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Résumé

IMMUNITÉ ANTI-BRUCELLA TRANSFÉRÉE PAR LES CELLULES LYMPHOIDES DE SOURIS VACCINÉES PAR UNE FRACTION DE LA PAROI BACTÉRIENNE. — Une immunité anti-Brucella peut être induite chez la souris soit par vaccin vivant, soit par vaccin bactérien total ou purifié. Il est connu que cette immunité peut être transférée adoptivement par les cellules lymphoïdes de souris vaccinées par le vaccin vivant ou passivement par un immun sérum dirigé contre certaines fractions bactériennes. Une fraction (PG) constituée de protéines liées à la paroi bactérienne a été utilisée pour vacciner des souris DBA/2 soit par voie intra-veineuse, soit par voie sous-cutanée plantaire. Les cellules spléniques ou ganglionnaires ont été transférées à des souris receveuses, éprouvées par voie veineuse avec la souche virulente *Brucella abortus* 544. La numération des Brucella dans la rate, et dans certains cas, dans le foie a été faite pour mesurer l'immunité, 15 jours après l'épreuve ou à plusieurs dates successives. Cette immunité augmente après le transfert pendant 21 jours. Les cellules ganglionnaires sont beaucoup plus efficaces que les cellules spléniques. L'immunité transférée est réduite, mais non supprimée, par le traitement des cellules par un sérum anti-Thy. Les deux types de cellules T et B séparés sur colonne de nylon transfèrent une immunité. Des expériences de transfert comparant deux lignées de souris, DBA/2 et CBA, deux modes de vaccination, vaccin vivant et fraction PG, et deux souches d'épreuve, l'une virulente (Ba 544), l'autre avirulente (souche vaccinale Ba 19) ont montré que ces trois facteurs interviennent dans l'induction et/ou l'expression de l'immunité. Les souris DBA/2 ont exprimé une meilleure immunité avec le vaccin fraction et l'épreuve virulente tandis que les souris CBA ont mieux répondu au vaccin vivant et à l'épreuve avirulente. Les souris F1 du croisement DBA/2 × CBA ont exprimé une bonne immunité aux deux types de vaccins à l'égard de l'épreuve virulente. Ainsi, deux types au moins d'immunité à médiation cellulaire doivent intervenir dans le transfert, dont l'expression plus ou moins bonne dépendrait d'un déterminisme génétique.

Brucellosis is an infectious disease of animals and man due to multiplication and survival of the facultative intracellular bacteria, *Brucella*, in several target organs and/or in reticulo-endothelial cells of specific organs such as liver and spleen. Immunity that follows natural disease may be reproduced by live low-virulence vaccine strain such as the *Brucella abortus* strain 19 (Ba 19) and is often considered as being essentially cell-mediated. Killed vaccines or bacterial fractions can however induce a good protective immunity : *eg* the proteins-bound cell wall peptidoglycan fraction (PG) was shown to protect mice as well as strain Ba 19 (Bosseray *et al* 1984). Immunity induced by PG fraction in mice was shown to be largely antibodies-mediated (Plommet and Plommet 1983), but cell-mediated immunity may also be considered since Brucella fractions may trigger several cell-mediated activities (Bascoul *et al* 1976, Vendrell *et al* 1980). Cell-mediated immunity was transferred from donor Ba 19 vaccinated to naive recipient mice by Pavlov *et al* (1982). Effectors were shown to be T-cells of the $Ly1^{+}2^{+}$ set, but not B-cells. Using similar transfer experiments, PG fraction was shown to induce an immunity transferable with lymphoid T- an B-spleen cells and T-lymph nodes cells. In contrast with live vaccine induced transferred immunity, this immunity was better expressed in DBA/2 than in CBA mice, and against a virulent than against an avirulent (vaccine strain) challenge.

Materials and Methods

Experimental design.

DBA/2J Orl unless otherwise stated, or, CBA/J Orl (Cesal, Orléans, France), or DBA/2×CBA F1 mice were used. The mice were bred in an isolated room of the air-conditioned animal building of the Institute and fed on sterilized chow and water *ad libitum*. At seven weeks of age, the mice were vaccinated either intravenously (iv) or subcutaneously into the hind footpads (scf) with the Brucella PG fraction or with the live Ba 19 strain. At different times, usually 28 days (90 days after Ba 19, splenic cells from 7 vaccinated and 7 control mice were extracted, cleared from plastic adherent cells or separated on

Table 1. – Association of adoptive immunity and the dose of transferred immune cells

splenic cells					lsd(b)
Normal	*Immune* (a)				P = 0.05
Brucella spleen count (log_{10} CFU)					
(number of cells $\times 10^7$)					
5.48 (12)	3.87 (15)	4.10 (4.4)	4.45 (1.4)	5.10 (0.4)	0.23

a : vaccine dose : PG fraction, 50 µg. Interval vaccination-transfer, 30 days, transfer-challenge, 15 days.
b : least significant difference between groups of 5 mice at level of P = 0.05.

nylon wool columns, and/or treated with anti-Thy serum plus complement (C'). The cells were then iv infused to 5 recipient naive mice, unless otherwise stated. In one experiment, recipient mice were treated with cyclophosphamide (200 mg/kg) the day before transfer. The recipient mice were then iv challenged with the virulent strain *B abortus* 544 (*Ba* 544), or in one experiment with either *Ba* 544 or *Ba* 19, at about 2 h post transfer or later as indicated. The number of transferred cells was estimated, eventually adjusted, by direct microscopic count on calibrated slides after Giemsa staining. Trypan blue exclusion test was used in preliminary experiments to scheck cells viability which was always higher than 90 % at transfer.

The mice were killed by cervical dislocation for splenic or hepatic *Brucella* counts as previously described 15 days or at stated times after challenge (Plommet and Plommet 1983). In brief, organs were glass-ground to homogeneity in buffer saline solution (BSS), diluted, plated on two Petri plates, incubated at 37 °C in CO_2 10 % for 5 days and enumerated. Results were expressed in log_{10} Colony Forming Unit (CFU) per organ and given by mean and standard error (SE) per group. The difference between bacterial number in control and immune cell transferred groups express the immunity.

Bacterial strains. PG vaccinal fraction

Virulent challenge strain Ba 544 was diluted in BSS from a standard lyophilised vial to about 2×10^5 CFU in 0.2 ml and iv injected (Plommet and Plommet 1983). Exact challenge number was a posteriori calculated by plating dilutions on six Trypticase Soy Agar plates (TSA, Biomerieux, Marcy l'Etoile, France).

The vaccinal Ba 19 strain was freshly prepared on TSA slants for 24 h and suspended in BSS. It was iv injected in 0.2 ml at 1-2 $\times$ 10^5 CFU as indicated.

The vaccinal PG fraction has been previously described in details (Bosseray *et al* 1978, Dubray and Bézard 1980). Briefly, Ba 19 cells grown in Trypticase Soy Broth, harvested in saline were inactivated by heating at 65 °C for 1 h, broken with glass beads in a Braun MSK homogenizer. Cell walls recovered by centrifugation were treated by boiling sodium dodecyl sulfate, washed in distilled water and lyophilized. The resulting white powder is the fraction, which yields about 5-10 % of the original bacterial dry weight. It contains about 30 % peptidoglycan in weight, 50 % proteins and a small amount of polysaccharides. Two major proteins bands of 36-38 K and 25-27 K molecular weight were considered as the main protective immunogens (Dubray and Bézard 1980, Dubray 1987). The white powder was weighed, ground with a glass grinder in BSS and either iv injected at dose 10-100 µg per mouse in 0.2 ml, or scf injected (10 µg in 0.05 ml into each hind foot pad).

Splenic and lymph node cells preparation

Spleens of iv vaccinated or normal mice immediately removed after killing were placed in 5-10 ml Earle BSS (Biomerieux, Marcy l'Etoile, France) added with 3 % heated (56 °C, 30 min) fœtal calf serum (FCS, Gibco, Painley, England) and heparin (calcium salt, 30 U/ml). The spleens were diced into small pieces, transferred onto a stainless steel gauze (12 $\times$ 0.3 mm wires), gently pushed through the gauze with a glass iston and washed with the cell suspension and fresh Earle BSS up to a total of 12 ml for 7 spleens (Henry 1980, Pavlov *et al* 1982).

The suspension was then either cleared of plastic adherent cells (macrophages) or enriched in nylon wool non-adherent (T-cells) or adherent (B-cells).

For plastic adherence separation, the suspension was pipetted (12 or 24 ml) into a tissue culture flask (Nuclon, A/S Nunc, Roskilde, Denmark), incubated at 37 °C in 5 % CO_2 for 2 h. The flask was gently shaked before the non-adherent cells were pipetted off, filtered throught a four-layered cotton sterile gauze into a centrifuge tube. The cells were washed twice by centrifugation at 250 *g* in Earle BSS, and finally suspended in 0.4 ml at appropriate concentration for iv infusion to mice or following treatment. This suspension was kept on crushed ice until injected to mice, usually for less than one hour. An aliquot was taken for examination and count under the microscope. Unless otherwise stated, cells from 7 spleens were transferred to 5 mice, about 1 $\times$ 10^8 per mouse. These cells were essentially free (< 5 %) of morphologically identified macrophages. In a preliminary experiment, plastic adherent cells were recovered by gentle scrapping with a glass rod and directly transferred to mice.

Popliteal lymph nodes from footpad vaccinated mice weighed about 6 mg *versus* 2 mg for controls one month

after vaccination. These nodes were suspended in BSS, cut into thin slices and pressed through the steel gauze. The cells were washed as above with care to discard drops of supernatant fat. About 2 to 3 x 10^6 cells, mostly lymphocytes, were obtained from one vaccinated mouse. Since no more than 10^5 cells were obtained from control nodes, no transfer were done in control group.

Separation of T- and B-cells on nylon wool

The enrichment method of Julius *et al* (1973) as described in (Henry 1980) was used. The splenic cells suspension from 16 mice (or 3 x 10^9 cells) was centrifuged and suspended in 50 ml Earle FCS warmed at 37 °C, filtered through cotton gauze, added to a rinsed, dried, prewarmed nylon wool pack of 20 g (Leuco Pak, Travenol Laboratories Inc, Deerfield, Ill). Earle FCS (20 ml) was added to fill up the pack. After a 45 min incubation at 37 °C, warmed Earle FCB (225 ml) was slowly added to exhaustivelly elute non-adherent cells at about one drop per second. This suspension was centrifuged and resuspended in appropriate volume of Earle for infusion to mice or following treatment. Adherent cells were eluted in the cold with 250 ml of cold (3 °C) saline. These cells were centrifuged, suspended in Earle FCS, and kept on ice until transfer.

Enrichment in T- and B-cells was tested in preliminary experiments by immunofluorescence staining with FITC anti-mouse IgG and FITC monoclonal Thy 1-2 (Miles-Yeda, Rehovot, Israel). In non-adherent cell fraction, there were 38 % Thy + cells and 11 % IgG + cells. In adherent, these were respectively 15 % and 80 %.

Treatment of immune cells by anti-Thy serum and complement

A batch of plastic non adherent cells or of nylon wool separated cells was divided into several aliquots respectively treated with (1) normal heated rabbit serum at 1:40, (2) rabbit anti-mouse T-cell serum (anti-mouse brain Thy 1.1 and 1.2, Cedarlane laboratories, Hornby, Ontario) at 1:40 (3) Low-Tox M rabbit complement (Cedarlane) at 1:12 diluted in cytotoxicity medium (RPMI 1640 Eagle medium with Hepes and 0.3 % BSA, Cedarlane) (4) successively with anti-Thy serum and complement.

Cells were treated as described (Pavlov *et al* 1982), with small modifications according to recommendations by Cedarlane and preliminary assays. Cells (1 ml, 1 x 10^7) were first incubated with gentle mixing at 4 °C for 1 h in anti-Thy serum at 1:40 in cytotoxicity medium. The cells were centrifuged at 250 *g* for 8 min, suspended in diluted complement and incubated at 37 °C for 1 h. The cells were then filtered thought cotton gauze, suspended in Earle BSS, counted and infused to mice. Control aliquots were sham-treated accordingly.

From preliminary assays it occurred that (1) some cells were lysed by complement alone, either low-Tox or guinea pig ; (2), some batches of anti-Thy serum alone were able to abate transferred immunity without cell lysis. Consequently, those groups were included in some assays.

Statistics

Each assay was considered as a particular experiment with a common variance from which the least significant difference (lsd) between two groups was calculated for probabilities P = 0.05 and P = 0.01.

Results

1. Capacity of splenic cells from PG vaccinated mice to transfer immunity

Preliminary experiments had shown that when donor mice were iv vaccinated with PG fraction (10-100 µg) 21 days at least before transfer, splenic cells depleted from plastic adherent cells (macrophages) confer an immunity conveniently estimated at day 15 post challenge. In these experiments, the challenge was injected either immediately or one day after transfer, but il was subsequently shown that the protection significantly increased when the interval from transfer to challenge was extended from 0 to 21 days (fig 1). In following experiments, the delay chosen was either 1 or 15 days, as indicated.

The transferred immunity evidenced four traits. (1) It was slightly dependent on the vaccine dose (10 *vs* 100 µg, $P < 0.05$). Later on, the 50 µg dose was usually chosen. (2) It was strongly dependent on the number of cells transferred, as shown in table 1. When 7 donor mice were taken for 5 recipients, about 1 x 10^8 cells were usually transferred per mouse. This number was usually lower after separation and treatment of cells. (3) It was not increased by treatment of recipients by cyclophosphamide. (4) Il was dependent on time from vaccination to transfer. Transferred immunity was nil before the 21st day after vaccination, then increased up to day 28, remained constant up to day 40, then slowly decreased but was still significant 90 days after vaccination. This decrease was concomitant with a decrease by half of the number of cells obtained from the donors.

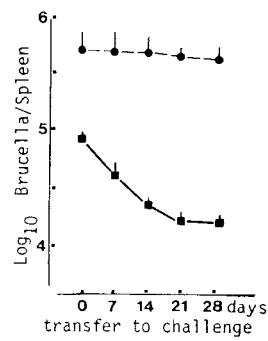

Fig 1. — Evidence that the protection conferred to mice by immune splenic cells (3.3 x 10^8 per mouse, ■) increased after transfer until the 21th day comparatively to normal cells transferred controls (3.9 x 10^8, ●). Spleen counts 15 days after challenge, vaccine dose, PG fraction 25 µg, ; mean ± SE, n = 5.

Table 2. – Brucella spleen counts in mice transferred with normal or immune splenic cells (a) treated or not with anti-Thy serum.

	Immune cells					
		treated with				lsd (b) P = 0.05 (P = 0.01)
Normal cells	Not treated	normal	serum anti-Thy	C'	anti-Thy + C'	
	Brucella spleen counts (log_{10} CFU) (number of normal or immune cells × 10^7)					
Experiment 61						
5.56 *(13)*	3.86 (18)	...	4.57 (15)	4.16 (10)	4.62 (8)	0.31 (0.42)
Experiment 62						
5.30 *(9)*	...	4.07 (16)	...	4.21 (13)	4.79 (13)	0.30 (0.40)
Experiment 69						
5.37 *(5)*	4.69 (4.4)	4.78 (4.7)	4.77 (4.7)	...	...	0.23 (0.32)
Experiment 71						
5.48 *(12)*	3.65 (15)	...	...	...	4.40 (10)	0.42 (0.57)

a : vaccine dose : fraction PG, 50 µg. Interval vaccination-transfer, 28 days ; transfert-challenge, 15 days.
b : least significant difference between groups of 5 mice at two levels of P.

2. Time course of splenic and hepatic infections in transferred mice

Groups of mice transferred with normal or immune splenic cells were challenged immediately after transfer and killed for spleen and liver counts at successive times after challenge (fig 2). In spleens, the difference between control and immune groups was already significant at day 2 post challenge then increased consistently with time. In livers, the difference took a longer time to be of any significance. It occurred on day 21 only, then increased thereafter.

3. Transfer of immunity by splenic cells treated with anti-Thy serum and/or separated on nylon wool

Splenic cells from control or from vaccinated mice were first depleted from plastic adherent cells (macrophages) then either transferred or treated with normal or anti-Thy serum, and/or complement, enumerated and transferred. Results of four successive experiments are shown in table 2. In experiment 61, protection conferred by immune cells was significantly ($P < 0.01$) abated but not abrogated by treatment with anti-Thy serum plus complement, concomitantly with a lysis of about half of the cells. Confirmation was obtained with a higher number of cells transferred in experiment

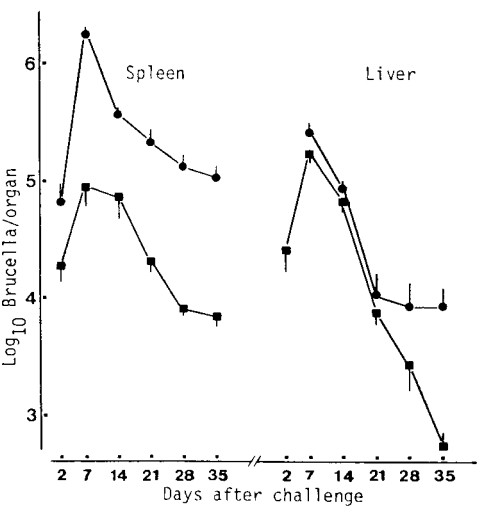

Fig 2. — Time course of splenic and hepatic infections in recipient mice transferred from control (●) or immunized (■) donors. Vaccine dose, PG fraction, 100 µg. No cell transferred, control ; 1.7 × 10^8, immune 2.6 × 10^8. Interval vaccination-transfer, 35 days and transfer-challenge, 1 day ; mean ± SE, n = 5.

Table 3. – Brucella spleen counts in mice transferred with normal or immune splenic cells (a) separated on nylon wool and treated or not with anti-Thy serum

	Immune cells					lsd (b) P = 0.05 (P = 0.01)
		T-nylon enriched		B-nylon enriched		
Normal cells	total	not treated	anti-Thy + C'	not treated	anti-Thy + C'	
	Brucella spleen counts (log_{10} CFU) (number of normal or immune cells × 10^7)					
Experiment 68						
4.98	4.46	3.91	...	...	...	0.33
(10)	(10)	(10)				(0.46)
Experiment 71						
5.48	3.65	4.06	...	4.53	...	0.42
(12)	(15)	(20)		(9)		(0.57)
Experiment 73						
5.53	...	4.48	4.87	4.45	4.53	0.39
(13)		(10)	(6)	(15)	(11)	(0.53)

a : vaccine dose : fraction PG, 50 µg. Interval : vaccination-transfer, 28 days ; transfer-challenge, 15 days.
b : least significant difference between groups of 5 mice at two levels of P.

62 and 71. Low tox complement alone decreased slightly the number of cells and immunity (Exp 61, 62). Anti-Thy serum without complement was able to reduce the transferred immunity in experiment 61 and in confirmatory assays (not shown) but this effect was not obtained with all batches of serum, as in experiment 69.

Because cells resistant to lysis were able to transfer immunity, and because in a preliminary experiment, plastic adherent cells (macrophages) were shown to be inactive, B-cells had to be considered. Immune splenic cells were enriched in T- and B-cells by filtration on nylon wool, then eventually treated with anti-Thy serum plus complement. It is shown in table 3 that

(1) enriched T-cell fraction conferred an immunity higher (Exp 68, $P < 0.01$) or about equal (Exp 71, $P = 0.05$) than total cells.

(2) enriched T-cell transferred immunity was abated but not abrogated again by anti-Thy serum plus complement, (Exp 73, $P < 0.05$)

(3) B-enriched fraction conferred a good immunity ($P < 0.01$ in Exp 71 and 73) and in two confirmatory experiments, that was not abated by anti-Thy serum plus complement (Exp 73).

In conclusion, the immunity conferred by splenic cells from PG fraction vaccinated mice depends on both anti-Thy serum sensitive T-cells and on insensitive, nylon adherent B-cells.

4. Transfer of immunity by lymph nodes cells

Popliteal lymph nodes and spleen cells were taken from mice vaccinated with the PG fraction in the foot pads. These cells were transferred directly or after treatment with anti-Thy serum plus complement. Results are shown in table 4.

Lymph node cells were very efficient to confer protection since 2.3×10^6 cells were more protective than 120×10^6 splenic cells. This protection increased with time after transfer, from 4.81 when the challenge was done 1 day after transfer to 3.78 when done after 15 days. The protection was strongly if not totally abated by treatment with anti-Thy serum. A monoclonal anti-Thy 1-2 (Cedarlane) was used in a last experiment with similar result.

5. PG fraction vs *living vaccine induced immunity transferred to recipient mice of strains DBA/2* vs *CBA, tested with virulent* vs *vaccinal Brucella strains*

The demonstration that anti-*Brucella* immunity induced by the live Ba 19 vaccine can be transferred in mice by T-cells was done by Pavlov *et al* (1982) with the low virulent vaccinal strain Ba 19 as challenge strain and with CBA mice, known to exhibit a somehow different time course infection than DBA/2 mice (Plommet and Plommet 1981, Ho and Cheers 1982). It was thus of interest to compare the immunity induced and expressed in both models. This was done in a complete factorial experiment, including the two strains of mice, the two vaccines, and the two challenge strains, as shown in table 5.

All mice transferred with immune cells were

Table 4. – Brucella spleen counts in mice transferred with lymph nodes or spleen cells from footpads vaccinated donor mice (a)

Control (no transfer)	*Immune cells from* lymph nodes total	lymph nodes Thy + C' treated	spleen total	lsd (b) P = 0.05 (P = 0.01)
	Brucella spleen counts (log_{10} CFU) (number of cells × 10^6)			
Experiment 65				
interval transfer-challenge, 1 day				
5.69	4.81 (2.3)	...	5.23 (120)	0.21 (0.30)
interval transfer-challenge, 15 days				
5.42	3.78 (2.3)	...	4.84 (120)	0.21 (0.30)
Experiment 75				
interval transfer-challenge, 15 days				
5.76	4.43 (5)	5.31 (2.5)	...	0.33 (0.45)

a : vaccination dose : fraction PG, 10 µg in each hind footpad. Interval vaccination-transfer, 28 days.
b : least significant difference between groups of 8 (exp 65) or 5 (exp 75) mice at two levels of probability.

Table 5. – Brucella spleen counts in two strains of mice transferred with splenic cells from mice vaccinated with either the live vaccine or the PG fraction, and challenged with either the vaccinal or the virulent strain.

Vaccination of donor mice (homologous strain)	*Challenge* Brucella strain (a) low virulent Ba 19 strain of mice CBA	DBA/2	high virulent Ba 544 strain of mice CBA	DBA/2
	Brucella spleen counts (log_{10} CFU) (number of cells transferred × 10^8)			
no vaccination				
	7.33 (0.6)	7.22 (0.8)	5.65 (0.6)	5.86 (0.8)
live vaccination : dose 2.1 × 10^5 CFU, vaccination-transfer 90 days				
	5.19 (1.4)	6.97 (1.5)	4.37 (1.4)	5.20 (1.5)
PG fraction : dose 100 µg, vaccination-transfer 35 days				
	7.09 (1.1)	6.90 (1.3)	4.99 (1.1)	4.60 (1.3)
lsd P = 0.05 (P = 0.01) (b)	0.22 (0.30)		0.44 (0.57)	

a : interval transfer-challenge, 1 day.
b : least significant difference between groups of 5 mice at two levels of probability.

Table 6. – Brucella spleen counts in DBA/2 × CBA F1 mice transferred with splenic cells from mice vaccinated with either the live vaccine or the PG fraction and challenged (a) with the virulent strain.

Vaccination of donor mice	*Normal cells*	*Immune cells*		lsd (b) P = 0.05 (P = 0.01)
		total	treated anti-Thy + C'	
	Brucella spleen counts (log_{10} CFU) (number of cells transferred × 10^8)			
no vaccination				
	5.50 (10)	...	...	...
live vaccination : dose 1 × 10^5 CFU, vaccination-transfer 90 days				
	...	4.10 (10)	5.07 (7.2)	0.22 (0.31)
PG fraction : dose 50 µg, vaccination-transfer 28 days				
	...	4.09 (10)	4.45 (6.7)	0.22 (0.31)

a : interval transfert-challenge, 15 days.
b : least significant difference between groups of 5 mice at two levels of probability.

significantly protected, with large differences however. Results with the Pavlov's model (CBA, live vaccine, Ba 19 challenge), and with our model (DBA/2, fraction vaccine, virulent challenge) were consistent with published or present results : protections were, respectively, of 2.14 (difference control-transferred) and 1.26. But, with the Ba 19 challenge, CBA mice were better protected by vaccination of donors by the live vaccine than by the fraction. DBA/2 evidenced a low protection only. With the virulent challenge, CBA mice were better protected by the live vaccine than by the fraction whereas DBA/2 mice were better protected by the PG fraction.

These results suggested that two mechanisms at least were involved in this transferred immunity, and that the two strains of mice had different capacity to respond in either induction or expression of each.

This hypothesis was tested in table 6 with DBA/2 × CBA F1 mice. Donors were vaccinated with the PG fraction or with the live vaccine and after appropriate time, respectively 28 and 90 days (in Pavlov's model, the delay from vaccination to transfer should be of about 3 months to avoid transfer of residual vaccine bacteria), splenic cells were transferred *in toto* or after treatment with anti-Thy serum and complement. The mice were then challenged with the virulent *Brucella*. Resulting immunity was equal in both groups transferred with total splenic cells, but was abated more deeply by anti-Thy serum in live vaccine immunized group.

In conclusion, F1 mice inherited from each parental strain an overall capacity to respond equally well to both types of vaccine. T-cells were however dominant, if not exclusive, in live vaccine induced immunity, whereas B-cells may be *a contrario* more important in PG fraction vaccine induced immunity.

Discussion

Transfer of spleen or lymph nodes cells from donor mice iv vaccinated with the protein-bound cell wall peptidoglycan PG fraction of *B abortus* adoptively confers to recipients an immunity that was evidenced by lower bacterial counts in spleen and liver of the iv challenged mice with the virulent *B abortus* 544 strain. This immunity was expressed in spleen from the 2nd day after challenge, then increased thereafter. In liver, it became significant 21 days after challenge only. This immunity depends on the number of cells transferred and on the vaccine dose. The weight of the spleen was not significantly increased by the vaccine. The immune activity of cells appeared about 21 days after vaccination to reach a maximum at 28-35 days, then decreased slowly. Treatment of recipients by cyclophosphamide did not increase the adoptive immunity. Cells from popliteal lymph nodes stimulated by a foot pad injection of PG were more active than spleen cells, but these cells were not stimulated by an iv vaccination. The adoptive immunity increased in recipients after transfer for about 21 days. Treatment of immune splenic and lymph nodes cells by anti-Thy serum plus complement reduced but did not abrogate transferred immunity indicating that in addition to Theta bearing T-cells, other cells were involved. Plastic adhe-

rent cells (macrophages) were inactive, but nylon wool adherent cells — a fraction containing about 80 % of IgG bearing cells — were able to confer a protection that was not abated by anti-Thy serum. Thus, B-cells should participate to a great extent to this protective immunity. Interestingly, the immunity conferred by lymph nodes cells was more sensitive to anti-Thy serum treatment than splenic cells (table 4), and immunity induced by the live vaccine in DBA/2 × CBA F1 mice was similarly more sensitive to anti-Thy serum than the immunity induced by the PG fraction (table 6). The comparison of the two strains of mice, DBA/2 and CBA, indicated that each strain may preferentially develop a largely B- or a T-cells dependent immunity, and that F1 mice may be endowed with both systems. The comparison between the two Brucella strains used as challenge indicated that the low virulence strain Ba 19, which multiplies very fastly in a first phase (table 5) but is eliminated in about 45 days, was more susceptible to the immunity conferred by the same live vaccine than by the PG fraction. In contrast, the virulent strain Ba 544, which persists a very long time in control mice (Bosseray *et al* 1982) was susceptible to both types of induced immunity, which are expressed at different levels in CBA and DBA/2 mice. Our results are thus in agreement with those of Pavlov *et al* (1982) who demonstrated that in CBA mice vaccinated and challenged with the strain Ba 19, the transferred immunity is mediated by T-cells but not by B-cells. It is clear however that in other models, cell-mediated immunity may be induced by a sub-cellular fraction such as the PG fraction and that B-cells are involved in addition to T-cells.

The immunity conferred to recipients by spleen or lymph nodes cells increases after transfer (fig 1, table 4). This may result from either (1) a carry over of antigen to recipients by the transferred cells, (2) an accumulation of active antibodies synthetized by activated B-cells, or (3) multiplication of sensitized T-cells. The first hypothesis is however unlikely since (a) the transferred cells were depleted from macrophages and (b) the hypothetical amounts of transferred antigen would be to small to induce an active immunity in less than 20 days, the shorter time needed to observe an active immunity in donors. The second hypothesis was tested by titration of antibodies directed against the PG fraction antigens in recipients 21 days transfer. A small increase was observed which is compatible on a titer basis with this hypothesis, since it was previously shown that antibodies administered before or after challenge may confer a good protection (Plommet and Plommet 1983). Lastly, the third hypothesis considers that T-cells may adoptively multiply after transfer. *Brucella* fractions are known to have several immunogenic activities (Bascoul *et al* 1976, Vendrell *et al* 1980, Vendrell *et al* 1985) which may last long enough after transfer, even without any contact with antigen, to stimulate an additional cell multiplication. From dose-responses of table 1 and time-responses from figure 1, it can be estimated that the increase of immunity from 1 to 15 days post transfer may result from a 15-fold increase in number of cells, or about 4 divisions. The fact that lymph node cells, highly sensitive to anti-Thy serum (table 2) were able to double the protection in 15 days substantiates this T-cell hypothesis.

The main purpose of this research was to study the immunity conferred to mice by the PG fraction. It was shown that this immunity can be transferred by splenic or lymph nodes cells. Several unanswered questions are now to be considered : (1) exact identification of subsets of cells involved, (2) hypothetical cooperation of T- and B-cells as recently suggested (Vendrell *et al* 1985), (3) direct inhibition, without lysis, of T-cell by some anti-Thy serum, which may be linked to inhibition of function adjacent to the Theta antigen as was shown for cytotoxic function by monoclonal antibody for the H-2 antigens (O'Neill 1986), (4) identification of the active antigen(s) of the PG fraction.

In addition to these points, the difference of responses to infection, immunization and transfer of the two strains of mice have to be considered. DBA/2 mice were known to express an earlier non specific resistance. CBA express an earlier specific response and a better antibodies response (Plommet and Plommet 1981, Ho and Cheers 1982, Cheers and Ho 1983). In contrast, positive effect of immune serum on spleen infection was similar in CBA (Ho and Cheers 1982) and in DBA/2 mice (Plommet and Plommet 1983, Plommet *et al* 1986). In the present paper, the two strains were shown to trigger and express different types or levels of cell-mediated immunity, that can be combined in F1 mice. It can thus be conclude that cell-mediated immunity can be induced and expressed in mice by both live and sub-cellular fraction vaccine on the condition that the mice have a proper genetic heritage.

Abstract

Immunity against *Brucella*, a facultative intracellular bacteria, can be induced in mice by live or by killed-whole-cell or fraction vaccine. This immunity can be adoptively transferred from live vaccinated donor to recipient mice, or passively with immune serum raised against bacterial fractions. A protein-bound

peptidoglycan fraction (PG) was used to immunize DBA/2 donor mice intravenously or subcutaneously in the hind footpads. Splenic or popliteal lymph nodes cells were transferred to recipients that were intravenously challenged with a *B abortus* virulent strain. *Brucella* spleen counts, and in some cases liver counts, were done 15 days later or at successive times. Spleen cells depleted from macrophages and lymph nodes cells conferred immunity to recipients. This immunity increased after transfer for about 21 days. Lymph nodes cells were more efficient to transfer immunity than spleen cells. This immunity was abated but not abrogated by treatment of cells by anti-Thy serum and was transferred by both nylon wool non adherent (T enriched) or adherent (B enriched) cells. Comparative transfer experiments with DBA/2 *versus* CBA mice, live vaccine *versus* PG fraction vaccine, virulent *versus* avirulent (vaccinal) challenge evidenced that the three factors were involved in induction and/or expression of immunity. DBA/2 mice responded better to fraction vaccine and virulent challenge whereas CBA responded better to live vaccine and to avirulent challenge strain. In contrast DBA/2 × CBA F1 responded equally well against the virulent challenge to transfer of cells from live vaccine or PG fraction vaccinated donor mice. Two cell-mediated immune mechanisms may thus be involved in transferred immunity the expression of which may be genetically determined.

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