Host parasite relationships in experimental staphylococcal infection

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One of the main features of staphylococcal infection is the wide range of sites that can be involved, from skin and soft tissue to bone, lungs and heart valves. Although some host defence mechanisms may be common to many of these sites it is a dangerous oversimplification to believe that the host-parasite balance in one site necessarily reflects the situation in another. This, together with the normal limitations of relating results obtained in one host species to infections in another, makes the task of developing useful experimental models of staphylococcal infection difficult. The lack of suitable models limits our understanding of host-parasite relationships in staphylococcal disease.

A good illustration of the way this vicious circle can be broken by a good animal model of infection is provided by the work on staphylococcal epidermolytic toxins which is discussed by Professor Arbuthnott and Dr Lyell. The infant mouse has provided an almost exact parallel to the disease caused by this toxin in young children and has led, through some elegant studies, to a very rapid understanding of this type of staphylococcal disease. In contrast our understanding of the more common staphylococcal boil or abscess in not nearly so complete.

The purpose of this paper is two-fold. Firstly to illustrate the importance of the route of infection in the study of the host-parasite relationship. Secondly to describe how ideas in the field of cellular immunology can be applied to the study of staphylococcal infection.

Staphylococcal Virulence Factors and Route of Infection

In mice the intraperitoneal and subcutaneous routes of infection have been used extensively in the study of staphylococcal infection. These two routes provide an interesting contrast with respect to both host defence mechanisms and staphylococcal virulence factors.

The major determination of staphylococcal virulence following subcutaneous infection is the ability to inhibit the early inflammatory response of the host. This was clearly shown by Agarwal (1967) using a model of staphylococcal infections developed by Noble (1965). This model, which employed a small cotton dust pellet on which the staphylococci could be injected subcutaneously, was itself a refinement of the original work of Elek & Conen (1957) who used a suture as a foreign body to enhance the severity of subcutaneous staphylococcal infection.

Hill (1969) demonstrated that the anti-inflammatory staphylococcal virulence factor was a protein-peptidoglycan complex which could be isolated from the cell walls of skin-virulent staphylococci by treatment with deoxycholate. The active principle remaining in the residue after deoxycholate extraction was termed DOCR (Deoxycholate Residue). Virulence in these experiments was defined arbitrarily as the ability of staphylococcal strains to produce dermonecrosis at a dose of 10^5 colony forming units (cfu). Hill found that *S. aureus* strains were either virulent or non-virulent, with no intermediate forms, and that virulence corresponded with the presence of DOCR. DOCR inhibits leucocyte chemotaxis (Weksler & Hill 1969) and may act by inhibiting the kinin system (Easmon, Hamilton & Glynn, 1973).

In contast, following intraperitoneal staphylococcal infection virulence is measured by mortality, and the major determinant of virulence is the ability to resist ingestion and intracellular killing by the phagocytic cells of the peritoneal cavity (Koenig, Melly & Rogers, 1962).

The rare strains of *S. aureus* which remain capsulated after subculture *in vitro* are the most virulent, virulence being related to the thickness of the antiphagocytic capsule (Melly *et al.*, 1974).

In both subcutaneous and intraperitoneal staphylococcal infection humoral immunity is protective. Easmon & Glynn (1975a) have shown that immunity to subcutaneous infection is primarily directed at producing an accelerated early local inflammatory response, the severity of the staphylococcal skin lesion being inversely proportional to the degree of early inflammation (Agarwal, 1967). In addition, the specific neutralizing action of antibodies against extracellular staphylococcal products cannot be entirely excluded. The protective immune response does not give complete

protection against staphylococcal skin lesions, but changes their character from dermonecrosis to chronic abscess. Interestingly there is no measurable increase in the killing of staphylococci in the skin lesions of immune mice and protection against dermonecrosis must therefore result from some more subtle change in the behaviour of organisms within the lesion (Easmon and Glynn, 1975a).

TABLE I

Virulence of *S. aureus* strains injected subcutaneously on cotton dust into mice

Staphylococcal strain	DOCR	Capsule	Necrotic Index	Viable counts (cfu $\times 10^6$) 4 h	24 h
PS80	+	–	28	1.0	200
Wood 46	–	–	4	2.0	200
Smith Diffuse	–	+	0	1.5	150
M	–	+	0	2.0	300

TABLE II

Virulence of *S. aureus* strains injected intraperitoneally into mice

Staphylococcal strain	Inoculum cfu	Mortality (per group of 20)
PS80	10^9	8
	10^8	0
Wood 46	10^9	14
	10^8	0
Smith diffuse	10^8	20
	10^7	6
M	10^7	20
	10^6	0

In intraperitoneal infection protective antibody is directed against the antiphagocytic polysaccharide capsule, and facilitates the ingestion and killing of capsulated staphylococcal strains by phagocytic cells. Several capsular types of *S. aureus* have been described and humoral immunity is type specific (Melly *et al.*, 1974).

The difference between the two routes of infection is striking. The intriguing question is how the skin-virulent and intraperitoneal-virulent strains of *S. aureus* compare when given by the 'wrong' route. This was studied by Easmon & Glynn (1976) who injected mice with capsulated *S. aureus* strains subcutaneously and DOCR positive strains intraperitoneally.

Tables I and II show that the capsulated strains M and Smith Diffuse do not produce dermonecrosis, whilst a DOCR positive strain such as PS80 is 'avirulent' in the peritoneal cavity. Without going into the relevance of the two models of infection to human staphylococcal disease it is clear that the use of only one of these models would give a very limited, indeed a possibly misleading view of staphylococcal virulence factors and their role in infection.

Cell Mediated Reactions to *S. aureus*

Cell mediated reactions to bacterial antigens involve thymus dependent (T) lymphocytes and macrophages and can result either in tissue damage (hypersensitivity) or in enhanced killing of the invading organism (immunity). T lymphocytes are not, as originally thought, a homogeneous population and while some act as effector cells to produce cell mediated immunity or hypersensitivity, others act as regulatory cells, 'switching off' humoral and cell mediated immune responses.

Although most staphylococcal infections are acute, a small group of patients suffer from chronic or recurrent staphylococcal sepsis, characterized by the formation of granulomata or cold abscesses. In this latter group delayed or cell mediated hypersensitivity might be implicated in the pathogenesis of the disease. Whereas no specific immune defect is found in many cases of chronic staphylococcal disease, defects of chemotaxis and phagocytosis, which result in the retention of large quantities of staphylococcal antigen in the tissues, are particularly associated with these chronic lesions (Hill *et al.*, 1974; Quie *et al.*, 1967).

The evidence for delayed hypersensitivity to *S. aureus* in experimental animal models goes back over 50 years. Panton & Valentine (1929) and later Johanovsky (1958) and Johnson, Cluff & Goshi (1961) all showed that in rabbits repeated staphylococcal infection resulted in delayed hypersensitivity with an increase in the severity of subsequent staphylococcal lesions. Taubler (1968) was able to demonstrate delayed hypersensitivity to *S. aureus* in mice, but again only after repeated skin infections as did Easmon & Glynn (1975b).

With mice too, cell mediated hypersensitivity to *S. aureus* was associated

with enhanced severity of subsequent staphylococcal skin lesions. This was in contrast to the protective effect of antibody. Interestingly the presence of the humoral response seemed to prevent the expression of the harmful effects of the cell mediated reaction (Easmon & Glynn, 1975b).

TABLE III

Effect of repeated staphylococcal skin infection on subsequent 24 h footpad reaction to the injection of 20 μg homologous cell walls

No. infections	Footpad swelling mm	(±SD)	Signifance [a] P
0	0.38	(0.03)	
1	0.45	(0.03)	NS
2	0.41	(0.06)	NS
3	0.50	(0.08)	NS
4	0.93	(0.04)	< 0.01
4 (+ATS)	0.59	(0.07)	NS

[a] Students *t*-test. NS = Not significant

TABLE IV

Passive transfer of delayed hypersensitivity with spleen cells and its inhibition by the removal of T-cells

Donor mice	Cells transferred	Footpad swelling mm	(±SD)	Significance[a]
Control—no	cell transfer	0.41	(0.04)	
Non-infected	None	0.34	(0.05)	NS
Infected × 1	None	0.38	(0.07)	NS
Infected × 4	None	0.87	(0.05)	< 0.01
Infected × 4	A.T.S. + C′	0.48	(0.03)	NS

[a] By students *t*-test. NS = Not significant.

In this work we again used the model of subcutaneous staphylococcal infection introduced by Noble (1965) where the organisms are injected on a cotton dust pellet. Delayed or cell mediated hypersensitivity was assessed 12 days after infection by measuring the 24 or 48 hour footpad response to the local injection of staphylococcal cell walls.

Significant delayed hypersensitivity was only seen after mice had been given 4 or more weekly skin infections (table III). Delayed hypersensitivity could be passively transferred to non-infected recipients with T lymphocytes from sensitive donors (table IV).

The question remained as to how the presence of immune serum acted to prevent the harmful consequences of cell mediated hypersensitivity to staphylococcal antigens. It was necessary to inhibit the capacity of the mouse to produce antibody whilst leaving it able to mount a cell mediated response. Several workers had already shown that the injection of the alkylating agent cyclophosphamide (CY) into animals 2 or 3 days before the administration of antigen depleted B lymphocytes to a greater extent than T lymphocytes, and short lived T lymphocytes to a greater extent than long lived recirculating T lymphocytes (Turk & Poulter, 1972; Dumont, 1974). Treatment with CY enhanced cell mediated reactions where these were normally suppressed by cells susceptible to the action of CY (Turk, Parker & Poulter, 1972).

TABLE V

Effect of CY (200 mg/kg) given two days before a single subcutaneous infection of 10^5 cfu *S. aureus* on the 24 h footpad response to homologous cell walls (20 μg)

CY treatment	No. infections	24 h footpad response mm (± SD)		Significance[a]
—	0	0.37	(0.04)	NS
+	0	0.35	(0.03)	NS
—	1	0.46	(0.05)	
+	1	1.13	(0.08)	< 0.01
—	4	1.04	(0.10)	< 0.01

[a] By students *t*-test. NS = Not significant

The injection of CY (200 mg/kg) 2 days before a single staphylococcal skin infection did in fact enhance the subsequent cell mediated response to staphylococcal antigen to levels only seen after 4 or more infections in mice not treated with CY (table V) (Easmon & Glynn, 1977). This suggested firstly that the cell mediated response to *S. aureus* was regulated by cells or products of cells sensitive to the action of CY, and secondly that repeated infection in the normal mouse was needed to override the inhibition of delayed hypersensitivity rather than to boost the cell mediated response.

If pretreatment with CY could enhance staphylococcal delayed hypersensitivity by removing natural control mechanisms, then it should be

TABLE VI

Cellular suppression of CY-enhanced delayed hypersensitivity to *S. aureus*

Source of cells	Day of transfer to relative infection	Net Footpad[a] Swelling mm (±SD)		Significance[b] P
Control	No cells	1.07	(0.05)	
Non-infected donor	0	1.12	(0.12)	NS
x2 infected donor	0	1.03	(0.11)	NS
x2 infected donor	3	0.99	(0.08)	NS
x2 infected donor	7	0.78	(0.04)	< 0.01
x2 infected donor	11	0.52	(0.06)	< 0.01

[a] Mice infected on day 0 and challenged with antigen on day 12.
[b] Students *t*-test, NS = Not significant.

TABLE VII

Humoral suppression of CY-enhanced delayed hypersensitivity to *S. aureus*

Source of Serum	Day of transfer[a] relative to infection	Net Footpad Swelling mm (±SD)		Significance[b] P
Control—no	serum	1.21	(0.12)	
Non-infected donor	0	1.14	(0.09)	NS
x2 infected donor	0	0.62	(0.05)	< 0.01
x2 infected donor	3	0.77	(0.04)	< 0.01
x2 infected donor	7	1.04	(0.10)	NS
x2 infected donor	11	1.01	(0.09)	NS

[a] Mice infected on day 0 and challenged with antigen on day 12.
[b] Students *t*-test, NS = Not significant.

possible to exploit this observation to analyse those same control mechanisms. In mice given one, two, or three infections without prior treatment with CY there must exist both active suppressive mechanisms, as well as the potential, albeit suppressed, for cell mediated hypersensitivity. A series of experiments was therefore planned in which either cells or serum were transferred from infected unresponsive mice into CY-treated infected recipients. The aim was to look for the suppression of CY-enhanced delayed hypersensitivity in these recipients by humoral or cellular factors from the unresponsive donors.

Two patterns of suppression were seen:

Spleen cells from unresponsive donors suppressed CY-enhanced delayed hypersensitivity to *S. aureus* only when given within four days of antigen challenge in the footpad (table VI). The cells responsible for this had the characteristics of B-lymphocytes (Easmon and Glynn, 1979).

Immune serum (or plasma) from infective donors, however, suppressed CY-enhanced delayed hypersensitivity only when transferred within 3 days of staphylococcal infection. It was wholly ineffective when given at the time of antigen challenge (table VII).

DISCUSSION

From this rather complex series of experiments it appears that cell mediated hypersensitivity to *S. aureus* in mice is a potentially harmful immune response which is only seen after repeated skin infection. This response is regulated by B lymphocytes which probably act on its expression, and by factors present in immune serum which act quite separately on the induction of the cell mediated reaction. These control mechanisms, which have been analysed by the use of CY, an agent which effectively destroys them, normally serve to limit the harmful effects of the uncontrolled cell-mediated response to *S. aureus*. Repeated *S. aureus* infection, can, however, override these controls in some way which is not yet understood.

At the beginning of the last section I mentioned that in staphylococcal disease in man chronic granulomatous lesions were sometimes seen when the underlying immune defect allowed the accumulation of staphylococcal antigen in the tissues. The best example is chronic granulomatous disease. The primary defect in this condition lies in the oxidative metabolism of polymorphonuclear and mononuclear phagocytes, which are unable to kill catalase positive organisms mainly *S. aureus* (Quie *et al.*, 1967). These organisms persist intracellularly and chronic granulomatous lesions, with the histological characteristics of delayed hypersensitivity reactions, occur in the skin, lymph nodes and viscera.

It may be that the failure to remove staphylococcal antigen in this disease leads to the formation of tissue destroying cell mediated hypersensitivity reactions because of an overriding of the control mechanisms which normally prevent the expression of this response.

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