Virulence and Coagulases of *Staphylococcus aureus.** (25761)

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The ability of *Staphylococcus aureus* to produce coagulase has customarily been taken as an *in vitro* reaction indicating potential virulence for this organism. Evidence for the existence of 2 different kinds of coagulase was first reported by Duthie(1). The soluble coagulase causes clotting of plasma (fibrinogen) in presence of an accessory factor, whereas the bound coagulase causes clumping of the organism by plasma or fibrinogen alone. It is of interest to determine if either kind of coagulase is of itself an essential virulence factor. This was done by selecting a strain of *S. aureus* known to be virulent for rabbits and possessing both kinds of coagulase. Mutants lacking either kind of coagulase were then derived from this strain and checked for virulence in rabbits. A simultaneous loss of virulence could possibly indicate a direct relationship with the particular missing coagulase, whereas if the mutant retained its virulence in the absence of a coagulase then this factor could not be essential for virulence. The parent strain, *S. aureus* 18Z, used in these experiments, was chosen because it possessed several possible virulence factors, including the soluble and bound coagulases, alpha and delta hemolysins, leucocidin activity, hyaluronidase and fibrinolysin. It was sensitive to penicillin, streptomycin, and other antibiotics and was of the phage type 42 B/52/80/81. This strain was originally isolated from the human nasopharynx and was virulent for rabbits.

Materials and methods. To isolate coagulase negative mutants, 6 hour old cultures of *S. aureus* strain 18Z grown in trypticase soy broth were washed with saline and subjected to UV irradiation sufficient to reduce the population to 1% survival. The irradiated suspensions were then diluted and spread on trypticase soy agar containing 15% human plasma. On this medium coagulase positive staphylococci have been reported(2,3) to produce an opalescent halo around the colonies, presumably due to deposition of fibrin in the agar. Attempts to isolate coagulase-lacking mutants were made by picking colonies that formed either no halos or halos of much lighter density. The colonies thus selected were individually checked for production of the coagulases by the slide and tube tests. In testing for bound coagulase, 0.1 ml of an overnight broth culture, concentrated 5-fold by centrifugation, was mixed with 0.05 ml of human plasma on a depression slide and the cells observed for evidence of clumping for at least 5 minutes. In the case of bound coagulase-positive cultures, the clumping was usually seen within 1 to 3 minutes. Production of soluble coagulase was detected by mixing in a Wassermann tube 0.1 ml of concentrated culture, 0.5 ml of human plasma, and 0.1 ml of trypticase soy broth. The tubes were incubated in a water bath for 3 hours at 37°C and periodically examined for signs of clotting. This usually became evident within 1 to 2 hours in the case of soluble coagulase-positive organisms. Mutants which appeared to lack one kind of coagulase by these tests were rechecked several times using different lots of plasma from humans and normal rabbits to minimize the chance of observing negative coagulase reactions due to inhibition by antibodies which might be present in a particular sample of plasma. Mutants were tested for virulence by injecting normal rabbits either intracutaneously or intravenously with 10^8 organisms. Animals inoculated intracutaneously were observed for several days to see if local lesions developed. Rabbits inoculated intravenously were held for 7 to 10 days and then sacrificed. Organs of these animals were then examined grossly for staphylococcal lesions. In addition, samples of liver and kidney were plated out on trypticase soy agar to determine presence of viable organisms. In some experiments the staphylococci recovered from these organs were reexamined

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to note whether their coagulase activities were the same as for the organisms originally inoculated into the animal. Various activities of the strains were determined as follows: hemolysin patterns according to the method of Elek and Levy(4), leucocidin by the method of Valentine(5), fibrinolysin by the procedure used by Lack and Wailling(6), and hyaluronidase by means of the ACRA test(7).

Results. The screening method used to isolate coagulase-negative mutants by means of the plasma-agar medium did not prove completely satisfactory. At least 53 "haloless" colonies had been picked during a number of experiments, but were later found to produce both bound and soluble coagulase. Only one such "haloless" isolate proved to be a soluble coagulase-negative mutant. However, a few randomly picked "halo-forming" colonies did lack either one or the other kind of coagulase. From among all these isolates 3 were chosen and tested for virulence in rabbits. 1) Mutant 18Z-B, a halo producer like the parent 18Z strain, produced soluble coagulase, but lacked bound coagulase. 2) Mutant 18Z-C did not form halos on the plasma-agar medium. It was negative for soluble coagulase, but possessed the same amount of bound coagulase as the parent 18Z strain. 3) Mutant 18Z-D was originally isolated as a "haloless" colony, but on subsequent rechecking always produced a halo on plasma agar. It produced both kinds of coagulase. These 3 mutants produced the same reaction, insofar as halo formation is concerned, when grown on trypticase soy agar containing fibrinogen (fraction I) as when grown on plasma-agar. To minimize the possibility that the 3 isolates were not mutants of the parent strain, but contaminating staphylococci, the other characteristics of these isolates were compared with those of the parent strain. With the exception of 18Z-C which lacked fibrinolysin in addition to soluble coagulase all mutants exhibited the same characteristics† as the parent strain.

The results obtained in rabbits inoculated with the mutants were consistent. Rabbits given 18Z-B (lacking bound coagulase) or 18Z-C (lacking soluble coagulase) intradermally developed localized abscesses of the same type and severity as those produced by a comparable dose of the parent 18Z strain. When these 2 mutants were administered by the intravenous route, renal and, usually also, liver abscesses were found. The mutants could be isolated in large numbers from these organs and subsequent testing revealed that coagulase activity of the staphylococci recovered from these organs was identical with that of the mutant originally inoculated into the animal. Mutant 18Z-D (possessing both kinds of coagulase) failed to produce lesions by the intradermal route and could not be recovered from kidneys, or livers, of animals inoculated intravenously 7-10 days previously.

Since mutant 18Z-D gave every indication of being avirulent for rabbits, a comparison of the characteristics of this mutant was made with those of the parent 18Z strain to see whether any "virulence factor" had been lost which might account for the observed loss in virulence. The results demonstrated that the avirulent 18Z-D mutant was indistinguishable from the parent virulent 18Z strain in every property thus far tested.

Discussion. These studies indicate that *S. aureus* mutants lacking either bound or soluble coagulase are just as virulent for rabbits as is the parent strain possessing both kinds of coagulase. Therefore, the coagulases do not appear to be essential for virulence of this organism. This is not to say that the coagulases, either singly or in combination with other factors, play no role whatsoever in pathogenesis of staphylococcal disease since our data cannot shed light on this question. Furthermore, the data do not negate the value of testing staphylococci for coagulase production since these are substances commonly produced by the species in question. In fact the rather high correlation of coagulase production, and for that matter, alpha hemolysin and hyaluronidase production with observed virulence of *S. aureus* may result from the fact that these are all common properties of this species and that most members of the species are virulent. Yet the mere presence of these characteristics would not necessarily imply

† Alpha and delta hemolysins, hyaluronidase, fibrinolysin, leucocidin, phage type, lactose and mannitol fermentation, and sensitivity to 13 antibiotics.

that they are responsible for virulence.

The 18Z-C mutant actually appears to be a double mutant since it lost ability to produce fibrinolysin in addition to losing ability to produce soluble coagulase. Since this mutant is fully virulent it would appear that fibrinolysin is also not essential for virulence.

Isolation of the avirulent 18Z-D mutant likewise offered the possibility of identifying a factor which if lost results in loss of virulence. However, this mutant is identical with the parent strain in all aspects thus far studied with the exception of virulence. Further studies comparing these 2 strains are in progress.

Summary. Three mutants were isolated from a single strain of *Staphylococcus aureus* of known virulence for rabbits. Two mutants, one lacking the soluble type of coagulase and the other lacking the bound type of coagulase, were still virulent for rabbits. The third mutant which produced both kinds of coagulase had lost its virulence for rabbits.

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Correlation of Tryptamine-Induced Convulsions in Rats with Brain Tryptamine Concentration. (25762)

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Tedeschi *et al.*(1) recently reported appearance of clonic convulsions of forepaws of rats following i.v. injection of tryptamine (TA). A good dose response relationship was obtained between quantity of TA injected and relative frequency and intensity of resulting convulsions. This correlation suggested that the observed pharmacologic responses were associated with increased levels of TA in the brain. On the assumption that amine in rat brain was metabolized chiefly by monoamine oxidase (MAO), Tedeschi *et al.* postulated that inhibition of enzyme activity should prolong the elevated brain TA levels and, therefore, potentiate convulsant properties of the amine. Although pharmacologic expectations were fulfilled and procedure shown to be suitable for screening and pharmacologic evaluation of MAO inhibitors, the question of relationship with brain biochemistry remained unanswered. The purpose of our study was to determine whether the TA-induced clonic convulsions in rats pretreated with a MAO inhibitor were correlated with concentration of amine in the brain.

Procedure and methods. Male albino rats, Wistar strain, weighing between 175 and 225 g were used. Tryptamine hydrochloride in physiologic saline was injected into tail vein in dose of 5 mg/kg, calculated as free base. Trans 2-phenylcyclopropylamine hydrochloride (tranylcypromine)(2) and 1-isonicotinyl-2-isopropyl hydrazine phosphate (iproniazid) were dissolved in H_2O and administered orally. Doses of drugs used refer to free base content. Following injection of TA the animals were observed for 60-75 seconds for signs of convulsive activity, then sacrificed by decapitation. Brains were immediately removed, rinsed free of adsorbed blood, blotted lightly, quickly weighed and placed in chilled glass tissue grinder of Potter-Elvehjem type fitted with teflon pestle. For determination of TA the brains were homogenized and analyzed according to method of Hess and Udenfriend(3). Separate brains were homogenized