Immunogenic Properties of Soluble Antigens or Whole Cells of Brucella abortus Strain 45/20 Associated with Immunoadjuvants II. Whole Cells

L. F. Woodard, N. M. Toone and C. A. McLaughlin*

ABSTRACT

Whole cells of Brucella abortus strain 45/20 were combined with trehalose dimycolate or muramyl dipeptide. These preparations were tested in guinea pigs for immunogenic properties. Both trehalose dimycolate and muramyl dipeptide were found to be effective adjuvants to whole cells when combined in oil emulsions. Although saline suspensions of whole cells or whole cells-muramyl dipeptide did not significantly reduce splenic infections, whole cells-muramyl dipeptide in water-in-oil emulsion or whole cells-trehalose dimycolate in oil droplet emulsion were both effective immunogens ($P < 0.05$). Oil emulsions of whole cells-muramyl dipeptide reduced mean splenic Brucella by 95.1% and those of whole cells-trehalose dimycolate reduced mean splenic Brucella by 99.3% as compared to the control animals.

RÉSUMÉ

Cette expérience consistait à combiner des bactéries intactes de la souche 45/20 de Brucella abortus avec du dimycolate de tréhalose ou du dipeptide de muramyl. On injecta ces antigènes à des cobayes, afin d'en préciser les propriétés immunogènes. Le dimycolate de tréhalose et le dipeptide de muramyl se révélèrent deux adjuvants efficaces, lorsqu'on les combinait aux bactéries intactes, dans une émulsion huileuse. Bien que les suspensions salines de bactéries intactes ou d'un mélange de bactéries intactes et de dipeptide de muramyl ne réduisirent pas de façon appréciable l'infection splénique par B. abortus, le mélange de bactéries intactes et de dipeptide de muramyl, dans une émulsion d'eau et d'huile, tout comme le mélange de bactéries intactes et de dimycolate de tréhalose, dans une émulsion huileuse, s'avérèrent des vaccins efficaces ($p < 0,05$). Les émulsions huileuses de bactéries intactes et de dipeptide de muramyl réduisirent l'infection splénique par B. abortus dans une proportion de 95,1% et celles de bactéries intactes et de dimycolate de tréhalose le firent dans une proportion de 99,3%, par rapport aux cobayes témoins.

In the companion paper (7), we described our experiences with soluble antigens of *Brucella abortus* 45/20 for use as possible immunogens. When lipid-conjugated or associated with defined adjuvants, these antigens were less immunogenic than heat-killed 45/20 whole cells (WC) in Freund's complete adjuvant. It was decided that an immunity trial of WC associated with trehalose dimycolate (P3) or muramyl dipeptide (MDP) would be desirable. When presented in oil emulsions, both of these adjuvants have been shown to stimulate cell-mediated immune responses (1, 3, 6). Such responses are known to be important in the clearance of intracellular organisms like *Brucella* (4). It was hoped that WC as-

*Department of Veterinary Science, Agricultural Experiment Station, University of Idaho, Moscow, Idaho 83843 (Woodard and Toone) and National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana 59840 (McLaughlin).

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TABLE I. Immunogenic Capacity of Whole Cells of *Brucella abortus* 45/20 in Various Adjuvants

Vaccine	# Infected	Mean *Brucella*/Spleen[a]	% Reduction
WC in saline	5/5	2.80×10^3	52.5
WC-MDP in saline	6/6	1.62×10^3	72.5
WC-MDP in oil emulsion	5/5	2.86×10^{2b}	95.1
WC-P3 in oil emulsion	3/5	3.60×10^{1b}	99.3
——(Controls)	5/5	5.89×10^3	—

[a]Guinea pigs were challenged with 5.88×10^3 CFU of *B.abortus* 2308
[b]$P < 0.05$

sociated with P3 or MDP would stimulate acceptable levels of protection in laboratory animals. Furthermore, use of rough strain 45/20 cells does not cause interference with standard tube or plate agglutination tests.

Female Hartley guinea pigs[1] (350-550 g) were used for all experiments.

Brucella abortus, strain 45/20,[2] was grown in trypticase-soy both[3]. After incubation for five days (37°C), the broth suspension was heated (80°C for one hour) to inactivate the bacteria. Whole cells (WC) were then collected by centrifugation, washed twice in saline, dialyzed against water and lyophilized. Strain 2308[2] of *B. abortus* was used for challenge studies.

Strain 45/20 WC were used at a level of 300 μg/dose for all vaccines. Muramyl dipeptide[4] (MDP, n-acetylmuramyl-L-alanyl-D-isoglutamine) or trehalose dimycolate[5] (P3, cord factor) were used for adjuvants at levels of 150 μg/dose. Saline suspensions of WC-MDP were given to one principal group, while oil emulsions of WC-MDP in Freund's incomplete adjuvant or WC-P3 in 1% oil and tween-saline were given to additional groups. Saline suspensions of WC without adjuvants were given to one principal group to measure adjuvant activity of MDP or P3. Control animals were inoculated with normal saline solution only. Vaccines were prepared in 0.2 mL dosages and were given subcutaneously.

Six weeks after vaccination, all guinea pigs were intramuscularly challenged with 5880 CFU of *B. abortus* 2308. Two weeks later, all animals were sacrificed and their spleens were cultured as previously described (7).

Data were expressed as $\log_e$ of *B. abortus* CFU/spleen. Duncan's new multiple range test was used to compare principal and control groups.

Results of protection studies with WC in saline or associated with defined adjuvants are presented in Table I. Oil emulsions of both WC-MDP or WC-P3 were effective in reducing ($P < 0.05$) splenic infections as compared to control animals. While WC or WC-MDP in saline solution tended to reduce splenic infections, these reductions were not significantly lower than those in control animals. Oil emulsions of whole cells without MDP or P3 are not effective immunogens (unpublished data). These data indicate that both MDP and P3 are effective adjuvants to 45/20 WC when administered in oil emulsions.

The WC-P3 vaccine was perhaps more effective in the prevention of splenic infection, since two of five animals in this group had no infection, while WC-MDP did not completely prevent infection in any animals. However, with the limited number of animals per group, no statistical differences between infection rates were found.

When combined with MDP or P3 in oil emulsions, 45/20 WC were effective in the reduction of splenic infection by *B. abortus* 2308. Protection was similar to that of WC in Freund's complete adjuvant (7). Although infection was not completely prevented, further clearance of the organism might have been seen if a longer period between time of infection and time of sacrifice had been observed. Trials are presently underway to determine what effects a longer period for clearance and revaccination have on acquired cellular resistance. It should be noted that whole cell vaccines with P3 or a combination of P3 and MDP

[1]Camm Research Institute, Wayne, New Jersey 07470.

[2]Veterinary Services Diagnostic Laboratory, USDA, Ames, Iowa 50010.

[3]Baltimore Biological Laboratory, Cockeysville, Maryland 21030.

[4]Calbiochem, LaJolla, California 92037.

[5]Rocky Mountain Laboratory, Hamilton, Montana 59840.

are effective in prevention of infections when guinea pigs are challenged with four times the number of *Brucella* used in the present study (unpublished data).

A subcutaneous route of administration was used in this trial rather than the intradermal inoculation used with soluble antigens. We felt this method of vaccination would be more desirable for use in field situations. Intradermal inoculations are more difficult to administer and would be less commercially acceptable.

Both MDP and P3 were effective adjuvants when mixed with WC in oil emulsions. The immunogenic properties of WC-MDP in saline was decidedly less effective and confirms previous reports that MDP must be administered in oil to induce cell-mediated immune responses to antigens (see refs. 1 and 6 for reviews).

Undesirable side effects or severe local reactions were not observed in the present study. Since both P3 and MDP produce granulomatous reactions when administered in oil (2, 5), studies are in progress to determine the extent and effects of these reactions. Hopefully, such lesions would not preclude the use of P3 or MDP in food animals.

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