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Immunity in mice to an intracerebral challenge of *Bordetella pertussis*

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INTRODUCTION

In most infective systems, increase in the number of organisms inoculated also increases the percentage of hosts responding. Virtually all the dosage-response curves of the infective systems reviewed by Meynell (1957) rose steadily with increasing dose, being either compatible with a curve derived from the first term of the Poisson series or rather less steep than this curve.

Unvaccinated mice are completely susceptible to *Bordetella pertussis* injected by the intracerebral route (Dolby & Standfast, 1961) so that the LD_{50} is 0·7 and the dose-response curve is Poissonian, i.e. a more or less symmetrical sigmoid curve is obtained when the percentage of mice dying is plotted against log mean number of viable organism inoculated. Vaccination in most systems merely shifts the dose-response curve along the dose axis towards a larger LD_{50}, without materially altering its shape. We found, however, that the dose-response curve in mice actively or passively immunized to intracerebral inoculation of *B. pertussis* differs substantially from that in normal mice. Irwin & Standfast (1957) were unable to establish any precise relation between the ImD_{50} of a vaccine and the ratio of the challenge dose to the LD_{50} with a series of vaccines tested against different challenges from 10 to 10,000 LD_{50}, though there was a tendency for ImD_{50} to be larger with the very large challenge doses.

METHODS

Active immunization

Groups of mice were inoculated with a single dose of 400 million organisms of each of four vaccines, British Provisional Standard, Lister Reference, Wright–Fleming Institute of Microbiology (W.F.I.M.) DS 1/59 and S-4, each of known ImD_{50} (dose of vaccine immunizing 50% mice). Fourteen days later the mice were challenged with doses of *B. pertussis* strain 18-323 varying from 20 to 2000 LD_{50}. One LD_{50} of this strain was *c.* 250 organisms, of which *c.* 10% were viable when the mice were challenged.

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Passive protection

Mice were passively protected with 1 PD_{50} of Lister anti-pertussis rabbit serum no. 20954, given by the intraperitoneal route 1 day before a challenge of 200 LD_{50} strain 18-323 given by the intracerebral route.

Detection of change in the blood-brain barrier

Groups of mice, both normal and passively protected, infected with *B. pertussis* were injected intraperitoneally, at various times after the infection, with 0·5 ml. of a 0·6 % solution of Pontamine sky blue in 0·75 % saline (Miles & Wilhelm, 1955). Eighteen hours later they were killed with chloroform and their brains removed and sectioned. Alternatively, 0·1 ml. of 5 % Pontamine sky blue was given intravenously and the mice killed 4hr later.

Other groups of mice, either untreated or infected with lethal doses of *B. pertussis* were given 1 ml. human serum intraperitoneally. The mice were killed 5 hr later and their brains removed. Each brain was homogenized in 1 ml. saline, centrifuged and the supernatants were assayed serologically for the presence of human serum. We are indebted to our colleagues Mr B. Weitz and Miss F. Lee-Jones of the Lister Institute for these estimations.

RESULTS

Infection in actively and passively immunized mice

The results with vaccinated mice (Table 1) and passively immunized mice (Table 2) in both cases indicate that the survival rate is independent of the size of the challenge dose between the limits of 20 and 2000 LD_{50}—corresponding to a variance of resistance far greater than 2—in fact, virtually infinity. The result is unexpected and clearly represents a response quite different from responses commonly observed in other instances of active and passive immunity.

This system is peculiar in that the infection is separated from circulating antibody by the relatively impermeable blood-brain barrier. The increased reistance of actively and passively immunized mice suggests, however, that the barrier becomes permeable to plasma antibody, at least at some time during the course of the infection.

During infection in intracerebrally inoculated mice the number of *B. pertussis* in the brain increases exponentially with time and mice die when it reached 10^7 to 10^8. In mice passively protected with antiserum given intraperitoneally at any time from 3 days before to 3 days after infection, the number of *B. pertussis* increases as in unprotected mice, reaching *c.* 10^5 on the 4th or 5th day. After this, in striking contrast to the events in non-immunized mice, the numbers at first remain at about this level, and then decrease. The events in actively immunized mice are similar. The period of about 4 days between infection and the effect of the antiserum is constant, no matter whether serum is given 3 days before or 3 days after the challenge (Dolby & Standfast, 1961).

It appears that circulating antibody reaches the infected brain tissue in effective concentrations only at the end of this period and it is at this time, therefore, that

Table 1. *Survival rates in groups of vaccinated mice challenged with different doses of* Bordetella pertussis

Dose of vaccine 400 million organisms given intraperitoneally in 0·5 ml. 14 days before challenge with strain 18-323.

	Vaccines											
	British provisional standard			Lister reference			W-F.I.M.DS 1/59			W.-F.I.M.S-4		
Challenge	S/T	χ^2/total	P	S/T	χ^2/total	P	S/T	χ^2/total	P	S/T	χ^2/total	P
$20 \times LD_{50}$	16/18	0·221	>0·5	14/18	0·83	>0·3	7/18	0·046	>0·8	10/35	1·948	>0·1
$200 \times LD_{50}$	17/18	1·215	>0·2	12/18	0	1	8/18	0·046	>0·8	19/35	1·048	>0·1
$1000 \times LD_{50}$	12/17	1·786	±0·2	9/18	1·722	+0·2	7/18	0·046	>0·8	16/35	0·205	>0·5
$2000 \times LD_{50}$	15/18	0·015	±0·9	13/18	0·203	>0·7	8/18	0·046	>0·8	13/35	0·226	>0·5
Control												
200 LD_{50}	1/16	—	—	1/16	—	—	1/11	—	—	2/16	—	—
Total χ^2 for heterogeneity		3·237			2·755			0·184			4·326	
D.F.		4			4			4			4	
P		0·5			>0·5	<·07		>0·99			0·25	

S/T = survivors/total number of mice.

Table 2. *Survival rates in groups of passively protected mice challenged with different doses of* Bordetella pertussis

Dose of serum (Lister no. 20954) constant at one PD_{50} (assayed against 200 LD_{50} challenge), inoculated intraperitoneally 1 day before challenge. Mice challenged with strain 18-323 and observed for 15 days. S/T = survivors/total challenged.

	Experiment 1				Experiment 2			
Challenge	S/T	χ^2/total	P	Controls S/T	S/T	χ^2/total	P	Controls S/T
$1 \times LD_{50}$	—	—	—	—	—	—	—	9/18
$10 \times LD_{50}$	11/18	0·102	>0·7	—	9/18	0·018	0·85	1/18
$100 \times LD_{50}$	7/18	1·886	±0·2	11/30	7/18	0·466	0·5	1/17
$500 \times LD_{50}$	11/18	0·102	>0·7	—	10/18	0·296	>0·5	—
$1000 \times LD_{50}$	12/18	0·562	±0·4	—	9/18	0·018	0·85	1/18
Total χ^2 for heterogeneity		2·653				0·80		
D.F.		4				4		
P		>0·5				0·9		

increased permeability of the blood-brain barrier to proteins, including antibody globulins, is to be expected.

Permeability of the blood-brain barrier

An attempt was made to demonstrate increased capillary permeability to dye and to proteins at this time by the Pontamine sky blue and the foreign serum protein techniques. With the dye (Table 3) the brains of normal mice are always unstained. In infected mice brains were not found to be stained before the 3rd day after infection. On the 4th day most brains were definitely blued, but whereas in all cases the brains of the non-immunized controls became progressively blue up to the time of death those of the serum-treated mice no longer blued on the 6th, 7th days—the barrier was no longer 'leaky'.

Table 3. *Blueing of brains in mice infected intracerebrally with c.* 200 LD_{50} Bordetella pertussis

Treated mice were given anti-pertussis rabbit serum 4 hr. before infection. All mice were given Pontamine sky blue intraperitoneally 18 hr. before necropsy.

	Days after infection					
	1	2	3	4	5	6
(1) Normal mice	.	0	0	0	0	.
(2) Infected, untreated	0	0	±	+	++	++
(3) Infected, and treated	0	0	±	+	+	± or 0

0, ±, +, ++ = degree of blueing of brain.

The blueing of the brain is regional and probably does not indicate staining in the usual sense. It is more likely due to a concentration of dye in the microglial cells.

The passage of serum through a 'leaky' blood-brain barrier was shown by giving human serum to normal and infected mice. Saline extracts of brains were made and titrated on the 1st, 2nd, 3rd and 4th days. There was no difference between normal and infected brains on the 1st, 2nd or 3rd days but on the 4th day the arithmetic mean titres of groups of 5 normal mice were 1/25, 1/10, 1/40, and of groups of 5 infected mice 1/120, 1/40 and 1/100, in 3 experiments.

DISCUSSION AND CONCLUSIONS

A dramatic change occurs in the brains of mice when the brain count reaches 10^5 to 10^6 live microbes at about the 4th day of infection. Nothing happens before this level is reached so the 'ultimate effective challenge dose' is constant at 10^5 to 10^6 live microbes or some 4000 LD_{50} and this is the constant-level measure of infection that has to be overcome for the animal to *recover*. This means that, whatever the number of organisms introduced, they are not affected by antibody until their numbers in the brain reach 10^5. It is therefore as if the mice in all dose-groups had been inoculated with the same number of organisms into specifically immune tissue. Consequently, the proportion of immunized mice surviving

inoculation is constant, regardless of the initial number of organisms administered and is dependent only on the degree of specific immunity. It is therefore, not surprising that increasing the number of organisms injected in the challenge from 5000 (20 LD_{50}) to 500,000 (2000 LD_{50}) caused no change in the percentage of mice dying which remained at values between 10 and 50 %, according to the protective vaccine or antiserum given (Tables 1, 2).

Friedeman, Zuger & Hollander (1939) showed that circulating tetanus antitoxin could neutralize intracerebrally injected tetanus toxin and Berenbaum, Ungar & Stevens (1960) showed that isotope-labelled human albumen injected parenterally was present in the brains of pertussis infected mice in the terminal stages of infection, and also showed that the ratio of radioactivity in the brain to the serum was certainly higher in mice 5–6 days after infection than in normal mice.

The experiments with Pontamine sky blue indicate either the development of a 'leak' in the capillary endothelium beginning on the 3rd day or a change in the infected brain cells at this time so that they fix Pontamine blue. This change continued to death on the 6th day in infected mice so that the brains became progressively bluer. In immune mice there was recovery on the 5th–6th days when the brains no longer blued.

A slow seepage of antibody would explain the slightly slower rise in bacterial count in the brains of highly immune mice than in the controls over the first 4 days of infection (Dolby & Standfast (1961)). At 3–5 days, however, there is a change and we suggest that this change in the brains of protected mice is due to a break down in the blood-brain barrier resulting in the circulating antibody becoming much more readily available to the brain tissue and so to the infecting organisms.

SUMMARY

The survival rate of mice actively or passively immunized against intracerebral challenge with *Bordetella pertussis*, was independent of the size of challenge dose within the range of 20–2000 LD_{50}.

This unexpected result appears to be due to the anatomical peculiarities of the infected organ, in which circulating antibody does not pass the normal blood-brain barrier easily. The pertussis infection does not cause sufficient inflammation to induce a pathological increase in the permeability of the barrier until the number of living microbes in the brain reaches 10^5 to 10^6. Since this stage in the brain consistently occurs after 4–5 days, independently of the size of the inoculum within the range 20–2000 LD_{50}, the outcome of infection in the immunized animal depends solely on the degree of specific immunity.

In the non-immune mouse, the increase in permeability of the barrier persists until death. In the immunized mouse, the elimination of the infection leads to a restoration of the normal barrier at about the 6th day.

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