Scand J Infect Dis, Suppl. 24: 82–85, 1980

Bacterial Interference and Competition[1]

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The struggle among microorganisms to survive and to establish an ecological niche in the human body has fascinated medical scientists almost as much as the struggle between a specific infectious agent and its human host. In our efforts to simplify, however, we often deal with bacteria in pure culture and consider the interactions of man and pathogen aside from the possible influences of other microorganisms in the environment. This review will focus on some aspects of bacterial interference and competition, with emphasis on streptococci and other gram-positive cocci.

Broadly speaking, the mechanisms of bacterial antagonism would include depletion of some essential substance (e.g. a substrate or a vitamin), alteration in the microenvironment (e.g. change in pH) or production of an antagonistic substance (e.g. an antimicrobial substance). The specific mediators that may play a role in bacterial antagonism range from rather complex entities or substances (such as bacteriophages, bacteriocins and bacteriolytic enzymes) to simple molecules (such as ammonia, lactic acid, free fatty acids, and hydrogen peroxide). This brief analysis will emphasize the bacteriocins, which at long last are being studied more intensively in gram-positive bacterial species (26). It will examine in particular the evidence for occurrence of bacterial interference in man or other mammalian hosts and the question of whether bacteriocins are related to bacterial virulence.

Since colicins have been the most exhaustively studied of all the factors possibly related to bacterial antagonism, it is rather ironic that attempts to demonstrate a role for colicins in the interactions of flora within the gut have been generally negative or at best ambiguous (10). The stability of the gastrointestinal flora and the prevention of invasion by exogenous bacteria are more likely related to competition for nutrients and to production of volatile fatty acids, bile acids, and low pH and Eh by the native flora. However, studies of mixed peritoneal infections in mice, of urinary tract infections in rats and of keratoconjunctivitis in guinea pigs strongly suggest that colicins may be significant ecological determinants outside the intestinal tract (15). For example, Braude and Siemienski (1) have shown that injection of a colicin producing strain of *E. coli* in one kidney of a rat and injection of a sensitive, non-colicin producing strain in the contralateral kidney results in elimination of the colicin-sensitive strain in urine collected from the bladder.

Clinical interest in bacterial interference in man was rekindled by the studies of Shinefield et al. (23) indicating that colonization with one strain of *Staphylococcus aureus* interfered with colonization by a second strain of the same species. In other studies Martin and White (17) showed that the presence of coagulase-negative staphylococci or diphtheroids prevented colonization of the nose by coagulase-positive staphylococci. The suppression of resident flora by antimicrobial agents increases the susceptibility of the nasal mucous membranes to colonization or recolonization by *Staphylococcus aureus* by natural or artificial means. Implantation of a strain of *Staphylococcus aureus* of low virulence (strain 502A) has been used to control outbreaks of staphylococcal infections in newborn nurseries and in the management of recurrent furunculosis (23). The mechanism of bacterial interference operative in these intervention efforts in humans is uncertain. Most *in vivo* models, including those employing experimental burns, have also failed to explain adequately the factors responsible for interference between strains of staphylococci. Although several staphylococcal bacteriocins have been identified and characterized, their possible role in human coloniza-

[1] The studies from the author's laboratory included in this publication were supported by grants from the National Institute of Allergy and Infectious Diseases, National Institutes of Health (Grants No. AI 09527 and AI 08724). The author is a Career Investigator of the American Heart Association.

tion or disease is not clear (26). However, studies of a model of impetigo in hamsters (7) have shown that mixed infections with a bacteriocin-producing staphylococcus and a sensitive Group A streptococcus often results in elimination of the streptococcal strain recovered from the experimental lesion.

The epidemiologic aspects of interactions between streptococci and other bacteria in the throat have been best studied by Sanders (22) and his collaborators. In prospective studies, these authors (3) showed that the children who subsequently became colonized with Group A streptococci had a lower percentage of throat cultures containing flora inhibitory or bactericidal for Group A streptococci than those who did not become colonized with these organisms. In further studies Sanders et al. (21) have demonstrated that oral treatment with penicillin (and to a less extent tetracycline) results in a decrease in the percentage of cultures with interfering organisms, a decrease which persisted for as long as three weeks after therapy. Other studies (20) suggested that interfering flora are more often recovered during the months with the highest prevalence of Group A streptococci and that the prevalence of bactericidal organisms increased with age, perhaps contributing to the resistance of adults to streptococcal infections as compared with children. The organisms showing the greatest ability to inhibit were alpha-hemolytic streptococci, non-hemolytic streptococci and neisseriae. The nature of the bacterial interference in these studies is uncertain, although some *in vitro* studies suggested that interference may have resulted from depletion of an unidentified substance in trypticase soy broth.

Evidence that alpha-hemolytic streptococci may play an important protective role against invasion of the oropharynx by gram-negative bacilli is provided in the studies of Spı unt et al. (25) who demonstrated that eradication of the alpha-hemolytic streptococci resulted in overgrowth by gram-negative enteric bacilli at this site.

The recent demonstration by Dajani and coworkers (5) that many strains of alpha-hemolytic streptococci produce bacteriocin-like substances raises the question of whether these substances contribute to bacterial interference in the oropharynx. These inhibitory substances have been called viridins and one of them viridin B has been partially purified and characterized in some detail (4, 6). This bacteriocin-like substance (and apparently other viridins as well) has some rather unusual properties that set it aside from many other well-established bacteriocins: 1) It is obtained only by mechanical disruption of the bacterial cell. 2) It is apparently virtually unique in that it inhibits many gram-negative as well as gram-positive species. 3) It is bactericidal for a *Neisseria sicca* strain but only bacteriostatic for a coagulase-negative staphylococcus. 4) It is heat labile. 5) It has a narrow pH range of activity. 6) Adsorption to susceptible bacteria apparently does not occur. 7) Viridin B is inactivated by mammalian hemoglobin. Although in our present state of knowledge, it has been recommended that the definition of a bacteriocin should be kept somewhat loose (26), it is clear that the viridins differ even more than other bacteriocins of gram-positive bacteria from the classical colicins and may in fact represent a new class of antibiotic substances. Further studies are needed to establish their ecological importance, which at present is only presumptive.

The fact that Group A streptococci generate peroxide, a potent bactericidal agent (16), has confounded the question of whether and how frequently the inhibitory effects produced by these organisms can be attributed to elaboration of a bacteriocin. However, the studies of Tagg et al. (27) clearly demonstrate that these organisms do produce a well-defined substance that fits most if not all of the criteria suggested for the definition of a bacteriocin. This bacteriocin of Group A streptococci, designated streptocin A or streptococcin A-FF22, is an extracellular protein which has been purified from supernates of tryptic soy broth cultures of the Group A streptococcal strain FF22.

Like the bacteriocin of phage group II staphylococci (staphylococcin C55), this bacteriocin of Group A streptococci is remarkably heat stable, resisting boiling for 30 minutes, and it is active against a number of gram-positive species. It is bactericidal but not lytic for susceptible strains of Group A streptococci. It is temperature dependent in its lethal effect and produces a striking array of metabolic abnormalities: 1) inhibition of synthesis of DNA, RNA, and protein; 2) degradation of RNA; 3) prevention of the uptake and incorporation of glucose by sensitive cells (27).

Streptococcin A-FF22 is produced by L-form derivatives of the parent strain (11). It is plasmid controlled and can be transduced (28).

This bacteriocin of Group A streptococci is the smallest of all bacteriocins so far described, with a molecular weight of only about 8 000 (27). This may

account for its failure to induce neutralizing antibody when injected into animals, a property which should favor its continued uninhibited activity in animal or human hosts. Despite the extensive characterization of this bacteriocin in the laboratory, almost no information is available concerning the possible significance of this or other bacteriocins of beta hemolytic streptococci in nature. One can only speculate that they may play a role in maintenance of the carrier state of Group A streptococci in the throat and of group B streptococci in the vagina and that they may contribute to the ability of these streptococci to colonize and to establish infection in human or animal hosts. A study by Kolesnichenko and Totolyan (14) indicated that strains of Group A streptococci producing bacteriocin-like activity were most prevalent during a period of high incidence of acute streptococcal disease and were characterized by a wide spectrum of activity and by striking zones of inhibition of indicator strains. These observations support the notion that bacteriocins may indeed have a function in the epidemiology of streptococcal infections.

An extracellular bacteriocin from *Streptococcus mutans* has been purified and characterized (19). Its activity is apparently limited to a variety of strains of the genus *Streptococcus*. In view of the role of *Streptococcus mutans* in the production of dental caries, it is of interest that studies of oral streptococci have revealed no correlation between cariogenicity and the production of bacteriocin-like activity (13).

With respect to the group D streptococci, Guze et al. (8) have produced evidence suggesting that bacteriocin-production may be an important factor in influencing the outcome of mixed urinary tract infections in rats. In other studies, Montgomerie et al. (18) have shown that strains of group D streptococci with bacteriocin-like activity were recovered in larger numbers from infected kidneys of rats than strains lacking this activity. The bacteriocins of group D streptococci have been well studied, particularly by Brock and Davie (2) and by Jackson (12) who have demonstrated that one of these, the type 1 bacteriocin produced by strains of *Streptococcus faecalis*, subspecies *zymogenes*, is probably identical with or related to its hemolysin.

Since this bactericidal agent of group D streptococci is both bacteriolytic and hemolytic it raises again the question of the limits of the definition of bacteriocins and the possible relationship of bacteriocins to other biologic or pathogenic properties of bacteria. This is often difficult to ascertain due to intrinsic problems of separation of complex mixtures of bacterial products and also to the fact that bacteriocin production and other characteristics may be controlled by closely linked genetic determinants on the same plasmid. The best evidence relating virulence to bacteriocinogenicity is that of Smith and Huggins (24) who showed that strains of *E. coli* producing colicin V survive better in inoculated animals than strains lacking this colicin, perhaps accounting for the increased virulence of these colicin-producing strains (for review see Konisky, ref. #15 and Hardy, ref. #9). As with virulence, attempts to relate bacteriocins to cytotoxic effects for mammalian cells has many problems of interpretation. As recently reviewed by Konisky (15), there is evidence that colicin ES inactivates ribosomes of mouse ascites cells and that a bacteriocin of a *Streptomyces* species is cytotoxic for Hela cells.

In conclusion, further studies are needed to understand and to evaluate the significance of bacteriocins and other bacterial interference factors in the ecology and pathogenicity of microorganisms in the animal and human host. In particular, better models for examining bacterial antagonism, prospective studies in animals and man, and further scrutiny of the possible contribution of bacteriocins to the virulence of bacteria would bring us closer to a confidential comprehension of the role of bacterial antagonism in nature.

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