J. Hyg., Camb. (1981), **87**, 437
Printed in Great Britain

Observations on the antigenic differences between the so-called SC and LC strains of *Mycoplasma mycoides* subsp. *mycoides*

BY G. R. SMITH AND JANET C. OLIPHANT

Nuffield Laboratories of Comparative Medicine, Institute of Zoology, The Zoological Society of London, Regent's Park, London NW1 4RY

(*Received* 27 *May* 1981)

SUMMARY

The so-called SC (small colony) and LC (large colony) strains of *Mycoplasma mycoides* subsp. *mycoides* are said to be indistinguishable by the *in vitro* serological tests generally used in mycoplasmology. In mice the immunity given by a single dose of killed LC-strain vaccine against challenge with SC strains is – unlike that given by SC-strain vaccine – only partial.

When multiple doses of killed or living vaccines were given, the majority of 13 LC strains still failed to immunize completely against a SC strain. This suggests that, although some protective antigens are shared between both types of strain, at least one of importance is present in the SC strains but absent from the majority of LC strains. The difference between the protective-antigen content of SC and most LC strains is thus qualitative, and not merely quantitative.

INTRODUCTION

Certain small-colony (SC) and large-colony (LC) mycoplasmas from goats and sheep are indistinguishable by *in vitro* serological tests from *Mycoplasma mycoides* subsp. *mycoides*, the cause of contagious bovine pleuropneumonia (CBPP) (Al-Aubaidi, Dardiri & Fabricant, 1972; Cottew & Yeats, 1978; see also Hooker, Smith & Milligan, 1979). CBPP strains are of the SC type.

Cross-protection tests in mice have shown that single doses of killed vaccines prepared from LC strains give only partial immunity against challenge with SC strains, whereas the immunity given by SC-strain vaccine against challenge with any SC strain is virtually complete (Hooker *et al.* 1979; Smith, Hooker & Milligan, 1980). The LC and SC strains must, therefore, differ to some extent in their protective-antigen content; but it is not known whether the difference is qualitative, or merely quantitative.

The following experiments were undertaken to examine this point. They were based on the reasoning that in cross-protection tests it should be possible to obliterate quantitative – but not qualitative – differences by the use of multiple doses of vaccine.

MATERIALS AND METHODS

The mouse protection tests were based on methods described by Smith (1967, 1969*a*, 1969*b*, 1971) and Hooker *et al.* (1979). Female Swiss white mice from an outbred closed colony were used; they weighed 16–18 g at the beginning of each experiment. Viable counts of colony-forming units (c.f.u.) were made as described by Smith & Oliphant (1981).

Mycoplasma strains

The Blenheim strain of *M. mycoides* subsp. *mycoides* originated from a case of contagious bovine pleuropneumonia (CBPP); like all CBPP strains it belonged to the SC type. Thirteen other mycoplasma strains, originating from goats or sheep, belonged to the LC type of so-called *M. mycoides* subsp. *mycoides;* their names are given in Table 2. For full details of all strains, including the method of cloning, see Smith & Oliphant (1981). A single dose of heat-killed vaccine prepared from each of the 13 LC strains had been shown earlier to immunize mice partially but not completely against challenge with strain Blenheim (Hooker *et al.* 1979; Smith *et al.* 1980).

Active immunization with killed vaccines

Mice were immunized intravenously with 0·25 ml volumes of 3- or 4-day BVF-OS (Turner, Campbell & Dick, 1935) culture, killed by heating at 56 °C for 30 min in a waterbath. Control mice received BVF-OS broth only. The number of doses given is stated in the description of each experiment. The optical opacity of the Blenheim (SC) vaccine was less than that of the LC-strain vaccines. In each experiment a single batch of the vaccines was prepared, and stored at −20 °C for use as required.

Active immunization with living vaccines

The method was as described above except that the cultures were not killed. Each vaccine was given in two doses separated by an interval of 21 days. The first dose contained 641–2250 million c.f.u. Blood cultures (see below) made 24 h after the first vaccination showed that all mice given living vaccine had mycoplasmaemia, except two of a group vaccinated with strain 222–69 N.Y. The mycoplasmaemia would have lasted no more than a few days. The second dose of vaccine contained 150–1182 million c.f.u. Despite the wide range of viable counts the vaccines differed little in optical opacity except that, once again, Blenheim vaccine was somewhat less opaque than the LC-strain vaccines. Control mice received BVF-OS broth only.

Challenge

The interval between injection of the final dose of vaccine and challenge is stated in the description of each experiment. Symptomless infections were produced by challenging the mice intraperitoneally with 0·5 ml volumes of a 3-day BVF-OS culture of the SC strain Blenheim, diluted in BVF-OS. The tests were assessed by the presence or absence of mycoplasmaemia 24 h after challenge.

Detection of mycoplasmaemia

The method of tail-blood culture in selective medium was as described by Smith & Oliphant (1981).

Table 1. *The effect of two doses of two killed LC-strain vaccines on challenge with the SC strain Blenheim*

Vaccine	Type of vaccine strain	Day(s) on which mice vaccinated	Mycoplasmaemia in groups of mice 24 h after challenge* on day 43
Blenheim	SC	1	5/13
		22	0/14
		1 and 22	0/13
Cov 2	LC	1	14/14
		22	12/14
		1 and 22	11/14†
74/2488	LC	1	14/14
		22	10/14
		1 and 22	5/13†
Broth medium		1 and 22	20/20

* Challenge dose = 242×10^6 c.f.u. of strain Blenheim.

† Protection significantly less ($P < 0{\cdot}013$) than that given by Blenheim vaccine.

RESULTS

Challenge with the SC strain Blenheim after immunization with one and two doses of two killed LC-strain vaccines

Vaccines were prepared from the LC strains Cov 2 and 74/2488 and also, for the purpose of comparison, from the challenge strain. Groups of mice were treated with each vaccine 3 weeks, 6 weeks, or 3 and 6 weeks before challenge. Unimmunized controls received two doses of BVF-OS medium. The results of blood cultures made 24 h after challenge are shown in Table 1.

There was some evidence (Blenheim and 74/2488; $P < 0{\cdot}04$) that vaccination 3 weeks before challenge was more effective than that 6 weeks before challenge. Two doses of the LC vaccines were little if any better than a single dose given 3 weeks before challenge ($P > 0{\cdot}05$); they failed, moreover, to immunize as effectively as two doses of Blenheim vaccine, or even as a single dose given 3 weeks before challenge ($P < 0{\cdot}013$). This failure led to the next experiment, in which the number of doses of killed vaccine was increased to the point of hyperimmunization.

The effect of four doses of 13 *killed LC-strain vaccines on challenge with the SC strain Blenheim*

The efficacy of the heterologous vaccines was compared with that of vaccine prepared from the challenge strain, in an experiment in which groups of mice were given four doses at intervals of 7 or 8 days. The mice were challenged 20 days after the final dose of vaccine.

Approximately 15% of the mice died from anaphylactic shock 10–90 min after challenge, probably because of the 10% content of bovine serum in BVF-OS medium. A few showed signs of anaphylaxis but rapidly recovered. Most were unaffected.

Table 2 shows the results of the experiment. Despite the use of multiple doses, six of the LC vaccines failed to immunize as effectively as vaccine prepared from the challenge strain ($P < 0{\cdot}05$). Because of the large challenge dose several of the

Table 2. *The effect of four doses of* 13 *killed LC-strain vaccines on challenge with the SC strain Blenheim*

Vaccine	Type of vaccine strain	Mycoplasmaemia in groups of mice 24 h after challenge*
Blenheim	SC	1/15
Y goat	LC	1/8
Ojo I	LC	3/9
Ojo II	LC	4/10†
Cov 2	LC	4/8†
74/2488	LC	4/11
2605-Razi	LC	2/9
Vom/Plum Island	LC	7/9†
222-69 N.Y.	LC	9/10†
143-A66 Conn	LC	12/12†
Mankefår 2833	LC	6/6†
F 30	LC	0/8
Ghaleh Morghi-16	LC	1/9
S-5-64	LC	0/9
Broth medium		19/19

* Challenge dose = 453×10^6 c.f.u. of strain Blenheim, given 20 days after the fourth dose of vaccine.

† Protection significantly less ($P < 0{\cdot}05$) than that given by Blenheim vaccine.

Table 3. *The effect of two doses of* 13 *living LC-strain vaccines on challenge with the SC strain Blenheim*

Vaccine	Type of vaccine strain	Mycoplasmaemia in groups of mice 24 h after challenge*
Blenheim	SC	1/20‡
Y goat	LC	1/9
Ojo I	LC	1/11
Ojo II	LC	0/10
Cov 2	LC	4/11†
74/2488	LC	4/10†
2605-Razi	LC	7/12†
Vom/Plum Island	LC	8/11†
222-69 N.Y.	LC	7/8†
143-A66 Conn	LC	5/10†
Mankefår 2833	LC	6/12†
F 30	LC	1/10
Ghaleh Morghi-16	LC	6/12†
S-5-64	LC	3/10
Broth medium		20/20

* Challenge dose = 56×10^6 c.f.u. of strain Blenheim, given 21 days after the second dose of vaccine.

† Protection significantly less ($P < 0{\cdot}025$) than that given by Blenheim vaccine.

‡ The result for a group (not shown) that received heat-killed instead of living Blenheim vaccine was 1/19.

six vaccines appeared to give no immunity but, as already stated, earlier work (Hooker *et al.* 1979; Smith *et al.* 1980) had shown all the LC strains used in this study to be capable of immunizing partially against challenge with strain Blenheim. The results obtained with the other seven LC vaccines were not significantly different from that obtained with homologous vaccine; had the challenge dose been higher it is possible that further differences might have emerged.

The effect of two doses of 13 *living LC-strain vaccines on challenge with the SC strain Blenheim*

Smith & Oliphant (1981) showed the value of intravenous injection of living mycoplasma culture as a potent immunizing procedure. Groups of mice were, therefore, vaccinated with living cultures in two doses separated by an interval of 21 days. They were challenged 21 days after the second dose. Approximately 9 % died from anaphylactic shock shortly afterwards. The results are given in Table 3. Eight of the 13 LC vaccines failed to immunize as effectively as vaccine prepared from the homologous challenge strain ($P < 0{\cdot}025$). They even failed ($P < 0{\cdot}023$) to immunize as effectively as killed homologous vaccine (see footnote to Table 3).

DISCUSSION

All 13 LC strains had been shown by earlier work to immunize partially but not completely against SC strains, including Blenheim.

In the present study, the majority failed to give complete cross-immunization against strain Blenheim, despite the use of multiple doses of killed or living vaccine. It seems clear, therefore, that the protective-antigen content of the SC strains differs qualitatively from that of many of the LC strains in respect of one or more antigenic constituents.

As already stated, the LC and SC strains are said to be indistinguishable serologically by the *in vitro* methods (see Kenny, 1979) generally used in mycoplasmology, e.g. the growth-inhibition, metabolism-inhibition, and immunofluorescence tests. Such tests usually distinguish between different mycoplasma species without difficulty. Possibly antibody-absorption tests would provide a means of differentiating LC and SC strains *in vitro*.

The conditions provided by the three experiments described succeeded in showing that nine of the 13 LC strains differed qualitatively from strain Blenheim in respect of protective antigens. Without further tests, the position of the remaining four strains must remain uncertain.

This work was supported by a grant from the Wellcome Trust.

REFERENCES

Al-Aubaidi, J. M., Dardiri, A. H. & Fabricant, J. (1972). Biochemical characterisation and antigenic relationship of *Mycoplasma mycoides* subsp. *mycoides*, Freundt and *Mycoplasma mycoides* subsp. *capri* (Edward) Freundt. *International Journal of Systematic Bacteriology* **22**, 155–164.

COTTEW, G. S. & YEATS, F. R. (1978). Subdivision of *Mycoplasma mycoides* subsp. *mycoides* from cattle and goats into two types. *Australian Veterinary Journal* **54**, 293–296.

HOOKER, J. M., SMITH, G. R. & MILLIGAN, R. A. (1979). Differentiation of *Mycoplasma mycoides* subsp. *mycoides* from certain closely related mycoplasmas by mycoplasmaemia and cross-protection tests in mice. *Journal of Hygiene* **82**, 407–418.

KENNY, G. E. (1979). In *The Mycoplasmas*, vol. I. (ed. M. F. Barile and S. Razin), pp. 351–384. New York, San Francisco and London: Academic Press.

SMITH, G. R. (1967). *Mycoplasma mycoides* var. *mycoides:* production of bacteriaemia and demonstration of passive immunity in mice. *Journal of Comparative Pathology* **77**, 203–209.

SMITH, G. R. (1969*a*). Effect of route of vaccination on the immune response of mice to a single dose of heat-killed *Mycoplasma mycoides* var. *mycoides*. *Journal of Comparative Pathology* **79**, 255–260.

SMITH, G. R. (1969*b*). A study of *Mycoplasma mycoides* var. *mycoides* and *Mycoplasma mycoides* var. *capri* by cross-protection tests in mice. *Journal of Comparative Pathology* **79**, 261–265.

SMITH, G. R. (1971). The use of mice in research on contagious bovine pleuropneumonia. *Tropical Animal Health and Production* **3**, 169–172.

SMITH, G. R., HOOKER, J. M. & MILLIGAN, R. A. (1980). Further studies on caprine and ovine mycoplasmas related to *Mycoplasma mycoides* subsp. *mycoides*. *Journal of Hygiene* **85**, 247–256.

SMITH, G. R. & OLIPHANT, J. C. (1981). The ability of *Mycoplasma mycoides* subspecies *mycoides* and closely related strains from goats and sheep to immunize mice against subspecies *capri*. *Journal of Hygiene* **87**, 321–329.

TURNER, A. W., CAMPBELL, A. D. & DICK, A. T. (1935). Recent work on pleuropneumonia contagiosa bovum in North Queensland. *Australian Veterinary Journal* **11**, 63–71.