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Development of an Adult-Like Cell-Mediated Immune Response in Calves After Early Vaccination with *Mycobacterium bovis* bacillus Calmette-Guérin*

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ABSTRACT

Effects of neonatal vaccination on antigen-specific cellular and humoral immune responses of dairy calves have not been well described. The purpose of this study was to characterize the ontogeny of the adaptive immune response in calves sensitized to the attenuated strain of *Mycobacterium bovis*, bacillus Calmette-Guérin. Holstein bull calves were nonvaccinated (n = 6, vaccination controls) or vaccinated subcutaneously (n = 6) with bacillus Calmette-Guérin at 1 and 7 wk of age. Composition and functional capacities of blood mononuclear cell populations from calves were evaluated at 1 (prevaccination), 3, 6, 7, 8, 9, and 12 wk of age. Young adults (nulliparous heifers, n = 4) vaccinated in an identical manner were sampled concurrently to evaluate effects of animal maturity on the development of the adaptive immune response. Responses of nonvaccinated calves to recall antigen (*Mycobacterium bovis* purified protein derivative) ex vivo and in vivo (i.e., cutaneous delayed-type hypersensitivity) were minimal or nonexistent. Responses of cells from vaccinated calves and young adults to recall antigen, however, were evident as early as wk 2 after primary vaccination. Antigen-induced T cell subset proliferation, and secretion of interferon-γ, nitric oxide, and tumor necrosis factor-α by cells from vaccinated calves were comparable to or greater than responses of vaccinated adults during the 11-wk study. Eleven weeks after primary vaccination, cutaneous responses of vaccinated calves and young adults to intradermal administration of antigen were pronounced and comparable, demonstrating the capacity of the bovine neonate to develop a vigorous cell-mediated immune response in vivo. Antibody responses (i.e., antibody concentrations in sera and in supernatants from antigen-stimulated cultures of blood mononuclear cells) of vaccinated calves, in contrast, were markedly lower than parallel responses of vaccinated adults. In conclusion, these results suggest that the bovine neonate can mount a vigorous, adult-like cell-mediated immune response when vaccinated at an early age.
(**Key words:** neonatal vaccination, adaptive immunity, *Mycobacterium bovis* BCG, calf)

Abbreviation key: BCG = bacillus Calmette-Guérin, **FBS** = fetal bovine serum, **PBMC** = peripheral blood mononuclear cells, **PBST** = PBS with Tween 80, **PBST-g** = PBST with gelatin, **PPD** = purified protein derivative, **PPDa** = *Mycobacterium avium*-derived PPD, **PPDb** = *Mycobacterium bovis*-derived PPD, **PWM** = pokeweed mitogen, **Th1** = T_{helper} 1-type response, **Th2** = T_{helper}2-type response, **TNF**: tumor necrosis factor, **WCS-PK** = proteinase K-digested whole cell sonicate of *Mycobacterium bovis* BCG.

INTRODUCTION

Effects of early vaccination on the adaptive immune response of the neonatal calf have not been well described. Protection afforded by early vaccination is critical given the diversity and number of potential pathogens in the calf's environment. The immune system of the neonate, however, is considered developmentally immature and incapable of mounting adult-like responses to antigenic stimulation (Adkins, 2000; Morein et al., 2002). Developmental immaturity of the calf's immune system may compromise the efficacy of vacci-

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nation protocols and exacerbate the calf's susceptibility to infectious disease.

The neonatal immune system is characterized by a T-cell population with a high proportion of naïve T cells that can suppress Ig production (Clement et al., 1990). Neonates also have higher proportions of antigen-presenting cells with defective costimulatory activity (Ridge et al., 1996) and a decreased capacity to produce cytokines, particularly those associated with $T_{helper}1$ (**Th1**) responses (Adkins, 2000; Siegrist, 2000). The Th1-biased response is necessary for protection against viruses and intracellular bacteria, and is characterized by the predominant production of IFN-γ. In infant mice, exposure to antigen leads to a $T_{helper}2$ (**Th2**)-biased response characterized by IL-4 secretion and antibody responses predominated by the Ig isotype, IgG_1. The ruminant animal (calf) is unique in that it is agammaglobulinemic when born and relies on ingestion of colostrum for acquisition of maternal immunoglobulin (passive immunity) and viable leukocytes (adoptive immunity) to afford protection against infection. Although maternally derived immune factors provide early protection, they may interfere with postnatal activation of the calf's own immune system and its capacity to mount a protective response to vaccination or infection. In humans, inhibition of the infant's responses to vaccination by maternal antibody is B-cell specific, depends on antibody titer and dose of vaccine antigen, and does not appear to influence responses of T cells (Siegrist, 2003).

Experiments demonstrating cell-mediated immunity frequently use animals immunized with *Mycobacterium bovis*. When challenged with mycobacterial antigens, a protective Th1 response is triggered in sensitized individuals. Infusion of tuberculin into the mammary glands of cows sensitized to mycobacteria results in antigen-specific mammary and peripheral responses characteristic of a cell-mediated immune response (Nickerson and Nonnecke, 1986; Nonnecke et al., 1986). Results from a recent study (Hope et al., 2002) indicate that in the neonate, $CD3^+/CD8^+$ natural killer cells produce IFN-γ in response to dendritic cells infected with attenuated *M. bovis* strain, bacillus Calmette-Guérin (**BCG**). Human newborns immunized with BCG develop Th1 responses of similar magnitude to those produced by adults (Marchant et al., 1999; Vekemans et al., 2001; Ota et al., 2002). These observations suggest that a BCG sensitization/purified protein derivative (**PPD**) challenge model might provide new information regarding the ontogeny of the adaptive arm of the immune system of the neonatal calf.

Objectives of this study were to characterize and compare adaptive (antigen-specific) immune responses of young and adult dairy cattle using a BCG sensitization and PPD challenge model. Of particular interest was the comparison of the magnitude and make-up of cellular and humoral responses of calves and young adults during the 11-wk period after primary vaccination with BCG.

MATERIALS AND METHODS

Animals and Treatments

Twelve age-matched male Holstein calves housed at the Land O'Lakes Research Farm, Webster City, IA, and 4 young adults (nulliparous heifers) housed at the National Animal Disease Center, U. S. Department of Agriculture, Agriculture Research Service, Ames, IA, were used. Calves were chosen for inclusion in the study if their serum gamma globulin concentrations were indicative of successful passive immunity. At the initiation of the study, calves were approximately 7 d of age (range: 3 to 10 d of age). Animal-related procedures were implemented following institutional guidelines for animal care. From 1 to 7 wk of age, calves were housed on elevated stalls in an indoor, ventilated environment. During this period they were fed milk replacer (0.57 kg/d, comprising 22% CP and 20% fat; Land O'Lakes, Inc.) and calf starter (ad libitum, 18% texturized CP; Land O'Lakes, Inc.) with lasalocid acid. Calves were weaned at 7 wk of age. During the postweaning phase of the study (7 to 12 wk of age), calves were housed outdoors in free-stalls bedded in straw and were fed a diet of 18% texturized crude protein (2.7 kg/d) with lasalocid (0.46 g/10 kg of CP). Hay was provided ad libitum.

Before vaccination, young adults were tested and confirmed negative for *M. bovis* and *Mycobacterium avium* exposure using a commercially available assay (Bovigam, CSL Ltd., Parkville, Victoria, Australia) to evaluate the responsiveness of blood lymphocytes to mycobacterial antigens. Adults were housed outdoors, received water ad libitum and a balanced ration consisting of pelletized alfalfa and grain.

Preparation and Administration of BCG Vaccine

The BCG (Pasteur strain) was grown in Middlebrook's 7H9 media supplemented with 10% oleic acid-albumin-dextrose complex (Difco, Detroit, MI) plus 0.05% Tween 80 (Sigma Chemical Co., St. Louis, MO) as described for virulent *M. bovis* (Bolin et al., 1997). Briefly, mid log-phase growth bacilli were pelleted by centrifugation at $750 \times g$, washed twice with PBS (0.01 *M*, pH 7.2), and diluted to the appropriate cell density in 2 mL of PBS. Bacilli were enumerated by serial dilution plate counting on Middlebrook's 7H11 selective media (Becton Dickinson, Cockeysville, MD).