of crude *C. pseudotuberculosis* culture supernatant associated to FIA. In addition, we assessed the capacity of CpG ODN to improve the immune response and the protection against challenge of an antigen obtained through concentration of crude culture supernatant. Finally, we observed the capacity of a living attenuated strain to protect against challenge with a wild strain. The *C. pseudotuberculosis* T1 strain used in this experiment was isolated from a goat's lymph node, in the state of Bahia (Paule et al., 2004a). It is considered a natural, attenuated strain due to a weak synergistic hemolytic activity when co-cultivated with *Rhodococcus equi* and the absence of pathologies when susceptible BALB/c mice were inoculated with the strain (Vale et al., unpublished data), and is considered as an alternative in the development of a vaccine against caseous lymphadenitis.

2. Methodology

2.1. Bacterial strains

Two different strains of *C. pseudotuberculosis* were employed in this experiment. The strain T1, had its identification confirmed by Gram staining, colony morphology, synergistic hemolytic activity with CAMP factor of *Rhodococcus equi*, urease and catalase production. A commercial kit to perform a more reliable identification was also used (API Coryne - BioMérieux). The pathogenic strain, employed as a challenge to vaccinated animals, was named VD57, and had a similar identification process as the attenuated strain. This pathogenic strain was isolated from a goat in the city of Juazeiro, Bahia State, Brazil.

2.2. Antigens

2.2.1. Secreted antigen

The *C. pseudotuberculosis* T1 strain was cultivated in Brain Heart Infusion (BHI) broth at 37 °C for 72 h. The culture was centrifuged for 30 min at 10,000 × *g*. Supernatant was filtered through a 0.22 μm membrane filter. This supernatant was kept at −20 °C until use. The amount of protein was determined by Lowry's modified method (Bio-Rad). The protein concentration determined was 6.2 mg/ml. This antigen was used in Group 1.

2.2.2. Secreted antigen concentrated using three phase partitioning method (TPP)

This antigen was obtained as previously described (Paule et al., 2004a), with some modifications. Briefly, the secreted antigen was prepared from culture supernatant saturated with 30% ammonium sulfate pH 4.0 (HCl) and *n*-butanol, under slow agitation at room temperature. The sample was homogenized, kept undisturbed for 60 min, and centrifuged for 10 min at 1350 × *g* at 4 °C. The resulting interface was dissolved in small volumes of 20 mM Tris buffer pH 7.4 (500 μl of buffer to 5 ml of supernatant extract) followed by dialysis in 50 mM phosphate buffer pH 7.4 for 48 h. Antigen was concentrated by ultra filtration with a