SPECIFICITY OF BRUCELLA PROTEIN ANTIGENS AND ROLE OF LIPOPOLYSACCHARIDE ANTIGENS IN ELICITING DELAYED HYPERSENSITIVITY REACTIONS IN SENSITIZED GUINEA PIGS

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SUMMARY

It was previously shown that protein antigens of *B. melitensis*, *B. ovis* and *B. canis* cannot be differentiated by immunoelectrophoresis. The present study showed that they also have similar specificity and potency in delayed hypersensitivity tests in guinea pigs.

Lipopolysaccharide antigen from *Y. enterocolitica* type 9, which is known to cross-react in serological tests with smooth *Brucella*, does not evoke cutaneous reactions in *Brucella* sensitized guinea pigs.

These studies suggest that a protein allergen prepared from one of the *Brucella* species could be used as a diagnostic aid for the detection of infection by any of the *Brucella* species. The allergic test could be useful in the differential diagnosis of brucellosis and infections by serologically cross-reacting organisms.

Brucella lipopolysaccharide antigen produced inflammatory reactions in normal guinea pigs and a somewhat greater response in *Brucella* sensitized guinea pigs. The latter was attributed to residual protein in the LPS antigen rather than to the polysaccharide component.

INTRODUCTION

Protein antigens prepared from a rough and a smooth strain of *Brucella melitensis* were shown to have very similar allergic activity in guinea pig skin tests (JONES, DIAZ and TAYLOR, 1973). As protein antigens prepared from the rough species, *Brucella canis* and *Brucella ovis*, had previously been shown by immunoelectrophoresis to be similar to protein antigens of rough and smooth *B. melitensis* (DIAZ, JONES and WILSON, 1968), this study was undertaken to see if their allergic activity was also similar. Most *Brucella* allergens used in medical and in veterinary diagnosis

contain both lipopolysaccharides and proteins. An attempt was made to determine the role, if any, of *Brucella* lipopolysaccharide in eliciting specific delayed hypersensitivity reactions.

MATERIALS AND METHODS

Protein antigens were prepared from *Brucella canis* strain RM 666, *Brucella ovis* strain REO and *Yersinia enterocolitica* type 9 in the same manner as previously employed for *Brucella melitensis* rough strain B 115 (JONES, DIAZ and TAYLOR, 1973). The method was a modification of the procedure of BHONGBHIBHAT, ELBERG and CHEN (1970) whereby acetone-killed cells were treated with 2.5 p. 100 sodium chloride for 3 days at 4°C, extracted material was precipitated with cold ethanol, dissolved in water, dialyzed, then centrifuged at 100 000 × *g* for 6 hours to remove large molecular weight material. The supernatant fluids were lyophilized.

Lipopolysaccharide (LPS) antigens were extracted from *Brucella melitensis* smooth strain 16 M and *Yersinia enterocolitica* type 8 and type 9 by the hot-phenol water method described by REDFEARN (1960) and LEONG *et al.* (1970). The LPS antigen was present in the phenol phase in the case of *B. melitensis* and *Y. enterocolitica* type 9 (DIAZ *et al.*, 1970, HURVELL, 1973) and it was in the aqueous phase in the case of *Y. enterocolitica* type 8 (DIAZ, unpublished). *B. melitensis* LPS was treated with pronase as described by LEONG *et al.* (1970).

The protein content of some antigens was determined by the LOWRY modification of the FOLIN-CIOCALTEU method (WILLIAMS and CHASE, 1968). The protein antigens extracted from *B. melitensis* B 115 and *Y. enterocolitica* both contained 56 p. 100 protein and the LPS antigen extracted from *B. melitensis* 16 M contained 2.8 p. 100 protein.

Guinea pigs of the Hartley strain were sensitized by various methods, the details of which will be given with the appropriate experiment. It had been shown previously that the species of *Brucella* employed for sensitization had little effect on the skin test response, but the method of sensitization, *i. e.* with living organisms or killed organisms in adjuvant, did affect the response (JONES, DIAZ and TAYLOR, 1973). Skin tests were performed as previously described (JONES, BERMAN and DIAZ, 1973). The intradermal dose was expressed as μg dry weight per 0.1 ml injected. Increase in skin thickness at injection site in comparison with surrounding area was measured with a Schnelltaster. The opposing diameters of erythema, measured with a vernier caliper, were used to determine the mean and the standard error of the mean.

RESULTS

Comparison of protein antigens of Brucellae

Studies in sensitized guinea pigs (table 1) showed that protein antigens prepared from *B. canis*, *B. ovis* and *B. melitensis* B 115 had approximately the same reactivity. Intradermal doses of 1 μg of each of the antigens produced erythematous reactions of about 15 mm in diameter in guinea pigs skin tested with one antigen only, and somewhat smaller reactions in guinea pigs given 3 or 4 antigens simultaneously. None of the protein antigens caused reactions in normal guinea pigs, whereas 10 μg of *Brucella* LPS antigen produced an erythematous reaction in normal animals.

In further experiments, the protein antigens were tested in guinea pigs which had been sensitized 6 to 7 weeks previously by the subcutaneous injection of 0.2 ml of vaccine containing 10^{10} heat-killed organisms of *B. canis*, *B. ovis* or *B. abortus* rough strain 45/20 in Freund's incomplete adjuvant (FIA) or Freund's complete adjuvant (FCA). They were also tested in guinea pigs which had been infected 12 weeks previously with 10^6 living organisms of *B. abortus* smooth virulent strain 544. Intradermal injection of a single dose per animal gave the results shown in table 2.

TABLE I

Skin reactivity of protein and lipopolysaccharide antigens of Brucella *species in sensitized guinea pigs* (1)

Réactivité cutanée des antigènes protéiques et lipopolysaccharidiques d'espèces de Brucella ***chez des cobayes sensibilisés*** (1)

Antigen	Dose (μg)	Mean diameter of erythema ± SE at 24 hours		
		Number of antigens tested per guinea pig		
		1	3	4
Protein				
B melitensis B 115	1	16.7 ± 1.1	13.2 ± 2.0	12.4 ± 1.2
B. canis	1	15.5 ± 1.7	13.0 ± 1.5	13.5 ± 0.6
B. ovis	1	14.1 ± 0.9	13.5 ± 1.1	13.5 ± 0.7
Lipopolysaccharide				
B. melitensis 16 M	10	12.4 ± 1.1	not done	10.2 ± 0.8

(1) Guinea pigs had been vaccinated with rough killed *Brucella* in FIA nine weeks and infected with virulent *B. abortus* five weeks prior to skin test. Five or 6 animals were tested per group.

(1) *Les cobayes ont été vaccinés avec des* Brucella *rough tuées en adjuvant de Freund incomplet (FIA) neuf semaines, puis infectés avec des* B. abortus *virulentes cinq semaines avant l'intradermoréaction. Cinq ou six animaux ont été testés dans chaque groupe.*

TABLE 2

Skin reactivity of protein antigens in sensitized guinea pigs (1)

Réactivité cutanée des antigènes protéiques chez des cobayes sensibilisés (1)

Sensitization method	Protein Antigen	Mean diameter of erythema ± SE 24 hours after injections of doses		
		10 μg	1 μg	0.1 μg
Killed *B. canis* + FCA	*B. canis*	14.8 ± 0.8	11.9 ± 1.4	
Killed *B. canis* + FIA	*B. canis*	17.5 ± 1.1	10.5 ± 0.9	
Living *B. abortus* 544	*B. canis*		20.3 ± 1.0	14.2 ± 1.0
Killed *B. ovis* + FIA	*B. ovis*	16.4 ± 0.5	10.4 ± 0.8	
Living *B. abortus* 544	*B. ovis*		21.9 ± 0.5	
Killed *B. abortus* 45/20 + FIA	*B. melitensis* B 115	15.7 ± 1.0	12.0 ± 1.6	8.2 ± 2.1
Living *B. abortus* 544	*B. melitensis* B 115		20.6 ± 0.5	15.6 ± 1.1

(1) Killed vaccines were given 6 to 7 weeks before skin test. Living organisms were given 12 weeks before skin test. A single dose of antigen was injected intradermally per animal. Five or 6 guinea pigs were tested per dose.

(1) *Les vaccins tués ont été injectés 6 à 7 semaines avant l'intradermoréaction ; les organismes vivants, 12 semaines avant. Une seule dose d'antigène a été injectée par voie intradermique par animal. Cinq ou 6 cobayes ont été testés pour chaque dose.*

With each of the antigens, 10 μg was required to give a skin test reaction of about 16 mm diameter in guinea pigs sensitized with killed adjuvant vaccines. Freund's complete adjuvant did not produce greater sensitivity than incomplete adjuvant. In contrast, in guinea pigs infected with *B. abortus*, 1 μg of each antigen was sufficient to give a skin test reaction of 20 mm.

These studies show that protein antigens prepared from the three species had the same potency in infected guinea pigs. There was no indication that sensitization with the homologous species resulted in greater sensitivity.

Comparison of protein and lipopolysaccharide antigens of Brucella

It was previously shown (JONES, DIAZ and TAYLOR, 1973) that *B. melitensis* LPS antigen evoked slightly larger cutaneous reactions in *Brucella* infected guinea pigs than in normal guinea pigs. *B. melitensis* LPS antigen is precipitated by unabsorbed sera from animals infected by any of the smooth *Brucella* species. In order to see if part of the inflammatory response to LPS was antibody mediated, groups of guinea pigs with and groups without high-titer circulating antibody to *Brucella* LPS antigen were employed and observations were made 4-5, 24 and 48 hours after the intradermal test. Groups with high titers had been inoculated with 10^6 living organisms of *B. abortus* smooth strain 544 fifteen weeks previously. Groups with little or no antibody to LPS antigen had been inoculated subcutaneously with killed *B. abortus* rough strain 45/20 in FIA fifteen weeks previously. The erythematous reactions observed 24 hours after the intradermal test are given in table 3 and the time course of the cutaneous response can be found in figures 1 and 2.

TABLE 3

Skin reactivity of protein and lipopolysaccharide antigens of B. melitensis *in normal and Brucella-sensitized guinea pigs* [1]

Réactivité des antigènes protéique et lipopolysaccharidique de B. melitensis *chez des cobayes normaux et sensibilisés* [1]

Antigen	Dose (μg)	Mean diameter of erythema ± SE at 24 hours in guinea pigs		
		Normal	Vaccinated	Infected
B. melitensis protein	25	< 4 (2)	18.7 ± 1.4 (5)	Not done
	5	< 4 (2)	20.0 ± 1.4 (5)	15.4 ± 2.1 (5)
	1	< 4 (2)	16.1 ± 1.2 (5)	21.0 ± 2.2 (4)
	0.2	< 4 (2)	Not done	19.7 ± 1.2 (4)
B. melitensis LPS	100	16.7 ± 1.0 (3)	18.4 ± 1.0 (5)	21.6 ± 2.1 (5)
	10	11.0 ± 2.4 (4)	15.4 ± 2.4 (4)	18.6 ± 1.4 (4)
	1	5.7 (3)	10.4 ± 1.5 (4)	15.9 ± 1.7 (4)

[1] All guinea pigs inoculated 15 weeks before skin test (see text). A single dose of antigen was injected intradermally per animal. The number of guinea pigs tested per dose is shown in parenthesis.

[1] *Tous les cobayes ont été inoculés 15 semaines avant l'intradermoréaction. Une seule dose d'antigène a été injectée par voie intradermique à chaque animal. Le nombre de cobayes testés par dose est indiqué entre parenthèses.*

The *Brucella* protein antigen produced no reation at 24 hours in normal guinea pigs. Maximum skin reactions were obtained with 5 μg of antigen in vaccinated guinea pigs and with 1 μg in infected animals.

The *Brucella* LPS antigen produced erythema in the skin of normal and sensitized guinea pigs. The erythematous response in vaccinated and infected guinea pigs was somewhat larger than the response in normal guinea pigs at each dose level. For example, 10 μg of LPS produced an erythematous lesion of 11 mm in normal guinea pigs, whereas 1/10th of this dose produced a comparable response in vaccinated.

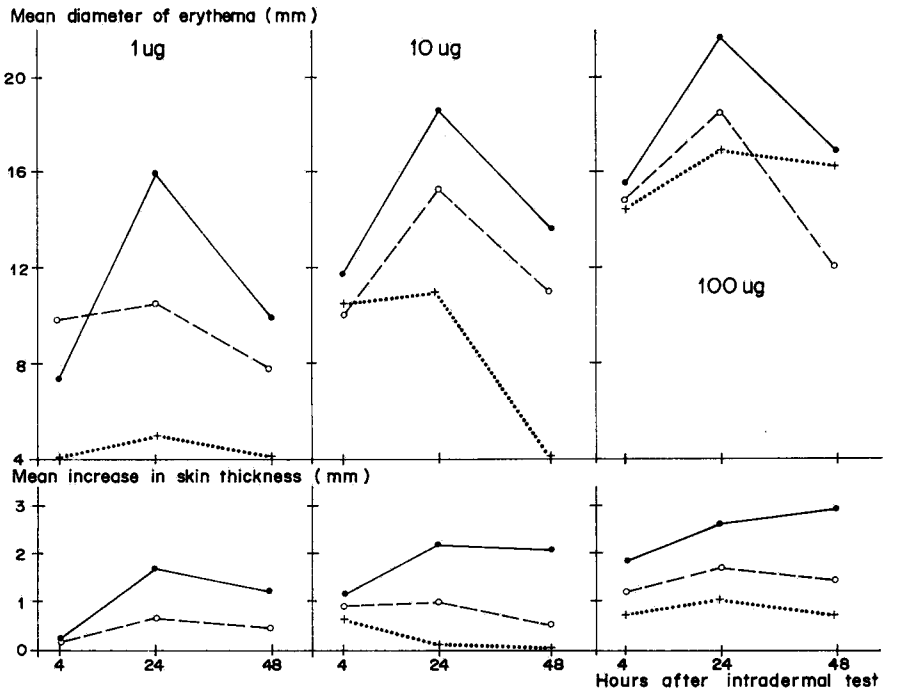

FIG. 1. — *Development of induration and erythema at 4-5, 24 and 48 hours after intradermal inoculation of various doses of* Brucella *LPS antigen into normal* (.....), *vaccinated* (— — —) *and infected* (———) *guinea pigs.*

Développement de l'induration et de l'érythème à 4-5, 24 et 48 heures après l'inoculation intradermique des différentes doses de l'antigène lipopolysaccharidique de Brucella *chez les cobayes normaux* (.....), *vaccinés* (— — —) *et infectés* (———).

Figures 1 and 2 show the development of erythema and induration following intradermal injection of varying doses of the two *Brucella* antigens. With LPS antigen (fig. 1) a definite response was seen 4-5 hours after the inoculation of 10 and 100 μg in all groups of animals. In normal animals the 24 hours response was not much greater. In sensitized animals the maximum response for both induration and erythema was observed at 24 hours and the reaction had diminished by 48 hours. As the dose of LPS increased 10-fold, the response increased in all groups of guinea pigs, at all reading times and by both parameters of reaction. There was no indication, within this dose range, that excess LPS antigen inhibited the skin reaction.

Five-fold differences in the doses of *Brucella* protein antigens were employed because it had been shown previously (JONES, BERMAN and DIAZ, 1973) that excess doses of B 115 allergen killed infected guinea pigs and inhibited the response of both infected and vaccinated guinea pigs. The results in table 3 and figure 2 confirm these findings and show in addition that the optimum dose of allergen varies with the method of sensitization. Infected guinea pigs showed the largest reactions with the 1 μg dose and a reduced reaction with 5 μg, whereas vaccinated guinea pigs showed the largest reactions with the 5 μg dose. Maximum reactions were observed at 24 hours with decreased reactions at 48 hours in all cases, except in infected guinea pigs given the 5 μg dose which inhibited the 24 hour reaction. Erythema was observed at 5 hours. Induration was observed at 5 hours in vaccinated guinea pigs only.

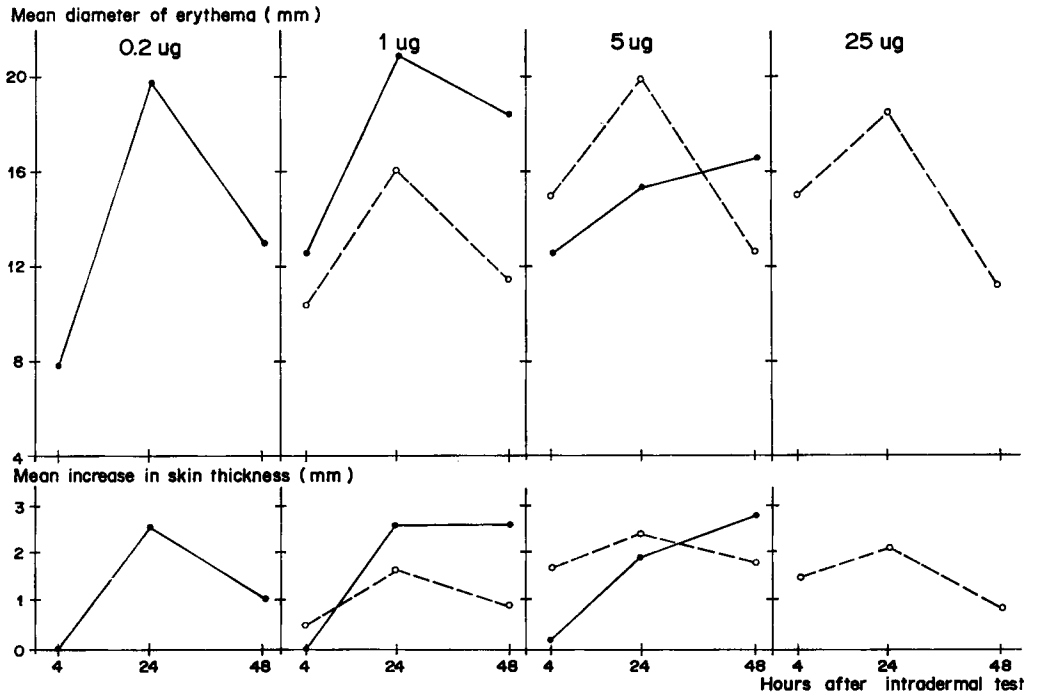

FIG. 2. — *Development of induration and erythema at 4-5, 24 and 48 hours after intradermal inoculation of various doses of* Brucella *protein antigen into vaccinated* (— — —) *and infected* (———) *guinea pigs.*

Développement de l'induration et de l'érythème à 4-5, 24 et 48 heures après l'inoculation intradermique des différentes doses de l'antigène protéique de Brucella *chez les cobayes normaux* (.....), *vaccinés*(— — —) *et infectés* (———).

If circulating antibody was complexing with LPS antigen and causing an Arthus-type reaction, then the response of infected guinea pigs at 4-5 hours would be expected to be significiantly greater than that of vaccinated guinea pigs. It was only slightly greater and the time course in the two groups of guinea pigs was similar to that seen with 1 μg of protein antigen in figure 2. Although the LPS had been treated with pronase, it contained 2.8 p. 100 protein as shown by the Folin-Ciocalteu reaction. On the basis of actual protein content of the two antigens, 10 μg of LPS

contained 0.28 μg protein and 1 μg of B 115 allergen contained 0.56 μg protein. Small doses of protein, such as these, were shown to produce significant delayed reactions if given as single doses per guinea pig (JONES, BERMAN and DIAZ, 1973). Figure 1 shows that 10 μg of LPS produced an erythematous reaction in normal guinea pigs at 4 hours which did not increase very much at 24 hours. Sensitized guinea pigs showed the same response at 4 hours as normal guinea pigs but the 24 hours (delayed) response was much greater.

Skin reactivity of Yersinia *antigens*

Lipopolysaccharide from *Y. enterocolitica* type 9 cross-reacts with anti-brucella serum whereas the lipopolysaccharide from *Y. enterocolitica* type 8 does not (DIAZ and BOSSERAY, 1974). Both *Yersinia* LPS antigens were tested in *Brucella* sensitized guinea pigs in order to see if the cross-reacting LPS component was responsible for the cutaneous reaction. In addition, protein antigen from *Y. enterocolitica* type 9 was tested in guinea pigs, although this antigen had shown no cross-reactivity with *Brucella* protein antigen in immuno-diffusion tests (DIAZ *et al.*, 1970).

TABLE 4

Skin reactivity of protein and lipopolysaccharide antigens of Yersinia enterocolitica *in normal and Brucella-sensitized guinea pigs* [1]

Réactivité cutanée des antigènes protéiques et lipopolysacharidique de Yersinia enterocolitica *chez des cobayes normaux et sensibilisés* [1]

Antigen	Dose (μg)	Mean diameter of erythema ± SE at 24 hours in guinea pigs		
		Normal	Vaccinated	Infected
Y. enterocolitica type 9, protein	100	15 ± 1.1 (3)	14.3 ± 0.7 (3)	13 (3)
	10	9.5 ± 0.3 (3)	8 ± 1 (3)	9.3 ± 1.3 (3)
	1	< 4 (4)	5 (4)	5 (4)
	0.1			< 4 (4)
Y. enterocolitica type 9, LPS	10	10 (1)	14.5 (2)	12.5 (2)
	1	11.5 (1)	8.0 (2)	7.0 (2)
	0.1	< 4 (3)	7.5 (3)	7.5 ± 1.5 (3)
Y. enterocolitica type 8, LPS	10	10.5 (1)	13.75 (2)	12.25 (2)
	1	11.0 (1)	12.0 (2)	9.0 (2)
	0.1	6.7 ± 1.5 (3)	8.8 ± 1 (3)	8.9 ± 2.5 (3)

[1] All guinea pigs inoculated 15 weeks before skin test. A single dose of antigen was injected intradermally per animal. The number of guinea pigs tested per dose is shown in parenthesis.

[1] *Tous les cobayes ont été inoculés 15 semaines avant l'intradermoréaction. Une seule dose d'antigène à été injectée par voie intradermique à chaque animal. Le nombre de cobayes testés par dose est indiqué entre parenthèses.*

The results of skin test with protein and LPS antigens of *Yersinia* are given in table 4. The erythema observed in normal guinea pigs was of the same diameter as that in *Brucella* vaccinated and infected guinea pigs at all dose levels and at all

times from 4 to 48 hours after injection. There was no indication of an increased inflammatory response to *Yersinia* antigens in guinea pigs sensitized with *Brucella*. The protein antigen from *Yersinia* produced greater reactions in normal guinea pigs than did the *Brucella* protein antigen (table 3). It is possible that the *Yersinia* protein antigen contained some LPS. The *Brucella* protein antigen had been prepared from a rough strain and no LPS was detected in the antigen (JONES, DIAZ and TAYLOR, 1973).

DISCUSSION

In immunoelectrophoretic studies of *Brucella* antigens, DIAZ, JONES and WILSON (1968) showed that the majority of protein antigens are common to all species within the genus *Brucella*. Protein antigens prepared from a rough and a smooth strain of *B. melitensis* were composed of a similar mixture of protein constituents as shown by immunoelectrophoresis, gel filtration on Sephadex G-75 and polyacrylamide gel isoelectric focusing (JONES, DIAZ and TAYLOR, 1973). These antigens had very similar skin reactivity in guinea pigs sensitized with living *B. abortus* or living *B. melitensis*.

The present study shows that protein antigens of three species of *Brucella*, *B. melitensis*, *B. ovis* and *B. canis*, also have the same ability to elicit delayed hypersensitivity reactions in sensitized guinea pigs, and again, there was no indication that sensitization with the homologous species resulted in greater sensitivity. This suggests that a single diagnostic allergen could be employed for the detection of infection by any of the *Brucella* species.

In contrast to the protein antigens which are common to all species of *Brucella* and both rough and smooth colonial phases, a lipopolysaccharide (LPS) component can be extracted from the surface of smooth species of *Brucella* but not from rough species (DIAZ *et al.*, 1968). A marked cross-agglutination between smooth *B. abortus* and *Yersinia enterocolitica* type 9 was reported by AHVONEN, JANSEN and AHO (1969) and studied extensively by HURVELL (1973) and HURVELL and LINDBERG (1973). DIAZ *et al.*, (1970) showed with gel diffusion that the LPS components of *Y. enterocolitica* type 9 and smooth *Brucella* cross-reacted, whereas the protein antigens showed no similarity. CORBEL (1972) and POP *et al.* (1972) reported cross-reactions in guinea pig skin tests with allergens prepared from *Brucella* and *Yersinia*, whereas JONES, DIAZ and TAYLOR (1973) could not demonstrate allergic reactions with *Brucella* protein antigens in guinea pigs sensitized with killed *Y. enterocolitica* type 9 in adjuvant. In the present study, guinea pigs sensitized with killed or living *Brucella* did not show delayed hypersensitivity reactions with either protein or LPS of *Y. enterocolitica* type 9.

The demonstration that the allergic test can differentiate betwen genera, such as *Brucella* and *Yersinia*, that show serological cross-reactivity, indicates that it could be helpful in the diagnosis of animal brucellosis in problem herds with serological reactions and no history of brucellosis.

Although the antigens involved in delayed hypersensitivy reactions are generally considered to be protein in nature, some workers believe polysaccharides are also

able to induce and elicit delayed reactions (GODFREY, BAER and CHAPARAS, 1969 ; GERETY, FERRARESI and RAFFEL, 1970). GODFREY *et al.* (1969) reported that a carbohydrate fraction of tuberculin had the same potency per µg as PPD in sensitized guinea pigs. HEILMAN *et al.* (1973), however, found that tuberculopolysaccharide was equally inhibitory for normal and tuberculin sensitive cells *in vitro* assays of tuberculin. Lipopolysaccharides from gram-negative organisms produce in normal animals a delayed inflammatory reaction which reaches maximum intensity in about 24 hours (STETSON, 1955).

Most *Brucella* allergens contain both LPS and protein components although the role of the LPS has not been established. We found that LPS antigens of *Brucella* provoke skin reactions in normal guinea pigs at concentrations only 10 × greater than their minimal skin reactivity in *Brucella* sensitized guinea pigs. In contrast, protein antigens are non-toxic in normal guinea pigs at concentrations 100 × greater than their minimal skin reactivity in sensitized guinea pigs. GALANOS *et al.* (1972) have reported that the Lipid A component and not the polysaccharide portion is responsible for *in vivo* toxic effects of gram-negative endotoxin. *Brucella* LPS contains Lipid A (LACAVE *et al.*, 1970) and this may be responsible for the skin reactions which *Brucella* LPS produced in normal guinea pigs.

Inflammatory reactions appearing 4 hours after intradermal injection of guinea pigs were considered to be due to antibody-mediated Arthus-type reactions rather than to delayed hypersensitivity reactions (SALVIN, 1958). The reactions obtained with *Brucella* LPS in *Brucella*-sensitized guinea pigs could not be attributed to antibody-mediated reactions directed to the polysaccharide portion of the molecule because :

1) Early reactions at 4-5 hours were not significantly greater with LPS than with protein antigens.

2) Guinea pigs sensitized with rough *Brucella* which had no circulating antibody to LPS also reacted to LPS antigen.

3) The serologically cross-reacting *Yersinia* LPS antigen did not elicit skin reactions in guinea pigs sensitized with smooth *Brucella*.

Although the LPS preparation had been treated with pronase, there was still sufficient protein present (2.8 p. 100 as shown by the Folin-Ciocalteu reaction) to produce a delayed reaction superimposed upon the toxic reaction apparent in normal guinea pigs.

It appears, therefore, that the delayed inflammatory skin reaction in *Brucella* sensitized guinea pigs elicited with *Brucella* LPS is a combination of a non-specific toxicity reaction due to Lipid A, and a specific delayed hypersensitivity reaction due to residual protein rather than to the polysaccharide portion of the antigen. It is possible that antibody-mediated reactions also occur but are masked by the non specific toxicity and the delayed hypersensitivity reactions. Further studies are in progress with a non-toxic polysaccharide antigen of smooth *Brucella* and these will be reported elsewhere.

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RÉSUMÉ

SPÉCIFICITÉ DES ANTIGÈNES PROTÉIQUES DE *BRUCELLA* ET RÔLE DES ANTIGÈNES LIPOPOLYSACCHARIDIQUES DANS LA MISE EN ÉVIDENCE DES RÉACTIONS D'HYPERSENSIBILITÉ RETARDÉE CHEZ DES COBAYES SENSIBILISÉS

Il a été démontré antérieurement que les antigènes protéiques de *Brucella melitensis*, *B. ovis* et *B. canis* ne peuvent pas être différenciés par électrophorèse. La présente étude a montré qu'ils ont aussi une spécificité et une activité semblables dans les tests d'hypersensibilité retardée chez les cobayes. L'antigène lipopolysaccharidique de *Yersinia enterocolitica* type 9, dont on sait qu'il donne une réaction croisée dans les tests sérologiques avec les *Brucella* « smooth », ne suscite pas de réactions cutanées chez les cobayes sensibilisés par *Brucella*.

Ces études suggèrent qu'un allergène protéique préparé à partir d'une espèce de *Brucella* pourrait être utilisé pour renforcer le diagnostic d'une infection par une espèce de *Brucella* quelconque. Le test allergique pourrait être utile pour le diagnostic différentiel entre la Brucellose et les infections par des organismes présentant des réactions sérologiques croisées avec le genre *Brucella*.

L'antigène lipopolysaccharidique de *Brucella* produit des réactions inflammatoires chez les cobayes normaux et une réponse un peu plus forte chez les cobayes sensibilisés. Cette dernière est attribuée à la protéine résiduelle de l'antigène LPS plutôt qu'au composant polysaccharidique.

РЕЗЮМЕ

Специфичность протеинических антигенов *Brucella* и роль липополисахаридических антигенов в выявлении реакции запоздалой повышенной чувствительности у сенсибилизированных морских свинок.

В предидущих работах было доказано, что антигены *Brucella meli tensis, B. ovis* и *B. canis* нельзя дифференцировать путем электрофореза. В данной работе мы показали, что они, к тому-же, имеют подобную специфичность и действие при тесте запоздалой повышенной чувствительности у морской свинки. Липополисахаридический антиген *Yersinia enterocolitica* типа 9, о котором известно, что он противодействует в серологических пробах с *Brucella* « *smooth* », и не вызывает накожных реакций у морских свинок сенсибилизированных при помоци *Brucella*.

Данные исследовании указывают, что для того чтобы подтвердить диагноз обнаружения заразы каким-нибудь *Brucella*, можно было бы употребить протеический аллерген, приготовленный каким-нибудь родом *Brucella*. Аллергический тест мог бы быть полезным для дифференциальной диагностики бруцеллёза и в разных заражениях организмами, представляющими противодействующие серологические реакции.

Липополисахаридический антиген *Brcuella* вызывает у нормальных морских свинок воспалительные реакции, и реакцию несколько более ясно выраженную у сенсибилизированных морских свинок. Последнюю приписывают резидуальному протеину антигена LPS скорее чем полиахаридному компоненту.

RÉFÉRENCES BIBLIOGRAPHIQUES

AHVONEN P., JANSSON E., AHO K., 1969. Marked cross-agglutination between *Brucellae* and a subtype of *Yersinia enterocolitica*. *Acta pathol. microbiol. scand.*, **75**, 291-295.

BHONGBHIBHAT N., ELBERG S. S., CHEN T. H., 1970. Characterization of *Brucella* skin-test antigens. *J. infect. Dis.*, **122**, 70-82.

CORBEL M. J., 1973. Immunological properties of an antigen from *Yersinia enterocolitica* serotype 9 cross-reacting with *Brucella* species agglutinogens. *Contrib. Microbiol. Immunol.* **2**, 150-156.

DIAZ R., BOSSERAY N., 1974. Influence de l'infection par *Brucella abortus* sur l'hypersensibilité et l'hyperréactivité de la souris aux endotoxines de *Brucella*, *Yersinia enterocolitica* et *Escherichia coli*. *Ann. Rech. vétér.*, **5** (1), 41-56.

DIAZ R., JONES L. M., WILSON J. B., 1968. Antigenic relationship of the Gram-negative organism causing canine mortion to s nooth and rough *Brucellae*. *J. Bacteriol.*, **95**, 618-624.

DIAZ R., JONES L. M., LEONG D., WILSON J. B., 1968. Surface antigens of smooth *Brucellae*. *J. Bacteriol.*, **96**, 893-901.

DIAZ R., LACALLE R., MEDRANO M. P., LEONG D., 1970. Immunological activities of the endotoxin from *Yersinia enterocolitica* strain M. Y. *Proc. Vth Internation. Congr. Infect. Dis. Vienna*, p. 11, **2**, Bacteria I.

GALANOS C., RIETSCHEL E. T., LUDERITZ O., WESTPHAL O., 1972. Biological activities of Lipid A complexed with bovine-serum albumin. *Europ. J. Biochem.*, **31**, 230-233.

GERETY R. J., FERRARESI R. W., RAFFEL S., 1970. Polysaccharide in delayed hypersensitivity. I. Pneumonococcal polysaccharide as inducer and elicitor of delayed reactivity in guinea pigs. *J. exper. Med.*, **131**, 189-206.

GODFREY H. P., BAER H., CHAPARAS S. D., 1969. Inhibition of macrophage migration by a skin reactive polysaccharide from BCG culture filtrates. *J. Immunol.*, **102**, 1466-1473.

HEILMAN D. H., AFFRONTI L. F., LEICHNER J. P., 1973. Comparison of the capillary tube and explant methods for the *in vitro* assay of tuberculin. *Infect. Immunity*, **7**, 704-710.

HURVELL B., 1973. Serological cross-reactions between different *Brucella* species and *Yersinia enterocolitica*. Biological and chemical investigations of lipopolysaccharides from *Brucella abortus* and *Yersinia enterocolitica* type IX. *Acta pathol. microbiol. scand., B.*, **81**, 105-112.

HURVELL B., LINDBERG A. A., 1973. Serological cross-reactions between different *Brucella* species and *Yersinia enterocolitica*. *Acta pathol. microbiol. scand., B.*, **81**, 113-119.

JONES L. M., BERMAN D. T., DIAZ R., 1973. Factors influencing biometric potency ssays of *Brucella* allergens in guinea pigs. *Brit. J. exper. Pathol.*, **54**, 368-379.

JONES L. M., DIAZ R., TAYLOR A. G., 1973. Characterization of allergens prepared from smooth and rough strains of *Brucella melitensis*. *Brit. J. exper. Pathol.*, **54**, 492-508.

LACAVE C., PROME J. C., ASSELINEAU J., ROUX J., SERRE A., 1970. Sur les fractions lipopolysaccharidiques extraites par le phénol de *Brucella melitensis*. *C. R. Acad. Sci., Paris, Ser. D*, **270**, 2380-2382.

LEONG D., DIAZ R., MILNER K., RUDBACH J., WILSON J. B., 1970. Some structural and biological properties of *Brucella* endotoxin. *Infect. Immunity*, **1**, 174-182.

POP A., CERBU A., POP A., DRAGHICI D., 1972. Parentés antigéniques entre les espèces classiques de *Brucella* et l'espèce *Yersinia enterocolitica* sérotype 9. I. Agglutinogènes et récepteurs phagiques. *Arch. roumaines Pathol. expér. Microbiol.*, **31**, 45.

REDFEARN M. S., 1960. *An immunochemical study of antigens of* Brucella *extracted by the* Westphal-*technique*. Ph. D. Thesis, University of Wisconsin.

SALVIN S. B., 1958. Occurrence of delayed hypersensitivity during the development of Arthus type hypersensitivity. *J. exper. Med.*, **107**, 109-124.

STETSON C. A., 1955. Studies on the mechanism of the Shwartzman Phenomenon. Similarities between reactions to endotoxins and certain reactions of bacterial allergy. *J. exper. Med.*, **101**, 421-436.

WILLIAMS C. A., CHASE M. W., 1968. *Methods in Immunology and Immunochemistry*, vol. 2, p. 273-275, Academic Press, New York.