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EXPERIMENTAL INFECTION WITH MYCOBACTERIUM AVIUM, SEROTYPE 2, IN PIGS

5. THE IMMUNIZING EFFECT OF BCG VACCINE AGAINST M. AVIUM INFECTION *

By
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JØRGENSEN, J. BERG: *Experimental infection with Mycobacterium avium, Serotype 2, in pigs. 5. The immunizing effect of BCG vaccine against M. avium infection.* Acta vet. scand. 1978, *19*, 430—440. — The immunizing effect of BCG vaccination against infection with M. avium was evaluated in pigs on the basis of clinical and pathological findings and numbers of acid-fast organisms in the tissues.

In experiments with small and large challenge doses i.v. (10^{-2} and 5 mg) the vaccinated animals were found to be partially protected. As compared to non-vaccinates, a reduction of viable organisms was found in vaccinates examined 28—31 or 70—73 days after challenge (Table 4), and fewer positive tissues were found in vaccinates than in non-vaccinates (Table 3). The most obvious results were seen in the experiment with a challenge dose of 10^{-2} mg i.v., where the number of organisms was consistently smaller in vaccinates than in non-vaccinates (Table 4). In contact infection experiments, the observations in both vaccinated and non-vaccinated pigs were limited and the results difficult to evaluate. There seemed to be a protection, as judged by histopathological and cultural findings.

Mycobacterium avium, Serotype 2; BCG vaccination; challenge, intravenous, oral; pigs.

In recent years many reports have appeared on infection with different serotypes of M. avium and M. intracellulare in pigs (*Tammemagi & Simmons* 1968, *Jørgensen et al.* 1972 a, a. o.). These infections can be of great economical importance, and as

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* This report is the result of a project planned and carried out in cooperation with dr. H. Chr. Engbæk and dr. A. Jespersen, both of the Tuberculosis Department, Statens Seruminstitut, Copenhagen, Denmark.

they are difficult to control, it was considered to be of interest to examine the immunizing effect of BCG (Bacillus Calmette-Guérin) against M. avium. The present work is a report of 4 experiments: 2 with i.v. challenge and 2 with contact challenge.

MATERIAL AND METHODS

Experimental animals. Table 1 shows the number of pigs, age at vaccination, vaccination periods, dose and route of challenge with M. avium. Vaccinated and non-vaccinated pigs were challenged, and in Exp. 7, 9 pigs were kept as vaccinated controls (no challenge).

Table 1. Survey of the experiments.

Experiment No.	Age at vaccination, days	Challenge, days after vaccination	Number of pigs				Duration of experiments, days
			donor pigs* for contact infection	only BCG	BCG and challenge	only challenge	
7	8	62		9	10** (5 mg i.v.)	10 (5 mg i.v.)	67—153
9	11	56			4 (5 mg i.v.)	4 (5 mg i.v.)	70—73
					5 (10^{-2} mg i.v.)	5 (10^{-2} mg i.v.)	28—31
15	13	51	5		6 (contact infect.)	6 (contact infect.)	147—154
15	9	73	4		5 (contact infect.)	5 (contact infect.)	136—178

* Pigs inoculated orally with M. avium, 0.5 mg daily for 5 days.
** Figures in brackets indicate dosage of M. avium.

Vaccination was performed with BCG vaccine, Strain Copenhagen, Batches 1935/70, 1967/70, 112/73 and 136/74, containing, respectively, 34.9×10^6, 23.6×10^6, 25.3×10^6 and 20.5×10^6 viable units per mg*. A dose of 0.1 ml (0.75 mg) was injected intradermally in the mandibular region.

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Challenge. In Exps. 7 and 9 M. avium, Serotype 2, strain SSC 1323 of pig origin (*Jørgensen et al.* 1972 b) was used for i.v. inoculation. For standard culture preparation, viable unit counts, and checking of colony morphology, see *Jørgensen* 1977 a. Viable unit counts varied between 10^7 and 10^8 units per mg wet weight. Colonies were mostly SmT with a few D and R variants. One rabbit and 1 hen, each given 1 mg organisms i.v., died after 20 and 42 days, respectively. In Exps. 15 and 16 M. avium, Serotype 2, strain SSC 1336 (ATCC 25291) of chicken origin (*Engbæk et al.* 1971) was used. Pigs inoculated by mouth with 0.5 mg daily for 5 days served as donors. Unit counts showed 32×10^6 and 51×10^6 viable organisms per mg, respectively.

Tuberculin tests. In Exps. 7 and 9 the pigs were tested with avian and human PPD, 1000 t.u. per dose (cf. *Jørgensen* 1977 a) before the experiments and on the days after vaccination and challenge indicated in Table 2.

Clinical observations. The pigs were inspected daily, and in Exps. 7 and 9 their weights were recorded once a week.

Duration of experiments are indicated in Table 1.

Post-mortem examination

Necropsy and histopathological and cultural examinations were performed as described previously (*Jørgensen* 1977 a, b). Mesenteric, hepatic and tracheobronchial lymph nodes, spleen, liver, lungs, kidneys, and in Exps. 15 and 16 also mandibular, parotid and medial retropharyngeal lymph nodes, musculus longissimus dorsi, tonsil and intestinal mucosa (Peyer patch) were examined histologically and by culture.

RESULTS

Clinical observations

In Exp. 7 the 3 groups had weight gains at the same level, except 21—42 days after challenge when a temporary lower weight gain was seen in the non-vaccinated, challenged group. In Exp. 9 no difference was seen between vaccinates and non-vaccinates. No differences in general condition and appetite was noticed in any of the experiments.

Tuberculin tests

The pigs showed no reaction before the experiments. Table 2 shows the mean results of the 24-hr. readings for each group in

Table 2. Comparative tuberculin tests with PPD avian and human, 1000 t.u. per dose, on BCG vaccinated pigs and on vaccinated and non-vaccinated pigs challenged with M. avium i.v. Mean reactions as read at 24 hrs.

Experiment No.	Number of pigs	Vaccinated + or —	M. avium dosage, mg	Days after vaccination	Days after challenge	Increase in skinfold thickness, mm H[1]	A[2]	H-A	s.e.m.	Erythema, diameter in mm H	A	H-A	s.e.m.
Infection alone													
7	10	—	5		26	2.1	3.2	—1.1	0.25	17.2	18.9	—1.7	1.20
9	4	—	5		22	1.9	2.3	—0.4	0.24	16.4	19.3	—2.9	2.31
9	5	—	10^{-2}		22	0.4	0.6	—0.2	0.20	8.6	8.8	—0.2	1.80
BCG alone													
7	9	+	0	46		4.7	3.7	1.0	0.63	18.7	17.4	1.3	0.74
		+	0	88		6.6	2.2	4.4	0.91	25.9	14.7	11.2	2.62
BCG and infection													
7	10	+		46		3.4	2.9	0.5	0.63	16.0	17.3	—1.3	1.33
		+	5	88	26	4.2	4.4	—0.2	0.63	23.7	19.0	4.7	2.54
9	4	+		50		2.8	1.8	1.0	0.20	17.5	13.4	4.1	0.88
		+	5	78	22	3.5	2.3	1.2	0.97	27.8	17.4	10.4	4.06
9	5	+		50		3.4	2.0	1.4	0.37	17.1	14.2	2.9	0.99
		+	10^{-2}	78	22	4.1	2.5	1.6	0.58	24.1	14.5	9.6	1.49

[1] human.
[2] avian.

Exps. 7 and 9. In the challenged, non-vaccinated animals the reactions were, on an average, greater to avian than to human tuberculin. For 14 animals on dosage 5 mg in Exps. 7 and 9 the average difference was clearly significant for skinfold thickness (—0.89, s.e.m. = 0.20, $P < 0.001$), but not for the erythema (—2.04, s.e.m. = 1.18, P: 0.05—0.1).

The 19 vaccinated animals in Exp. 7 showed no clear differences between avian and human reactions 46 days after vaccination. After 50 days, the human reactions in the 9 vaccinated animals in Exp. 9 were, on an average, greater than the avian, and the mean difference were significantly greater than 0 in all 4 groups of vaccinated animals.

On the second tuberculin test the human reactions were clearly greater than the avian in the vaccinated, unchallenged group, while the difference was less pronounced in the vaccinated and challenged groups.

Details about the tuberculin tests will be published in a subsequent paper (*Jørgensen & Weis Bentzon*, in preparation).

Necropsy

Exp. 7.

Table 3 shows the number of tissues with macro- and microscopic lesions. No lesions were found in the unchallenged, vaccinated pigs.

In non-vaccinates the macroscopic lesions varied in size from 1 to 5 mm. At Days 67—68 after challenge (early stage) the lesions were fleshy or caseated, in the later stages (103—153 days) caseo-calcareous. Histopathologically, early lesions were granulomatous, possibly caseated and calcified. Older lesions were well-demarcated, caseo-calcareous, and sometimes obliterated by fibrous tissue. In livers, lungs and kidneys, infiltrations were found of fibrous tissue and lymphocytes, probably consequent to tuberculous inflammation. There was no systematic reduction in the number of macro- and microscopic lesions from 67 to 153 days after challenge. Of a total of 70 tissues 54 and 58 were positive by macro- and microscopic examination, respectively (Table 3).

Table 3. Pathological findings in Exps. 7 and 9. Number of tissues positive by macro- and microscopic examination.

Experiment No. (dosage)	Number of pigs		Number of tissues in each group	Number of tissues positive			
				vacc.		non-vacc.	
	vacc.	non-vacc.		Ma.	Mi.	Ma.	Mi.
7 (5 mg)	10	10	70	26	30	54	58
9 (5 mg)	4	4	28	18	19	23	28
9 (10^{-2} mg)	5	5	35	0	11	5	21

Ma. = Positive macroscopically.
Mi. = Positive microscopically.

In the vaccinates the macroscopic lesions were similar to those of the non-vaccinates. Histologically a greater tendency was seen to caseation, calcification and demarcation, especially in the early stages. At Days 67 and 68 the number of lesions was about the same as in non-vaccinates, but from 103 to 147 days after challenge both lesions and affected tissues were

fewer. Positive tissues numbered 26 by macroscopic and 30 by microscopic examination (Table 3).

Exp. 9

In pigs challenged with 5 mg M. avium, post-mortem findings were of the same nature as in the pigs in Exp. 7 that were killed 67—68 days after challenge. In the vaccinates there were fewer lesions and affected tissues than in the non-vaccinates (Table 3). In non-vaccinates challenged with 10^{-2} mg M. avium, miliary lesions were found, especially in the lungs. Histological examination showed epithelioid cells and granulomas, most pronounced in the non-vaccinates, in which 21 specimens were positive as against 11 in the vaccinates (Table 3).

Exps. 15 and 16

From contact infection none of the pigs showed gross lesions. Microscopically, solitary giant cells were found in vaccinates, while in non-vaccinates also small giant-cell granulomas were seen. Affected were lymph nodes of the digestive tract, intestinal mucosa, tonsils, lungs, liver and kidney. Of a total of 143 tissues examined, 8 were positive in vaccinates, 21 in non-vaccinates.

For details about the contact infection experiments, see *Jørgensen* 1978.

Culture

The number of viable organisms varied greatly between animals of the same group and examined at the same time after challenge, as well as between comparable tissues in different animals. On the other hand, the ratio between numbers of organisms in different tissues varied relatively little from animal to animal, and the variation between tissues could therefore be illustrated by calculation of the geometric mean for each tissue. The ratios between these figures are independent of the variation between animals. Table 4 shows the results of these calculations in the case of Exp. 9, which included, respectively, 8 and 10 animals on doses 5 and 10^{-2} mg. Except for the hepatic lymph nodes, dosage 5 mg, the geometric means are greater for non-vaccinates than for vaccinates. Generally the differences are greater in pigs on dosage 10^{-2} mg. In both groups of pigs in Exp. 7, a decrease was seen in the number of viable organisms from Day 103 to

Table 4. Bacteriological findings in Exp. 9. Viable units for each tissue calculated as geometric means.

Tissue	M. avium, dosage 5 mg Survival time 70—73 days			M. avium, dosage 10^{-2} mg Survival time 28—31 days		
	non-vacc.	vacc.	ratio	non-vacc.	vacc.	ratio
Ln. mesentericus	2805	1047	2.7	52	0	>52.0
Ln. tracheobr. sin.	2985	701	4.3	473151	34277	13.8
Ln. hepaticus	11749	128233	0.1	1399587	13772	101.6
Spleen	213	39[a]	5.5	373[a]	7[a]	53.3
Liver	817	19[a]	43.0	26[a]	0	>26.0
Lung	126	14	9.0	339	35[a]	9.7
Kidney	4.4	0	>4.4	0	0	—

[a] In the calculation of the geometric mean 1 was used instead of 0.

Day 153 after challenge. Viable unit counts were considerably higher for non-vaccinates than for vaccinates.

In Exps. 15 and 16 (contact infection) organisms were recovered from 1 of 11 vaccinates (parotid and hepatic lymph nodes) and from 4 of 11 non-vaccinates (tonsils, mandibular and tracheobronchial lymph nodes).

DISCUSSION

Reports on BCG vaccination of pigs are few, and most of them deal with its effect on M. bovis infection. While experimental results have been disappointing (*Jundell & Magnusson* 1931, 1934, *Hayes et al.* 1932), field results have been satisfactory (*Sanz* 1930, *Girard* 1948). More recently, cross-protection has been observed between phylogenetically unrelated mycobacterial species (*Fenner* 1957, *Youmans et al.* 1961, *Palmer & Long* 1966, *Collins* 1971, a. o.), and in experiments on rabbits, guinea pigs and mice, *Engbæk & Jespersen* (1966) found that the initial multiplication of M. avium was inhibited in BCG vaccinated animals and that the time and rate of survival of such animals were increased. These observations suggested that BCG vaccination might be used to reduce the incidence of avian tuberculosis in infected herds of swine.

In an experimental model comparable to the culture method used in potency determination of BCG vaccines (*Dubos et al.* 1953), it was found in the present study that with a small challenge dose (10^{-2} mg i.v.) and an experimental period of about

30 days an obvious reduction of viable units could be attained in BCG vaccinated animals, the ratio non-vaccinated/vaccinated being from 9.7 to 101.6, depending on the tissues examined (Table 4). In the same experiment about twice as many tissues with microscopic lesions were found in non-vaccinates as in vaccinates, which also indicates protection of the vaccinates (Table 3).

The 2 experiments with challenge dose 5 mg M. avium i.v. was planned in accordance with the survival-of-lethal-challenge method (*Jespersen* 1971). As the non-vaccinates failed to die by the time expected (about 65 days) the effect of the vaccination was judged by evaluation of viable organisms and macro- and microscopic lesions. In animals killed at about Day 70 viable unit counts were from 2.7 to 43.0 times higher in non-vaccinates than in vaccinates. Yet, in 1 tissue (hepatic lymph node) the ratio was 0.1 (Table 4). The number of lesions at 70 days was about the same in both groups, but there was a greater tendency to demarcation in the vaccinates. From Day 103 to 153 a reduction was observed in the number of positive tissues in the vaccinates.

As appears from the results of challenge i.v., and most clearly in the experiments with a small challenge dose, BCG had induced a protection against M. avium infection, but the protection was not complete, as found in similar experiments with small animals (*Engbæk & Jespersen*).

In a previous experiment, 5 mg M. avium proved lethal to pigs within about 65 days after inoculation i.v. (*Jørgensen* 1977 a). In the present experiments the only clinical effect of 5 mg M. avium i.v. was a slight and transient decrease in weight gain in 1 group of non-vaccinated animals. The reason for this may be variations in the virulence of the M. avium strain, or more favourable environments (housing, temperature, humidity) for the pigs in the BCG experiments. In any event, a dose of 5 mg M. avium i.v. is lethal to pigs under certain conditions only.

In the contact infection experiments the results are difficult to evaluate. There seems to have been an effect of vaccination, since in non-vaccinates about twice as many tissues were positive histologically and 4 times as many animals by culture as in vaccinates. The results are comparable to those published by *Tammemagi & Simmons* (1970) who found some protection in BCG vaccinated pigs challenged orally with 0.2 mg M. intracellulare, Serotype 6.

The allergenicity of BCG was clearly demonstrated, in that all the vaccinated pigs were positive to human PPD, and in a somewhat lesser degree to avian PPD (1000 t.u.) (Table 2). It is interesting that challenge with M. avium did not alter the type of reactivity induced by BCG. The same phenomenon was observed by *Bang* (1917) in pigs naturally infected with M. avium and later exposed to M. bovis infection. *Abrahams* (1970) referred to this as "original mycobacterial sin" and suggested that the first mycobacterial infection may set the "antigenic reaction pattern".

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SAMMENDRAG

Infektionsforsøg på svin med Mycobacterium avium, Serotype 2.

5. BCG vaccinens beskyttende effekt mod M. avium infektion.

I forsøg med lille og stor challenge dosis, i.v. (10^{-2} og 5 mg) fandtes en beskyttende effekt af vaccinen. Ved dyrkningsundersøgelser fandtes færre bakterier hos vaccinerede dyr undersøgt 28—31 eller 70—73 dage efter challenge (tabel 4), og patologisk-anatomiske undersøgelser viste færre positive organer hos de vaccinerede dyr (tabel 3). De mest overbevisende resultater fandtes i forsøget, hvor 10^{-2} mg

anvendtes som challenge, idet der her sås systematisk færre bakterier hos de vaccinerede dyr (tabel 4). I forsøg med kontaktsmitte var fundene meget begrænsede både hos vaccinerede og ikke-vaccinerede dyr, og en sikker konklusion kan ikke udledes af forsøgene. Der synes dog at være en beskyttende effekt af BCG vaccinen bedømt på resultater af dyrkning og patologisk-anatomiske undersøgelser.

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