The Journal of Medical Microbiology

Vol. 2 No. 1

PROTECTION OF MICE AGAINST INFECTION BY *STAPHYLOCOCCUS AUREUS*

M. J. HILL

Wright-Fleming Institute, St Mary's Hospital Medical School, London

STRAINS of *Staphylococcus aureus* differ in their ability to cause lesions when injected subcutaneously into mice along with cotton dust. From studies of the evolution of these lesions, Agarwal (1967*a, b* and *c*) postulated that "mouse-virulent staphylococci", that is to say, strains that produce a necropurulent lesion when injected in relatively small numbers, possess a factor or factors that inhibit the acute inflammatory response. Staphylococci were virulent in this sense only if they inhibited both the production of oedema and the migration of leucocytes into the lesion site; strains that inhibited only one or neither of these components of the acute inflammatory response did not produce necropurulent lesions. Later we isolated from such mouse-virulent strains a cell-wall component that stimulated lesion production by a dose of *Staph. aureus* that alone would not have caused a severe lesion (Hill, 1968). This "cell wall aggressin" inhibits the production of oedema, and preliminary results indicate that it also inhibits the migration of leucocytes *in vitro* (Weksler, unpublished).

This paper describes experiments on mice immunised by repeated subcutaneous injections of the aggressin. The effect of such immunisation on the severity of subsequent infections was investigated in terms of the gross appearance of the lesion, the count of viable organisms at the lesion site and the rate of influx of oedema fluid to the lesion site.

MATERIALS AND METHODS

Organisms and conditions of growth

The strains of *Staph. aureus* used have been described previously (Hill, 1968). All were grown at 37°C with gentle agitation in a medium consisting of 10 g Oxoid Nutrient Broth no. 2 with 10 g Oxoid Yeast Extract per l.

Isolation of staphylococcal aggressin

The aggressin was isolated by a method described previously (Hill, 1967, 1968); staphylococcal cell walls were extracted with deoxycholate and the aggressin remained in the residue.

Received 18 July 1968; accepted 24 Sept. 1968.

500 ml of an overnight culture was added to 5 l of fresh broth medium warmed to 37°C; after 3 hours' incubation this culture was harvested and the bacterial cells were washed in physiological saline. The cocci were disrupted by Mickle disintegration, and the cell walls were deposited by centrifugation at 6000*g* for 30 min., washed twice in physiological saline and then suspended at a concentration of 50 mg per ml in 1 per cent. sodium deoxycholate solution for 6 hr at 0°C. The residual undissolved material was sedimented at 6000*g* for 30 min., washed twice with physiological saline and twice with distilled water, and finally freeze-dried. This deoxycholate-resistant fraction (DOCR) of the cell walls contained the aggressin if the cell walls used were from a mouse-virulent strain. DOCR from mouse non-virulent strains had no aggressin activity.

Production of antiserum against aggressin in rabbits

DOCR (2 mg) from strain PS80 was thoroughly mixed with complete Freund's adjuvant (5 ml) and 0.5 ml was injected into each flank of two rabbits. The rabbits were bled from the ear 4 wk later. Test bleedings were taken before the injection and used as normal rabbit serum controls.

Measurement of lesions

Staphylococcal lesions were initiated by the subcutaneous injection of cocci mixed with cotton dust, as described by Agarwal (1967*a*). The methods used for scoring the lesions, assaying oedema production and counting the bacteria in the lesions were those described previously (Agarwal, 1967*a, b* and *c*; Hill, 1968).

TABLE I

Effect of rabbit antiserum on mean lesion score after subcutaneous injection of Staphylococcus aureus mixed with cotton dust

Staphylococcus		Mean lesion score * in mice given			
strain	dose (c.f.u.) †	no serum	0.1 ml normal rabbit serum	0.1 ml antiserum from	
				rabbit A	rabbit B
PS80	10 ⁵	2.2	2.0	1.3	1.6
PS80	10 ⁶	5.3	4.7	3.2	2.3
7634	10 ⁵	...	2.0	2.0	...
Wood 46	10 ⁶	...	1.7	...	2.0

* Mean scores of lesions in groups of 6 mice.

† Colony-forming units.

RESULTS

Passive protection of mice by means of rabbit antiserum

Mice in groups of 6 were given 0.2 ml of the rabbit antiserum prepared against aggressin into the lateral tail vein and were then challenged with a subcutaneous injection of 10⁵ colony-forming units (c.f.u.) of strain PS80 mixed with cotton dust. Control groups of mice were given normal serum or no serum. Further experiments were performed with the challenge strains 7634 (a mouse-virulent strain) and Wood 46 (which is much less virulent for mice

than strain PS80) and also with strain PS80 at a dose of 10^6 c.f.u. The lesions were observed at 24 and at 48 hr and the mean lesion score was determined.

Injection of normal rabbit serum led to a very small and probably insignificant decrease in lesion score with both doses of strain PS80 (table I). Treatment with rabbit antiserum reduced the severity of the lesions due to strain PS80 but not those due to strains 7634 and Wood 46.

TABLE II

Effect of active immunisation with aggressin on lesion score after subcutaneous injection of strain PS80 mixed with cotton dust

Expt no.	Dose of staphylococci (c.f.u.) †	Mean lesion score * in mice given the following number of weekly injections ‡					
		0	1	2	3	4	5
1	10^6	4.4	...	3.5	3.2	1.6	...
2	10^6	4.2	...	4.0	2.0	1.5	...
3	10^6	4.6	...	...	...	...	0.5
4	10^5	3.3	...	...	...	...	0

* Mean scores of lesions in groups of 6 mice.

† Colony-forming units.

‡ 50 μ g DOCR subcutaneously.

TABLE III

Lesion scores after challenge with various staphylococci of unimmunised mice and of mice immunised with DOCR from strain PS80 or from strain Wood 46

Challenge strain of staphylococcus	Mean lesion score * in		
	untreated control mice	mice immunised with DOCR from	
		strain PS80	strain Wood 46
PS80	2.5	0.5	3.0
1531	3.0	1.0	3.0
7641	2.5	0.5	2.5
Wood 46	1.0	0.5	1.5

* Each value based on a group of 6 mice.

Passive protection of mice with human serum

Sera from 11 patients with chronic staphylococcal infections were tested for passive protection by the same technique. Here the situation was less clear, because most of the sera had elevated titres of anti-staphylolysin and this reduced the dermonecrosis. Nevertheless, the sera failed to reduce the size of the lesions; the viable count of staphylococci in the lesions 24 hr after infection was not significantly different from that in the untreated controls.

Active immunisation of mice

Mice given repeated subcutaneous injections of aggressin resisted challenge with quite high doses of virulent staphylococci. In general, mice given 3 weekly subcutaneous injections each of 50 μ g DOCR in 50 μ l of $\frac{1}{4}$ -strength Ringer's solution were clearly protected against infection by strain PS80 (table II) and 5 injections gave almost complete protection. In all subsequent experiments, mice were given a course of 5 weekly injections of 50 μ g DOCR.

Mice immunised with DOCR from strain PS80 were protected against infection not only by the homologous strain but also against mouse-virulent heterologous strains (table III). A similar course of injections of DOCR from strain Wood 46 (which contains no demonstrable aggressin) had no protective effect.

TABLE IV

Viable counts (VC) at 24 hr and mean lesion scores (MLS; mean for 3 mice) in control mice and in mice immunised with aggressin

Staphylococcus challenge		Untreated control mice		Mice immunised with DOCR of PS 80 *		Mice immunised with DOCR of Wood 46 *	
strain	dose	VC at 24 hr	MLS	VC at 24 hr	MLS	VC at 24 hr	MLS
PS80	5×10^5	2×10^8	4	4×10^5	0	5×10^7	6
7641	6×10^5	1×10^8	5	5×10^6	1	2×10^8	5
1531	1×10^6	7×10^7	6	3×10^6	2	5×10^7	6
Wood 46	8×10^6	2×10^7	2	2×10^7	1	2×10^7	3

* Five weekly subcutaneous injections.

The effect of immunisation on the viable count in the lesions

The viable staphylococci at the lesion site 24 hr after infection were enumerated in immunised and in unimmunised control mice. Mouse-virulent strains did not multiply as freely in immunised mice as they did in normal mice, and the viable counts at 24 hr were similar to those expected from a non-virulent strain in normal mice (table IV). The viable counts for strain Wood 46 were not significantly different in control and immune mice. This is not surprising, since strain Wood 46 contains no demonstrable aggressin and antibody to aggressin would be irrelevant in these lesions. Mice given repeated injections of DOCR from strain Wood 46 had viable counts not significantly different from those in untreated control mice after infection by the virulent strains PS80, 7641 and 1531 and by the non-virulent strain Wood 46.

With strain PS80, the variation in viable count with time after infection was studied more closely (table V). The counts in immunised mice did not differ substantially from those in control mice for the first 4 hr after infection, and differed significantly only at 24 hr. In this respect infection with a mouse-virulent strain in immunised mice is similar to infection with a non-virulent strain in normal unimmunised mice.

*Effect of immunisation of mice on the oedema-inhibiting
action of aggressin*

In a previous paper (Hill, 1968) it was shown that DOCR from mouse-virulent strains inhibited the accumulation of oedema fluid at the injection site, whereas DOCR from mouse non-virulent strains stimulated this component of

TABLE V

Viable counts in the lesions at various times after infection with strain PS80 in immune and in non-immune mice

Expt	Mice	Viable count * at the following time (hr) after infection					Mean lesion score †
		0	2	4	7	24	
A {	non-immune	2×10^5	9×10^2	2×10^5	9×10^5	2×10^7	3.8
	immune ‡	2×10^5	7×10^2	4×10^4	2×10^4	1×10^5	0.8
B {	non-immune	9×10^4	3×10^2	2×10^3	3×10^5	2×10^7	4.6
	immune ‡	9×10^4	1×10^2	8×10^2	1×10^4	8×10^4	0.5
C {	non-immune	3×10^5	4×10^3	8×10^4	2×10^6	2×10^8	3.3
	immune ‡	3×10^5	8×10^2	4×10^4	4×10^5	9×10^5	0

* Mean of counts in groups of 3 mice.

† Mean scores of lesions in groups of 6 mice.

‡ Five weekly subcutaneous injections of 50 µg DOCR of strain PS80.

TABLE VI

Oedema response to DOCR from various strains of Staphylococcus aureus in immune and non-immune mice

Mice	Oedema index (I) * for DOCR of strain								
	PS80	Orbach	7625	7634	8628	8640	8668	8669	8690
Non-immune	0.40	0.40	0.40	0.40	0.42	0.40	0.33	0.42	0.41
Immune †	2.95	3.25	2.00	2.17	2.93	2.10	3.16	3.96	2.25

* $I = \frac{\text{area of blue zone around test substance}}{\text{area of blue zone around cotton alone}}$ (see Hill, 1968).

† Five weekly subcutaneous injections of 50 µg DOCR.

the acute inflammatory response. The oedema response to DOCR of mouse-virulent strains was tested in immunised mice and compared with that in normal mice. In immune mice the aggressin not only failed to inhibit the accumulation of oedema but actually stimulated it (table VI).

DISCUSSION

Virtually all substances derived from staphylococci and known to be antigenic have antibody counterparts in most adult human sera (Rogers and Melly, 1965). Nevertheless, repeated staphylococcal infections are a common occurrence despite this impressive array of antibodies; clearly these antibodies do not confer protection on the host. The results of Agarwal (1967*a*) showed that "mouse-virulent" strains of *Staph. aureus* possess a factor (or factors) that suppresses the acute inflammatory response to the infection that results from the subcutaneous introduction of staphylococci mixed with cotton dust. If the inflammatory response could be "derepressed", the "virulent" strains would lose their advantage and become benign under normal circumstances. We have, therefore, isolated the aggressin responsible for suppression of the acute inflammatory response, used it to immunise mice, and shown that mice immunised in this way are protected against infection not only by the homologous strain but also by a range of heterologous strains. In immune mice, virulent strains no longer suppressed the influx of oedema fluid and behaved like non-virulent strains.

Agarwal (1967*c*) showed that repeated infection of mice with live *Staph. aureus* did not lead to protection against subsequent infection either by the homologous or by heterologous strains, although the necrotising effect of α -lysin was neutralised. Infection of mice with live organisms thus apparently failed to stimulate the production of antibodies against aggressin. Human serum from patients with long-established staphylococcal infection, when used in passive protection tests in mice, also failed to reduce the lesion severity. Therefore it may be concluded that in man, too, little or no antibody to aggressin is produced during infection with live staphylococci. Rogers and Melly noted that "containment of staphylococci within a fibrinous or fibrous capsule may prevent antigens from escaping to reach antibody forming centers". They further demonstrated that injection of a staphylococcal antigen into a sterile abscess-like cavity in rabbits led either to no antibody formation or to a greatly delayed immune response, compared with a subcutaneous injection.

The studies reported here indicate that it is possible to immunise an experimental animal and confer on it protection against heterologous strains of *Staph. aureus*. Further experiments are needed with a wider range of staphylococci both as sources of aggressin and as challenge strains. The effects of immunisation should also be investigated in other experimental models and with different species of laboratory animals. If the results obtained confirm the value of active immunisation, trials in human subjects could usefully be begun.

SUMMARY

Mice immunised with an aggressin from cell walls of a virulent strain of *Staphylococcus aureus* were protected against the effects of a subcutaneous injection of the homologous strain and of a range of heterologous strains of *Staph. aureus*. Three subcutaneous injections of 50 μ g DOCR at weekly

intervals led to a significant reduction in the severity of the lesion, and 5 such injections conferred almost complete protection. The superficial lesion was abolished and the viable count at 24 hr considerably reduced. Aggressin from a number of strains that inhibited the exudation of oedema in normal mice did not do so in immunised mice. Immunisation with a similar cell-wall fraction from a non-virulent strain gave no protection against infection either with respect to the severity of the surface visible lesion, the count of viable staphylococci 24 hr after infection or the effect on the exudation of oedema fluid.

I wish to thank the Medical Research Council for their financial support of this work, and Mrs Jennifer Williams and Miss Rosalind Stewart for their invaluable technical assistance.

REFERENCES

- AGARWAL, D. S. 1967a. *Br. J. Exp. Path.*, **48**, 436.
 „ 1967b. *Ibid.*, **48**, 468.
 „ 1967c. *Ibid.*, **48**, 483.
 HILL, M. J. 1967. *Nature, Lond.*, **214**, 1152.
 „ 1968. *J. Med. Microbiol.*, **1**, 33.
 ROGERS, D. E., AND MELLY, M. ANN . 1965. *Ann. N.Y. Acad. Sci.*, **128**, 274.