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Recombinant bovine interleukin 2 enhances immunity and protection induced by *Brucella abortus* vaccines in cattle

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Abstract

Augmentation of immunization of cattle *Brucella abortus* S19 or a *B. abortus* soluble protein extract (SPEBA) vaccine through administration of recombinant bovine IL 2 (rBoIL 2) was evaluated. Seventy-five heifers were divided among 6 groups that were treated with the following: Group 1, no treatment; Group 2, rBoIL 2 (1 μg/kg) on day 0; Group 3, SPEBA (2 mg) on day 0 and week 9; Group 4, SPEBA + rBoIL 2 on day 0, SPEBA on week 9; Group 5, S19 (10^7 CFU) on day 0 and week 9; Group 6, S19 + rBoIL 2 on day 0, S19 only on week 9. Approximately, 6 months after vaccination, cattle were bred by natural service, and at mid-gestation pregnant cattle were challenged intraconjunctivally with 9.1×10^5 CFU of virulent *B. abortus* S2308. Pre- and post-challenge antibody responses were measured by an enzyme-linked immunosorbent assay, a particle concentration fluorescence assay, and the card test. Lymphoproliferation (LP) responses to γ-irradiated *B. abortus* and SPEBA antigens were measured in peripheral blood mononuclear cells. After vaccination, antibody responses to *B. abortus* elevated rapidly in SPEBA- and S19-vaccinates with and without rBoIL 2, however, these responses were significantly ($P < 0.05$) higher in vaccinates which also received rBoIL 2. Antibody levels for all vaccinated groups had returned to those of negative control groups by the challenge date with the exception of the SPEBA/rBoIL 2 group. In general, LP responses were higher in vaccinated or rBoIL 2-treated cattle than for unvaccinated controls. Challenge of 48 pregnant heifers resulted in abortions in 4/9 of Group 1, 0/9 of Group 2, 4/8 of Group 3, 2/9 of Group 4, 1/7 of Group 5, and 0/6 of Group 6 cattle. Treatment with rBoIL 2 alone (Group 2) provided significant ($P < 0.05$) protection from infection, abortions and induction of sero-positive status compared to untreated (Group 1) cattle. Co-administration of rBoIL 2 with S19 resulted in significant ($P < 0.05$) augmentation in onset, duration and magnitude of LP responses to *B. abortus* antigens following challenge. Characterization of the cytokine response of bovine monocyte-derived macrophages by real-time polymerase chain reaction indicated that in vitro stimulation of these cells with rBoIL 2 resulted in a profound up-regulation of genes encoding tumor necrosis factor-α, IL 12p40, and interferon-γ reflecting activation of the cells.

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Overall, rBoIL 2-treatment was associated with fewer infections, sero-conversions and a significant ($P = 0.02$) level of protection against abortion as compared to vaccination alone or no treatment.

Keywords: Interleukin 2; *Brucella*; Vaccine; Bovine; Immunity

1. Introduction

Bovine brucellosis continues to be a regulatory disease problem in certain regions of the USA despite past widespread use of live *Brucella abortus* strain 19 (S19) vaccine and current use of *B. abortus* RB51 as successful portions of a national eradication program. Vaccination of cattle with these live vaccines increases resistance to natural and experimental infection with *B. abortus* (Deyoe et al., 1979; Confer et al., 1985). The degree of vaccine-induced protection, however, varies with such factors as challenge dose, age at immunization, vaccine dose, interval between immunization and challenge, and reproductive status at challenge. Searches for an efficacious subunit or killed *B. abortus* vaccine are ongoing (Winter et al., 1983; Montaraz and Winter, 1986; Confer et al., 1987; Smith et al., 1990; Dzata et al., 1991a,b).

The mechanism(s) of live vaccine-induced immunity are not entirely understood but appear to be primarily associated with cell-mediated immunity (CMI, Confer et al., 1985; Splitter and Everlith, 1989) including macrophage (MΦ) activation and killing of infected MΦ by $CD4^+$ and $CD8^+$ T lymphocytes (Wyckoff, 2002). Conversely, we recently reported that S19-vaccination induced strong T suppressor (T_S) cell responses in some cattle which may down-regulate immunity to *B. abortus* challenge and result in reduced immunity in certain cattle (Wyckoff and Confer, 1990). Despite its stimulatory effects on T_S cells, S19-vaccination also stimulates interleukin 2 (IL 2) production by peripheral blood mononuclear cells (PBMC) following specific antigenic stimulation (Wyckoff and Confer, 1990). IL 2 is a glycoprotein, secreted mainly by activated T helper (T_h) lymphocytes, that can enhance cytotoxic defenses, stimulate interferon-γ production, enhance B lymphocyte responses (Gillis et al., 1978; Smith, 1988) and activate MΦ to some degree (Malkovsky et al., 1987, 1988; Panelli et al., 2002). Administration of recombinant IL 2 can enhance immunity to experimental vaccines (Weinberg and Merigan, 1988; Reddy et al., 1989; Karvashima and Platt, 1989; Hughes et al., 1991) and limit various experimental bacterial infections in mice (Chong, 1987; Iizawa et al., 1988; Jeevans and Asherson, 1988; Haak-Frendscho et al., 1989).

Since IL 2 has been shown to enhance immune function, we vaccinated heifers in the present study with S19 or a soluble protein extract of γ-irradiated S19 (SPEBA) with or without the administration of recombinant bovine IL 2 (rBoIL 2). Pregnant heifers were subsequently challenged at mid-gestation with virulent *B. abortus* S2308. SPEBA was tested in the experiment because it previously induced low primary antibody responses in standard assays, yet induced measurable CMI responses when incorporated with appropriate adjuvants (Dzata et al., 1991a).

2. Materials and methods

2.1. Cattle

Seventy-five crossbred beef heifers were obtained at 9–10 months of age. These heifers were obtained from a herd that had no clinical signs of brucellosis and, when tested serologically (days −7 and 0), were negative for *B. abortus*. They were transported to the Livestock Health Research Center in Hugo, Oklahoma, maintained on a Bermuda grass pasture, and fed a protein supplement (Confer et al., 1985). At the time of challenge and thereafter, the cattle were placed in pens and fed hay with protein supplement.

2.2. Serologic tests

The standard brucellosis card test was used. For evaluation of the card test, the criteria of Deyoe et al. (1979) was used, i.e., any degree of readily visible agglutination was considered to be positive. The enzyme-linked immunosorbent assay (ELISA) was