Br. J. exp. Path. (1973) **54**, 416

THE ROLE OF POLYMORPHONUCLEAR LEUCOCYTES IN PROTECTING MICE VACCINATED AGAINST *PSEUDOMONAS AERUGINOSA* INFECTIONS

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Received for publication March 20, 1973

Summary.—In the presence of serum from normal unvaccinated mice polymorphonuclear leucocytes taken from mice 1, 2, 3 and 4 days after one injection of a pseudomonas vaccine phagocytosed progressively more bacteria on each successive day. More bacteria were phagocytosed by polymorphs from vaccinated mice in serum taken 3, 4 or 7 days after vaccination than in serum taken earlier.

Polymorphs from vaccinated mice ingested more of the strains of bacteria against which vaccinated mice were protected than other pseudomonas strains.

MICE were successfully protected against intraperitoneal challenge of *Pseudomonas aeruginosa* as early as 2 days after an injection of a pseudomonas vaccine (Jones, 1971). Investigations into mechanisms of early resistance of vaccinated mice showed that the protection could be passively transferred to unvaccinated mice by serum from vaccinated mice (Jones, Lilly and Lowbury, 1971) and that IgM played an important part in early protection of mice against pseudomonas infections (Jones, Hall and Ricketts, 1972).

In this report polymorphonuclear leucocytes from vaccinated mice have been studied to see whether they become involved in the early protection of mice against *Ps. aeruginosa* infection. Polymorphs taken from the blood of vaccinated mice at different times after vaccination were compared with polymorphs from unvaccinated mice in respect of their abilities to phagocytose various strains of *Ps. aeruginosa*. Serum obtained at different times after vaccination was also tested to see how it influenced the phagocytosis of *Ps. aeruginosa* by polymorphs from both vaccinated and unvaccinated mice.

MATERIALS AND METHODS

Polymorphonuclear leucocytes.—Groups of 5 or 10 mice (Schofield, albino male weighing 25 g) each anaesthetized by intraperitoneal injection of 1·0 ml of 1/20 Nembutal in saline, were exsanguinated by cardiac puncture using 2·0 ml Steristar plastic syringes. The syringes were primed with 0·1 ml of a sterile solution of 300 units of heparin (Boots Pure Drug Co. Ltd.) in 1·5 ml of Hank's basal salt solution (BSS) to prevent the blood from clotting. Blood was gently drawn from the heart and placed in a 10 ml plastic tube containing 1·0 ml of warmed (37°) sterile Hanks BSS and 100 units of heparin. A total WBC count was made using the improved Neubauer chamber.

The pooled blood was centrifuged at low speed (mark 2 in MSE minor centrifuge) for 12 min. After the supernatant had been removed the cells were washed once in warm (37°) Hank's BSS and used immediately. A blood film was made of the packed cells before mixing with serum and bacteria to see if they had been damaged in preparation.

Serum.—Groups of 40 mice were given a single intraperitoneal injection of 0·1 mg/kg body weight/mouse of a pseudomonas vaccine (XR-123). This vaccine was made from *Ps. aeruginosa* (P14) by Dr Milerova at the Wellcome Research Laboratories. Groups of 40 mice were exsanguinated by cardiac puncture 1, 2, 3, 4 and 7 days after vaccination. Blood was obtained in a similar way from control mice. Serum was separated from the clot after centrifugation and kept at − 21° until used.

Bacteria.—Strains of *Ps. aeruginosa* with serotypes 1, 4, 5C, 6B, 10, 11 and 2A2B5C were obtained from patients with burns; serotype 6C was obtained from the National Collection of Type Cultures. Bacteria were grown overnight on horse blood agar and suspended in saline to give a turbidity equivalent to tube 2 on the Brown's opacity scale (Wellcome) ≡ $1{\cdot}4 \times 10^9$ bacteria/ml.

Phagocytosis.—To 0·03 ml of serum in a sterile plastic tube were added 0·2 ml of washed packed mouse blood and 0·2 ml of a 1/10 dilution of bacterial suspension. After gentle mixing the tube was put into a 37° waterbath.

At half-hourly intervals from ½–3 hours, blood films were made from the mixture with a concave spreader (Wright and Colebrook, 1921). This spreader concentrates the leucocytes at one end of the film. Films were air dried, fixed for 5 min in absolute methanol and stained by May–Grunwald–Giemsa technique (Gurr, 1963).

The number of bacteria in each of 50 polymorphs was counted. From the results an estimate of the average number of bacteria ingested by the 50 polymorphs at each sampling was made.

RESULTS

Phagocytic activity of polymorphs from vaccinated mice in control serum

The experiments summarized in Fig. 1 show the average number of *Ps. aeruginosa* P14 phagocytosed by polymorphs taken from mice at different times

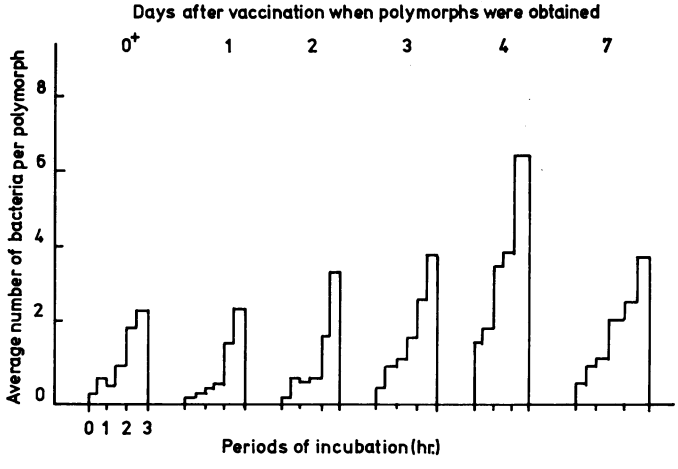

FIG. 1.—Phagocytosis of *Ps. aeruginasa* P14 by polymorphs taken from vaccinated mice in serum from control unvaccinated mice (combined results of 3 experiments).

(1, 2, 3, 4 and 7 days) after vaccination in serum from the same pool of unvaccinated mice. Average counts of bacteria ingested by 50 polymorphs are shown for samples taken every half-hour for 3 hours for each group of polymorphs from vaccinated mice.

After 3 hours' incubation, polymorphs taken from mice 4 days after vaccination had phagocytosed more bacteria than polymorphs taken 1, 2 or 3 days after

vaccination. Polymorphs taken from mice 1, 2, 3 and 4 days after vaccination phagocytosed more bacteria with each successive day, but polymorphs taken 7 days after vaccination were comparatively less active.

Phagocytic activity of polymorphs from vaccinated mice in serum from vaccinated mice

Figure 2 summarizes experiments in which polymorphs obtained from mice 2, 4 and 7 days after vaccination were tested for their abilities to phagocytose *Ps. aeruginosa* P14 in sera taken 1, 2, 3, 4 and 7 days after vaccination. The phagocytic activity of polymorphs from unvaccinated mice in the same sera is also shown.

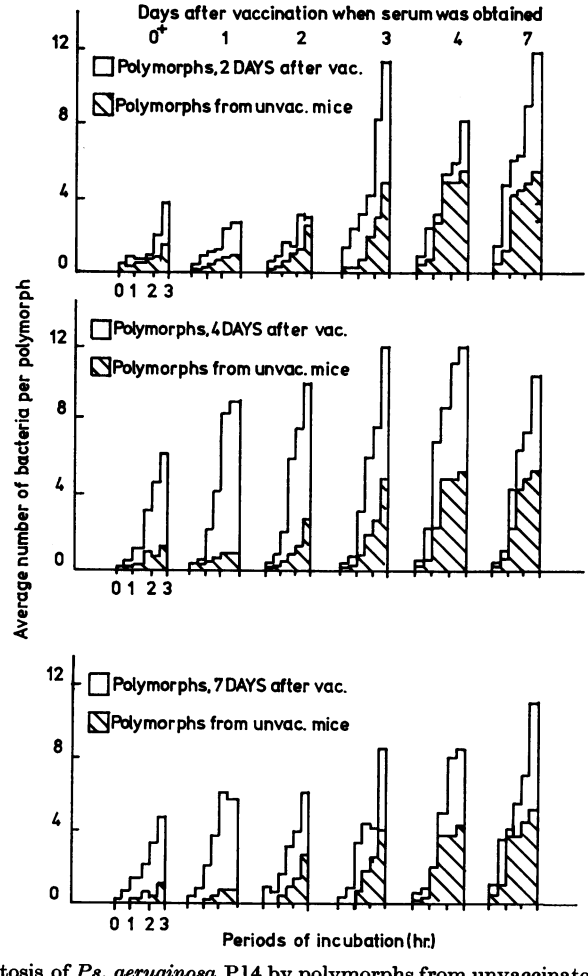

FIG. 2.—Phagocytosis of *Ps. aeruginosa* P14 by polymorphs from unvaccinated mice and from mice 2, 4 and 7 days after vaccination in serum taken from control mice and mice 1, 2, 3, 4 and 7 days after vaccination (combined results of 3 experiments).

Polymorphs from vaccinated mice (white blocks) phagocytosed more *Ps. aeruginosa* P14 in each of the 6 sera than polymorphs from unvaccinated mice (shaded blocks), irrespective of the time after vaccination when the polymorphs were obtained.

There was no apparent difference in the numbers of bacteria phagocytosed by polymorphs taken from mice 2 days after vaccination in serum from unvaccinated mice or in serum obtained 1 or 2 days after vaccination. However, the phagocytic properties of polymorphs from the same pool of vaccinated mice was considerably increased (2–3 times) by the addition of serum taken from mice 3, 4, or 7 days after vaccination. These 3 sera also enhanced the phagocytic properties of polymorphs taken 4 and 7 days after vaccination as well as polymorphs taken from control mice.

Polymorphs taken from mice 7 days after vaccination differed in their phagocytic properties from those taken 2 days after vaccination only in that they ingested more bacteria in control serum and in serum taken from mice 1 or 2 days after vaccination.

Of the 3 groups of polymorphs examined, those taken 4 days after vaccination phagocytosed the largest number of bacteria in each of the sera.

Phagocytosis of serologically different strains of Ps. aeruginosa

Fig. 3 shows the phagocytosis of 8 different strains of *Ps. aeruginosa* by polymorphs from vaccinated mice. The polymorphs were obtained from mice 4 days after vaccination—a time when mice were protected against strains P6C, P11, P6B and P1, but not against strains P10, P4, P2A2B5C and P5C (Jones, 1972).

The polymorphs were found to be more effective in phagocytosing bacteria against which vaccinated mice were protected than bacteria against which vaccinated mice were not protected. Polymorphs were able to phagocytose more of the bacteria against which vaccinated mice were protected in serum taken from vaccinated mice than in control serum. The serum from vaccinated mice, moreover, did not enhance the uptake of bacteria against which vaccinated mice were not protected, resulting in similar numbers of these bacteria being phagocytosed both in control sera and in sera from vaccinated mice.

DISCUSSION

In 1953 Fox and Lowbury provided early evidence that opsonins in type-specific antisera increased the uptake of *Ps. aeruginosa* by polymorphs. The present study has shown not only that the phagocytosis of *Ps. aeruginosa* by mouse polymorphs was influenced by the serum in which the tests were made but also by the time after vaccination when polymorphs were taken and the immunotype of the bacteria. *In vitro*, each factor was found to be capable independently of influencing the phagocytosis of pseudomonas: serum obtained 4 days after vaccination enhanced the uptake of pseudomonas by polymorphs from unvaccinated mice, polymorphs taken 4 days after vaccination phagocytosed considerable numbers of pseudomonas in control serum and those pseudomonads against which vaccinated mice were protected were more readily phagocytosed than other strains of pseudomonas.

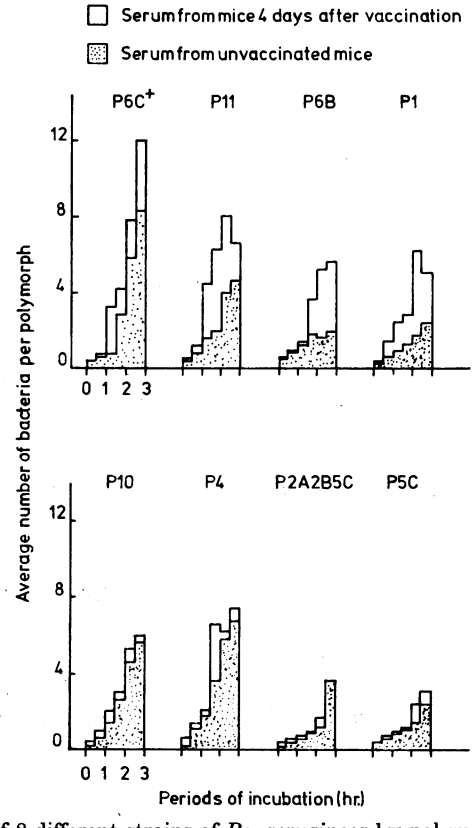

FIG. 3.—Phagocytosis of 8 different strains of *Ps. aeruginosa* by polymorphs from vaccinated mice. Vaccinated mice are protected against strains P6C, P11, P6B and P1 but are not protected against strains P10, P4, P2A2B5C and P6C. The polymorphs used in the experiment were obtained from a pool of mice vaccinated 4 days previously.

Experiments illustrated in Fig. 2 showed that polymorphs taken 2 days after vaccination, which were capable of ingesting few bacteria in homologous serum, could be made to ingest larger numbers of bacteria provided a suitable serum (taken 3, 4 or 7 days after vaccination) was present. The relevance of serum to phagocytosis was shown by other experiments in which polymorphs (taken 4 days after vaccination) with an ability to ingest large numbers of bacteria and polymorphs from control mice with no special ability to ingest bacteria can be encouraged to phagocytose considerably more bacteria by serum taken 4 or 7 days after vaccination.

Previous experiments (Jones *et al.*, 1971, 1972) showed that serum taken 3, 4 and 7 days after vaccination can passively protect mice against pseudomonas challenge, and it seems that one possible mechanism by which these sera protect mice is by enhancing the phagocytic properties of the polymorphs in the blood of the passively immunized mice.

Although the experiments show the value of certain sera in promoting phagocytosis, they did not show that polymorphs taken as early as 2 days after vaccination had any enhanced phagocytic properties.

Implications from these experiments must be tentative, as only part of the protective response is represented in *in vitro* tests, but it seems that since neither polymorphs nor serum show enhanced phagocytic or phagocytosis-promoting properties 2 days after vaccination, yet vaccinated mice are protected against lethal challenge at this time, some other part of the mouse's defence system must have been stimulated by the vaccine. Projected studies on the effect of pseudomonas vaccines on the bactericidal activity of leucocytes and of the reticuloendothelial system may help to clarify the role of cellular factors in early protection following vaccination.

REFERENCES

FOX, J. E. & LOWBURY, E. J. L. (1953) Immunity and Antibody to *Ps. pyocyanea* in rabbits. *J. Path. Bact.*, **65**, ii, 533.

GURR, G. T. (1963) *Biological Staining Methods.* 7th Ed. Gurr.

JONES, R. J. (1971) Early Protection by Vaccines in Burns. *Br. J. exp. Path.*, **32**, 100.

JONES, R. J. (1972) Specificity of Early Protective Responses induced by Pseudomonas Vaccines. *J. Hyg., Camb.*, **70**, 343.

JONES. R. J., HALL, M. & RICKETTS, C. R. (1972) Passive Protective Properties of Serum Fractions from Mice Inoculated with an Antipseudomonas Vaccine. *Imunology*, **23**, 889.

JONES, R. J., LILLY, H. A. & LOWBURY, E. J. L. (1971) Passive Protection of Mice against *Pseudomonas aeruginosa* by Serum from Recently Vaccinated Mice. *Br. J. exp. Path.*, **52**, 204.

WRIGHT, A. E. & COLEBROOK, L. (1921) *Technique of the Teat and Capillary Glass Tube.* 2nd Ed. London: Constable & Co. Ltd.