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Necrobacillosis and immunity in mice

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SUMMARY

The study arose from the recent finding that sub-lethal numbers of certain bacterial species greatly enhanced the infectivity of *Fusobacterium necrophorum*.

A severe *F. necrophorum* infection in mice, cured with metronidazole, produced significant though slight resistance, which was demonstrable by challenge with a minute dose of *F. necrophorum* (< 20 organisms) suspended in a sub-lethal dose of *Escherichia coli* (300×10^6 organisms) to enhance fusobacterial infectivity. In an earlier comparable experiment, challenge with *F. necrophorum* alone, in necessarily large doses ($\geqslant 3 \times 10^6$ organisms), failed to demonstrate that a single cured fusobacterial infection gave rise to resistance; such an infection neither protected against the fatal necrobacillosis produced by challenge nor prolonged survival.

A sub-lethal *E. coli* infection was also shown by challenge with a minute dose of *F. necrophorum* (< 10 organisms), suspended in a sub-lethal dose of *E. coli* (152×10^6 organisms), to produce significant though slight protection against necrobacillosis.

The degrees of resistance demonstrated were too slight to give any encouragement to the prospect of an effective necrobacillosis vaccine.

INTRODUCTION

Necrobacillosis, which affects many animal species including man, is caused by *Fusobacterium necrophorum*, but other bacteria are often also present in the lesions.

The literature on immunization against necrobacillosis contains many conflicting opinions, but a recent study in mice (1) suggested that *F. necrophorum* immunized against itself only with great difficulty. Thus a severe *F. necrophorum* infection, cured with metronidazole, produced no trace of resistance to challenge by the subcutaneous route. Slight but significant protection resulted, however, from two such infections given in rapid succession. The method of challenge used in these experiments had to take account of the high minimum infective dose ($> 10^6$ organisms) of virulent strains of *F. necrophorum* when used as pure cultures.

Subsequently, it was discovered (2) that the infectivity of *F. necrophorum* was greatly enhanced by the presence of sub-lethal numbers of certain other bacteria. Thus fatal necrobacillosis, with profuse fusobacterial multiplication, was produced by the subcutaneous injection of < 10 *F. necrophorum* cells suspended in 0·1 ml of *Escherichia coli* broth culture.

It seemed possible that this new-found ability to produce necrobacillosis with minute doses of *F. necrophorum* might provide a challenge method of improved sensitivity for demonstrating protection. This report describes experiments in which small doses of *F. necrophorum* suspended in *E. coli* culture were used to challenge mice previously immunized by infecting them with either *F. necrophorum* or *E. coli*.

MATERIALS AND METHODS

The mice, culture media, anaerobic methods and viable count technique were essentially as already described (3).

Organisms

F. necrophorum strain A42, isolated from a wallaby with necrobacillosis, has been fully described (2). *Escherichia coli* NCTC 10418 was supplied by the National Collection of Type Cultures.

Immunization of mice

Mice were immunized by infecting them with either *F. necrophorum* (Table 1, experiment 1) or *E. coli* (Table 1, experiment 2). The inocula, which consisted of 0·1 ml volumes of undiluted 18 h culture in BM broth (4), were injected subcutaneously on the outer aspect of the left thigh.

Animals that received *F. necrophorum* were each given a potentially lethal dose (256×10^6 viable organisms) and 3 days later, when severely affected (1), were treated with the first of eight daily doses of metronidazole 0·5%, w/v. This drug (Torgyl; May and Baker Ltd), given intraperitoneally in daily doses of 0·8 ml/mouse (*c.* 200 mg/kg) effected a complete cure. Uninfected control mice were also given metronidazole.

Animals that received *E. coli* were each given a dose of 105×10^6 viable organisms. This produced a subcutaneous infection which lasted more than 7 days and resulted in a local lesion that within 14 days of inoculation had ulcerated and begun to heal. The weights of the mice, which fell sharply as a result of the infection, had become comparable with those of uninfected control mice by the time of challenge.

Challenge of mice

In both experiments 1 and 2 (Table 1) mice were challenged subcutaneously on the outer aspect of the right thigh with 0·1 ml volumes of an 18 h BM culture of *E. coli* in which were suspended graded small doses of *F. necrophorum*, also derived from an 18 h culture in BM broth. The numbers of *E. coli* and *F. necrophorum* in the various challenge inocula are given in Table 1. In experiments 1 and 2 the animals were challenged 31 and 34 days respectively after the immunizing inoculation. Challenge either had no apparent effect or produced potentially fatal necrobacillosis (2) which necessitated euthanasia to prevent suffering.

Table 1. *Failure of immunization by infection with* F. necrophorum (*FN*) *or* E. coli (*EC*) *to produce more than a trace of resistance to challenge with minute doses of FN suspended in EC culture*

Experiment	Challenge dose of FN	Immunizing agent	Fatal infections in groups of: vaccinated mice	Fatal infections in groups of: unvaccinated mice
1	19500*	FN	11/12 (5)	12/12 (12)
	1950*	FN	9/12 (7)	12/12 (9)
	195*	FN	6/12 (3)	7/12 (6)
	19*	FN	3/12† (1)	8/12 (7)
2	2900*	EC	12/12 (9)	16/16 (16)
	290*	EC	12/12 (10)	16/16 (16)
	29*	EC	12/12 (4)	16/16 (15)
	3*	EC	3/12† (0)	11/16 (9)
3‡	312×10^6§	FN	14/14	14/14
	31×10^6§	FN	14/14	13/14
	3×10^6§	FN	3/6	1/6

* Suspended in 0·1 ml of EC culture, containing 300×10^6 and 152×10^6 viable EC in experiments 1 and 2 respectively.

† Statistical evaluation (5) showed a significant ($P < 0{\cdot}05$) difference from unvaccinated mice.

‡ Results of an earlier experiment (1), for comparison with those of experiments 1 and 2.

§ Suspended in 0·1 ml of BM medium.

Numbers in brackets represent mice showing clinical signs 2 days after inoculation.

Controls: in experiment 1, groups of 8 and 12 mice given 0·1 ml of EC culture and $1{\cdot}95 \times 10^6$ FN respectively all survived; in experiment 2, 19 of 20 mice given 0·1 ml of EC culture and 12/12 mice given $0{\cdot}29 \times 10^6$ FN survived.

RESULTS

Immunization by means of a cured F. necrophorum *infection*

The results are given in Table 1 (experiment 1). The numbers of mice clinically affected (lame) 2 days after challenge showed that immunization delayed to some extent the onset of symptoms. Immunization protected significantly ($P < 0{\cdot}05$) against challenge with a minute dose of *F. necrophorum* (19 organisms) suspended in a sub-lethal dose of *E. coli* culture. This protection was so slight, however, that it was obliterated by a dose of 195 fusobacteria, which in the presence of *E. coli* produced necrobacillosis equally well in immunized and control mice. Lesions from a representative selection of mice yielded on culture both *E. coli* and a heavy growth of *F. necrophorum*.

As an indicator of immunity the challenge method was apparently more sensitive than that used earlier (1), in which mice were given *F. necrophorum* alone, in necessarily large doses ($\geqslant 3 \times 10^6$ organisms); this more orthodox method of challenge failed to demonstrate protection in a comparable experiment (Table 1, experiment 3).

Immunization by means of a sub-lethal E. coli *infection*

The results are given in Table 1 (experiment 2). The method of immunization, like that in the previous experiment, delayed the onset of necrobacillosis; it also

protected significantly ($P < 0{\cdot}025$) against a minute dose of *F. necrophorum* (three organisms) suspended in a sub-lethal dose of *E. coli* culture, but not against a larger though still minute dose of 29 fusobacteria.

It would appear that the method of immunization ameliorated the *E. coli* infection – and thereby the *F. necrophorum* infection – produced by subsequent challenge, without rapidly abolishing it. Thus, as part of the same experiment, 20 immunized mice (not shown in Table 1) were each challenged with 0·1 ml of the *E. coli* culture (152×10^6 organisms) alone. The mean weights/mouse 2 days before *E. coli* challenge, and 1, 2 and 3 days afterwards were 27·2, 26·8, 26·9 and 27·1 g respectively. The comparable figures for a group of 20 unimmunized mice challenged in the same way were 27·9, 24·9, 25·9 and 27·2 g. This suggested that immunization exerted some effect on a further *E. coli* infection. However, the necrobacillosis lesions of a representative selection of mice in experiment 2, killed 4 days after challenge (dual infection), all yielded *E. coli* as well as a heavy growth of *F. necrophorum*.

DISCUSSION

A new method of challenge, with minute doses of *F. necrophorum* suspended in a sub-lethal dose of *E. coli* to enhance infectivity, demonstrated that a single infection with *F. necrophorum*, cured by metronidazole treatment, produced slight resistance. In this the challenge method was more successful than one used previously (1), in which mice were given a minimal, but necessarily large, dose of pure *F. necrophorum*.

A single infection with a sub-lethal dose of *E. coli* also protected slightly against the necrobacillosis produced by the new method of challenge. This protection was apparently an indirect effect, resulting from the partial suppression of the *E. coli* element of the dual challenge infection and consequently of its ability to enhance the infectivity of *F. necrophorum*.

The methods of immunization used, although extreme, protected only slightly against what was probably a highly sensitive method of challenge. The results therefore offer no prospect of an effective necrobacillosis vaccine. As pointed out earlier (1), it seems probable that the main virulence factors of *F. necrophorum* are only weakly antigenic.

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