Clot Formation by Group A Streptococci

HAIG DONABEDIAN* AND MICHAEL D. P. BOYLE
Medical College of Ohio, Toledo, Ohio

Received 24 November 1997/Returned for modification 21 January 1998/Accepted 25 February 1998

Group A streptococci of several different M serotypes can cause human plasma to clot in nutrient-poor media. Addition of glucose to the medium prevents clot formation. Once formed, clots are stable for several days and can be lysed on addition of exogenous streptokinase or urokinase. Clot lysis can also be achieved by addition of glucose to a clot containing wild-type group A streptococci but not clots containing an isogenic mutant in which the ska gene was inactivated.

During experiments designed to analyze the interaction of group A streptococci with the human plasmin(ogen) system, we noted that certain streptococcal isolates incubated in chemically defined medium (20) without glucose or sodium bicarbonate (CDM) and 30% citrated human plasma caused the formation of a clot which persisted for several days. The ability of streptococci to induce clotting has not been reported previously, most probably due to the efficiency of the potent bacterial plasminogen activator streptokinase (12). Since the ability to induce coagulation of human plasma by Staphylococcus aureus is well characterized (4) and has been suggested as a potential virulence-enhancing strategy (3, 5, 7, 14), we investigated the phenomenon of clot formation by Streptococcus pyogenes.

A series of representative S. pyogenes isolates were grown to stationary phase (20 to 26 h) at 37°C in Todd-Hewitt broth. Bacteria were harvested by centrifugation, washed twice in phosphate-buffered saline (PBS), and resuspended to 10¹¹ CFU/ml in PBS by using an optical density standard nomogram (15). One-tenth of a milliliter of the streptococcal suspension in PBS was added to 0.3 ml of citrated human plasma and 0.6 ml of CDM in a 6-ml polypropylene tube. The mixture was incubated for various times at different temperatures, and 0.1 mM CaCl₂—divalent-cation concentrations roughly determined were 10 mM magnesium chloride and 0.1 mM CaCl₂—divalent-cation concentrations roughly equivalent to those in CDM (20). Clotting was detected when 10¹⁰ or 10¹¹ CFU/ml was used, but no clotting was seen for up to 72 h when 10²⁰ CFU/ml or less was tested.

Initiation of clotting of human plasma can occur as an active process due to the production of a bacterial factor, e.g., staphylocoagulase (11), or by virtue of a surface activation phenomenon (6). If clotting were solely a result of a surface effect, then heat-killed bacteria would be predicted to be as efficient as live bacteria and the addition of glucose to live bacteria should have no effect on clotting. We carried out a series of studies comparing the efficacy of clot formation by selected live or heat-killed group A isolates by using the Tris-plasma medium. In all cases, live bacteria were more effective in generating stable clots than were heat-killed bacteria. Addition of glucose to heat-killed streptococci had no effect on clot formation, but it completely blocked clot formation by live bacteria. Addition of glucose to 10 mg/ml had no effect on the clotting of plasma when exogenous thrombin or S. aureus Cowan was added (data not shown). Taken together, these studies suggested that the ability of S. pyogenes to mediate clot formation was influenced by some unrecognized bacterial factor.

To analyze the clotting phenotype further, eight isolates, including representative M types 1, 3, 12, 18, 49, and 55 and M-nontypeable isolate 64/14, were tested. The results of these...
studies are presented in Fig. 2. Variability was noted in the abilities of different isolates to generate clots. Isolates CS101 (M49), 64/14 (M nontypeable), and A928 (M55) routinely resulted in clot formation, while for isolates B930 (M3), T12 (M12), and A992 (M18), no clotting was observed under any temperature condition within a 142-h time period. When glucose was added at a final concentration of 10 mg/ml at the beginning of the incubation, none of the eight strains exhibited any clotting for up to 142 h. The time course of clotting was quite variable among the strains, and variation from experiment to experiment was also noted. The fact that some strains do not produce clotting further argues against a simple surface-mediated clotting mechanism. Those isolates that mediated clotting of human plasma when incubated in 0.15 M Tris containing divalent cations generally did so within 20 h, and the clot was stable for at least 60 h at 37°C and for greater than 160 h when incubated at 22°C. Clots rarely formed within 8 h and never within 1 h, as was seen with coagulase-positive S. aureus Cowan. Clot formation was always more apparent at 22°C and was least efficient at 37°C, and incubation at 33°C produced an intermediate effect. There was no obvious association between clot formation and SpeB production, expression of opacity factor, or the antigenic class of M protein. Both skin and throat isolates were able to induce clotting.

Since clot formation could be prevented by addition of glucose, we investigated the ability of preformed clots containing bacteria to lyse on addition of this carbon source. For all of these studies, clots were first generated at 22°C and then tested for stability on addition of glucose and subsequent incubation at three different temperatures. Clot lysis was observed on addition of glucose, suggesting that when the bacteria were provided with an appropriate nutrient, they could secrete adequate streptokinase to hydrolyze the clot.

To determine the importance of production of functional streptokinase in this system, these experiments were repeated with M49 serotype isolate NZ131 and an isogenic mutant in which the ska gene was inactivated (19). The results of these studies, presented in Fig. 3, demonstrate that the presence of NZ131 in a fibrin clot prevented or reversed clot formation upon addition of glucose. This occurred when the clot-bacterium mixture was incubated at 22, 33, or 37°C. The most efficient clot dissolution was observed at 37°C after addition of glucose. Evidence of clot solubilization was observed even in the absence of added glucose when the bacterium-clot mixture was shifted from 22 to 37°C. Glucose and temperature thus seem to act additively in clot dissolution. The observation that addition of glucose to streptococci can reverse or prevent the clotting process in vitro may be relevant to clot dissolution when nutrient conditions improve in vivo.

A comparison of the results obtained with the isogenic NZ131 mutant in which the ska gene was inactivated demonstrated no significant difference in clot formation. However, when glucose was added or when the temperature was elevated, the clots containing the ska mutant remained intact for 43 h at 37°C. Addition of exogenous streptokinase to these clots could solubilize the clots within 2 h at 37°C. These studies indicate that the process of clot lysis is dependent on streptokinase production.

FIG. 2. Eight strains of S. pyogenes, including wild-type (wt) CS101 and a streptokinase deletion mutant (sk−), were incubated at three different temperatures in the plasma-Tris medium described in the text and scored after 43 h.
and that under the nutrient conditions used to generate the clot, sufficient functional streptokinase was not present.

The ability of streptococci to form clots under nutrient-poor conditions and at temperatures below 37°C may be an adaptive mechanism to allow survival in host tissues when there are less-than-ideal conditions for invasion. Certain S. aureus isolates can mediate clotting through production of a staphylocoagulase and promote clot lysis by production of staphylokinase (2).

Staphylococcal coagulase is regulated by the agr system, which also negatively and positively regulates other potential virulence genes but not staphylokinase (1, 9). Nutrients and pH have been shown to affect agr expression (8, 13, 16–18). To date, the mechanism of regulation of staphylocoagulase production has not been reported. It is intriguing that the two most important gram-positive pathogens of humans can mediate both clot formation and clot lysis. The ability of strains of streptococci to produce a plasminogen activator or to induce clotting under certain growth conditions implies that there may be a regulation of thrombogenic versus thrombolytic activity in both S. aureus and group A streptococci. The ability of streptococci to induce clotting could contribute to the array of pathogenic mechanisms that can be used by these versatile human pathogens.

We thank Joseph Ferretti for providing wild-type NZ131 and the ska isogenic mutant.

This work was supported in part by NIH grant HL41898.

REFERENCES