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A new *Pseudomonas* vaccine: Preliminary trial on human volunteers

By R. J. JONES, E. A. ROE and E. J. L. LOWBURY
M.R.C. Industrial Injuries and Burns Research Unit, Birmingham Accident Hospital
J. J. MILER and J. F. SPILSBURY
The Wellcome Research Laboratories, Beckenham, Kent

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SUMMARY

Fifteen healthy volunteers were given three weekly subcutaneous injections of a new polyvalent *Pseudomonas aeruginosa* vaccine (PEV-01). Four doses – 1·0 RHD (manufacturer's recommended human dose), 0·75 RHD, 0·5 RHD and 0·1 RHD – were used in separate groups of volunteers. Blood samples taken before each of the injections and one taken 7 days after the last injection were examined for immune response to the vaccine and for possible adverse clinical, biochemical and haematological effects.

Raised titres of antibody in serum of volunteers given 0·5–1·0 RHD vaccine were shown, often by the seventh day, in passive haemagglutination tests against all of the 16 serotypes of *Ps. aeruginosa* represented in the vaccine; serum from volunteers who received 0·1 RHD usually showed a reduced antibody titre. Tests of mouse protection by serum against intraperitoneal challenge with *Ps. aeruginosa* P14 showed increased titres of mouse protective antibody in the blood of volunteers given 1·0, 0·75 or 0·5 RHD of vaccine but a reduced mouse-protective titre in two out of three sera from volunteers given 0·1 RHD vaccine. There was a suggestion of enhanced phagocytic ingestion and intracellular killing of two strains of *Ps. aeruginosa* by the blood of vaccinated volunteers, and more definite enhancement of ingestion of inert latex particles, which were less well ingested than were the bacterial cells by phagocytes from unvaccinated volunteers.

Apart from slight or moderate local reactions and a transient rise of temperature in some volunteers, there were no clinical, biochemical or haematological abnormalities in the vaccinated volunteers.

INTRODUCTION

Prophylaxis against invasive *Pseudomonas aeruginosa* infection in burned or immunodeficient patients has special importance because of the relatively limited value of chemotherapy in such infections. In burns, defence against contamination by the local application of silver compounds, mafenide and other antimicrobial agents has proved highly effective. There is evidence, too, that immunological protection against invasion may provide a valuable second line of defence. This

has been demonstrated repeatedly in animal experiments showing the value of active and passive immunization (Lowbury & Jones, 1975), the former associated with the early appearance of antibodies in the IgM fraction of plasma immunoglobulins (Markley & Smallman, 1968; Jones, 1971; Jones, Hall & Ricketts, 1972) which makes it feasible to use the method in prophylaxis of patients with severe burns. There is some evidence of the value of active and passive immunization against *Ps. aeruginosa* in such patients (Feller, 1966; Alexander & Fisher, 1973).

Work initiated in this Unit (Jones, 1968; Jones, Jackson & Lowbury, 1966) led to the development, at the Wellcome Research Laboratories, of an improved polyvalent vaccine (Miler *et al.* 1976) which has been shown to protect burned mice, from about 48 hr. after the first dose of vaccine, against challenge by intraperitoneal injection, or by application of *Ps. aeruginosa* to the surface of the burn (Jones & Lowbury, 1972); protection by a previously developed vaccine against early intraperitoneal challenge had already been demonstrated (Jones, 1971). We report here a preliminary trial of the new vaccine in human volunteers, to assess its safety and potential effectiveness.

MATERIALS AND METHODS

The vaccine

The polyvalent vaccine (PEV-01) was prepared from surface antigens of a selected strain from each of the 12 Habs serotypes and from four additional strains, as described by Miler *et al.* (1976). It was provided freeze-dried in vials and re-constituted immediately before use with 2·5 ml. of pyrogen-free isotonic saline, then warmed at 37° C. to ensure a clear solution; this solution was discarded if not used within 2 hr.

The vaccine had been approved for use in volunteers by Wellcome Quality Control Division.

Volunteers

Members of laboratory staff between the ages of 18 and 65 years were chosen if they were in good health and available for vaccination and blood sampling over a period of 21 days (and possibly for a longer period of follow-up in some individuals). Female volunteers were admitted only if they were not pregnant and willing not to become pregnant for 3 months after the first dose of vaccine. Volunteers were not admitted if they had allergies, diabetes, immunodeficiency or skin diseases, or were taking routine medication.

Dosage

To establish an optimum effective dosage of vaccine, the first three volunteers received 0·5 ml. of vaccine containing one-tenth of the manufacturer's recommended human dose (RHD). The vaccine was given, by the subcutaneous route, on days 0, 7 and 14. After completion of the observations on these volunteers (who suffered no ill-effects), three further volunteers were given 0·5 ml. volume sub-

cutaneously on days 0, 7 and 14. This was followed by tests on another three volunteers who were given 0·75 RHD; and finally six volunteers were given 1·0 RHD of the vaccine in 0·5 ml. at the same times as those used for the other doses.

Clinical observation

The site of inoculation was inspected before inoculation and at 1 hr., 3 hr., 6 hr., 24 hr., 48 hr. and 1 week after inoculation. Volunteers recorded their temperature hourly for 6 hr. after vaccination, and then their morning temperature only for 5 days after each injection; they also kept records of erythema, irritation, swelling, induration and pain at the site of injection, hourly for the first 6 hr., then at 12 hr. and daily for 6 days.

Laboratory examination of blood of volunteers

Samples of blood (30 ml.) taken before each injection of vaccine were divided in three portions, 15 ml. being heparinized, 10 ml. allowed to clot and 4 ml. placed in a plastic bottle coated with EDTA ('Greyward'). In addition to these samples, a final sample was taken from each volunteer 3 weeks after the first dose of vaccine.

Immunological tests. The following tests were made on serum and on leucocytes (from packed cells of heparinized blood and from whole heparinized blood), to assess the potential effectiveness of immunization with the vaccine:—

(*a*) Titration of antibodies against all 16 components of the polyvalent vaccine in fresh serum, and in serum treated with 2-mercapto-ethanol (2 ME) to inactivate IgM, by passive haemagglutination of sensitized sheep cells, as described by Jones *et al.* (1972) and by Jones & Roe (1975).

(*b*) Passive protection of mice against a virulent strain of *Ps. aeruginosa* (P14) by injection of volunteers' serum, as described by Jones, Lilly & Lowbury (1971).

(*c*) Tests of leucocyte function, as shown by the ingestion and killing of two strains of *Ps. aeruginosa*, P14 (serotype 6) and UK1 (serotype 1), and by the ingestion of inert latex particles.

Phagocytosis (ingestion): equal volumes (0·1 ml.) of whole blood and of a suspension of bacteria or latex particles (7×10^6 per ml.) were incubated at 37° C. for 30 min. A smear was prepared on a slide from 0·02 ml. of the mixture of leucocytes and bacteria or latex particles; this was stained and counted as described by Roe & Jones (1971).

Intracellular killing: 0·4 ml. of packed cells from heparinized blood was mixed with 0·1 ml. of bacterial suspension (7×10^6 bacteria) in a plastic tube. A 0·1 ml. sample was removed and lysed in 1·0 ml. of distilled water, and the number of viable bacteria in the sample was counted by the method of Miles, Misra & Irwin (1938). After sampling, the mixture was incubated at 37° C. for 30 min., and a further sample was then taken and counted. The percentage of bacteria killed during 30 min. incubation was calculated from the two counts.

Nitroblue tetrazolium (NBT) tests were made using the cytocentrifuge technique described by Gordon *et al.* (1975).

Myeloperoxidase tests were made by the staining method described by Lillie (1965).

(*d*) Globulin fractions: IgG and IgM levels were measured by an immunodiffusion technique (Hoechst Pharmaceuticals, 1972). Total globulin in plasma was measured, with other biochemical examinations (see below), at the Wolfson Laboratories, Clinical Chemistry Department, Queen Elizabeth Medical Centre, Birmingham University.

Haematological tests. Total and differential leucocyte counts, erythrocyte count, platelet count, haemoglobin, erythrocyte sedimentation rate (ESR), clotting time, packed cell volume (PCV) and mean corpuscular haemoglobin concentration (MCHC).

Biochemical tests. (At the Wolfson Laboratories, Queen Elizabeth Medical Centre, Birmingham University.) Sodium, potassium, urea, creatinine, urate, calcium, albumin, globulin, bilirubin, alkaline phosphatase, aspartic acid transaminase, alanine transaminase, cholesterol, glucose, lactate dehydrogenase.

Tests for endotoxin. The Limulus lysate test (Levin & Bang, 1968) was carried out in the manner described by Jones, Roe & Dyster (1975).

RESULTS

Immunological tests

Passive haemagglutination

Table 1 shows the largest increase in titre, after vaccination, to each of the 16 component antigens of the vaccine in each of the 15 volunteers. Most of the volunteers showed the presence, in the pre-vaccination specimen, of antibodies to most of the antigens. After vaccination with the lowest dose of vaccine (0·1 RHD) there was usually a fall in antibody titre. With the higher doses there was an increase in most volunteers to most antigens; these increases varied greatly, the same volunteer showing much greater increases in antibody to some antigens than to others, while response to the same antigen was much greater in some volunteers than in others.

Table 2 shows examples of the actual titres, in fresh serum and in 2ME-treated serum (showing IgG), from three volunteers given 0·75 RHD and three given 1·0 RHD of vaccine, of haemagglutinin to three commonly found serotypes of *Ps. aeruginosa*. This shows the variety of response in different volunteers, ranging from the absence, in volunteer 7, of any increase either of total antibody or of IgG to serotype 1, to the large increase of antibody in volunteers 8 and 13 to serotype 6. Some volunteers showed maximum response of total antibody in the first post-vaccination blood sample (e.g. in volunteer 13 to serotype 6); in others the response was more delayed. IgG titres were often much lower than the total antibody titres. Volunteer 14 had a very high pre-vaccination titre against serotype 6; the absence of any increase in antibody to this antigen may have been due to absorption of the immunizing antigen by the circulating antibody.

The greatest antibody response was to antigens 6 and 7, the smallest was to antigens 1, 3 and 14. Volunteer 15 failed to respond to antigen 13 and volunteer 10 failed to respond to antigen 2.

Table 1. *Largest increase in haemagglutinin titre after immunization with polyvalent vaccine, PEV*-01 (*expressed as multiple of pre-vaccination titre*)

Volunteer			Serotype of *Ps. aeruginosa* in polyvalent vaccine															
No.	Sex	RHD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	M	0·1	<1	<1	<1	<1	<1	<1	<1	2	—	2	<1	4	2	2	<1	<1
2	F	0·1	<1	<1	<1	<1	<1	<1	<1	<1	<1	4	<1	2	<1	16	2	<1
3	M	0·1	<1	<1	<1	<1	<1	<1	<1	<1	—	<1	<1	2+	<1	<1	<1	<1
4	M	0·5	4	16+	16	16	8	2	16+	2+	8+	64+	4	16	16	2	16+	64+
5	F	0·5	4	128+	4	128	64	64	32	4	64+	64	64	64	1024	<1	64	128
6	F	0·5	1	128	2	2	256	128	4	2	16	64+	4	4	4	1	2	1028
7	F	0·75	1	8	4	4	8	8	64	1	8	64	2	2	16	2	4	8
8	F	0·75	8	32	2	128	2	128	32	8	4	4	2	2	128	4	8	16
9	F	0·75	2	64	4	2	2	2	1	2	16+	16	4	8	8	<1	<1	4
10	M	1·0	4	—	4	8+	4	2	64+	16+	4+	8+	16	16+	16+	4	32+	8+
11	F	1·0	8	64+	2	8+	4	4	32+	16+	8+	128+	2	512+	4+	4	32	16
12	M	1·0	2	8	2	2	1	1	4	4	8+	32	2	32	16	2	8	2
13	M	1·0	8	64	2	8	4	128	256	8	16	256+	32	4	4	2	8	32
14	M	1·0	2	2048	<1	128	32	4	32	4	8+	256	64	256	256	2	4	4
15	M	1·0	8	128+	16	4	32	8	256+	16	32+	256	16	32	<1	4	16	128

Where the pre-vaccination sample showed no detectable antibody, the sign '+' is placed after the number shown, meaning that this is *at least* the increase in titre compared with the pre-vaccination titre. The sign '—' means no antibody detected. The sign '<' means that no post-vaccination titre was greater than the pre-vaccination titre, and one or more (usually 2 or 3) were smaller.

Table 2. *Examples of titres of haemagglutinin in fresh serum and in 2 ME-treated serum (showing IgG)*

Volunteer	Dose (RHD)	Serum	Titre in samples taken at times: Pre	1	2	3	Pre	1	2	3	Pre	1	2	3
			Serotype 1				Serotype 6				Serotype 11			
7	0·75	Fresh	32	32	32	32	16	64	64	128	32	64	64	64
		2 ME-treated	8	8	8	8	16	16	16	16	8	8	16	16
8	0·75	Fresh	8	16	64	64	32	256	4096	4096	64	64	128	128
		2 ME-treated	8	8	16	8	—	—	16	8	8	8	16	16
9	0·75	Fresh	64	64	128	128	32	32	64	64	32	64	16	128
		2 ME-treated	64	128	64	128	16	64	64	64	64	64	64	64
13	1·0	Fresh	16	32	128	128	32	4096	4096	4096	32	64	1024	1024
		2 ME-treated	—	—	8	8	—	—	—	—	—	—	128	128
14	1·0	Fresh	32	32	64	64	4096	4096	4096	4096	64	64	4096	4096
		2 ME-treated	8	16	16	32	—	4	16	4	8	8	64	64
15	1·0	Fresh	16	16	128	64	16	32	128	64	16	32	512	256
		2 ME-treated	32	8	8	16	—	—	—	—	—	—	8	8

Table 3. *Passive protection of mice against challenge by* Ps. aeruginosa *P14 (serotype 6)*

Volunteer	Dose of vaccine (RHD)	Titre of serum that protected mice in sample: Pre-vaccination, Fresh serum	Pre-vaccination, 2 ME-treated	Post-vaccination 1, Fresh serum	1, 2 ME-treated	2, Fresh serum	2, 2 ME-treated	3, Fresh serum	3, 2 ME-treated
1	0·1	15	0	0	0	0	0	10	0
2	0·1	50	0	10	0	0	4	10	8
3	0·1	0	0	0	0	0	4	0	4
4	0·5	0	0	10	4	0	8	0	4
5	0·5	10	0	50	0	10	4	10	0
6	0·5	0	0	0	0	10	8	0	4
7	0·75	10	0	100	4	100	8	100	16
8	0·75	10	0	50	0	100	4	100	4
9	0·75	10	0	50	0	100	4	50	8
10	1·0	10	0	10	4	50	8	64	16
11	1·0	8	0	50	0	50	4	100	4
12	1·0	50	0	50	0	100	8	512	32
13	1·0	10	0	50	0	100	0	10	0
14	1·0	0	0	100	0	100	8	10	4
15	1·0	0	0	100	0	100	4	100	4

Passive protection of mice by serum from vaccinated volunteers

Table 3 shows the dilution of serum, both fresh and 2ME-treated, which protected mice against challenge by intraperitoneal injection of *Ps. aeruginosa* P14 (which killed 100 % of unprotected controls). Fresh serum from unvaccinated volunteers (the pre-vaccination samples) often had appreciable protective action. After receiving the lowest dose of vaccine (0·1 RHD) two volunteers had less mouse-protective antibody in their serum than did the pre-vaccination samples; with higher dosage of vaccine, in particular 0·75 and 1·0 RHD, there was an almost consistent increase in the mouse-protective titre of the volunteer's serum, which was often seen in the first post-vaccination sample. These results corresponded strikingly with the haemagglutination titres, both in respect of the negative response to 0·1 RHD and positive response to 0·75 and 1·0 RHD of vaccine.

Leucocytic activity

Fig. 1 shows the mean percentage of neutrophils containing bacteria (*Ps. aeruginosa* P14 and *Ps. aeruginosa* UK1), and the mean numbers of these bacteria and of latex particles per neutrophil in the blood of volunteers after incubation of packed cells for 30 min. with bacteria or particles; also the percentage of ingested *Ps. aeruginosa* killed after 30 min. incubation of cells, following the counts of ingested bacteria.

Pre-vaccination samples showed bacteria ingested by most of the neutrophils;

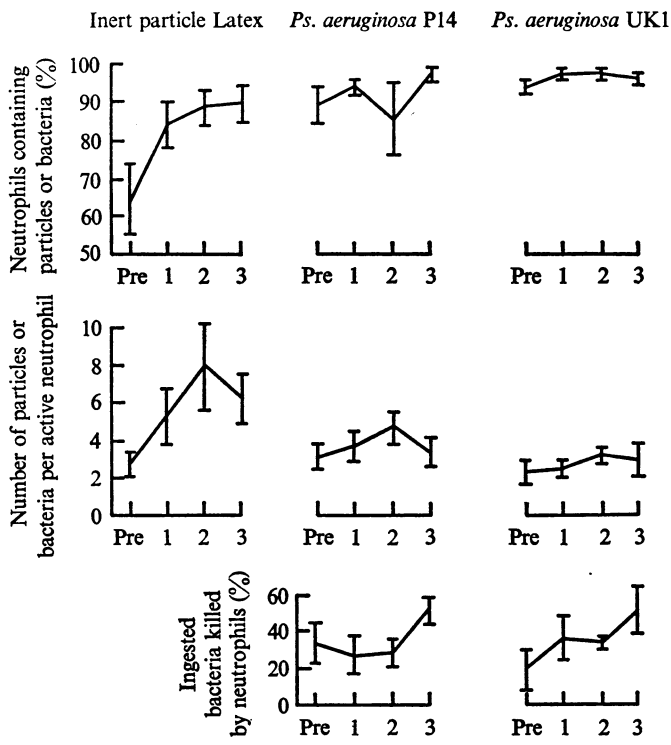

Fig. 1. Results of tests on phagocytic ingestion of two strains of *Ps. aeruginosa* (P14 and UK1) and of latex particles, and on killing of the ingested bacteria by neutrophil granulocytes in blood of volunteers before and after vaccination with PEV-01. The graphs show mean percentage of neutrophils containing bacteria or latex particles, mean numbers of bacteria or particles per neutrophil and mean percentage of bacterial cells killed after ingestion. Parentheses show range of observations.

nevertheless there was a suggestion of an increase, after vaccination, in the proportion of neutrophils containing bacteria, in the numbers of bacteria ingested by neutrophils and in the numbers of bacteria killed after ingestion by neutrophils. Ingestion of latex particles by neutrophils in pre-vaccination samples of blood was much less active (shown by the low proportion of neutrophils ingesting latex particles) than that of bacteria, and there was an appreciable increase in the ingestion of latex particles by neutrophils (both in proportion of active neutrophils ingesting latex and number of particles ingested) in the post-vaccination samples.

Myeloperoxidase tests showed that neutrophils from all samples from all volunteers were capable of phagocytic function.

The NBT test showed a range (2–16 %) of NBT positive neutrophils which were within the normal limits for healthy volunteers.

Total globulin and immunoglobulins G and M

There were minor fluctuations, within normal limits, of total globulin, and no consistent change in relation to vaccination. Both IgG and IgM concentrations rose in some volunteers after receiving higher doses of vaccine; e.g. 4 out of 6

volunteers given 1·0 RHD showed an increase in IgM after the first dose of vaccine; 3 out of 3 volunteers given two injections of vaccine at 0·5 RHD showed an increased concentration of IgG and IgM. However, these increases were not consistent, and several volunteers showed lower immunoglobulin concentrations after vaccination.

Haematological and biochemical tests

Apart from a high ESR in volunteer 8 (present in the pre-vaccination sample as well as after vaccination), there were no results which fell outside normal limits in samples from volunteers given 0·5, 0·75 or 1·0 RHD doses of vaccine. Volunteer 2, given the 0·1 RHD dose, showed a transient neutropenia; she developed an upper respiratory virus infection shortly after the experiment, and may have been in the incubation period at the time of sampling.

Limulus test

Endotoxin was found at very low concentrations (0·1 ng./ml.) in plasma samples from 4 of the 15 volunteers; in one of these it was found in the pre-vaccination sample.

Clinical observations

Volunteers given 0·1 RHD of vaccine showed no rise in temperature of more than 0·1° C., and no redness, tenderness or induration at the site of injection. Some of those given 0·5, 0·75 or 1·0 RHD of vaccine showed small increases in temperature, ranging from 0·4 to 1·1° C., between 3 and 6 hr. after vaccination, and also redness, for periods of 1–72 hr., tenderness, for periods of 1–48 hr., and induration, for periods up to 48 hr. None of the reactions were severe, and no volunteer felt ill.

DISCUSSION

These studies on human volunteers have shown a regular, though variable, response to vaccination with the polyvalent pseudomonas vaccine, PEV-01. The increase in haemagglutinins, which sometimes reached a high titre in 1 or 2 weeks, appeared against almost all the component antigens in nearly all of the volunteers, provided the dose of vaccine was adequate, and vindicated the decision to include in the vaccine representatives of all the serotypes. It had previously been shown (Jones, 1972) that a vaccine containing a selection of serotypes, which between them could stimulate the production of antibody active against a full range of serotypes, stimulated immunity in mice against all strains of the serotypes represented in the vaccine, but against only some of the strains of serotypes not represented; the vaccine PEV-01 did, to some extent, avoid this difficulty by including all the serotypes.

A low dose (0·1 RHD) of the vaccine caused a drop in the volunteers' normal pre-vaccination titre of pseudomonas antibody, probably due to absorption of circulating antibody onto the vaccine and the absence of any antigenic stimulus. Changes in haemagglutinin titre – both the increase after recommended dose of vaccine and the paradoxical fall after a low dose – were paralleled by the results

of mouse protection tests with a strain of *Ps. aeruginosa* P14. It cannot be assumed that the protection against other serotypes would be as good as that shown with strain P14, but from the association between the pattern of haemagglutinin titres and mouse protection titres it seems likely that the former may be regarded as giving a useful indication of probable protective value. In view of the dilution of injected antibody in the blood of the mouse, some individuals whose serum has failed to protect mice in passive protection tests may have had enough circulating antibody to protect themselves against infection.

Pseudomonas antibodies have been shown to protect by enhancement of leucocytic action (Jones & Roe, 1975; Jones & Dyster, 1973; Fox & Lowbury, 1953; Young & Armstrong, 1972). There was a suggestion, in the vaccinated volunteers, of slightly increased phagocytic ingestion (which was also very active in leucocytes from the unvaccinated) and of increased intracellular killing of *Ps. aeruginosa*; there was a more definite increased ingestion of latex particles, showing that the effect of vaccination on leucocytic action was, at least in part, non-specific and not dependent on pseudomonas antigen in the particles ingested. This finding suggests the possibility of a useful enhancement of non-specific resistance by the vaccine.

The trial on volunteers was a first step towards controlled trials of the vaccine in patients with severe burns. The early appearance of protective antibody after vaccination of volunteers augurs well for the use of the vaccine in preventing invasive pseudomonas infection in these patients. In the event of very early contamination of extensive burns with *Ps. aeruginosa*, however, even such early immunity as that obtained with the vaccine PEV-01 cannot be expected to prevent potentially fatal pseudomonas invasion. For such patients an antiserum or specific immunoglobulin is more likely to be useful (Alexander & Fisher (1973)), and could be prepared by immunization of volunteer donors with the polyvalent vaccine.

The absence of appreciable toxic effects was an outstanding feature of the vaccine; apart from some redness, tenderness and induration (usually slight or moderate) at the injection site in many of the volunteers, and a small very transient increase in temperature in some volunteers, there were no adverse clinical, biochemical or haematological changes; the volunteers felt well throughout the period of vaccination.

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