PATHOLOGY

Staphylococcal Interference in Infections in Embryonated Eggs

THE protection afforded by infection with avirulent virus against subsequent challenge with a second lethal virus[1] has led to the demonstration of interferon production by infected cells *in vitro* and *in vivo*[2,3]. Despite clinical evidence of the importance of bacterial interference, and experimental demonstration that avirulent staphylococci are capable of inhibiting nasal colonization with virulent staphylococci[4], bacterial interference has not been extensively investigated in experimental models. Investigations of experimental staphylococcal infections in embryonated eggs have demonstrated that infection with strains of staphylococci that are not virulent for eggs affords significant protection against subsequent challenge with virulent staphylococci.

Ten-day-old embryonated eggs were injected with from 10 to 100 colony forming units (c.f.u.) of avirulent coagulase-negative *Staphylococcus epidermidis* or coagulase-positive *Staphylococcus aureus* intra-allantoically, and one-half of each group were challenged with 10–100 c.f.u. of virulent *Staphylococcus aureus* 48 h later. The remainder of the eggs infected with avirulent staphylococci served as controls and were injected with 0·1 ml. of saline two days later. Virulence controls received 0·1 ml. of saline on day 10 followed by intra-allantoic injection of 0·1 ml. of a broth culture of virulent *S. aureus*, diluted to contain 10–100 c.f.u., 48 h later. Eggs were candled daily to determine viability. Table 1 demonstrates the fatality rates in each of these experimental groups. Infection with either *S. epidermidis* or avirulent *S. aureus*, lines 1 and 4, produced fatality rates ranging from 18 to 20 per cent. The fatality rates observed after saline injection followed by challenge with virulent *S. aureus*, lines 3 and 6, approximated 80 per cent. Prior infection with avirulent strains, lines 2 and 5, afforded significant protection against subsequent challenge with virulent staphylococci (lines 2 and 5 versus lines 3 and 6 : $\chi^2 = 40$; $P < 0{\cdot}001$). The fatality rates of eggs infected with avirulent staphylococci followed by virulent staphylococci (28–30 per cent) did not differ materially from those observed in eggs infected with avirulent strains alone. Similar protection was afforded using four other strains of avirulent *S. epidermidis* and *S. aureus* for the initial infection. Significant protection was also observed using different challenge strains of virulent *S. aureus*. In addition, prior infection with avirulent staphylococci afforded significant protection against challenge with egg virulent strains of *Salmonella typhimurium*, *Diplococcus pneumoniae* and *Streptococcus pyogenes*.

The mechanism of protection could not be explained on the basis of alterations in the chorio-allantoic fluid or exhaustion of nutrient materials essential for the growth of the challenge strain. Allantoic fluid taken from uninfected controls and from eggs infected with avirulent staphylococci, sterilized by filtration through a Millipore filter (0·45μ), were inoculated with the challenge strain of *S. aureus* for determination of growth curves. The growth of virulent *S. aureus* was identical in both types of allantoic fluid, indicating that previously infected allantoic fluid was capable of supporting optimal growth of the challenge strain and that no filterable substance capable of inhibiting the extra-cellular growth of the challenge strain was produced in the allantoic fluid as a result of prior infection. *In vivo* growth of the challenge strain in the presence of active infection with a non-virulent strain varied with different strains. One strain of virulent staphylococci attained similar population densities ($\pm 10^9$ bacteria/ml.) in the allantoic fluid of infected eggs to those observed in uninfected eggs. A second challenge strain, however, failed to multiply well in the presence of active infection with an avirulent strain.

The protective effect of active bacterial infection with avirulent staphylococci against super-infection in these experimental infections appears to be well established from these investigations. The results support the clinical observations of the protective effect of the normal body flora. In addition, the findings closely parallel the experimental demonstration of the ability of avirulent staphylococci to inhibit nasal colonization and staphylococcal infection in infants[4]. The mechanism of protection in this experimental system remains undefined and the subject of further investigation. Several features suggest that bacterial interference in this system reflects a phenomenon different from that of viral interference and interferon production.

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Table 1. PROTECTIVE EFFECT OF INFECTION WITH AVIRULENT STAPHYLOCOCCI AGAINST SUBSEQUENT CHALLENGE WITH VIRULENT STAPHYLOCOCCI IN EMBRYONATED EGGS

Protective strain (day 10)	Challenge (Day 12)	Fatalities No. deaths/No. eggs infected	%
S. epidermidis (±100 c.f.u.)	Saline	7/40	18
S. epidermidis (±100 c.f.u.)	Virulent *S. aureus* (±100 c.f.u.)	11/40	28
Saline	Virulent *S. aureus* (±100 c.f.u.)	31/40	78
Avirulent *S. aureus* (±100 c.f.u.)	Saline	8/40	20
Avirulent *S. aureus* (±100 c.f.u.)	Virulent *S. aureus* (±100 c.f.u.)	12/40	30
Saline	Virulent *S. aureus* (±100 c.f.u.)	32/40	80

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Non-specificity of Thioflavine-*T* as an Amyloid Stain

IT has been claimed by several workers[1,2] that thioflavine-*T* is more sensitive and consistent than congo red and methyl violet as an amyloid stain. Vassar and Culling[3] found no substances which gave false positive reactions apart from myeloma casts and keratin, which do not present a diagnostic problem. McAlpine, Radcliffe and Friedman[4] support these findings but point out that no extensive control investigation has been carried out on 'hyaline' substances with thioflavine-*T*.

The fluorescent technique described by Culling[5] was therefore adopted as a routine staining method for amyloid at the Royal Free Hospital. It was then discovered that there was often a marked variation in the intensity of

Table 1

Case No.	Aetiology	Tissue	Congo red	Methyl violet	Thioflavine-*T*	Autofluorescence
1	Primary amyloidosis	Kidney	±	±	+++	−
2	Amyloidosis secondary to tuberculosis	Adrenal	++	++	+++	−
3	Amyloidosis secondary to tuberculosis	Kidney	+	±	±	−
4	Amyloidosis secondary to tuberculosis	Kidney	++	++	±	−
5	Amyloidosis secondary to tuberculosis	Kidney	++	++	+	−
6	Amyloidosis secondary to myeloma	Kidney	±	±	+	±
3	Amyloidosis secondary to tuberculosis	Spleen	++	++	−	±