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Comparison of Sensitivity and Specificity of Purified Lymphocyte and Whole-Blood In Vitro Lymphocyte Stimulation Assays in Detection of *Brucella abortus* Infection in Cattle

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A study was conducted to compare the sensitivity and specificity of purified lymphocyte and whole-blood in vitro lymphocyte stimulation assays in detection of *Brucella abortus* infection in cattle. Cattle used were infected with *B. abortus* field strains or strain 19. Peripheral blood was collected, and lymphocytes for the purified lymphocyte stimulation assay were prepared by the Ficoll-diatrizoate technique. The blood for the whole-blood lymphocyte stimulation assay was diluted 10-fold with RPMI 1640 medium (without additional serum supplement) and cultured. The two tests were run simultaneously, and *B. abortus* soluble antigen or concanavalin A was added to the cultures. The cultures were incubated for 6 days and assayed for [^{3}H]thymidine incorporation into their DNA. Generally, cultures of the purified lymphocyte stimulation assay had higher counts per minute than those of the whole-blood lymphocyte stimulation assay, but the stimulation ratios for the two tests were comparable. The two assays were comparable in terms of their sensitivity and specificity as applied to detection of brucella infection in cattle.

In our previous study (5) the whole-blood lymphocyte stimulation (WBLS) assay was described. It was pointed out that this assay had several advantages over the pure lymphocyte assay (PLS) in that it was simpler, faster, and less costly. It was also pointed out that the whole-blood test was reproducible and correlated with infection. These last two attributes, reproducibility and correlation with infection, were also reported in our previous studies on the PLS assay (4, 6; J. M. B. Kaneene, R. K. Anderson, D. W. Johnson, R. D. Angus, C. C. Muscoplat, D. E. Pietz, L. C. Vanderwagen, and E. Sloane, Am. J. Vet. Res., in press). The question of comparison of sensitivity and specificity of these two assays has been given minimal consideration in the literature. This experiment was designed to compare sensitivity and specificity of these two assays in blood samples from cattle with different exposure experiences to *Brucella abortus*.

MATERIALS AND METHODS

Design of the study. The study was a single-blind study and was conducted in two parts.

(i) Experiment A. Table 1 shows the type and status of the dairy cattle used in this part of the study. Animals 21, 51, and 74 in group I were kept in separate isolation units of the College of Veterinary Medicine, University of Minnesota. Animal 611 in group I was in a Florida dairy herd. Animals in group IV were from a brucellosis-free herd of the Department of Animal Science, University of Minnesota. The animals in groups II and III were from a dairy herd in Florida, except animal 22 (housed at the University of Minnesota), which was being used for *B. abortus* strain 19 vaccination trials in adult cattle. The animals in this experiment were bled twice within a 3-month period.

(ii) Experiment B. Table 2 shows the type and status of the dairy cattle used. Animals in group I and animal 22 in group III were from the same source as described in experiment A. Animals in group I and animal 473 were from a second herd from Florida that was being used for *B. abortus* strain 19 vaccination trials in adult cattle. Animals in group IV were kept in the same herd as group IV in experiment A. Animals in experiment B were tested twice within a 1.5-month period.

Bleeding and testing schedule. In both experiments, on the same day that animals in Florida were bled, those in St. Paul were also bled, and blood was kept at ambient temperature and tested the next morning together with samples from Florida.

Collection of blood. Approximately 20 ml of blood was collected by jugular venipuncture from each ani-

TABLE 1. *Daily cattle used in experiment A*[a]

Group	Animal no.	Method of *B. abortus* exposure	Date of exposure	*B. abortus* isolated
I	21	Naturally infected	Not known	Biotype 1
	51	Naturally infected	Not known	Biotype 1
	74	Naturally infected	Not known	Biotype 1
	611	Naturally infected	Not known	Biotype 1
II	33	Adult vaccinated, 0.1 ml i.c.	10 June 1976	Strain 19
	38	Adult vaccinated, 0.1 ml i.c.	10 June 1976	Strain 19
	337	Adult vaccinated, 5 ml s.c.	9 Feb. 1976	Strain 19
	438	Adult vaccinated, 5 ml s.c.	5 Feb. 1976	Strain 19
	561	Adult vaccinated, 0.1 ml i.c.	10 Feb. 1976	Strain 19
III	22	Calfhood vaccinated, 5 ml s.c.	1972	Strain 19
	412	Calfhood vaccinated, 5 ml s.c.	1972	Strain 19
IV	920	Not exposed	Not applicable	Not applicable
	921	Not exposed	Not applicable	Not applicable
	922	Not exposed	Not applicable	Not applicable
	923	Not exposed	Not applicable	Not applicable
	924	Not exposed	Not applicable	Not applicable
	930	Not exposed	Not applicable	Not applicable

[a] Animals were grouped according to the method of exposure to *B. abortus* and culture isolation results. i.c., Intracutaneously; s.c., subcutaneously.

TABLE 2. *Dairy cattle used in experiment B*[a]

Group	Animal no.	Method of *B. abortus* exposure	Exposure date	*B. abortus* isolated
I	21	Naturally infected	Not known	Biotypel
	51	Naturally infected	Not known	Biotype 1
	74	Naturally infected	Not known	Biotype 2
II	61	Adult vaccinated, 5 ml s.c.	July 1976	Strain 19
	306	Adult vaccinated, 5 ml s.c.	June 1976	Strain 19
	1429	Adult vaccinated, 5 ml s.c.	June 1976	Strain 19
	6004	Adult vaccinated, 5 ml s.c.	July 1976	Strain 19
III	22	Calfhood vaccinated, 5 ml s.c.	1972	Strain 19
	473	Calfhood vaccinated, 5 ml s.c.	1973	Strain 19
IV	931	Not exposed (control)	Not applicable	Not applicable
	932	Not exposed (control)	Not applicable	Not applicable
	934	Not exposed (control)	Not applicable	Notapplicable
	987	Not exposed (control)	Not applicable	Not applicable
	988	Not exposed (control)	Not applicable	Not applicable
	989	Not exposed (control)	Not applicable	Not applicable

[a] Animals were grouped according to the method of exposure to *B. abortus* and bacterial culture results. s.c., Subcutaneously.

mal and placed into two sterile tubes containing heparin (50 U/ml; The Upjohn Co., Kalamazoo, Mich.). One tube was used for the WBLS assay, and the second was used for the PLS assay.

Preparation of blood cultures for the WBLS assay. Blood culture preparation was conducted as reported in our earlier studies (5). Briefly, heparinized blood was diluted 10-fold, using RPMI 1640 (Grand Island Biological Co., Grand Island, N.Y.) culture medium containing penicillin (100 U/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM). No other supplements were added.

Preparation of cultures for the PLS assay. Lymphocyte suspensions were prepared by the Ficoll-Hypaque technique as described previously (4).

Culture medium, mitogen, antigen, and culturing conditions. RPMI 1640 medium was used to culture lymphocytes in both assays, the only difference being that no added serum was necessary for the WBLS assay. Concanavalin A (ConA; Miles-Yeda, Rehovot, Israel) was used at concentrations of 1.0 μg/culture for the WBLS assay and 2.0 μg/culture for the PLS assay. *B. abortus* soluble antigen (BASA) was used at a concentration of 2.2 μg of protein per culture in the WBLS assay and at twice that concentration in the PLS assay. All culture samples for both assays were cultured for 6 days. A concentration of 2.2 μg of protein per culture and a 6-day incubation period were selected because they were found to induce optimal lymphocyte stimulation responses (LSR) in our

earlier studies (4, 5). The rest of the conditions for culture, [^{3}H]thymidine (Schwarz/Mann, Orangeburg, N.Y.) labeling, harvesting, and scintillation spectrometry counting, were as previously described (5, 6).

Statistical evaluation. The differences in lymphocyte stimulation responses between each group were evaluated by using Student's *t* test.

RESULTS

Expression of lymphocyte stimulation results. The lymphocyte stimulation results are expressed in two ways: (i) counts per minute = mean counts per minute of triplicate cultures with or without either ConA or BASA; (ii) stimulation index (SI) = mean counts per minute of triplicate cultures with ConA or BASA divided by mean counts per minute of triplicate cultures without either ConA or BASA. An SI ≥3.0 was classified as infection, whereas an SI ≥3.0 was classified as not presently infected.

Results of experiment A. (i) Lymphocyte stimulation results. Figures 1 and 2 and Table 3 show the results of experiment A. Results show the following points. (i) BASA induced positive LSR in samples from animals in groups I, II, and III on both PLS and WBLS assays (Fig. 1 and 2). The LSR on both assays in group I were statistically higher ($P < 0.01$) than in either group II or group III. There was no statistical difference between the LSR of groups II and III ($P > 0.05$). (ii) BASA induced no response in samples from group IV on both tests. (iii) Generally, BASA induced higher counts per minute (Table 3) in lymphocytes from groups I, II, and III on the PLS assay than on the WBLS assay; however, it may be of more value to evaluate the SI for these two assays as in Fig. 1. (iv) Counts per minute from cultures where there was neither ConA nor BASA were lower on the WBLS assay than on the PLS assay. (v) ConA (Table 3) induced significant counts per minute from all samples, but the counts for PLS were generally higher than for the WBLS assay.

(ii) Results of *B. abortus* culture attempts. *B. abortus* organisms were isolated from cattle (Table 1) in groups I, II, and III, and no isolation attempts were made in animals in group IV, which were nonexposed controls.

Results of experiment B. (i) Lymphocyte stimulation results. Results of this part of the experiment are presented in Fig. 3 and 4 and Table 4. Results show the following points. (i) BASA induced LSR ≥3.0 in samples from animals in groups I, II, and III on both PLS and WBLS assays (Fig. 3 and 4). (ii) BASA induced

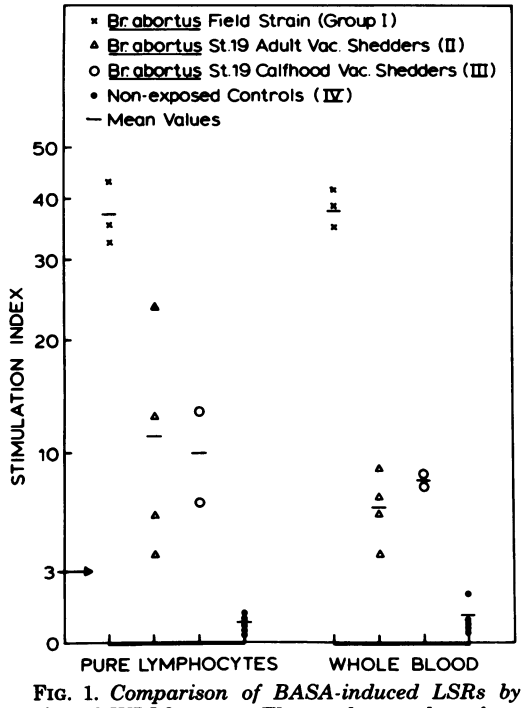

FIG. 1. *Comparison of BASA-induced LSRs by PLS and WBLS assays. The results are those from experiment A, first test.*

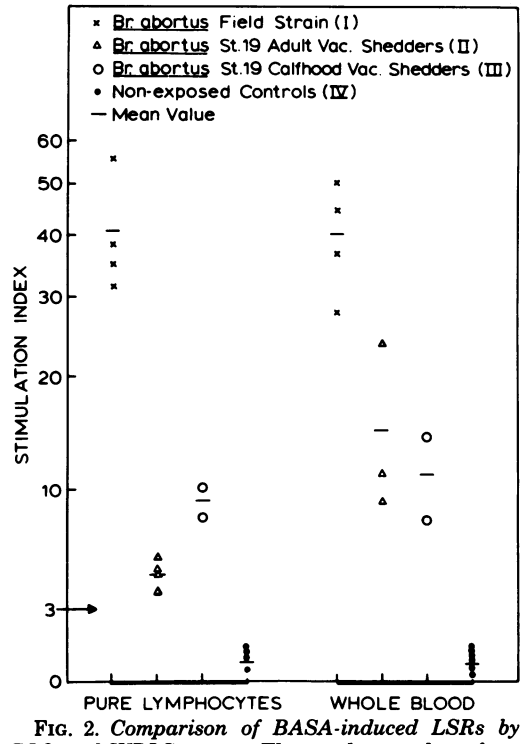

FIG. 2. *Comparison of BASA-induced LSRs by PLS and WBLS assays. The results are those from experiment A, second test, approximately 2 months later.*

TABLE 3. *Experiment A: comparison of lymphocyte stimulation induced by ConA and BASA in pure lymphocytes and whole blood from cattle with different exposure experiences to B. abortus and nonexposed cattle*

Group	No. in group	Stimulator	Pure lymphocytes		Whole blood	
			Lowest-highest cpm	Mean cpm ± SEM[a]	Lowest-highest cpm	Mean cpm ± SEM
I	4	Con A	129,534–170,033	151,025.5 ± 5,522.2	49,261–99,144	67,704.4 ± 5,637.1
		BASA	24,701–78,234	44, 384.6 ± 5,961.5	5,007–9,566	7,658.0 ± 620.6
		None	670–1,314	1,041.1 ± 92.4	175–433	270.9 ± 41.6
II	5	ConA	100,778–388,248	174,159.4 ± 28,259.7	10,834–105,729	59,240.7 ± 11,062.1
		BASA	590–3,593	1,986.1 ± 305.5	527–4,498	2,350.6 ± 501.0
		None	165–634	412.7 ± 67.6	121–321	206.7 ± 25.3
III	2	ConA	55,960–70,001	64,453.0 ± 3,079.4	24,004–106,875	78,647.5 ± 18,591.7
		BASA	2,817–3,107	3,002.3 ± 64.8	1,383–1,500	1,425.3 ± 98.7
		None	236–342	282.0 ± 24.4	201–328	272.3 ± 30.1
IV	6	ConA	105,806–155,993	127,613.3 ± 8,114.5	23,736–54,146	42,165.8 ± 4,821.6
		BASA	255–440	386.7 ± 42.0	570–761	676.8 ± 33.7
		None	175–865	440.8 ± 95.1	516–1,334	942.2 ± 177.3

[a] SEM, Standard error of the mean.

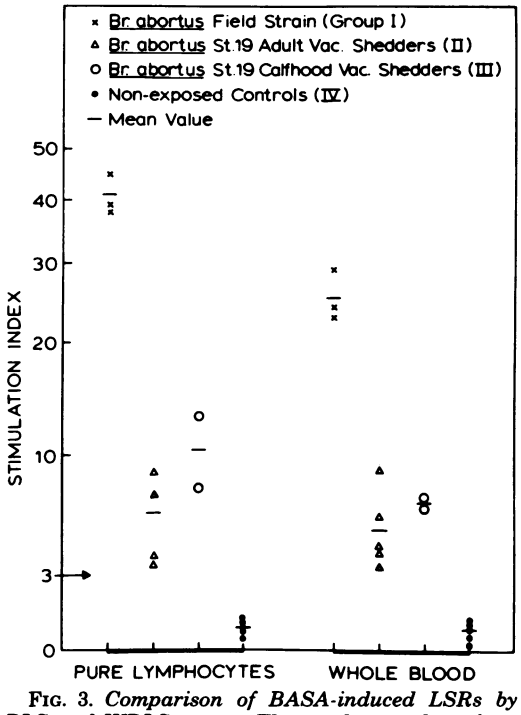

FIG. 3. *Comparison of BASA-induced LSRs by PLS and WBLS assays. The results are those from experiment B, first test.*

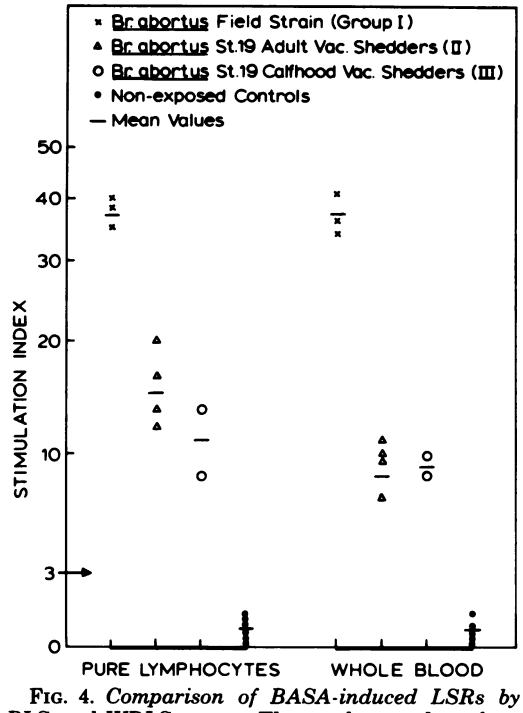

FIG. 4. *Comparison of BASA-induced LSRs by PLS and WBLS assays. The results are those from experiment B, second test, approximately 1.5 months after initial test.*

no detectable LSR in samples from group IV animals on both tests. (iii) BASA induced higher counts per minute (Table 4) in lymphocytes from groups I, II, and III on the PLS than on the WBLS assay. (iv) Counts per minute from cultures where there was neither ConA nor BASA were lower on the WBLS than on the PLS assay.

(ii) *B. abortus* culture attempts. *B. abortus* organisms were isolated (Table 2) from cattle

TABLE 4. *Experiment B: comparisons of lymphocyte stimulation induced by ConA and BASA in pure lymphocytes and whole blood from cattle with different exposure experiences to B. abortus and nonexposed cattle*

Group	No. in group	Stimulator	Pure lymphocytes		Whole blood	
			Lowest-highest cpm	Mean cpm ± SEM[a]	Lowest-highest cpm	Mean cpm ± SEM
I	3	ConA	279,395-301,759	290,087.0 ± 6,474.5	29,685-51,667	41,451.7 ± 6,392.9
		BASA	43,966-87,102	60,921.7 ± 13,279	2,672-5,002	3,837.0 ± 1,165.0
		None	964-1,595	1,207.7 ± 195.8	213-490	324.7 ± 84.3
II	4	ConA	156,236-276,376	229,976.0 ± 26,727.2	16,164-53,820	27,639.0 ± 8,502.0
		BASA	3,039-94,421	29,356.8 ± 22,126.5	1,370-2,051	1,729.0 ± 149.3
		None	529-901	786.8 ± 86.6	230-538	394.8 ± 72.2
III	2	ConA	556,007-509,736	532,871.5 ± 23,135.5	107,091-272,923	190,007.0 ± 82,916.0
		BASA	3,561-8,796	6,178.5 ± 2,617.5	1,623-1,998	1,810.0 ± 187.5
		None	443-501	472.0 ± 29.0	201-300	250.0 ± 49.5
IV	6	ConA	15,717-271,515	172,311.0± 38,553.0	43,511-130,504	69,500.2 ± 13,077.2
		BASA	229-734	471.3 ± 83.5	249-406	323.5 ± 23.1
		None	318-872	512.3 ± 81.5	419-595	507.2 ± 36.3

[a] SEM, Standard error of the mean.

in groups I, II, and III but were not isolated from group IV.

Sensitivity and specificity of the two assays. Sensitivity and specificity were calculated using the formula:

$$\text{Sensitivity} = \frac{\text{true positive}}{\text{true positive + false negative}} \times 100 \quad (1)$$

$$\text{Specificity} = \frac{\text{true negative}}{\text{true negative + false negative}} \times 100 \quad (2)$$

where true positive = isolation of *B. abortus*, and true negative = no exposure to *Brucella* spp. With these formulas, the two assays were devoid of false positives and false negatives.

Summarizing the results from both experiments, it can be stated that: (i) both PLS and WBLS assays were comparable in sensitivity and specificity; (ii) BASA induced a significantly higher LSR in samples from *B. abortus* field strain-infected cattle (group I) than in *B. abortus* strain 19 shedders (group II or III) ($P \leq 0.01$); (iii) BASA did not induce any significant LSR in samples from control animals; (iv) generally, the counts per minute were higher in the PLS assay than in the WBLS assay, but their SIs were comparable (Figs. 1 through 4).

DISCUSSION

The results of this study show that the PLS and WBLS assays are comparable in terms of their sensitivity and specificity. It is important to point out that these two assays cannot be compared directly by using counts per minute since the counts per minute for the WBLS assay are usually lower than those of the PLS assay. Using an SI, however, gives a better comparison since the SI is a ratio obtained by division of one factor into another. Thus, when calculating SI, the numerator in the PLS assay is higher than that in the WBLS assay but so is the denominator. Mathematically, then, the ratio obtained in both assays could rationally be compared.

The counts per minute for the WBLS assay are generally lower than those for the PLS assay and may be due to a number of factors. (i) There is a smaller lymphocyte population (and probably less macrophages) in the amount of blood used for WBLS. Normal leukocytes in cattle range from 80,000 to 10,000 per ml^3, and about 60% of these are lymphocytes. Assuming that the cattle used had 10,000 leukocytes/ml^3 each, 60% of that would be 6,000 lymphocytes/ml^3. In the WBLS assay a 10-fold dilution of the whole blood was used. The number of lymphocytes used per 1 ml can be estimated to have been 600. In contrast, a 1.5×10^6-lymphocyte/ml concentration was standardized in the PLS assay. Thus, per a given volume of blood, the WBLS assay used less lymphocytes. It should be pointed out, however, that absolute total whole blood cells and lymphocytes were not determined. (ii) In the WBLS assay, there are other cells like erythrocytes and platelets in addition to the lymphocytes and macrophages. These erythrocytes and platelets may physically prevent complete interaction among lymphocytes, macrophages, and antigen. These two factors may explain the lower counts per minute obtained with the WBLS assay.

Several investigators have reported that the PLS assay is sensitive in detecting several infections (2, 3, 8–10, 13–15). Our findings (4, 6; Kaneene et al., in press) are in agreement with those investigators. The WBLS assay was found to be sensitive and specific in detecting brucella infection (5), which was in agreement with other investigators reporting on other diseases (1, 10–12). However, no reported studies were found where the PLS and WBLS assays were compared directly. With the many advantages of the WBLS assay over the PLS assay (simple, fast, and less costly), the findings that these assays were comparable in terms of sensitivity and specificity should encourage further evaluation of the two assays and may lead to wider application of the WBLS assay as an aid to disease investigation and diagnosis.

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