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PROTECTIVE PROPERTIES AND HAEMAGGLUTININS IN SERUM FROM HUMANS AND IN SERUM FROM MICE INJECTED WITH A NEW POLYVALENT PSEUDOMONAS VACCINE

R. J. JONES AND E. A. ROE

From the MRC Industrial Injuries and Burns Research Unit, Accident Hospital, Bath Row, Birmingham B15 1NA

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Summary.—Mice given single injections of a polyvalent pseudomonas vaccine produced anti-pseudomonas haemagglutinins against the 16 component immunogens of the multivalent vaccine. Mice passively immunized with sera from vaccinated mice were protected against lethal challenge by 8/10 strains of *Ps. aeruginosa* of homologous serotype. Protection by the serum was inversely proportional to the virulence of the challenge strains.

Anti-pseudomonas haemagglutinins were always present in sera which passively protected mice against pseudomonas infection. Low levels of anti-pseudomonas haemagglutinins were present in some sera which failed to passively immunize mice against pseudomonas infection. Anti-pseudomonas haemagglutinins and antibodies involved in passive protection were mainly in the IgM fractions of mouse serum.

Control human sera contained anti-pseudomonas haemagglutinins against most serotypes of *Ps. aeruginosa*. Sera from patients with burns contained high levels of anti-pseudomonas haemagglutinins against some but not all serotypes of *Ps. aeruginosa*. Sera from both controls and patients with burns passively protected mice against pseudomonas infection.

PSEUDOMONAS AERUGINOSA is a pathogen of patients whose immune responses are impaired (Kefalides *et al.*, 1964; Feller, 1966; Alexander, 1971) *e.g.* patients with burns (Alexander *et al.*, 1969) or cystic fibrosis (Doggett and Harrison, 1972) or cancer (Young and Armstrong, 1972). The use of anti-pseudomonas vaccines and immune sera has been found to improve a patient's resistance to pseudomonas infection and also his chances of survival.

The usual method for monitoring resistance to pseudomonas infections in patients is to measure changes of anti-pseudomonas antibody levels in serum. Anti-pseudomonas antibodies can be measured by agglutination tests (Fox and Lowbury, 1953; Feller, 1966) but the most widely used test has been the passive haemagglutination test (Gaines and Landy, 1955; Alexander and Fisher, 1970; Young, Yu and Armstrong, 1970; Johnston and Sycklocha, 1972).

In this study a passive haemagglutination test, and also a passive protection test, were used to try and detect changes in humoral responses in mice injected with a new pseudomonas vaccine (Milerova and Spilsbury, unpublished). The vaccine, prepared from a strain from each of the 16 different serotypes of *Ps. aeruginosa* described by Homma (personal communication) gives protection to mice challenged by several strains of all 16 serotypes (Jones and Roe, unpublished). Haemagglutination titres against all 16 serotypes of *Ps. aeruginosa* were also measured in normal human sera and in sera from patients with burns.

Sera from humans and vaccinated mice were treated with 2-mercaptoethanol

to determine the kind of antibodies involved in protection against pseudomonas infection.

MATERIALS AND METHODS

Passive haemagglutination test

Erythrocytes.—Fresh sheep erythrocytes (defribinated, Wellcome), were washed once in haemagglutination buffer (HA buffer: NaCl, 90 g; Na_2HPO_4, 13·7 g; $NaH_2PO_4.2H_2O$, 2·4 g in 1 litre of distilled water, pH 7·2); once in HA buffer containing 3% formalin and twice in HA buffer. After the final washing sheep erythrocytes were resuspended in HA buffer to give a 10% suspension.

Antigen.—Antigens from the cell walls of 16 different serological types of *Ps. aeruginosa* (Milerova and Spilsbury were kindly supplied by Wellcome Research Laboratories. Each antigen was prepared from a representative strain of *Ps. aeruginosa* from the Habs serotypes 1–12, Veron 13, Meitert 10, Homma 11 and Homma 13. The antigens as supplied were diluted 1 in 4 with HA buffer before use.

Sensitization of erythrocytes with pseudomonas antigens.—1 volume of washed, formalin treated sheep erythrocytes was mixed with 4 volumes of diluted antigen in 10 cm × 1 cm glass tubes. The erythrocytes were incubated for 90 min at 37° and during this time the tubes were shaken frequently. After sensitization the sheep erythrocytes were washed twice in HA buffer and resuspended in HA buffer to give a final concentration of 0·2%.

Mouse serum.—Groups of 20 mice were exsanguinated by cardiac puncture on the 4th or 7th day after one injection of pseudomonas vaccine. In separate experiments mice were injected with either the polyvalent vaccine or one of each of the 16 components of the vaccine. Sheep haemagglutinins were absorbed from the mouse serum by addition of 1 volume of fresh, washed formalin treated sheep erythrocytes to 6 volumes of serum for 30 min at room temperature. After centrifugation, serum was kept at − 20° until used.

Human serum.—10 ml of venous blood was taken from 15 healthy individuals, 50 patients with burns and from 18 patients with other wounds. Serum was heated at 56° for 30 min to destroy complement and was absorbed with sheep erythrocytes (vide infra) to remove agglutinins.

Haemagglutination test.—Mouse or human sera from which normal sheep agglutinins had been absorbed were diluted 1/2–1/1024 in HA buffer in 7·5 cm × 1 cm glass tubes and transferred in 0·06 ml amounts to Stayne "A" plastic trays (Stayne Laboratories). An equal volume of 0·2% suspension of sensitized erythrocytes was added. After thoroughly mixing serum and erythrocytes together, the trays were covered and left for 3 h at room temperature. A small, smooth button of erythrocytes which was difficult to resuspend when the tray was shaken was recorded as negative: positive haemagglutination was recorded when the agglutinated erythrocytes formed either an evenly spread deposit of cells which resuspended easily on shaking or rafts of agglutinated erythrocytes.

Treatment of serum with 2-mercaptoethanol

Two-fold dilutions of serum (0·5 ml) in HA buffer were mixed in equal volumes with 0·2 mol/l 2-ME in 10 cm × 1 cm capped, glass tubes, incubated for 60 min at 37° and then left overnight at room temperature (Huebner and Gengozian, 1965).

Absorption of anti-pseudomonas haemagglutinins from serum

The titre of anti-pseudomonas haemagglutinins in 0·06 ml of serum was determined and the volume of sensitized erythrocytes required to absorb out the anti-pseudomonas haemagglutinins was then calculated; *e.g.* a serum with a titre of haemagglutinins of 1/16 was found to require 16 × 0·06 ml of 0·2% sensitized erythrocytes to absorb out the haemagglutinins completely. In practice, sera were absorbed 3 times (30 min at room temperature) with twice the calculated volume of sensitized erythrocytes.

Absorption of protection inducing factor from vaccine 06

One of the component vaccines, vaccine 06, made from strain 6 of *Ps. aeruginosa* serotype 6, (1·0 ml) diluted 1 in 4 with HA buffer was absorbed 3 times with 0·1 ml of packed, washed, formalin treated sheep erythrocytes. Passive haemagglutination tests with the erythrocytes used for absorption showed that after 3 absorptions the vaccine was no longer sensitizing the erythrocytes. The absorbed vaccine was then diluted to 10^{-6}, 10^{-7} and 10^{-8} with saline and groups of 60 mice were injected (1 ml per mouse) with each dilution. On Days 0, 1, 2, 3, 4, 7 and 10 after injection groups of 10 mice from each dilution were challenged i.p. with 1 LD_{100} of the autologous strain of *Ps. aeruginosa* (strain from which the vaccine was made). A control series of mice injected with the original unabsorbed 06 vaccine were challenged on the same days after vaccination with the same saline suspensions of pseudomonas.

Passive protection tests in mice

Groups of 3 mice were passively immunized by i.p. injection of 0·2 ml (1/2–1/64) of serum 2 h

before i.p. challenge with 1 LD_{100} of *Ps. aeruginosa*. Deaths were recorded 24 h after challenge.

RESULTS

Haemagglutinin response to 16 pseudomonas vaccines

Table I shows reciprocals of haemagglutination titres in sera from groups of mice injected with 16 pseudomonas vaccines. Sera from the mice were obtained 4 and 7 days after one injection of vaccine and the haemagglutination titres were measured with erythrocytes sensitized with the same vaccines as were used for making the antisera. Haemagglutination titres in sera from which IgM had been inactivated by 2-ME are also shown.

None of the sera from 100 control unvaccinated mice contained anti-pseudomonas haemagglutinins to any of the 16 groups of sensitized erythrocytes. Sera taken 4 or 7 days after vaccination showed a wide range of anti-pseudomonas haemagglutinin (2–1024). In sera taken 4 days after vaccination the anti-pseudomonas haemagglutinins were all destroyed by treatment with 2-ME, but 6/16 sera taken 7 days after vaccination contained some anti-pseudomonas haemagglutinins which resisted the action of 2-ME. The levels of haemagglutinins were highest in 10/16 sera taken 7 days after vaccination compared with sera taken 4 days after vaccination.

Haemagglutinin response to polyvalent vaccine

Sera from mice given a single injection of polyvalent vaccine (Table II) contained lower levels of anti-pseudomonas haemagglutinins against groups of erythrocytes sensitized with the 16 component vaccines than sera induced by injection of the individual component vaccines (Table I): the haemagglutinins induced by the polyvalent vaccine 4, 7 and 14 days after vaccination were all sensitive to treatment with 2-ME.

Table III shows the haemagglutinin titres in sera from mice given 2 or 3 injections of polyvalent vaccine. The

TABLE I.—*Haemagglutinins in Sera from Mice Injected with 16 Different Pseudomonas Vaccines*

Antigen used for sensitizing erythrocytes	Reciprocal HA titres in autologous* sera from groups of 20 mice 4 and 7 days after one injection of pseudomonas vaccine				Haemagglutinin titres in sera from control unvaccinated mice (100)
	4 days		7 days		
	−2ME	+2ME	−2ME	+2ME	
01	512	—	1024	64	—
02	256	—	256	4	—
03	256	—	64	—	—
04	512	—	32	—	—
05	64	—	32	4	—
06	8	—	16	—	—
07	64	—	32	4	—
08	2	—	8	—	—
09	8	—	32	—	—
10	16	—	64	—	—
11	32	—	128	4	—
12	32	—	128	—	—
13	32	—	16	—	—
14	2	—	4	—	—
15	4	—	16	—	—
16	8	—	1024	32	—

* The pseudomonas vaccine used for preparing the antiserum was also used as the antigen for sensitizing the erythrocytes.
−2 ME serum not treated with 2-mercaptoethanol.
+2 ME serum pretreated with 2-mercaptoethanol.

TABLE II.—*Haemagglutinins in Sera from Mice Injected with a Polyvalent Pseudomonas Vaccine*

Antigen used for sensitizing erythrocytes	Reciprocal of HA titres in mouse sera taken on following days after one injection of vaccine							
	4		7		10		14	
	−2ME	+2ME	−2ME	+2ME	−2ME	+2ME	−2ME	+2ME
01	32	—	2	—	2	—	2	—
02	32	—	2	—	2	—	2	—
03	8	—	—	—	—	—	—	—
04	32	—	2	—	2	—	2	—
05	8	—	—	—	—	—	—	—
06	4	—	—	—	2	—	2	—
07	8	—	—	—	2	—	2	—
08	2	—	—	—	2	—	2	—
09	4	—	2	—	—	—	2	—
10	2	—	2	—	—	—	2	—
11	2	—	2	—	—	—	2	—
12	2	—	—	—	2	—	2	—
13	2	—	—	—	—	—	2	—
14	—	—	—	—	—	—	2	—
15	2	—	—	—	2	—	4	—
16	2	—	—	—	2	—	2	—

−2ME serum not treated with 2-mercaptoethanol.
+2ME serum pretreated with 2-mercaptoethanol.

TABLE III.—*Haemagglutinins in Sera from Mice Injected with 2 or 3 Injections of Multivalent Pseudomonas Vaccine*

Antigen used for sensitizing erythrocytes	Reciprocal of HA titres in mouse sera taken after							
	2 injections of vaccine				3 injections of vaccine			
	2 days*		4 days		2 days		4 days	
	−2ME	+2ME	−2ME	+2ME	−2ME	+2ME	−2ME	+2ME
01	256	8	256	16	64	32	256	32
02	128	—	512	—	64	—	256	—
03	8	—	128	—	32	—	512	—
04	1024	8	1024	32	64	16	256	32
05	2	—	16	—	2	—	64	—
06	—	—	4	—	32	—	64	—
07	1024	—	512	4	256	4	128	—
08	16	—	128	—	64	—	64	—
09	64	—	128	—	64	—	256	—
10	8	—	128	8	64	—	128	32
11	64	—	512	—	64	—	256	4
12	64	—	16	—	16	—	32	—
13	16	—	64	—	16	—	32	—
14	16	—	32	—	64	—	32	—
15	32	—	64	—	64	—	32	—
16	64	—	1024	32	128	32	1024	128

* Days after last injection of polyvalent pseudomonas vaccine.

polyvalent vaccine was administered at 5-day intervals. Haemagglutination titres were determined in sera taken 2 and 4 days after the last injection of vaccine. There was a higher level of anti-pseudomonas haemagglutinins against most of the 16 component antigens of the polyvalent vaccine in sera taken 4 days after the last injection of vaccine than in sera taken 2 days after vaccination. In all 4 groups of sera most haemagglutinins were inactivated by 2-ME. After 3 injections of polyvalent vaccine the anti-pseudomonas haemagglutinins against the 16

TABLE IV.—*Cross-haemagglutination Patterns of* 16 *Pseudomonas Antigens*

Antigen used for sensitizing erythrocytes	Serum															
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16
01	+	+	±	−	+	+	−	+	±	+	±	±	−	−	−	−
02	−	+	−	−	+	+	−	−	−	−	±	−	−	−	−	−
03	−	−	+	+	+	+	−	−	−	+	−	±	−	−	−	−
04	−	±	−	+	+	+	+	+	+	−	+	+	−	+	+	+
05	−	±	−	−	+	+	−	+	−	−	−	−	−	−	−	−
06	−	−	−	−	−	+	−	+	+	−	+	−	−	−	−	−
07	−	−	−	±	−	−	+	+	+	−	+	−	−	−	−	−
08	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
09	−	±	−	−	−	+	−	±	+	+	+	−	−	−	−	−
10	−	±	−	±	−	+	−	−	−	+	+	−	−	−	−	−
11	−	+	−	+	+	+	−	−	−	−	+	+	−	−	−	−
12	−	+	−	+	±	+	−	−	−	−	−	+	+	−	−	−
13	−	+	−	−	±	+	−	−	−	−	−	−	+	−	−	−
14	−	−	−	+	±	+	+	−	−	−	+	+	−	+	−	−
15	−	±	−	+	±	+	+	−	−	−	+	+	−	+	+	+
16	−	−	−	+	+	+	+	+	+	±	+	+	−	+	+	+

\+ positive reaction.
± weak positive reaction.
− negative reaction.

component antigens of the polyvalent vaccine were of a consistently higher level than those in sera for mice given 2 doses of vaccine, or 1 dose of vaccine (Table II).

Cross-haemagglutination

Table IV shows the cross-haemagglutination patterns of 16 different pseudomonas antigens in serum obtained from groups of mice 4 days after one injection of the 16 pseudomonas antigens.

Each of the 16 groups of sensitized erythrocytes was strongly agglutinated by several sera, including the autologous serum; weak haemagglutination was also found. The range of sera which agglutinated the sensitized erythrocytes was different for each of the 16 antigens. Only one of the sera, serum 01, was monospecific; sera prepared against vaccines 2–16 contained autologous antibody and antibodies of different specificities. However, erythrocytes sensitized with 01 were agglutinated by several sera, showing that some of the other vaccines contained 01 antigen. All vaccines were shown to contain at least one antigen other than their own specific antigens: the most commonly shared antigens were 04 and 16.

In an experiment similar to the one described in Table IV where serum was taken 7 days after a single injection of individual vaccines, 3 sera (01, 15 and 16) gave monospecific haemagglutination reactions but in other sera reactions were as varied and numerous as those shown in Table IV.

Heat lability of sensitizing antigens

Attempts were made to reduce by heating the number of cross-haemagglutination reactions produced by the 16 antigens in sera from vaccinated mice. Antigens were heated before sensitization to 56° for 30 min or to 125° for 20 min by autoclaving. Gentle heating hardly changed the number of cross-reactions in autologous sera taken 4 or 7 days after vaccination, while autoclaving destroyed 10 out of 16 of the autologous reactions and some of the cross-reactions, and produced several new reactions.

Haemagglutination and passive protection

Table V shows the haemagglutination titres and passive protective properties of serum from vaccinated mice. Serum was obtained from groups of 40 mice 4 and 7 days after one injection of vaccine 01.

TABLE V.—*Haemagglutinins and Passive Protective Factors in Serum from Vaccinated Mice*

Serum			Reciprocal titre	
Sample number	Day after vaccination when taken	Treated with 2-ME	HA	Passive protection*
1	4	—	512	160
	4	+	—	—
2	7	—	1024	80
	7	+	64	—

* Lowest dilution of serum which passively protected 3/3 mice against i.p. challenge with 1 LD_{100} of autologous strain of *Ps. aeruginosa*.
2-ME 2-mercaptoethanol.
HA Haemagglutinins.

Serum taken 4 days after vaccination was twice as protective as serum taken from mice 7 days after vaccination even though the haemagglutinins to antigen 01 were twice as high in the 7-day serum as in the 4-day serum.

Treatment of the 2 sera with 2-ME removed the passive protective properties from both sera and destroyed the 01 haemagglutinins in the serum taken 4 days after vaccination. Haemagglutinins against 01 antigen in the sample taken 7 days after vaccination were only partially destroyed by 2-ME treatment (a titre of 1/64 2-ME resistant haemagglutinins remained), nevertheless the passive protection test failed to detect protective antibodies in the serum.

Protection was induced by passively immunizing mice with a serum prepared by injection of a monovalent vaccine (Table VI). The serum was obtained from 80 mice 4 days after one injection of 01 vaccine, prepared from a serotype 1 strain of *Ps. aeruginosa*. Injection of 0·2 ml of serum per mouse protected mice against LD_{100} challenge (i.p.) of 8/10 different strains of *Ps. aeruginosa*, serotype 1. Protection against some strains of *Ps. aeruginosa* (2, 5, 6, 7 and 9) was achieved by as little as 0·02 ml of serum per mouse. Protection against the most virulent strain (10) of pseudomonas was achieved by the smallest amount of serum used (0·2 ml of 1/32 dilution) while attempts to protect

TABLE VI.—*Passive Protection of Mice against* 10 *Different Strains of* Ps. aeruginosa (*Serotype* 1) *with a Serum from Mice Injected with a Vaccine Prepared from Strain of* Ps. aeruginosa, *Serotype* 1

Challenge strain			Reciprocal titre of serum which passively protected mice against i.p. challenge
No.	Serotype	LD_{100} ($2 \cdot 1 \times 10^9$)	
1*	1	0·2	16
2	1	0·4	2
3	1	0·2	16
4	1	0·4	16
5	1	0·3	2
6	1	1·0	0
7	1	0·3	2
8	1	0·2	16
9	1	0·2	0
10	1	0·1	32

*Autologous strain of *Ps. aeruginosa*.

mice against the least virulent strain (6) failed with the largest volume of serum used (0·2 ml of 1/2 dilution).

When mice were passively immunized with serum from mice given 3 doses of polyvalent vaccine (Table VII) they

TABLE VII.—*Passive Protection in Mice Immunized with Serum taken from Mice* 4 *days after one or three Injections of Polyvalent Pseudomonas Vaccine*

Challenge strain		Mortality Death in groups of 3 mice 24 h after i.p. challenge of passively immunized mice (0·2 ml of 1/2 dilution of serum)	
Serotype	LD_{100} ($2 \cdot 1 \times 10^9$)	A*	B*
1	0·2	0	0
2	0·2	1	3
3	0·2	2	1
4	0·25	1	0
5	0·2	3	3
6	0·03	0	0
7	0·25	2	2
8	0·2	2	3
9	0·1	0	0
10	0·3	2	3
11	0·1	2	0
12	0·7	2	0
13	0·4	3	0
14	0·5	3	2
15	0·7	2	2
16	0·25	2	3

A* serum from mice given 1 injection of vaccine.
B* serum from mice given 3 injections of vaccine.

received 100% protection against more challenge strains of *Ps. aeruginosa* (7/16) than mice passively immunized with serum from mice given 1 dose of polyvalent vaccine (3/16).

Absorption of protective property from serum

Haemagglutination titres against erythrocytes sensitized with vaccine 06 and passive protective properties of 2 sera (mouse and human) against challenge with *Ps. aeruginosa*, strain 6 (Table VIII), were determined. The mouse serum was prepared by immunizing 40 mice with 06 vaccine and bleeding them 4 days after vaccination. The human serum was from a normal individual.

TABLE VIII.—*Absorption of the Protective Property from the Serum of Vaccinated Mice and from Human Serum*

Serum	Absorption* of serum	Reciprocals of titres in Serum	
		HA	Passive protection†
Mouse	—	16	64
(Day 4)	+	2	4
Human	—	256	8
	+	8	0

* Absorption with sheep erythrocytes sensitized with vaccine 06.

† Mice challenged with 1 LD_{100} of homologous strain of *Ps. aeruginosa*, 6.

After the 2 sera were absorbed with 06 sensitized erythrocytes, the haemagglutination titre of the mouse serum was reduced by 8-fold and the passive protective property of the serum by 16-fold; in the human sera absorption reduced the haemagglutination titre more (32-fold) than the passive protective property (8-fold).

Absorption of protection inducing factor from vaccine

In the experiment summarized in Table IX, vaccine 06 was absorbed with normal sheep erythrocytes before injection into mice to see if the antigen in vaccine 06 which sensitized the erythrocytes was the same as the antigen which induced protection.

The unabsorbed vaccine (2 dilutions 10^{-6} and 10^{-7}) induced good protection against lethal challenge in mice 2, 3, 4, 7 and 10 days after vaccination. Vaccine dose 10^{-7} failed to protect any mice challenged one day after vaccination but the larger dose of vaccine (10^{-6}) protected mice on that day.

There were more deaths after challenge with *Ps. aeruginosa* 6 in mice injected with the vaccine which had been absorbed with sheep erythrocytes than in mice challenged at different times after vaccination in the group injected with unabsorbed vaccine. The total number of deaths in mice challenged at different times after vaccination in the group injected with erythrocyte

TABLE IX.—*Absorption of Vaccine* 06 *with Sheep Erythrocytes*

Vaccine injected	Vaccine dose	Deaths in groups of 3 mice challenged with 1 LD_{100} of strain 6 on the following days after vaccination					
		1	2	3	4	7	10
Vaccine absorbed 3× with sheep erythrocytes	10^{-6}	3	1	2	2	0	1
	10^{-7}	1	2	1	1	3	2
Control unabsorbed vaccine	10^{-6}	0	0	0	0	0	0
	10^{-7}	3	0	0	0	0	1
No vaccine injected	—	3	3	3	3	3	3

10^{-6} $3{\cdot}3 \times 10^4$ microbial equivalents per ml.

10^{-7} $3{\cdot}3 \times 10^3$ microbial equivalents per ml (Milerova and Spilsbury, unpublished).

TABLE X.—*Haemagglutinins in Human Sera*

	Haemagglutinin titres in sera from								
	Control volunteers (15)			Patients with burns (50)			Other patients (18)		
Antigen	%*	Range	Mean	%	Range	Mean	%	Range	Mean
01	100	2–128	30	98	8–1024	163	100	32–1024	177
02	66	0–16	10	53	0–128	10	33	0–256	26
03	93	0–64	12	46	0–256	26	66	0–128	23
04	100	2–32	13	98	2–2048	155	94	0–2048	322
05	100	4–64	20	92	0–128	38	83	0–512	51
06	100	8–128	42	100	4–1024	134	100	4–4028	271
07	100	4–128	23	87	0–1024	66	83	0–512	58
08	93	0–128	15	82	0–1024	43	72	0–512	62
09	47	0–16	3	34	0–128	14	22	0–32	2
10	73	0–32	9	34	0–64	6	12	0–16	1
11	100	4–128	40	100	4–1024	109	100	16–1024	174
12	93	0–64	14	78	0–512	55	89	0–256	55
13	87	0–32	9	90	0–1024	53	94	0–1024	124
14	100	2–512	54	35	0–0124	55	94	0–1024	131
15	93	0–512	54	66	0–128	17	—	—	—
16	93	0–64	13	89	0–128	35	—	—	—

* % Percentage of samples tested which showed titres to antigens.
— Not tested.

absorbed vaccine at a dose of 10^{-6} was 9/18 and at a dose of 10^{-7} was 11/18. In the corresponding group of mice given unabsorbed vaccine the deaths were 0/18 and 4/18 respectively.

Table X summarizes haemagglutination titres found against 16 pseudomonas antigens in human sera from 15 healthy individuals, 50 patients with burns and 18 patients from a major injuries unit. The column on the left hand side of each group of sera shows the percentages of samples in each of the 3 groups of sera which agglutinated the 16 antigens. The centre column shows the range of titres found against each antigen and the column on the right hand side shows the average titre against each antigen.

The range of antibody specificities found in each of the 3 groups of sera was wide: antibodies to all 16 antigens were found in half of the sera from controls but in sera from patients only 1/16 of the sera had antibodies to all 16 antigens. In sera from controls, antibodies to 7 of the antigens (1, 4, 5, 6, 7, 11 and 14) were found in all sera: in sera from patients with burns, antibodies to only 2 of the antigens (6 and 11) were found in all sera; in the other group of patients 3 antigens (1, 6 and 11) were agglutinated by all 18 sera.

Although there appears to be a wider range of anti-pseudomonas antibody in sera from controls than in sera from patients, the columns showing the mean titres indicate that the antibody levels in the controls were usually lower than those of patients. The highest haemagglutination titres were found in patients, especially those with burns; these titres were often over 1000.

When serum from controls was treated with 2-ME haemagglutinins against all 16 pseudomonas antigens were removed from 6/15 of the sera; 9/15 sera showed haemagglutinins against only one or 2 antigens. The range of haemagglutinins remaining in serum from patients with burns after 2-ME treatment was wider than in serum from controls; haemagglutinins against 8 of the pseudomonas antigens still remained in some sera.

DISCUSSION

Unvaccinated mice have no naturally occurring anti-pseudomonas haemagglutinins in their serum and usually die within 24 h of a suitable i.p. challenge dose of *Ps. aeruginosa* (Jones and Roe, un-

published). Injection of monovalent or polyvalent pseudomonas vaccine protects mice against lethal doses of pseudomonas within a day of vaccination (Jones, 1971), and induces detectable levels of anti-pseudomonas haemagglutinins within 3–4 days of vaccination (Jones, Hall and Ricketts, 1972).

It was the purpose of this study to determine whether anti-pseudomonas haemagglutinins were suitable for measuring protection against *Ps. aeruginosa* infection in mice injected with a newly developed polyvalent pseudomonas vaccine (Milerova and Spilsbury, unpublished).

Each of the 16 components of the polyvalent vaccine, as well as the polyvalent vaccine, was found to induce haemagglutinins in mice. The passive haemagglutination tests showed that each component vaccine made from a single strain of *Ps. aeruginosa* from each of 16 different serological groups contained minor antigens which cross-reacted with sera with different serological specificities as well as with major antigens of its own serological type. Mice injected with these vaccines produced antibodies to both the major and minor antigens. Thus, the antibody level measured in the serum of mice injected with polyvalent vaccine may originate from several sources of antigen, so making it difficult to determine the part played by each vaccine in inducing an antibody response in mice injected with the polyvalent vaccine.

Attempts to remove cross-reacting minor antigens from the component vaccines by heat were unsuccessful, suggesting that both the minor and major antigens were of similar heat lability.

After injection of similar amounts of component vaccines into groups of mice, a range of different levels of anti-pseudomonas haemagglutinins was found, suggesting that individual vaccines varied in antigenicity assuming that there was no variation in host response. Lower levels of haemagglutinins were found after injection of the polyvalent vaccine than after injection of the individual component vaccines, but experiments showed that comparable levels were achieved by several injections of the polyvalent vaccine.

Passive protection and absorption experiments with mouse sera containing anti-pseudomonas haemagglutinins showed that sera containing high levels of anti-pseudomonas haemagglutinins gave better protection against strains of *Ps. aeruginosa* of homologous serotype than sera containing low levels of haemagglutinins. Reduction or removal of anti-pseudomonas haemagglutinins from mouse serum by absorption lowered the passive protective properties of the serum. The amount of serum needed to passively protect mice against strains of the same serotype but with different virulence (as judged by i.p. injection) was inversely proportional to the virulence of the strains. These results suggest that the sera have probably been prepared against one of the factors responsible for the virulence of these bacteria in mice, as virulent strains which presumably contain more of the factor were controlled more easily by the antisera than avirulent strains which contain less or none of the virulence factors.

Since the vaccinated mice are protected against direct challenge by all strains of homologous serotype (Jones and Roe, unpublished), the above experiments show that serum gives full protection against some strains of pseudomonas however, it seems that only part of the protection induced by vaccination is present in the serum. It follows therefore that anti-pseudomonas haemagglutinins monitor only part of the full protective response of a vaccinated animal.

Attempts to remove the protection inducing factor from the vaccine by several absorptions with fresh sheep erythrocytes was only partly successful because the absorbed vaccine still contained a protection inducing factor. This means that only part of the protection inducing property of the vaccine is absorbed on to the erythrocytes and thus the passive

haemagglutination test measures only agglutinins against that part.

Sera from patients and healthy concontrols contained a wide range of anti-pseudomonas haemagglutinins. Normal human sera showed low levels of anti-pseudomonas haemagglutinins to a wide range of pseudomonas antigens. Patients with burns had much higher titres against some of the pseudomonas antigens, but the range of antibody titres against the 16 pseudomonas antigens was narrower than those of sera from healthy individuals. This suggests that injury or infection influences antibody production against some pseudomonas antigens. Antibodies to either 06 or 11 antigen were found in sera from most patients; thus it might be possible to develop a test for screening patients' sera using these 2 antigens instead of all 16. Further studies will show whether responses to these 2 antigens are representative of the overall response to injection with the polyvalent vaccine.

The studies have shown something of the complexity of protective humoral responses following vaccination. Even though anti-pseudomonas haemagglutinins measure only part of the protective responses following vaccination, they were shown to correspond to a large degree with protective properties in the serum, thus measurement of anti-pseudomonas haemagglutinins in serum after vaccination should be indicative of the protective status of the vaccinated host.

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