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NOTES

Blastogenic Transformation by Lipopolysaccharide of Blood Leukocytes from Immunized but not Normal Cattle

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Escherichia coli lipopolysaccharide produced blastogenic transformation in whole-blood leukocytes from heifers that had been infected and immunized with *Campylobacter fetus*, but not in cells from control animals. This suggests that lipopolysaccharide does not function as a B-cell mitogen in cattle and that its stimulation of cells from immunized animals occurred through another mechanism.

The effect of endotoxin lipopolysaccharide (LPS) as a B-cell mitogen has been established in mice (1, 2, 3, 7, 8) and reported to occur also in guinea pigs (6) and cattle (9). In the course of studies aimed at elucidating the nature of protective immunity in *Campylobacter fetus* infection of cattle, we performed tests on whole blood to determine the presence of immune cells after genital infection and systemic immunization. Phytohemagglutinin (PHA) and LPS were evaluated on these preparations as markers for T and B cells, respectively, for subsequent use with purified lymphocyte fractions. PHA produced marked stimulation in all samples, but stimulation by LPS occurred only in samples of animals that had been infected and immunized with *C. fetus*.

The protocol for these experiments was the same as that previously used (10), in which heifers were infected at estrus with *C. fetus* and then immunized systemically 14 and 24 days thereafter, resulting in termination of infection in the majority of animals (10). Immunizations were performed with killed cells or warm-water extracts of *C. fetus* in incomplete Freund adjuvant. Killed cell vaccine was prepared as before (10). The water extract was produced by incubation at 40°C for 50 min of a suspension of cells in distilled water (1 g [wet weight]/ml), followed by centrifugation at 98,000 × *g* for 3 h at 4°C. The supernatant was concentrated by ultrafiltration to 5.3 mg of protein per ml, calculated using the Lowry method (5) with bovine serum albumin as standard, and emulsified with an equal volume of incomplete Freund adjuvant.

Blood samples were collected by venipuncture in heparinized Vacutainer tubes, and 0.1-ml quantities of whole blood were cultured in 2-ml, flat-bottomed plastic trays (Limbro 96 CV-TC) with 1.0 ml of RPMI-1640 alone (control) or containing 0.03 ml of PHA-M (Difco), 12 ng of *Escherichia coli* O128:B12 LPS extracted with hot phenol (Difco), or 10 or 100 ng of *C. fetus* water extract protein. Medium contained penicillin (100 U/ml), streptomycin (100 ng/ml), and amphotericin B (Fungizone; 0.25 ng/ml). Plates were covered with sterile plastic lids and incubated for 72 h at 37°C in a humidified chamber of 95% air and 5% CO_2. Cultures were pulsed with 1 μCi of [^{3}H]thymidine (5,000 μCi/mmol, New England Nuclear Corp.) for an additional 18 h. Cells were collected with an automated sample harvester, and samples were counted for radioactivity in a liquid scintillation spectrometer, using Aquasol Universal LSC cocktail (New England Nuclear Corp.) as scintillation fluid. The levels of lymphocyte transformation are expressed as a stimulation index, which was calculated by dividing the mean counts per minute based on three replicate wells containing whole blood cultured in the presence of a given mitogen or antigen by the mean counts per minute of three replicate wells containing whole blood cultured in the absence of mitogen or antigen (control).

Cells from all animals responded to PHA stimulation. Only cells from immunized animals responded to *C. fetus* water extract and to *E. coli* LPS, whereas cells obtained from noninfected, nonimmunized animals did not (Table 1). These results suggest that *E. coli* LPS does not function as a B-cell mitogen in cattle. The

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TABLE 1. *PHA-, E. coli LPS-, and C. fetus antigen-induced blastogenesis of whole bovine blood lymphocytes*

Group	Heifer no.	Stimulation index[a]				Mean absolute counts of unstimulated cultures
		PHA	LPS	*C. fetus* extract		
				10 ng	100 ng	
Infected, vaccinated with whole cells	08	214.1	ND[b]	9.2	2.9	400
	16	23.2	40.1	41.9	26.7	3,717
	49	158.4	0.7	30.8	31.0	1,012
	62	12.8	10.1	8.9	7.4	19,482
Infected, vaccinated with water extract	01	146.5	93.0	77.7	78.1	1,111
	04	200.1	256.8	173.0	234.5	862
	40	131.4	96.2	71.1	62.7	1,679
	53	13.8	16.3	15.8	18.3	11,571
	10	46.8	46.9	30.3	25.7	7,234
	09	151.8	2.8	8.3	12.4	1,304
	50	54.6	7.7	3.6	2.8	5,611
	54	40.0	26.1	19.1	31.4	5,790
Not infected, not vaccinated	01	99.4	0.5	2.0	0.4	3,297
	02	79.0	0.4	1.5	0.8	3,052
	03	10.9	0.1	0.3	0.2	20,676
	04	86.9	0.6	1.8	0.8	3,950
	05	24.8	0.1	0.2	0.1	7,015
	06	102.4	0.6	1.2	0.9	2,980

[a] See text for calculation of stimulation index.
[b] ND, Not done.

basis for LPS stimulation after immunization is not certain. It is possible that the *C. fetus* preparations used for immunization shared antigens with *E. coli* O128 LPS sufficient to produce cross-reactions detectable in the blastogenic test. Cross-reactions between O antigens of other *E. coli* serovars and *C. fetus* have been shown to occur in immunodiffusion tests (11). Such a cross-reaction was not demonstrable in this study, nor were antibodies to the *E. coli* LPS detected in sera of immunized animals by immunodiffusion tests. Blastogenesis by *E. coli* LPS may also have arisen as a nonspecific effect after immunization with *C. fetus* antigens, comparable to that noted by Graybill and Alford (4) in humans after immunization with vaccinia. Such an effect might account for the apparent difference in our results from those of Rouse and Babiuk (9), in that they determined LPS stimulation only on cells of cattle that had been immunized with infectious bovine rhinotracheitis virus. The possiblity must be considered that in infected or immunized animals, blastogenic transformations resulting from such antigenic cross-reactions or nonspecific phenomena may be mistaken for general mitogenic effects.

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