HAL
open science

Immunity to Brucella abortus induced in mice by popliteal lymph node restricted strain 19 vaccination

M. Plommet, A.M. Plommet

To cite this version:

M. Plommet, A.M. Plommet. Immunity to Brucella abortus induced in mice by popliteal lymph node restricted strain 19 vaccination. Annales de Recherches Vétérinaires, 1989, 20 (1), pp.73-82. hal-00901845

HAL Id: hal-00901845

https://hal.science/hal-00901845

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Ann. Rech. Vét. (1989) 20, 73-82
© Elsevier/INRA

Original article

Immunity to *Brucella abortus* induced in mice by popliteal lymph node restricted strain 19 vaccination

M. Plommet and A.M. Plommet

Station de Pathologie de la Reproduction, INRA, Centre de Tours-Nouzilly, 37380 Nouzilly, France

(received 21-4-1988, accepted 20-6-1988)

Summary — Living vaccines induce immunity by lymphoid organs colonization that persists for a while before subsiding. *Brucella abortus* 19 vaccine injected into the footpad of mice was shown to colonize the popliteal lymph node and the spleen for at least 15 days before progressive disappearance. Administration of an immune serum before footpad vaccination restricted the colonization to the popliteal lymph node. The serum did not interfere with immunity when this immunity was measured by the virulent challenge spleen count method at least 90 days after vaccination. Immunity induced by lymph node restricted vaccination was very efficient 90 or 180 days after vaccination and was not boosted by a second injection administered 90 days after the first. This immunity was however about 20% lower, expressed in $\log_{10}$ spleen counts, than immunity induced by a non-restricted systemic vaccination which in addition was boosted by a recall. Restricted vaccination may mainly trigger the T-cell limb of immunity, whereas systemic vaccination triggers both humoral and cellular effectors.

***Brucella abortus* - brucellosis - mouse - B19 vaccine - immunity**

Résumé — Immunité induite chez la souris contre *Brucella abortus* par restriction de la souche vaccinale 19 au ganglion poplité. *Les vaccins vivants provoquent une colonisation limitée dans le temps de certains organes lymphoïdes qui induit l'immunité. La souche vaccinale* Brucella abortus *19 injectée dans le coussinet plantaire de la souris colonise le ganglion poplité et la rate pendant 15 jours au moins avant d'être progressivement éliminée. La colonisation peut être restreinte au ganglion par l'injection d'un sérum antibrucella avant la vaccination. Le sérum n'interfère pas notablement avec l'immunité mesurée par le niveau de l'infection splénique après épreuve virulente, si celle-ci a lieu au moins 90 jours après la vaccination. L'immunité induite par la colonisation restreinte du ganglion mesurée 90 ou 180 jours après la vaccination est très efficace. Exprimée en $\log_{10}$ du nombre de bactéries dans la rate, cette immunité est toutefois inférieure de 20%, à celle résultant de la vaccination systémique non restreinte. De plus, contrairement à celle-ci, elle n'est pas renforcée par une injection de rappel. La vaccination restreinte stimulerait préférentiellement les effecteurs de l'immunité cellulaire alors que la vaccination systémique stimulerait les deux effecteurs, humoraux et cellulaires.*

Brucella abortus - *brucellose - souris - vaccin B19 - immunité*

Introduction

The facultative intracellular bacteria *Brucella* experimentally develops in mice a 3-phases infection which may be accurately quantified by splenic and hepatic bacterial count time courses (Mackaness, 1964; Plommet and Bosseray, 1977; Plommet and Plommet, 1981, 1983; Bosseray *et al.*, 1984). Immunity evidenced by lower counts and/or earlier recovery of challenged mice can be transferred from vaccinated donor to recipient mice either by immune sera (Plommet and Plommet, 1983) or by splenic T- and B-lymphoid cells or by lymph node T-cells (Pavlov *et al.*, 1982; Plommet and Plommet, 1987). Both living or bacterial fraction vaccines may induce this transferable immunity : the lipopolysaccharide fraction (LPS) was thus shown to convey a high degree of immunity in mice by monoclonal antibody transfer (Limet *et al.*, 1987).

In large animals, living vaccines are usually recommended, as they are more regularly efficient than fractions or total cell vaccines (Joint FAO/OMS Committee, 1986). However, living vaccines, such as *B. abortus* strain 19 or *B. melitensis* Rev1, induce long-lasting serological responses, mostly directed toward the LPS antigen (Diaz and Levieux, 1972) that may disturb diagnostic tests used in surveillance control programs. These responses can be limited to below significant values by administration of the vaccine by the conjunctival route instead of the usual subcutaneous route (Fensterbank and Plommet, 1979). Conjunctival vaccination induces a vaccine strain colonization mostly restricted to the lymph nodes of the head (Plommet and Plommet, 1976), whereas subcutaneous vaccination systemically extends colonization to other lymphoid organs including spleen; hence a higher serological response. Conjunctivally-vaccinated cows and ewes were protected against an experimental challenge as well as subcutaneously vaccinated counterparts (Fensterbank and Plommet, 1979; Fensterbank *et al.*, 1982, 1985). Thus, anti-LPS antibodies should convey a marginal protection only in these animals. In contrast, cell-mediated immunity should be of paramount importance.

In mice vaccinated with a bacterial fraction, T-cells extracted from locally stimulated popliteal lymph nodes were more efficient in transferring immunity than total or T-splenic cells (Plommet and Plommet, 1987). It can therefore be hypothesised that a lymph node restricted vaccination may mostly induce T-cell dependent immunity. Restriction to the popliteal lymph node can be obtained by administration of an immune serum prior to injection of the vaccine strain into the hind footpad (Pardon, 1977; Plommet and Plommet, 1983). By this method, lymph node restricted vaccination was shown to induce a very efficient immunity at a lower level, however, than systemic (non-restricted) vaccination.

Materials and Methods

Mice

Female mice, 5-6 week old, born at the Station controlled animal building (filtered air at 21°C and relative humidity 60%) from parental outbred CD-1 mice (Charles River, Elbeuf), were randomly allotted to experimental groups of 6, 10 or 12 as indicated.

Bacterial strains

Vaccine strain 19 was originally obtained from the National Animal Diseases Laboratory (Ames, Iowa) and kept in a lyophilized stock. The strain was freshly prepared from a lyophilized ampoule on Trypcase soya gelose slants (TSA) (BioMerieux, Marcy l'Etoile), harvested in saline buffer after 24 h incubation, photometri-

cally ajusted and diluted to the required concentration ≈ 1 x 10^5 colony forming unit (CFU) in 0.2 or 0.05 ml for subcutaneous or footpad injection respectively. Exact doses were retrospectively corroborated by dilution and plating on at least 5 TSA plates.

Virulent challenge strain *B. abortus* 544 was obtained from the Central Veterinary Laboratory (Weybridge, England). It was kept and prepared as strain 19 except that it required 10% CO_2 for growth. It was used at dose ≈ 1 x 10^6 in 0.2 ml and injected intraperitoneally. As strain 19 was inhibited by the addition of erythritol (1 mg/ml) to TSA, differential counts of both strains in doubly infected mice were carried out by double-plating on TSA, with and without erythritol, and double incubation with or without CO_2 respectively.

Spleen counts

At appropriate times after vaccination or challenge, the mice were killed by cervical dislocation and the spleens were aseptically taken, defatted, weighed and homogenized with a glass grinder in 9 volumes of saline buffer. This first 1/10 suspension was then either spread (0.2 ml) onto 2 or 4 TSA or TSA-erythritol plates, then diluted and seeded as required. When small numbers of *Brucella* were expected, the entire first suspension was spread onto 5-8 plates. After incubation with and/or without CO_2 for 5 days, colonies were enumerated. When no colony was found, one was considered present on one plate to compute individual $\log_{10}$ values and mean.

Popliteal lymph node counts

In footpad vaccinated mice, survival of the vaccine strain was followed by successive enumerations in the corresponding popliteal lymph node. For this, the node was finely dissected, defatted, and ground in a small saline buffer volume and seeded onto 2 TSA plates. After incubation, CFU were enumerated or estimated when > 200 per plate.

Immune serum

An immune serum obtained from a large group of mice chronically infected (3 months) with strain 544 (Plommet and Plommet, 1983) was used to restrict colonization by the vaccine strain to the popliteal lymph node. For this, 0.1 ml was intravenously injected one day before footpad vaccination. This serum was also used to measure the passive immunity thus transferred and to test a hypothetical effect of this passive immunity upon active immunization.

Expression of results; statistics

Vaccine strain footpad injection was followed in popliteal node and spleen by enumeration. Results were expressed by frequency (positive/total), and by the mean $\log_{10}$ CFU per group. When these values were very low, the mean of arithmetic values was used.

Vaccine immunity was measured by a slightly modified standard method (Plommet and Bosseray, 1977; Bosseray *et al.*, 1984) : mice were intraperitonealy challenged at a convenient time after vaccination and killed for differential counts 15 days later. Individual spleen count (x) was transformed as follows : $\log_{10}$ ($x/\log_{10} x$) before computation of mean and standard error (SE) per group in order to normalize data distribution and avoid bias in variance analysis with low values (Bosseray and Plommet, 1976). To avoid too many negative values which could be predicted in some groups with the standard challenge, the standard dose was increased 5-fold to ≈ 1 x 10^6 CFU.

Results

Vaccine strain colonization restricted to the popliteal lymph node by immune serum (Table I)

Restriction of colonization by the vaccine strain to the lymph node was first tested in groups of 6 footpad-injected mice after being infused or not with immune serum (Table I, Exp. *A*). The vaccine strain was found in high number in the spleen of all control mice up to 28 days whereas 1-3 CFU were found in the spleen of only 5 serum-transferred mice. Lymph node restriction was thus near total in this system.

Length and level of lymph node colonization resulting from this manoeuvre were tested in a second experiment (Table I, Exp. *B*) : mice vaccinated as above were

Table I. Restriction of the vaccine strain colonization to the popliteal lymph node by infusion of an immune serum before footpad vaccination, established by comparison of vaccinal infection in spleen and lymph node.

	Experiment A [1]		*Experiment B* [1]			
	Spleen [a]		*Spleen* [a]		*Popliteal node* [a]	
Days post vaccination	*Control mice*	*Serum infused mice*	*Control mice*	*Serum infused mice*	*Control mice*	*Serum infused mice*
7	6 (4.2)	0	6 (3.0)	0	6 (2.7)	6 (1.6)
14	6 (3.4)	2 *(2)* [b]	6 (2.6)	0	6 (2.3)	6 (1.7)
21	6 (3.1)	2 *(1.5)*	5 (2.1)	1 *(1)*	5 (1.7)	3 (0.5)
28	6 (3.0)	1 *(2)*	5 (1.1)	1 *(1)*	3 (0.8)	3 (0.7)
42	—	—	1 *(1)*	0	3 (0.3)	3 (0.4)
59	—	—	0	0	3 (0.4) [c]	0
75	—	—	1 *(2)*	0	0	0
90	—	—	0	0	0	0

[1] Vaccine dose : 1.1 x 10^5 and 1.0 x 10^5 CFU respectively. [a] Number of infected organs out of 6 and (mean $\log_{10}$ CFU). [b] Italicized number indicates the arithmetic CFU means of infected spleens. [c] One value > 1000 CFU not included in mean.

killed for spleen and lymph node counts as indicated. All lymph nodes were infected for at least 14 days, then the infection progressively disappeared from some mice. Lymph node restricted mice had on average a lower level of colonization and recovered earlier. As before, the spleens of restricted mice were not colonized by the vaccine strain. Therefore immunity resulting from lymph node restricted vaccination should come from this lymph node stimulation only.

Effects of passive immunity on active immunization

Because immune sera can transfer passive immunity measured in the model, and because feedback regulation may modify active immunization, it was necessary to estimate both hypothetical effects.

Direct effect was estimated in a preliminary experiment in which 5 groups of 7 mice were infused with immune serum or saline, then challenged after 1, 30, 60 or 90 days. Spleen counts indicated that passive immunity which was quite high the first day (3.4 *vs* 4.3 in control) decreased with time but was still slightly positive on day 90 (3.9 *vs* 4.3, $P < 0.05$). Thus, 2 delays of 90 and 180 days after vaccination were chosen to compare restricted to systemic vaccination.

The indirect effect of immune serum on active immunization was tested in mice infused or not with immune serum then intravenously vaccinated : in intravenous vaccination, no lymph node restriction could occur. Since immunity was expected at about 2.0 (Bosseray *et al.*, 1984) a 30-day vaccination-challenge delay was

considered sufficient to avoid direct effect of immune serum. Spleen counts of the challenge strain (Table II) indicated that the transferred immune serum did not interfere with active immunization. It was also observed that the vaccine strain survived in large numbers in the spleens of non-infused mice, hence a significant splenomegaly. To avoid this problem, the subcutaneous route was adopted for systemic vaccination in following experiment.

Immunity induced by lymph node restricted vaccination

Immunity induced by lymph node restricted vaccination was compared to immunity induced by systemic vaccination injected either subcutaneously or into the footpad in 3 protocols (Table III). Differential spleen counts 15 days after challenge showed that :

(1) One systemic vaccination conferred a good protection independent of route (subcutaneous or footpad) and time from vaccination to challenge (90 or 180 days).

(2) A second systemic vaccination significantly boosted the immune protection (one injection groups *vs* 2 injections groups, $P < 0.05$).

(3) Lymph node restricted vaccination induced a good protection — significantly lower, however, than in systemic groups (all systemic groups *vs* all restricted groups, $P < 0.05$). This vaccinial (restricted) protection was not boosted by a second vaccination. On the spleen count scale this protection amounted to 80% of the one of systemic groups.

(4) The vaccine strain was found in high numbers (from 2.0-6.0, mean 3.6) in 10 out of 70 mice in the systemic footpad and in the restricted groups. This observation, in contrast with preliminary observations (Table I), may be a biased estimation of the immune protection : vaccine strain positive mice were on average more infected by the challenge strain than negative mice (Table III). To avoid this bias, the immune protection was also esti-

Table II. Absence of interference of passive immunity upon active immunization induced by systemic (intravenous) vaccination.

	Spleen [b]		
Groups	*Weight (mg)*	*Challenge strain* [c] *(m ± SE)*	*Mice still harboring vaccine strain/ total*
Intravenous vaccination [a]	357	1.42 ± 0.15	10/10
Intravenous vaccination after injection of serum [a]	125	1.61 ± 0.17	3/10
No vaccination	393	4.64 ± 0.12	—

[a] Vaccine : 1 x 10^5 CFU strain 19. [b] Challenge : 0.9 x 10^6 CFU strain 544 in 0.2 ml intraperitoneally injected 30 days after vaccination. [c] Expressed by $\log_{10}$ ($x/\log_{10} x$), with x = CFU per spleen.

Table III. Evidence that lymph node restricted vaccination induced significant immunity-lower, however, than systemic.

Time (days) and number of vaccination before challenge	*Spleen weight mean (mg)*	*Mice still harboring vaccine strain/ total*	*Challenge [b] strain counts [c] (mean ± SE)*		
				In vaccine strain (non-restricted) vaccination	
			in all mice [d]	*negative mice*	*positive mice*
Systemic vaccination : subcutaneous in back[a]					
180	107	0/12	1.93 ± 0.20	1.93 ± 0.20	—
90	109	0/12	2.31 ± 0.15	2.31 ± 0.15	—
180 and 90	117	0/12	1.76 ± 0.14	1.76 ± 0.14	—
Systemic vaccination : footpad injection[a]					
180	121	2/11	2.09 ± 0.13	1.95 ± 0.11	2.71
90	120	1/12	2.32 ± 0.40	1.95 ± 0.14	6.45
180 and 90	139	0/12	1.64 ± 0.16	1.64 ± 0.16	—
Restricted vaccination : footpad injection after administration of serum [a,]					
180	138	2/12	2.73 ± 0.21	2.48 ± 0.23	4.00
90	136	3/12	2.70 ± 0.22	2.37 ± 0.19	3.66
180 and 90	140	2/11	2.72 ± 0.28	2.42 ± 0.26	4.02
No vaccination					
—	361	0/12	4.90 ± 0.14	—	—
—	304	0/12	4.65 ± 0.09	—	—

[a] Vaccine dose : 1 x 10^5 CFU, strain 19. [b] Challenge : 0.95 x 10^6 CFU intraperitoneal, strain 544. [c] CFU per spleen : *x*. Expressed as mean log (*x*/log *x*). [d] $N = 12$ at beginning. Least significant difference : 0.59 for $P = 0.05$. [e] Serum : 0.1 ml i.v. one day before each vaccination.

mated from negative mice only : it was again better in systemic than in restricted groups (Table III, mean : 1.83 *vs* 2.42, $P < 0.05$).

Discussion

Immunity against brucellosis may be induced in mice by living or by killed vaccines and can be transferred to recipients with either immune sera or immune lymphoid cells. While proteic antigens linked to the cell wall are the best putative candidates for T-cell mediated immunity (Dubray, 1987; Winter, 1987) LPS was shown to be largely involved in humoral immunity (Limet *et al.,* 1987). Because in veterinary medicine serological reactions following vaccination are disavantageous, efforts along several lines were attempted to devise new "non-agglutinogenic" vaccines. Among them, conjunctival administration of living vaccines was shown to induce good protection but low serological responses (Plommet and Plommet, 1975; Fensterbank and Plommet, 1979; Fensterbank *et al.,* 1982, 1985). Vaccine strains administered by the conjunctival

route have to cross the mucosae before successively colonizing several nodes of the lymphatic chain. As a consequence, only a few bacteria are able to reach systemic lymphoid organs — hence the low serological responses, contrasting with those occurring after a subcutaneous vaccination. Cell-mediated immunity, in contrast, may be triggered at a high level by colonization of one — or a limited number of — lymph nodes, as in the tuberculosis model (Lefford, 1983).

Twelve years ago, we developed a mouse model to control immunogenicity of *Brucella* vaccines: spleen counts carried out 15 days after a standard intraperitoneal challenge gave a relevant immunogenic index of living or fraction vaccines (Plommet and Bosseray, 1977; Bosseray *et al.,* 1984). In the model, both immune sera and immune splenic cells may transfer dose-dependent protection (Plommet and Plommet, 1983, 1987). Because *Brucellae* cannot cross the mucosal barrier in mice (Verger, 1971), conjunctival vaccination cannot be studied in this animal. However, colonization restricted to one lymph node can be obtained by transfer of immune sera before injection of *Brucella* into the hind footpad (Pardon, 1977). In proper experimental conditions, the vaccine strain can be totally trapped inside the popliteal node. The immune serum does not interfere significantly with: (1) level and duration of lymph node colonization; (2) induction of immunity, when no restriction upon colonization is imposed; (3) and on vaccinial immunity provided that it is measured 3 and 6 months after transfer. Under those conditions, comparisons between systemic and restricted vaccinations can be carried out: immunity induced by restricted vaccination was high, but about 20% lower when expressed on the spleen count log scale, than by systemic vaccination. In addition, this immunity was not boosted by a restricted recall, contrary to systemic vaccination.

In T-cell mediated immunity directed against facultative intra-cellular bacteria such as *Mycobacterium tuberculosis,* long lasting sensitized memory cells elicited from a local lymph node infection express a generalized immunity, even after complete resolution of the bacterial infection (Lefford, 1983). In brucellosis, immunity differs from this tuberculosis model in at least 2 aspects: (1) antibodies play an important role; (2) cell wall fractions may induce, at least in mice, a cell-mediated transferable immunity. Nevertheless, when living vaccines are used in such a way as to suppress or limit antibody response, T-cell mediated immunity is expected to play the major role. As locally stimulated lymph node cells were shown to be very efficient at transfer of immunity (Plommet and Plommet, 1987), we assumed that in restricted groups, effective immunity was mostly T-cell dependent. In contrast, in systemic groups, both immune effectors may act simultaneously but independently because in transfer experiments, no additive effects were observed (Plommet *et al.,* 1986). This predominant T-cell mediated hypothesis in restricted vaccination was reinforced by absence of a recall effect since memory T-cells are long lasting (Lefford 1983), whereas antibody synthesis is usually boosted by a secondary injection.

Survival of the vaccine strain was not observed in spleen or node of control mice after the 75th day (Table I). In contrast, it was observed in footpad vaccinated and challenged groups 90 (6 mice) and 180 days (4 mice) after vaccination. The vaccine strain may thus survive in small numbers in nodes or in other deep-seated foci from which it cannot be reisolated in control mice but may be reactivated by the challenge. Reactivation results in a secondary growth of the strain by a

temporary break in the equilibrium between bacteria and host under addition of antigen(s) or of a virulent challenge (Plommet and Plommet, 1988). A secondary colonization of the spleen by the vaccine strain may thus occur concomitantly with colonization by the virulent challenge. A differential count between vaccine and challenge strains was therefore indispensable to avoid confusion between both phenomena.

In conclusion, colonization of only one lymph node by the *Brucella* vaccine strain induces good and long lasting immunity, probably mostly T-cell dependent. It may be used as a model for other vaccination systems for limiting the serological response by restriction of colonization to some lymph nodes, by association of a dose and a route of administration such as in conjunctival vaccination.

Acknowledgments

We are grateful to Nicole Bosseray and Bernard Poutrel for valuable suggestions with the manuscript and to Evelyne Desnoë for the typing.

References

Bosseray N. & Plommet M. (1976) Transformation normalisant la distribution du nombre de *Brucella* dans la rate de souris inoculées par voie intra-péritoneale. *J. Biol. Stand.* 4, 341-351

Bosseray N., Plommet A.M. & Plommet M. (1984) Theoretical, practical and statistical basis for a general control method of activity for anti-*Brucella* vaccines. *Dev. Biol. Stand.* 56, 257-270

Diaz R. & Levieux D. (1972) Role respectif en sérologie de la brucellose bovine des antigènes et des immunoglobulines G1 et G2 dans les tests d'agglutination, de Coombs et au Rose Bengale ainsi que dans le phénomène de zone. *CR Acad. Sci. Paris* 274D, 1593-1596

Dubray G. (1987) Protective antigens in *Brucellosis. Ann. Inst. Pasteur/Microbiol.* 138, 84-87

Fensterbank R. & Plommet M. (1979) Vaccination against bovine brucellosis with a low dose of strain 19 administered by the conjunctival route. 4. Comparison between two methods of vaccination. *Ann. Rech. Vét.* 10, 131-139

Fensterbank R., Pardon P. & Marly J. (1982) Comparison between subcutaneous and conjunctival route of vaccination with Rev. 1 strain against *Brucella melitensis* infection in ewes. *Ann. Rech. Vét.* 13, 295-301

Fensterbank R., Pardon P. & Marly J. (1985) Vaccination of ewes by a single conjunctival administration of *Brucella melitensis* Rev 1 vaccine. *Ann. Rech. Vét.* 16, 351-356

Joint FAO/WHO Expert Committee on Brucellosis (1986) Sixth report. Technical Report Series No. 740, WHO, Geneva

Lefford M.J. (1983) Immunity to facultative intracellular parasites. *In: The Reticuloendothelial System. A Comprehensive Treatise* (N.R. Rose & B.V. Siegel, eds.), Plenum Press, New York, 4, 103-143

Limet J., Plommet A.M., Dubray G. & Plommet M. (1987) Immunity conferred upon mice by anti-LPS monoclonal antibodies in murine brucellosis. *Ann. Inst. Pasteur/Immunol.* 138, 417-424

Mackaness G.B. (1964) The immunological basis of acquired cellular resistance. *J. Exp. Med.* 120, 105-120

Pardon P. (1977) Resistance against a subcutaneous *Brucella* challenge of mice immunized with living or dead *Brucella* or by transfer of immune serum. *Ann. Inst. Pasteur/Immunol.* 128C, 1025-1037

Pavlov H., Hogarth M., Mckenzie I.F.C. & Cheers C. (1982) *In vivo* and *in vitro* effects of monoclonal antibody to Ly antigens on immunity to infection. *Cell. Immunol.* 71, 127-138

Plommet M. & Plommet A.M. (1975) Vaccination against bovine brucellosis with a low dose of strain 19 administered by the conjunctival route. I. Protection demonstrated in guinea pigs. *Ann. Rech. Vét.* 6, 345-356

Plommet M. & Plommet A.M. (1976) Vaccination against bovine brucellosis with a low dose of strain 19 administered by the conjunctival route. II. Determination of the minimum dose leading to colonization of the regional lymph

nodes of cattle. *Ann. Rech. Vét.* 7, 1-8

Plommet M. & Bosseray N. (1977) Le contrôle des vaccins antibrucelliques par le dénombrement des *Brucella* dans la rate de souris, vaccinées ou non, inoculées par voie intrapéritonéale. *J. Biol. Stand.* 5, 261-274

Plommet M. & Plommet A.M. (1981) Evolution de l'infection splénique de souris de quatre lignées, inoculées par voie veineuse, par trois doses de *Brucella abortus*. *Ann. Rech. Vét.* 12, 345-351

Plommet M. & Plommet A.M. (1983) Immune serum mediated effects on brucellosis evolution in mice. *Infect. Immun.* 41, 97-105

Plommet M., Hue I. & Plommet A.M. (1986) L'immunité anti-*Brucella* transférée par sérum immun et l'immunité transférée par les lymphocytes spléniques ne s'additionnent pas. *Ann. Rech. Vét.* 16, 169-175

Plommet M. & Plommet A.M. (1987) Anti-*Brucella* cell-mediated immunity in mice vaccinated with a cell-wall fraction. *Ann. Rech. Vét.* 18, 429-437

Plommet M. & Plommet A.M. (1988) Reactivation of a residual *Brucella abortus* 19 vaccine infection in mice by a virulent challenge or by injection of brucellin or of *Brucella* lipopolysaccharide. *Ann. Rech. Vét.* (in press)

Verger J.M. (1971) Comparaison des doses infectieuses 50 p. 100 (DI 50) de *Brucella melitensis* inoculée par les voies conjonctivale, intragastrique et intrapéritonéale à la souris. *Ann. Rech. Vét.* 2, 185-196

Winter A.J. (1987) Outer membrane proteins of *Brucella*. *Ann. Inst. Pasteur/Microbiol.* 138, 87-89