Antigen-Specific B Cell Responses of Vaccinated, Neonatal Calves

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Monica Foote, Ph.D. student, Dept. Animal Science, ISU, currently a postdoctoral researcher, Cornell U, Ithaca;
Brian Nonnecke, National Animal Disease Center, Ames;
Donald Beitz, Dept. Animal Science, ISU;
Ray Waters, National Animal Disease Center, Ames

Summary and Implications

The immune response of newborn calves to early vaccination is often variable and frequently characterized by marginal or nonexistent antibody responses. The B cell subpopulation of immune cells is pivotal in the production of antibody and has not been characterized completely in the newborn calf. Results from this research describe the composition and antigen-specific responses of B cell populations in preruminant calves vaccinated at an early age. Although preliminary, these data indicate that the responsiveness of B cell population in young calves is dependent on the nature of the vaccine and less on animal maturity. This research provides important new information regarding the immune responsiveness of the neonatal calf to vaccination.

Introduction

Economic losses associated with infectious diseases in preruminant calves are substantial. The mortality rate for pre-weaned dairy calves is approximately 8 to 11% and the morbidity rate is approximately 37%.

The calf is born without measurable serum immunoglobulin and relies on ingestion of colostrum for establishment of passive immunity. Ingestion of colostrum is necessary for acquisition of protective maternal Ig; however, it has been suggested that colostral Ig compromises adaptive immune responses of vaccinated calves. For example, neonatal calves fail to develop antigen-specific Ig (i.e. antibody, Ab) responses to *Brucella abortus* if antigen-specific maternal Ab is present at the time of vaccination. In the absence of colostral Ab, calves develop vigorous Ab responses to *B. abortus*. Preruminant calves sensitized to ovalbumin (**OVA**) (an antigen to which there was no maternal Ig) at birth mount humoral responses similar to older cattle, regardless of colostral status. Likewise, neonatal calves vaccinated with an attenuated strain of *Mycobacterium bovis* (strain BCG) fail to mount measurable Ab responses; however, they do develop vigorous cell-mediated immune responses. In addition, calves given a Bovine Viral Diarrhea virus vaccine develop antigen-specific $CD4^+$, $CD8^+$, and γδ TCR^+ cell responses and generate memory T and B cells, despite primary humoral responses being blocked by maternal Ab. These results suggest that although maternal Ab may block the vaccinated calf's antibody response, its B cell population retains the capacity to differentiate into memory B cells.

The present study characterized the composition and function of B cell populations from neonatal calves vaccinated at an early age with OVA and BCG. Ovalbumin was selected because it is not encountered in the natural environment of cattle and it has been shown that the magnitude of Ab responses of neonatal and adult cattle to this antigen is similar. BCG was utilized because it induces an Ab response in adult cattle but not in colostrum-fed neonatal calves, presumably due to blocking maternal Ab resulting from exposure of dams to environmental mycobacteria. Potential effects of BCG vaccination on OVA-specific Ab responses were also considered.

Materials and Methods

Vaccination Schedule for Experiment 1: Six Holstein bull calves were acquired at <4 d of age. Calves in this experiment and Experiment 2 (see below) each received 3.9 L of fresh colostrum within 6h of birth. At 3 wk of age, all calves were vaccinated subQ in the mid-cervical region, left side of the neck, with OVA/incomplete Freund's adjuvant (IFA). On the same day, 3 calves were vaccinated subQ in the right mid-cervical region of the neck with BCG. All calves were revaccinated with OVA/IFA at 5 wk of age.

Vaccination Schedule for Experiment 2: Holstein bull calves (24) were acquired at <5 d of age. At 3 wk of age, all were vaccinated subQ in the left mid-cervical region of the neck with OVA. On the same day, all calves were vaccinated with BCG (subQ in the right mid-cervical region). All calves were revaccinated with OVA at 5 wk of age (14d after initial sensitization). Six calves (n = 6) were euthanized at 7 wk of age. Superficial cervical lymph nodes from both sides of the neck were harvested.

Antigens: Recall antigens were OVA and *M. bovis* purified protein derivative (**PPD**). A proteinase K-digested whole cell sonicate of *M. bovis* BCG (**WCS-PK**) was used as capture antigen.

Measurement of Ig in Serum and in Culture Supernatants: Antigen-specific IgG_1 and IgG_2 concentrations were determined for serum samples collected weekly before and after vaccination. OVA-specific and WCS-PK-specific Ig concentrations in supernatant from non-stimulated and antigen-stimulated immune cell cultures were evaluated using a capture ELISA.

Activation Molecule Expression on B cells Isolated from Lymph Nodes: Mononuclear cells recovered from superficial cervical lymph nodes were used to establish cultures in microtiter plates. Cultures were not stimulated

(media only), stimulated with OVA, and stimulated with PPD and subsequently incubated for 3 or 6 d at 39°C in a 5% CO_2 atmosphere. Cells were phenotyped by flow cytometry (Table 1). Cells exhibiting light scattering properties consistent were bovine mononuclear cells were analyzed.

Table 1. Primary and secondary antibodies used in flow analysis of cervical lymph node cells from vaccinated calves.

Primary antibody specificity	Clone	Isotype	Source	Secondary antibody
sIgM	Big73A	IgG_1	VMRD	αIgG_1-PerCP
sIgM	PIG45A	IgG_{2b}	VMRD	αIgG_{2b}-Cy5
CD5	B29A	IgG_{2a}	VMRD	αIgG_{2a}-PE
B-B2	BAQ44a	IgM	VMRD	αIgM-FITC
CD25	LCTB2A	IgG_3	VMRD	αIgG_3-FITC
CD21	GB25A	IgG_1	VMRD	αIgG_1-PerCP
CD11a/18	BAT75A	IgG_1	VMRD	αIgG_1-PerCP
CD40		IgG_1	D.M. Estes	αIgG_1-PerCP
CD80	BBI	IgM	Pharmingen	αIgM-FITC

Results and Discussion

Vaccination of 3-wk old calves with OVA elicited measurable Ab responses (Figure 1) that were amplified by revaccination supporting previous research demonstrating that the neonatal calf possesses the capacity to produce Ab responses to antigens not present in the natural environment. OVA-specific recall responses of B cells from lymph nodes draining the OVA vaccination site (Table 2) were characterized by decreased CD5, CD21, and CD40 expression and increased BB2, CD25, and CD80 expression. Vaccination with BCG, in contrast, did not augment existing levels of mycobacteria-specific Ab (Figure 2). PPD-induced recall responses of B cells from lymph nodes draining the site of BCG vaccination site (Table 3) were characterized by increased CD25 and CD80 expression suggesting that although maternal Ab may block endogenous Ab production, the calf's B cells are still activated by BCG vaccination. Interestingly, B cells from lymph nodes draining the vaccination site, but not from the opposing side of the body, responded to antigenic stimulation, suggesting initiation and maturation of adaptive immune responses are localized within nodes draining the vaccination site. Overall, these data indicate that the responsiveness of B cells in neonatal calves is dependent on the nature of the vaccine and less on animal maturity. Future studies will consider the effects of the colostral Ab on responses of calves to early vaccination and infection.

Table 2. Expression of activation molecules on IgM^+ B cells and $CD5^+IgM^+$ B cells isolated from left superficial cervical lymph nodes draining the OVA vaccination site.

		Stimulation	
Cell Phenotype	Nonstimulated	OVA	PPD
IgM^+ B cells			
% CD5	66.2 ± 2.6[a]	49.4 ± 4.3[b]	61.7 ± 2.2[a]
CD5 MFI	67.8 ± 10.9[a]	25.3 ± 6.2[b]	45.3 ± 6.4[ab]
% B-B2	37.6 ± 2.9[a]	54.0 ± 3.2[b]	42.0 ± 2.0[a]
B-B2 MFI	7.5 ± 0.7[a]	12.0 ± 1.3[b]	8.5 ± 0.6[a]
CD21 MFI	4.9 ± 0.2[a]	4.2 ± 0.2[b]	4.7 ± 0.2[ab]
CD25 MFI	4.0 ± 0.3[a]	7.0 ± 0.8[b]	4.9 ± 0.3[a]
MHC-II MFI	5.6 ± 0.7	9.2 ± 1.7	7.0 ± 1.0
CD11a/18 MFI	11.1 ± 0.9	8.6 ± 0.9	10.5 ± 1.0
CD80 MFI	4.5 ± 0.5[a]	6.8 ± 0.5[b]	5.3 ± 0.4[a]
CD40 MFI	5.8 ± 0.4[a]	4.5 ± 0.3[b]	5.1 ± 0.3[ab]
$CD5^+IgM^+$B cells			
% B-B2	17.2 ± 1.96[a]	30.5 ± 2.5[b]	22.7 ± 1.0[a]
B-B2 MFI	3.7 ± 0.2[a]	4.8 ± 0.2[b]	4.0 ± 0.2[a]
CD21 MFI	4.9 ± 0.3[a]	4.0 ± 0.2[b]	4.5 ± 0.3[ab]
CD25 MFI	3.0 ± 0.2[a]	5.0 ± 0.6[b]	3.7 ± 0.2[a]
MHC-II MFI	2.8 ± 0.1[a]	4.1 ± 0.5[b]	3.4 ± 0.2[ab]
CD11a/18 MFI	14.1 ± 0.9	12.4 ± 1.2	14.4 ± 1.2
CD80 MFI	3.3 ± 0.3[a]	4.1 ± 0.2[b]	3.8 ± 0.2[ab]
CD40 MFI	5.2 ± 0.3[a]	4.2 ± 0.3[b]	4.8 ± 0.4[ab]

[1] Left superficial cervical lymph node cells from calves vaccinated with OVA on the left side of the neck at 3- and 5-wk of age and with BCG on the right side of the neck at 3 wk of age. At 7-wk of age lymph nodes were harvested and cultured in vitro in absence and presence of antigens (OVA and PPD). Mean fluorescence intensities (MFI; ± SEM) and percent of IgM^+B cells and $CD5^+IgM^+$ B cells positive for each phenotype are shown.
[ab] Means within a row with different superscripts differ ($P < 0.05$).

Table 3. Expression of activation molecules on IgM^+ B cells and $CD5^+IgM^+$ B cells from right superficial cervical lymph nodes draining the BCG vaccination site.

		Stimulation[1]	
Cell Phenotype	NS	OVA	PPD
IgM^+ B cells			
% CD5	66.3 ± 3.7	66.2 ± 2.8	60.0 ± 3.6
CD5 MFI	64.7 ± 15.1	54.7 ± 8.5	39.4 ± 7.4
% B-B2	40.2 ± 4.4	43.3 ± 2.8	45.3 ± 2.0
B-B2 MFI	7.8 ± 0.7	8.3 ± 0.6	9.6 ± 0.9
CD21 MFI	5.0 ± 0.1	4.9 ± 0.2	4.8 ± 0.2
CD25 MFI	4.2 ± 0.4[a]	4.6 ± 0.3[a]	6.1 ± 0.5[b]
MHC-II MFI	6.1 ± 1.0	6.3 ± 0.8	8.9 ± 1.8
CD11a/18 MFI	10.8 ± 1.1	9.9 ± 0.5	11.0 ± 1.4
CD80 MFI	4.9 ± 0.4[a]	5.4 ± 0.4[ab]	6.3 ± 0.5[b]
CD40 MFI	5.4 ± 0.1	5.0 ± 0.2	5.1 ± 0.2
$CD5^+IgM^+$B cells			
% B-B2	20.5 ± 3.3	24.7 ± 2.5	27.0 ± 2.1
B-B2 MFI	4.0 ± 0.3	4.5 ± 0.2	4.4 ± 0.2
CD21 MFI	4.9 ± 0.2	4.8 ± 0.3	4.6 ± 0.3
CD25 MFI	3.1 ± 0.2[a]	3.5 ± 0.2[a]	4.9 ± 0.4[b]
MHC-II MFI	2.9 ± 0.1[a]	3.3 ± 0.2[a]	4.2 ± 0.4[b]
CD11a/18 MFI	13.3 ± 1.0[ab]	12.1 ± 0.6[a]	15.0 ± 0.8[b]
CD80 MFI	3.7 ± 0.2[a]	4.1 ± 0.2[ab]	4.6 ± 0.3[b]
CD40 MFI	5.0 ± 0.2	4.8 ± 0.2	4.7 ± 0.3

[1] See Table 1 footnote

Table 4. Effects of lymph node location on expression of activation molecules by OVA-stimulated sIgM$^+$ cells. Calves were vaccinated with OVA on left side only at 3 and 5 wk of age.

	Location of lymph node	
Cell Phenotype	Left	Right
OVA-stimulated		
% CD5 positive	49.4 ± 4.3	66.2 ± 2.8*
CD5 MFI	25.3 ± 6.2	54.7 ± 8.5*
% B-B2 positive	54.0 ± 3.2	43.3 ± 2.8*
B-B2 MFI	12.0 ± 1.3	8.3 ± 0.6*
CD21 MFI	4.2 ± 0.2	4.9 ± 0.2*
CD25 MFI	7.0 ± 0.8	4.6 ± 0.3*
MHC-II MFI	9.2 ± 1.7	6.3 ± 0.8
CD11a/18 MFI	8.6 ± 0.9	9.9 ± 0.6
CD80 MFI	6.8 ± 0.5	5.4 ± 0.4
CD40 MFI	4.5 ± 0.3	5.0 ± 0.2

* Means within a row differ ($P \leq 0.05$)

Figure 1. OVA and WCS-PK specific Ig in sera from vaccinated calves. Calves were vaccinated with OVA only at 3- and 5-wk of age (OVA, n = 3) or vaccinated with BCG + OVA at wk 3 and OVA at wk 5 (OVA+BCG; n = 3). Serum was collected before vaccination at 1- and 3-wk of age, and after vaccination at 5, 6, 7, and 8 wk of age. Absorbances (mean ± SEM) are shown.