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Brucella Fractions Behave as Nonspecific Mitogens and Polyclonal B-Cell Activators for Human Lymphocytes

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Two lipid-A-free fractions which were extracted from *Brucella melitensis* and were designated PI and SF stimulated human unsensitized mononuclear cells to proliferate and to secrete immunoglobulins. Both of these effects were observed in cultures of peripheral blood, tonsils, and cord blood lymphocytes. Neither B cells nor T cells alone proliferated in the presence of these fractions, whereas the proliferative response of T cells plus B cells was largely independent of accessory cells. Polyclonal activation was estimated by counting the cells which secreted immunoglobulins of different isotypes into culture supernatants. This phenomenon was strongly T dependent.

Two lipid-A-free vaccinating fractions extracted from *Brucella melitensis* (fractions PI and SF) induce thymidine uptake by lymphocytes from brucellosis patients and from vaccinated subjects and thus are useful tools for studying cellular anti-*Brucella* immune responses (2).

Lymphocytes from some donors which are free from any stigmata of brucellosis are sometimes also stimulated by fractions PI and SF. Since fractions PI and SF have been shown to be nonspecific mitogens and polyclonal activators for murine B cells (26), it was tempting to study whether this was the case with human lymphocytes. We found that, under optimal conditions, lymphocytes from all unsensitized donors are indeed activated by fraction PI or SF to proliferation and polyclonal antibody synthesis.

In this paper we describe the optimal conditions for high [^{3}H]thymidine uptake and for optimal plaque-forming cell (PFC) responses. We also show the absolute requirement for T cells plus B cells and the relative adherent cell independence of these phenomena.

MATERIALS AND METHODS

Preparation of fractions PI and SF. The extraction of fractions PI and SF from *B. melitensis* has been described previously (19). Briefly, fraction PI is the phenol-insoluble phase obtained after three phenol water extractions at 65°C by the Westphal procedure from *B. melitensis* biotype I strain M15. Further purification of fraction PI by treatment with DNase, RNase, pepsin, papain, pronase, and sodium dodecyl sulfate leads to fraction SF. Fraction PI contains many proteins, lipoproteins, sugars, amino sugars, and nucleic acid residues, whereas fraction SF contains only two or three proteins and lipoproteins (19). The presence of *Brucella* lipopolysaccharide or lipid A has been ruled out by (26): (i) the absence of sugars, glucosamine, and phosphorus in fraction SF and the absence of β-hydroxymyristic acid and 2-keto-3-deoxyoctulosonic acid in both fractions; (ii) the absence of fraction PI- and SF-induced leucopenia; (iii) the results of toxicity tests in adrenalectomized mice; and (iv) the results of the *Limulus* test showing that endotoxin contamination is lower than 0.1 and 0.005% in fractions PI and SF, respectively. Homogenized suspensions of fractions PI and SF were sterilized by heating them for 1 h at 65°C three times, controlled for sterilization, and stored at 4°C.

Preparation of mononuclear cells. Heparinized blood (10 U/ml; Seromed, Lille, France) was obtained from healthy adult volunteers having negative humoral anti-*Brucella* tests and negative melitin skin tests or from the cord blood of neonates. Diluted with 2 volumes of Hanks balanced salt solution, the blood was layered over 10-ml Ficoll-Hypaque gradients (Seromed), and the mononuclear cell fraction (designated PBMC) was suspended in complete medium consisting of RPMI 1640 medium (Biomérieux, Lyon, France) supplemented with 2 mM L-glutamine (Biomérieux), 100 U of penicillin (Seromed) per ml, 20 μg of gentamicin (Seromed) per ml, and 10% heat-inactivated fetal calf serum (lot 732; IBF, Villeneuve, France).

Tonsil lymphocytes were extracted from the tonsils of *Brucella*-negative patients with a history of repeated tonsilitis but no infection at the time of tonsillectomy. Tonsil lymphocytes were teased with forceps in RPMI 1640 medium, recovered after centrifugation on Ficoll-Hypaque gradients, and suspended in complete medium containing vincamycin (20 μg/ml) and amphotericin B (1 μg/ml; Seromed).

Preparation of T-cell- and B-cell-enriched populations. T and B cells were isolated by two successive rosettings with sheep erythrocytes treated with 2-aminoethylisothiouranium dibromide (Sigma Chemical Co., St. Louis, Mo.) as described by Falkoff et al. (9).

Rosette-positive cells were considered the T cell-enriched population, and rosette-negative cells were considered the B cell-enriched population.

Preparation of adherent depleted populations. Monocyte depletion was achieved by adherence on Sephadex G-10 beads (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) as described by Jerrels et al. (14).

Cell identification. A total of 2×10^5 washed cells were incubated with 100 μl of the appropriate dilution of mouse monoclonal antibody OKT3 or B1 (Coultronics, Margency, France) for 30 min on ice. The cells were washed twice, suspended in 50 μl of an appropriate dilution of F(ab')^2 goat anti-mouse immunoglobulin G (IgG) (Coultronics), and incubated again for 30 min on ice. After washing, samples were assayed for immunofluorescence by using a model 50H cytofluorograph (Ortho Instruments, Westwood, Mass.). In

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TABLE 1. Stimulation of PBMC by various concentrations of fraction PI or SF[a]

Fraction	Δ cpm with the following concn:				
	0.1 μg	1 μg	10 μg	50 μg	100 μg
PI	1,503 ± 210[b]	2,130 ± 196	6,748 ± 398	18,204 ± 2,125	14,920 ± 1,102
SF	998 ± 118	1,120 ± 128	3,560 ± 420	16,588 ± 1,118	16,290 ± 2,039

[a] A total of 5×10^5 PBMC per well were cultured for 5 days.
[b] Mean ± standard error of the mean for three experiments.

some cases, T cells were also identified by overnight E rosetting (13), and B cells were identified by direct membrane fluorescence, using fluorescein-treated $F(ab')^2$ fragments of a goat anti-human immunoglobulin serum (Cappel Laboratories, Cochranville, Pa.) and evaluation of monocytes by counting esterase-staining cells (16). T-cell-enriched PBMC populations usually contained 90 to 95% E^+ cells, 94 to 98% $T3^+$ cells, 2% IgS^+ or $B1^+$ cells, and ≤ 1% esterase-staining cells; T-cell-depleted B-cell-enriched populations contained 40 to 80% IgS^+ or $B1^+$ cells, 1 to 3% $T3^+$ cells, and 10 to 50% esterase-staining cells. T-cell-depleted B-cell-enriched tonsil populations contained 85 to 90% IgS^+ cells and <3% esterase-positive cells.

Culture conditions for proliferation assays. Samples (200 μl) of a lymphocyte suspension in complete medium (see above) were cultured at 37°C in an atmosphere containing 5% CO_2 in triplicate wells of 96-well microtiter plates (type 3042; BD Labware, Oxnard, Calif.). Fractions PI and SF, phytohemagglutinin, and pokeweed mitogen (PWM) (Difco Laboratories, Detroit, Mich.) were diluted in complete medium and added in a volume of 10 μl/well. At 18 h before cell harvesting, 1 μCi of tritiated thymidine (specific activity, 1 Ci/mmol; C.E.A., Saclay, France) was added to each well. Cells were harvested by using glass fiber disks, which were counted for radioactivity in 5 ml of scintillant [5% 2,5-diphenyloxazole (PPO) and 0.01% 1,4-bis-(5-phenyloxazolyl)benzene (POPOP) in toluene] with a Packard minivial liquid scintillation fluid spectrometer. Results were expressed either as the mean counts per minute of at least three cultures or as the changes in counts per minute (Δcpm) by subtracting the control value.

Culture conditions for PFC. A total of 2×10^6 cells were cultured in type 3047 plates (Falcon, Grenoble, France) with appropriate doses of fractions PI and SF and PWM. At different times, activated cells were recovered, washed in complete medium (see above), and counted for viability.

Detection of reverse protein A PFC. PFC were detected by using a previously described reverse hemolytic plaque assay (11, 12) that made use of staphylococcal protein A (IBF)-coated sheep erythrocytes. Plaques were developed with a polyvalent rabbit anti-human immunoglobulin (IgA plus IgM plus IgG) serum (Cappel) diluted 1:100. In some experiments IgM-, IgG-, and IgA-secreting cells were detected by using heavy chain-specific rabbit anti-human IgM-IgG-IgA serum (Cappel) diluted 1:50. The complement source used was a 1:12 dilution of guinea pig serum (Biomerieux) previously absorbed with sheep erythrocytes. All data were expressed as the number of PFC per 10^6 cells recovered.

Quantitation of immunoglobulin secreted in culture supernatants. The amounts of IgG, IgM, and IgA in culture supernatants were determined by laser nephelometry (22, 27). The light-dispersing properties of supernatants were reduced by passing them through a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.), and the supernatants were examined without dilution by using a commercially available system provided by Hyland Laboratories, Inc., Costa Mesa, Calif., with slight modifications. The calibration standards were diluted 1:100 before they were mixed with antisera, and the cell culture supernatants were assayed undiluted, resulting in a 100-fold relative increase in the sensitivity of the method. In the case of IgG determinations, 100-μl samples of the cell culture supernatants and only 50-μl portions of the calibration standard were used. This provided an additional twofold increase in sensitivity.

RESULTS

Mitogenic activity of fractions PI and SF: optimal conditions for nonspecific mitogenic effect of fraction PI or SF. Incorporation of [^{3}H]thymidine by normal unsensitized lymphocytes depended on (i) the doses of fraction PI or SF added to a culture (Table 1), with maximal responses for a concentration of 50 μg/ml and minimal responses for concentrations under 10 μg/ml; (ii) the cellular concentration in the culture wells (Table 2), with maximal responses for a concentration of 5×10^5 cells per well; and (iii) the kinetics of the cellular responses (Table 3), with optimal responses on days 5 to 7.

Under the optimal conditions positive results were obtained in all cases. Table 4 shows the results of assays done with the PBMC of 16 different patients. This table shows that fraction PI-induced [^{3}H]thymidine incorporation ranged from 10,000 to 30,000 cpm and that there were a few cases of low-fraction PI responses (experiment 5, 1,220 cpm [= background value × 3.5]; experiment 10, 4,520 cpm [=

TABLE 2. Mitogenic effect of fraction PI or SF on different concentrations of PBMC cultured for 5 days

Concn (no. of PBMC per well)	Δ cpm with:	
	Fraction PI (50 μg/ml)	Fraction SF (50 μg/ml)
10^5	6,795 ± 396[a]	2,139 ± 324
2×10^5	12,935 ± 1,312	18,318 ± 2,121
5×10^5	25,596 ± 3,421	21,118 ± 918
10^6	10,218 ± 1,062	19,464 ± 2,426

[a] Mean ± standard error of the mean for three experiments.

TABLE 3. Kinetics of [^{3}H]thymidine incorporation after stimulation of 5×10^5 PBMC by fraction PI or SF

Days of culture	Δ cpm with:	
	Fraction PI (50 μg/ml)	Fraction SF (50 μg/ml)
3	2,263 ± 198[a]	1,469 ± 235
5	14,258 ± 1,630	24,569 ± 3,048
7	12,191 ± 2,129	21,512 ± 1,241
9	10,251 ± 541	12,569 ± 962

[a] Mean ± standard error of the mean for three experiments.

TABLE 4. Mitogenic activity of fraction PI or SF on PBMC

Expt[a]	[3H]thymidine incorporation (Δcpm) with:[b]		
	Phytohemagglutinin (50 μg/ml)	Fraction PI (50 μg/ml)	Fraction SF (50 μg/ml)
1	55,357 ± 6,101	6,723 ± 501	5,699 ± 1,041
2	69,589 ± 6,192	17,152 ± 391	9,950 ± 616
3	100,038 ± 9,104	13,477 ± 1,092	7,082 ± 801
4	55,925 ± 3,406	28,884 ± 1,493	5,416 ± 348
5	28,132 ± 3,542	1,220 ± 98	1,315 ± 116
6	91,538 ± 8,515	30,240 ± 2,743	11,642 ± 845
7	96,053 ± 3,918	32,124 ± 2,164	13,520 ± 901
8	76,618 ± 7,112	26,920 ± 3,100	7,220 ± 887
9	61,127 ± 5,492	21,118 ± 1,916	9,912 ± 617
10	27,989 ± 3,115	4,520 ± 164	2,980 ± 178
11	81,633 ± 6,531	16,880 ± 2,140	13,220 ± 1,018
12	89,486 ± 7,024	18,420 ± 1,540	19,670 ± 1,125
13	41,232 ± 1,562	12,795 ± 910	16,525 ± 1,940
14	98,986 ± 2,120	18,320 ± 1,220	15,575 ± 2,120
15	36,220 ± 3,145	11,240 ± 2,120	14,525 ± 978
16	110,274 ± 8,762	16,100 ± 1,200	13,749 ± 1,585

[a] For each experiment there was a different donor.
[b] The subtracted background value varied from 348 cpm (experiment 5) to 4,258 cpm (experiment 2). The results are the averages of triplicate cultures.

background value × 5.8]). Similarly, the values for fraction SF-stimulated cultures ranged from 5,000 to 20,000 cpm and there were low responses in the same patients (experiment 5, 1,315 cpm [= background value × 3.8]; experiment 10, 2,980 cpm [= background value × 3.8]).

In a more extensive study of 35 donors (16 of which are recorded on Table 4), the uptake of [3H]thymidine after fraction PI or SF stimulation ranged from 2 to 50 times the background values of nonstimulated cultures. Moreover, although there was no significant correlation between the levels of [3H]thymidine incorporation induced by phytohemagglutinin or PWM compared with fraction PI or SF, PI and SF responses were significantly correlated (K = 0.92). Tonsil lymphocytes were included in this study because they are good responders to polyclonal B-cell activators (PBA) and because their monocyte contamination is minimal. Cord blood lymphocytes were included because their previous *Brucella* sensitization was excluded. The optimal responses of these two types of lymphocyte populations to fraction PI or SF depended on the same experimental conditions as the responses of PBMC. Under these conditions, the levels of uptake of [3H]thymidine are comparable to the levels for PBMC (Tables 5 and 6).

TABLE 5. Mitogenic activity of fraction PI or SF on tonsil lymphocytes

Expt	[3H]thymidine incorporation (Δcpm) with:[a]		
	Phytohemagglutinin (20 μg/ml)	Fraction PI (50 μg/ml)	Fraction SF (50 μg/ml)
1	70,845 ± 8,163[b]	13,332 ± 1,712	20,285 ± 2,164
2	65,528 ± 6,116	15,628 ± 1,203	19,118 ± 2,164
3	27,229 ± 2,104	75,318 ± 7,804	43,618 ± 4,822
4	54,546 ± 6,174	15,329 ± 1,512	14,268 ± 1,264
5	76,304 ± 8,748	14,792 ± 1,719	22,062 ± 2,172
6	34,328 ± 3,216	27,128 ± 2,761	29,198 ± 3,015
7	54,203 ± 3,298	15,187 ± 978	13,740 ± 1,461

[a] The subtracted background values varied from 1,426 ± 231 cpm (experiment 5) to 6,796 ± 914 cpm (experiment 2).
[b] Average ± standard deviation for three cultures.

Cellular requirements. T-cell- and B-cell-enriched populations were compared with total peripheral or tonsil lymphocytes and cultured with fraction PI or SF, phytohemagglutinin, and PWM. Figure 1 shows that [3H]thymidine incorporation by T-cell-enriched populations, although normal or slightly enhanced for phytohemagglutinin-stimulated cultures and notably decreased for PWM stimulation, was strongly decreased in fraction PI- or SF-stimulated cultures. On the other hand, T-cell-depleted populations did not proliferate. Proliferative responses were restored by mixing back T-cell- and B-cell-enriched preparations, and these responses were maximal when cultures contained approximately 50 to 70% T cells and 30% B cells (Fig. 2). When unfractioned cells were passed on Sephadex G-10 beads, no reduction in the proliferative responses occurred (although the number of monocytes decreased to less than 1% under our experimental conditions). Table 7 shows this phenomenon and also shows that, in some but not all cases, a slight enhancement of [3H]thymidine incorporation was observed after adherent cell depletion.

Polyclonal activating effect of fractions PI and SF. Activation of lymphocytes by fraction PI or SF induced PFC. An optimal number of PFC was observed when the concentrations (Table 8) of fraction PI or SF exerted their optimal mitogenic effects and was reached on day 5 of the culture. The numbers of PFC obtained after stimulation of PBL, tonsil lymphocytes, or cord blood lymphocytes varied from 10 to 100 times the background values. Studies on the 35 aforementioned PBL cultures showed a significant correlation between the numbers of PFC induced by fractions PI and SF (K = 0.96) but not between the number of PFC obtained with fraction PI or SF and the number obtained with PWM ($K < 0.02$). In three cases where isotype-specific PFC were studied, IgM, IgG, IgA, PFC were all augmented (Table 9). The PFC responses to stimulation by fraction PI or SF were T cell dependent and were strongly reduced in T-cell- and B-cell-enriched populations (Table 10). Identical results were obtained when stimulation was done with tonsil lymphocytes (Table 10).

In 13 cases the immunoglobulin concentration in the culture supernatant was measured on day 8 by laser nephelometry (Table 11). A significant increase (approximately of the same amplitude as the increase obtained with PWM) was found in cultures stimulated by fraction PI or SF.

DISCUSSION

Our results clearly show that fractions PI and SF may behave as nonspecific mitogens for human lymphocytes; these fractions induce the proliferation of lymphocytes from

TABLE 6. Mitogenic effect of fraction PI or SF on cord blood lymphocytes

Expt[a]	[3H]thymidine incorporation (Δcpm) with:[b]		
	Phytohemagglutinin (50 μg/ml)	Fraction PI (50 μg/ml)	Fraction SF (50 μg/ml)
1	142,704 ± 6,129[c]	76,529 ± 5,574	68,494 ± 6,120
2	99,526 ± 11,205	16,529 ± 1,800	13,412 ± 1,202
3	64,314 ± 2,128	23,515 ± 2,041	25,530 ± 1,520

[a] A total of 5 × 10^5 cells were cultured with mitogens for 4 days in experiment 1 and for 6 days in experiments 2 and 3.
[b] The background values subtracted varied from 1,289 ± 174 cpm (experiment 1) to 7,865 ± 556 cpm (experiment 2).
[c] Average ± standard deviation for three cultures.

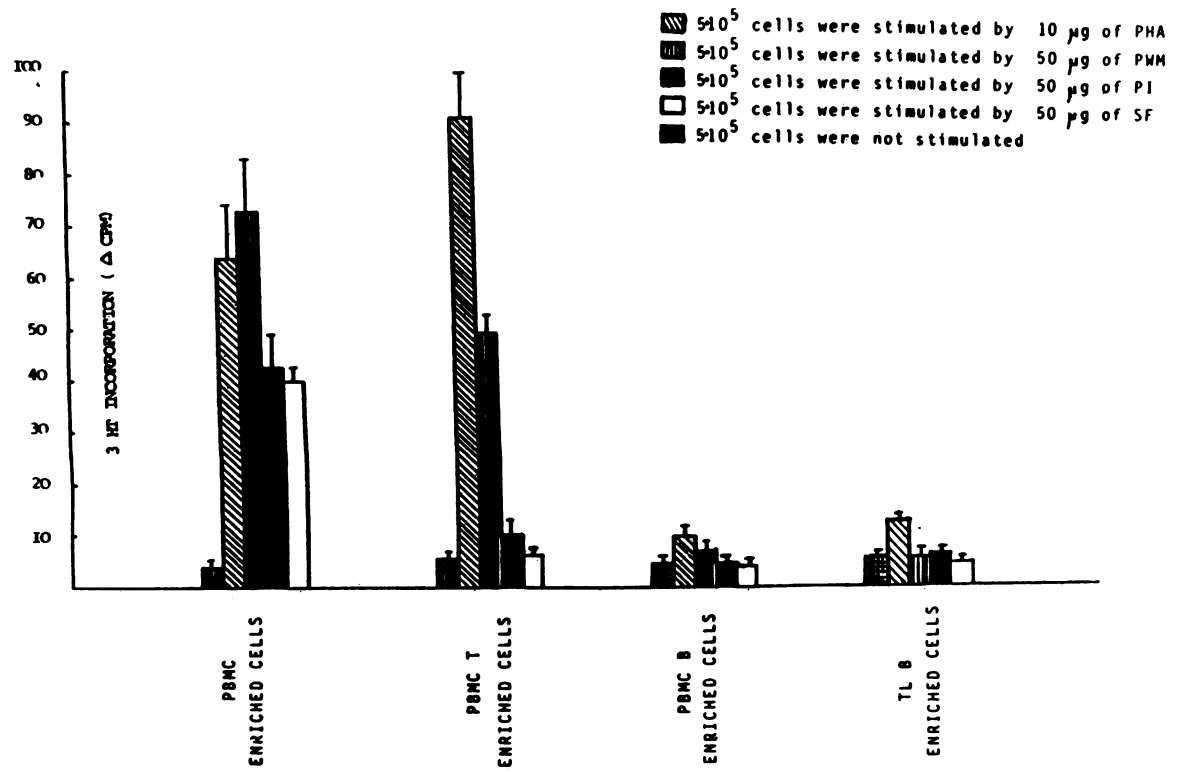

FIG. 1. Mitogenic activity of fractions PI and SF on T-cell- and B-cell-enriched PBMC separated by 2-aminoethylisothiouronium dibromide–sheep erythrocyte rosetting. The bars indicate the standard error of the mean for three experiments. PHA, Phytohemagglutinin; 3 HT, [^{3}H]thymidine; TL, tonsil lymphocytes.

subjects with no previous history and no biological evidence of *Brucella* infection (and even of lymphocytes from the cord blood of neonates). They also induce maturation of B cells to immunoglobulin-secreting cells and to polyisotypic immunoglobulin secretion and thus are considered new human PBA. The precise biochemical composition of fractions PI and SF is not known. However, the following points should be stressed. (i) Fractions PI and SF do not contain the bacterial lipopolysaccharide or lipid A (see above). Therefore, this ubiquitous bacterial component, known as a B mitogen for murine cells and reported to activate human B cells under certain experimental conditions (20), is not responsible for

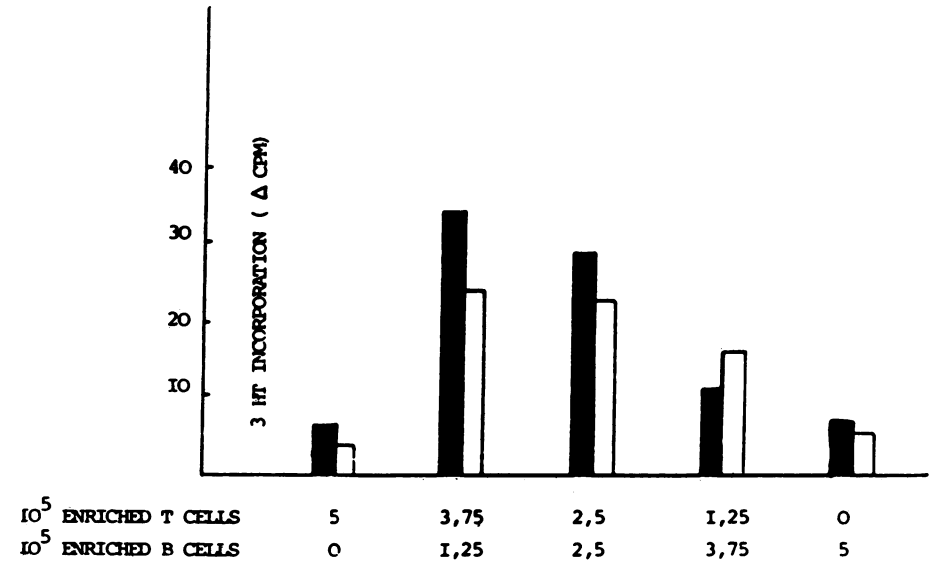

FIG. 2. Effects of the addition of T lymphocytes to B-cell-enriched PBMC populations activated with 50 µg of fraction PI (solid bars) or 50 µg of fraction SF (open bars). [^{3}H]thymidine (3 HT) incorporation was measured on day 5.

TABLE 7. [^{3}H]thymidine incorporation by PBMC depleted of adherent cells[a]

Expt	Treatment	[^{3}H]thymidine incorporation (cpm) by:	
		PBMC	PBMC depleted of adherent cells
1	Control	3,320 ± 410[b]	1,620 ± 180
	PI (50 μg/ml)	9,140 ± 1,010	10,935 ± 910
	SF (50 μg/ml)	8,516 ± 420	9,403 ± 1,030
2	Control	3,210 ± 510	1,502 ± 110
	PI (50 μg/ml)	12,740 ± 1,298	30,837 ± 2,120
	SF (50 μg/ml)	16,180 ± 1,820	34,533 ± 3,220
3	Control	3,210 ± 121	106 ± 20
	PI (50 μg/ml)	9,166 ± 980	25,830 ± 3,210
	SF (50 μg/ml)	9,673 ± 192	12,243 ± 1,359

[a] Experiments 1, 2, and 3 were done with PBMC from differents donors; 2.5 × 10^6 cells per ml were cultured for 5 days.
[b] Mean ± standard deviation.

our results. (In addition, we never obtained under our conditions any proliferation of human lymphocytes with preparations of *Brucella* lipopolysaccharide). (ii) Proliferative and B-cell maturation responses to fractions PI and SF are correlated; since fraction SF is a purified fraction of fraction PI, it is possible that the same mitogenic structure(s) and the same corresponding cellular receptor(s) are involved. This is currently being studied by using a large panel of monoclonal antibodies. This may shed some light on the phenomenon of *Brucella* adherence to B cells previously described by Teodorescu and Mayer (25) and may explain the fact that whole *Brucella* cells are also capable of stimulating human B lymphocytes (unpublished data). (iii) Since fraction SF is devoid of the *Brucella* peptidoglycan present in fraction PI, this mitogenic moiety may not be responsible for fraction PI-induced stimulation. (iv) The fact that responses to PWM and to fractions PI and SF are not correlated may indicate either that PWM and fractions PI and SF trigger two different B-cell populations (a phenomenon previously described for *Nocardia opaca* and PWM [4]) or that the same B-cell population could be differently regulated after stimulation with different mitogens.

Numerous other human PBA, with different cellular requirements, have been reported. These include PWM, *N. opaca*, *Staphylococcus aureus* Cowan I, and several other bacterial PBA (3, 5, 7, 8, 10, 15, 18, 21–23). Fractions PI and SF are not able to directly stimulate B cells; B-cell-enriched suspensions neither proliferate nor mature to ISC in the absence of T cells. This distinguishes them from *N. opaca* (3, 5, 28) and strain Cowan I (10), which have been shown to induce largely T-cell-independent B-cell proliferation, whereas plasmatic maturation is more or less T cell dependent (3, 10, 18). Another possibility is that the lack of response of B-cell populations to fractions PI and SF is due to the excessive number of monocytes (up to 50%) in these

TABLE 8. PFC responses induced by fraction PI or SF

Cells	Expt	No. of PFC per 10^6 lymphocytes recovered with the following prepn:			
		None	PWM (50 μg/ml)	Fraction PI (50 μg/ml)	Fraction SF (50 μg/ml)
PBMC	1	50	2,100	5,200	2,120
	2	100	2,000	15,000	12,000
	3	750	21,730	7,130	15,000
	4	50	1,176	2,410	2,625
	5	50	1,000	944	679
	6	150	26,470	3,610	3,330
	7	230	15,000	13,125	12,000
	8	125	4,083	3,120	1,923
	9	70	4,060	2,533	3,440
	10	80	17,391	7,653	5,888
	11	30	6,930	3,545	2,680
	12	100	7,860	12,570	1,285
	13	200	35,000	3,140	3,820
	14	100	20,700	28,500	5,625
	15	70	18,560	20,350	2,350
	16	80	40,560	55,500	45,500
Tonsil lymphocytes	1	400	18,500	12,500	8,500
	2	250	6,500	3,500	5,400
Cord blood lymphocytes	1	60	7,600	2,900	3,200
	2	320	4,860	1,980	2,480

TABLE 9. Isotype distribution of PFC responses to fraction PI or SF

Expt[a]	Anti-serum[b]	No. of PFC per 10^6 lymphocytes recovered with the following prepn:			
		None	PWM (50 μg/ml)	Fraction PI (50 μg/ml)	Fraction SF (50 μg/ml)
1	Total	270	19,200	6,650	4,000
	IgM	90	13,845	4,660	900
	IgG	60	3,000	1,110	1,800
	IgA	70	615	444	580
2	Total	180	8,100	9,300	7,610
	IgM	80	3,250	1,800	5,480
	IgG	50	4,550	5,300	6,200
	IgA	55	350	720	780
3	Total	240		7,300	6,800
	IgM	120		1,900	3,620
	IgG	90	ND[c]	3,560	6,900
	IgA	110		1,120	880

[a] Experiments 1, 2, and 3 were done with PBMC from differents donors.
[b] The values for total antiserum are the numbers of reverse protein A PFC obtained with polyvalent rabbit anti-human immunoglobulin. IgG, IgA, and IgM values are the numbers of PFC obtained with heavy-chain-specific antisera.
[c] ND, Not done.

TABLE 10. PFC responses of mononuclear cell subpopulations activated by fraction PI or SF

Cells	Expt	Cell sub-population[a]	No. of PFC per 10^6 lymphocytes recovered with the following prepn:			
			None	PWM (50 μg/ml)	Fraction PI (50 μg/ml)	Fraction SF (50 μg/ml)
PBMC	1	N	80	2,900	5,400	7,500
		E^+	85	105	700	120
		E^-	110	110	680	350
	2	N	50	14,000	4,800	6,200
		E^+	100	500	100	180
		E^-	120	220	250	200
Tonsil lympho-cytes	1	N	400	18,500	12,500	8,500
		E^+	500	500	350	2,100
		E^-	350	220	50	180
	2	N	220	6,240	6,920	3,900
		E^+	125	580	410	160
		E^-	160	1,250	390	300

[a] N, Unfractionated cells; E^+, E-rosette lymphocytes (90 to 95% overnight rosettes, <3% IgS^+ cells, and <1% esterase-positive cells); E^-, nonrosetting lymphocytes (85 to 90% IgS^+ cells, <2% E overnight rosettes, and <3% esterase-positive cells).

TABLE 11. Induction of immunoglobulin (IgG, IgM, and IgA) secretion in supernatants of PBMC cultured with fraction PI or SF

Treatment	Immunoglobulin concn (μg/ml)[a]		
	IgG	IgM	IgA
PWM (20 μg/ml)	2.26 ± 1.6[b]	8.55 ± 4	4.8 ± 1.48
PI (50 μg/ml)	2.52 ± 1.65	7.22 ± 5.3	3.35 ± 1.63
SF (50 μg/ml)	3.6 ± 1.4	11.12 ± 7.25	5.53 ± 2.4
None	0.4 ± 0.2	2.1 ± 1.4	1.85 ± 1.65

[a] The amounts of immunoglobulins in culture supernatants were measured by laser nephelometry on day 8 for 2.5×10^6 lymphocytes per culture.
[b] Mean ± standard deviation for 13 cultures from different donors.

cell fractions; excessive numbers of monocytes are indeed known to inhibit lymphocyte proliferation and differentiation (16). This possibility is supported by the higher responses to *Brucella* fractions seen in monocyte-depleted cultures (Table 7). On the other hand, T-cell-depleted tonsil populations which contain less than 3% monocytes do not proliferate, thus supporting the true T cell dependence of B-cell responses to fractions PI and SF. Whether this T cell dependence means that fractions PI and SF activate both T and B cells or whether the B-cell stimulation indirectly results from T-cell activation is currently under study, together with a comparative identification of the B-cell subpopulations activated by fraction PI and by other available PBA. Preliminary results show that although they do not stimulate T-cell proliferation, fractions PI and SF do activate T cells to interleukin 2 secretion.

As previously shown for other PBA, such as purified protein derivative (24) fraction PI or SF induces both specific and nonspecific proliferation. The dose requirements for optimal expression of these two functions do in fact differ. However, there is an ambiguity in certain patients or experiments or both. The exact nature of the ligand(s) and receptor(s) involved in specific and nonspecific responses and the cellular and lymphokine requirements of these two functions are currently being studied because a better discrimination between specific and nonspecific cellular immune responses would indeed enhance the clinical significance of in vitro stimulation assays. Moreover, if, as shown in mice (6), these two PBA are able to induce human auto-antibodies and circulating immune complexes, the study of fractions PI and SF may shed some light on some of the pathological manifestations of human brucellosis.

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