Influence of Coagulases and Route of Injection on Staphylococcal Virulence in Mice.* (26186)

SAMIH Y. ALAMI† AND FLORENE C. KELLY (Introduced by Martin M. Cummings)
Dept. of Microbiology, University of Oklahoma School of Medicine, Oklahoma City

The rare strains of staphylococci which lack either free coagulase or bound coagulase (the clumping factor responsible for agglutination and compact colony formation in fibrinogen), but not both these properties, have been considered to be of doubtful pathogenicity(1). However, Johanovsky(2) suggested that the clumping property rather than free coagulase may allow survival of staphylococci within phagocytes. Hunt and Moses (3) reported that only one of 2 serologically distinct variants of the Smith strain of *Staphylococcus aureus* was virulent for mice although both were coagulase positive. The mouse virulent variant formed diffuse colonies in plasma soft agar, whereas the colonies of the avirulent variant were compact in this medium. Alami and Kelly(4) concluded that the compacting of staphylococcal colonies in fibrinogen-containing soft agar is an expression of the clumping factor and need not be due exclusively to antibody action. The present study was initiated to compare free coagulase and bound coagulase as virulence factors and to determine whether route of injection affected the results in mice.

Materials and methods. Twelve strains representing typical coagulase positive and coagulase negative staphylococci as well as variants which lack either free or bound coagulase (hereafter referred to as coagulase and clumping factor, respectively) or both these properties were selected for study. The compact colony (S) and diffuse colony (Sv) forming variants of the Smith strain were kindly supplied by Dr. G. A. Hunt. Others were isolated locally from hospital personnel and out-patients or were variants of these strains. Coagulase and clumping factor activities were determined by the soft agar technic(4) as well as by conventional methods. Sterile filtrates of 18 hour 10% serum brain heart infusion cultures were used in coagulase titrations(5). Strains were also examined for other properties usually associated with pathogenicity, particularly hemolysins and staphylokinase. Four coagulase positive, clumping factor positive strains (S, V, E33 and H) comprised Group I. The 3 coagulase positive, clumping factor negative strains of Group II included the Sv strain and 2 previously unreported strains, K6 and K93, which have remained stable as to these properties since 1956. Strain K6 was isolated from the nasopharynx of a healthy carrier and K93 from a cutaneous ulcer. Three coagulase negative, clumping factor positive strains, Hvl, E35 and E35s, made up Group III. Hvl first appeared as a variant in soft agar cultures of the H strain. The other 2 are rough (E35) and smooth (E35s) forms of the same strain. Also tested as Group IV were 2 coagulase negative, clumping factor negative staphylococci, Hv2 and E33v, which are variants of H and E33, respectively.

Intravenous and intraperitoneal median lethal doses (LD_{50}) were determined by injecting an equal number of male and female, 20 to 30 g albino mice of CFW Webster Swiss origin with washed cells from 24 hour trypticase soy broth cultures. Cells were twice washed by centrifuging (2400 g) and resuspending the sediment in phosphate buffered saline. For each strain, 0.3 ml of each of 5 dilutions of a buffered saline suspension was injected intravenously into each of 6 mice and intraperitoneally into another group of 6 mice. Similarly, intraperitoneal challenge was repeated with cells suspended in 5% hog gastric mucin. Suitable controls included intravenous and intraperitoneal injection of 0.3 ml amounts of a sterile broth culture supernatant and a heat killed cell suspension of the highest challenge dose of each strain. The mice were observed for 14 days. At autopsy organs and body fluids

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† Present address: Dept. of Bacteriology, School of Med., American Univ., Beirut, Lebanon.

TABLE I. Intravenous and Intraperitoneal Median Lethal Doses (LD_{50}) of Typical Staphylococci and Strains Which Lack Either Coagulase or Clumping Factor Activities.

Group	Strain	Coagulase titer	Clumping factor	LD_{50} Intrav.	LD_{50} Intraper. Without mucin	LD_{50} Intraper. With mucin
I	S	1:1024	+	4.5×10^8	1.1×10^9	1.2×10^8
	V	1:256	+	2.7×10^8	3.2×10^8	1.0×10^8
	E33	1:128	+	2.5×10^8	4.2×10^9	1.7×10^9
	H	1:64	+	6.4×10^8	2.8×10^9	1.6×10^8
II	Sv	1:256	—	4.0×10^7	1.1×10^7	1.0×10^4
	K6	1:8	—	1.8×10^9	2.1×10^8	6.3×10^5
	K93	1:8	—	1.7×10^9	1.8×10^8	1.1×10^5
III	Hv1	0	+	9.7×10^8	3.6×10^9	1.0×10^9
	E35	0	+	1.5×10^9	1.6×10^9	3.8×10^8
	E35s	0	+	8.5×10^8	2.8×10^9	3.9×10^8
IV	Hv2	0	—	9.4×10^9	3.2×10^9	3.2×10^8
	E33v	0	—	1.3×10^{10}	4.5×10^9	1.8×10^8

were examined microscopically and culturally.

Results. When injected intraperitoneally in 5% mucin not only the diffuse colony variant of the Smith strain (Sv), but also the other 2 coagulase positive, clumping factor negative strains of Group II (K6 and K93), were more virulent than organisms of other groups regardless of coagulase titer (Table I). There was no evidence that other properties of these strains explained their unusual pathogenicity.

The Sv strain proved to be the most virulent of all strains tested whether introduced intravenously or intraperitoneally, with or without mucin. The other 2 strains of Group II were no more virulent when injected intravenously than those of other groups, nor were their mucin-free intraperitoneal LD_{50} values significantly lower when all strains were compared by this challenge. However, small, though consistent differences were observed between the intravenous and mucin-free intraperitoneal LD_{50} of certain strains, notably those of Group II (Fig. 1). An overall analysis of variance of strain, group, route of injection, replicate and route of injection by group interaction indicates that only the last comparison is of statistical significance with $P < 0.01$. Although LD_{50} could not be correlated regularly with coagulase titer (*e.g.*, strains S and Sv), the coagulase negative, clumping factor negative variants Hv2 and E33v were slightly less virulent intravenously, but not intraperitoneally, than their coagulase positive counterparts H and E33. None of the control mice died.

Post-mortem cultures indicated that the character of the staphylococci injected remained stable in the infected mice. However, during the study, a mucoid variant (K93m) with a demonstrable extracellular slime layer was recovered occasionally, together with the non-mucoid parent organism, from mice injected with K93. The intraperitoneal LD_{50}

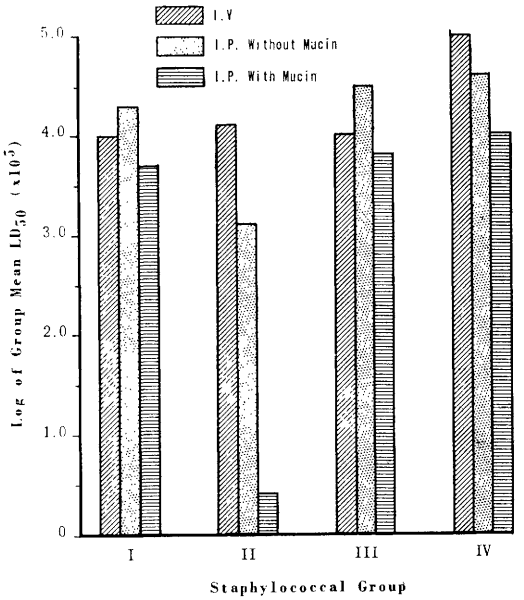

FIG. 1. Influence of route of inj. and mucin on mouse virulence of 12 strains of staphylococci grouped according to coagulase (C) and clumping factor (CF). Group I, C+, CF+; Group II, C+, CF−; Group III, C−, CF+; Group IV, C−, CF−.

of K93m was greater (6.2×10^8 without mucin; 1.2×10^7 with mucin) than that of K93 (1.8×10^8 without mucin; 1.1×10^5 with mucin), whereas intravenous LD_{50} values of both organisms were almost identical.

Discussion. The mouse is relatively resistant to infection with staphylococci and large numbers of these organisms are required to kill the animal. From the above results it is apparent that the intravenous and intraperitoneal median lethal dose was not significantly influenced by free coagulase, clumping factor or, indeed, by any other recognized property of the staphylococci. Kapral and Li (6) recently reported that *S. aureus* mutants which lacked either bound or soluble coagulase were just as virulent for rabbits when injected by the intradermal or intravenous route as was the parent strain which possessed both bound and free coagulase.

Reports of the influence of route of injection on susceptibility of mice to staphylococcal infection have been controversial(7). Unfortunately, this study does not provide conclusive evidence on this point. The fact that small differences between intravenous and mucin-free intraperitoneal LD_{50} were observed consistently in the case of certain strains, particularly those of Group II, suggests that similar studies should be extended to a larger series of staphylococcal strains and possibly to other genetic lines of mice. The clumping factor negative variant of the Smith strain (Sv) was the most virulent of all strains tested by either route of injection despite a lower coagulase titer than that of the clumping factor positive variant (S) of the same strain. Undoubtedly the "mouse virulent" Smith variant possesses some factor or factors yet to be identified.

The enhancing effect of mucin on virulence of coagulase positive, clumping factor negative staphylococci is in sharp contrast to that of the other strains tested. This finding agrees with that of Hunt and Moses with respect to the diffiuse and compact colony forming variants of the Smith strain and it appears to be true of other coagulase positive staphylococci which lack the ability to agglutinate in presence of fibrinogen. These observations, together with the fact that mucin was more effective in decreasing LD_{50} of K93 than that of its mucoid variant, suggest the existence of a property or cellular component other than bound coagulase or extracellular slime which interacts with mucin in intraperitoneal challenge to affect virulence of staphylococci in mice.

Summary. Intraperitoneal and intravenous LD_{50} values of 12 strains of staphylococci, grouped according to free coagulase and the so-called clumping factor or bound coagulase, indicate that mouse virulence of *S. aureus* is influenced less by route of injection than by nature of the organisms injected. Mucin markedly enhanced mouse virulence for intraperitoneally injected coagulase positive, clumping factor negative staphylococci but not that of other strains. Neither free nor bound coagulase *per se* appeared to be a critical factor of virulence for mice.

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