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Serological Reactions Associated with the Clumping Factor of *Staphylococcus aureus*

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ABSTRACT

ROTTER, JOAN (University of Oklahoma Medical Center, Oklahoma City), AND FLORENE C. KELLY. Serological reactions associated with the clumping factor of *Staphylococcus aureus*. J. Bacteriol. **91**:588–594. 1966.—Evidence that the substance which causes staphylococci to clump in the presence of fibrinogen (clumping factor) is antigenically similar in strains which are serologically diverse according to agglutination reactions has been obtained from fibrinogen-cell clumping inhibition tests. Antisera for clumping factor (CF)-positive strains inhibited the clumping reaction of all strains tested. After adsorption with homologous cells or with cells of other CF-positive strains, the antisera no longer inhibited clumping. When antisera were adsorbed with trypsin-treated, CF-positive cells, or with cells of CF-negative mutants, the ability to inhibit the clumping reaction persisted. Antibody to CF activity was not associated with coagulase. Latex coated with extracts derived from the cells of five CF-positive and six CF-negative strains was, in each instance, agglutinated by sera from rabbits immunized with CF-positive cells. After adsorption with trypsinized, CF-positive cells, antisera still agglutinated latex which had been treated with the CF-positive extracts, but not with the CF-negative extracts. Similar results were obtained after antisera were adsorbed with the cells of CF-negative mutants. Cell agglutination titers of sera from rabbits immunized with CF-negative staphylococci were significantly lower than those produced in response to CF-positive cells, regardless of their coagulase activity. If the CF-inhibiting antibody also functions as an agglutinin, it apparently is not solely responsible for this difference.

Among strains of *Staphylococcus aureus*, the existence of antigenically distinct coagulases is well established (2, 3, 8). Duthie (4, 5) reported that antibodies to free coagulase did not inhibit the activity of bound coagulase, the name he applied to the factor which causes staphylococci to clump in the presence of fibrinogen. Conversely, he found that antibodies produced in response to injection of a partially purified preparation obtained from broth culture supernatant fluids of *S. aureus* inhibited the fibrinogen-cell clumping reaction, but did not interfere with plasma-clotting activity. He concluded that the substance responsible for the clumping reaction was antigenically identical in different strains. The number and character of strains examined were not reported by Duthie, and, to date, confirmation of his conclusion is lacking.

Kato and Omori (6) reported that a phenol extract of a free coagulase-negative, bound coagulase-positive variant of the Newman strain of *S. aureus* adsorbed antibodies which inhibited the clumping reaction, as well as agglutinins, present in serum of rabbits immunized with the bound coagulase-positive cells. Adsorption of the serum with cells of a bound coagulase-negative variant of the same strain did not remove the clumping inhibiting antibody.

In the present investigation, the aim was to compare cells and cell extracts of a number of staphylococcal strains to determine the serological identity of their clumping factor. (The term clumping factor, not bound coagulase, is used here because there is no evidence that the fibrinogen-cell clumping reaction is related to coagulase activity.)

MATERIALS AND METHODS

Staphylococcal strains. Among the strains used were representatives of typical coagulase (C)-positive, clumping factor (CF)-positive *S. aureus* mutants isolated from cultures of *S. aureus* or naturally occurring strains which lacked C or CF, and typical C-negative, CF-negative *S. epidermidis* organisms. Strains origi-

nally obtained from outside this Medical Center were: the C-positive, CF-positive Smith (S) strain and its CF-negative (Sv) mutant (G. A. Hunt); J17, a C-positive, CF-positive strain used as a source of antigen A by K. Jensen and supplied by him; the C-positive, CF-positive Wood 46 strain (G. M. Dack). Two C-positive, CF-negative strains (K6 and K93), the C-positive, CF-positive Hutton (H) strain, and all others used were isolated from patients or personnel at this Medical Center. Strains Hv1 (C-negative, CF-positive) and Hv2 (C-negative, CF-negative) are mutants which occurred in cultures of the Hutton (H) strain. Positive CF strains were selected for differences in degree of CF activity based on the concentration of fibrinogen required to cause clumping of their cells.

Fibrinogen-cell clumping activity. The CF activity of each batch of cells was checked before use in experiments. Growth from the surface of an 18-hr (37 C) Trypticase Soy Agar (BBL) plate culture, which had been inoculated with a 6-hr (37 C) Trypticase Soy Broth, was harvested and diluted in saline to a standard density of 10% transmittance with a Bausch & Lomb Spectronic-20 colorimeter (approximately 10^9 cells per milliliter). Fibrinogen, in a series of twofold dilutions beginning with 1.0% fibrinogen in saline, was added to an equal volume (0.2 ml) of the cell suspension. Saline was substituted for fibrinogen in the control. Tubes were shaken at medium speed on a Burrell wrist-action shaker for 5 min at room temperature and were observed immediately for clumping. CF activity was recorded according to the least concentration of fibrinogen which was required to clump the cells.

Preparation and preliminary testing of antisera. Antisera were prepared in rabbits against eight strains (S, Sv, H, Hv1, Hv2, J17, K6, and K93) by four or more weekly intravenous injections of 2×10^9 heat-killed cells each. Sera were checked periodically for homologous cell agglutinins, and booster doses were given as indicated by agglutination titers. Other rabbits were injected with extracts of each of five strains (S, Sv, H, Hv2, and J17). Before use, the protein content of each extract was measured by a modification of the Folin-Ciocalteau phenol reaction (7). A 0.5-ml amount of the extract, containing 0.5 mg of protein per ml, was mixed with an equal volume of incomplete Freund's adjuvant and was injected intradermally at multiple sites. After 2 weeks, the animals were similarly reinjected with the extract-adjuvant mixture. One week after this injection, sera were examined, and, when no extract precipitating antibodies could be demonstrated by the agar plate gel diffusion method, 0.5 ml of extract was administered intravenously. One week later, sera were still negative for precipitating antibodies, and the extract-adjuvant mixture was again injected intradermally. One week after the last injection of cells or extract, rabbits were bled. All sera were heated at 56 C for 30 min and were frozen until used. Adsorbed antisera were prepared by mixing an equal volume of packed cells and serum, incubating the mixture at 37 C for 2 hr with occasional agitation, and then holding overnight at 4 C.

Trypsinization of staphylococci. Duthie (4) first observed that trypsin-treated cells of *S. aureus* no longer clumped in the presence of fibrinogen. Kato and Omori (6) reported that fibrinogen was not adsorbed by the trypsinized cells. The trypsinization procedure of the latter workers was used. Equal volumes of packed cells and 0.4% trypsin (Difco) in saline were mixed, incubated at 37 C for 2 hr, and held in a boiling-water bath for 15 min to inactivate the trypsin.

TABLE 1. *Extract-latex agglutinations in unadsorbed and trypsinized cell-adsorbed antistaphylococcal sera*

Strain (source of extract)	C/CF[a]	Homologous cell titer	Extract-latex agglutinating titers			
			S antiserum[b]		H antiserum[b]	
			Unadsorbed	Adsorbed with trypsinized S cells	Unadsorbed	Adsorbed with trypsinized H cells
S	+/+	1:5,120	1:20	1:20	1:40	1:80
Sv[c]	+/−	1:320	1:80	0	1:80	0
H	+/+	1:10,240	1:20	1:20	1:40	1:40
Hv1[d]	−/+	1:20,480	1:40	1:40	1:80	1:40
Hv2[d]	−/−	1:320	1:40	0	1:20	0
E35	+/+	—	1:80	1:80	1:80	1:40
HA	+/+	—	1:40	1:40	1:40	1:20
K6	+/−	—	1:20	0	1:40	0
FR	−/−	—	1:80	0	0	0
CA	−/−	—	1:80	0	1:20	0
HI	−/−	—	1:80	0	1:20	0

[a] Abbreviations: C = coagulase; CF = clumping factor.

[b] Serum from rabbits immunized with C+, CF+ cells of the S(Smith) and H(Hutton) strains, respectively.

[c] Mutant of the S (Smith) strain.

[d] Mutants of the H (Hutton) strain.

Preparation of staphylococcal extracts. Phenol-ether extracts of cells of the 11 strains listed in Table 1 were prepared according to the method used by Kato and Omori (6) for their P-extract. Without further treatment, the amount of extract obtained from 20 g of lyophilized cells was dialyzed against phosphate-buffered saline (*p*H 7.4), with two changes of 14 liters each, for 24 hr. The dialysate was concentrated by further dialysis against 2.0% polyvinyl pyrrolidone until it contained 1.5 to 2.0 mg of protein per ml. Extracts were frozen until used.

Extract-latex agglutination tests. Essentially, the method of Singer and Plotz (9) was followed in latex-fixation tests. A 1-ml amount of latex (Difco) was added to 4.0 ml of isotonic borate buffer (*p*H 8.2). After mixing, the suspension was filtered through Whatman no. 5 filter paper. The staphylococcal extract was diluted with borate buffer to contain 2.0 to 3.0 mg of protein per ml. A 3-ml amount of this extract was added to 1.5 ml of latex suspension, and the

TABLE 2. *Extract-latex agglutinations in anti-staphylococcal sera before and after adsorption with homologous (CF-positive) cells and with cells of a CF-negative mutant*

Strain (source of extract)	C/CF[a]	Extract-latex agglutinating titers					
		S antiserum[b]			H antiserum[b]		
		Unadsorbed	Adsorbed with S cells	Adsorbed with Sv cells	Unadsorbed	Adsorbed with H cells	Adsorbed with Hv2 cells
S	+/+	1:80	0	1:20	1:40	0	1:20
Sv[c]	+/−	1:80	0	0	1:80	0	1:80
H	+/+	1:80	0	1:160	1:80	0	1:40
Hvl[d]	−/+	1:160	0	1:180	1:180	0	1:80
Hv2[d]	−/−	1:20	0	0	1:120	0	0

[a] Abbreviations: C = coagulase; CF = clumping factor.

[b] Serum from rabbits immunized with C+, CF+ cells of the S (Smith) and H (Hutton) strains, respectively.

[c] Mutant of the S (Smith) strain.

[d] Mutants of the H (Hutton) strain.

mixture was incubated at room temperature (25 C) for 2 hr. Antiserum, in twofold dilutions beginning with a 1:10 dilution, was mixed with an equal volume (0.1 ml) of extract-treated latex in buffer. Controls consisted of extract-treated latex in antiserum. Tubes were held at 56 C for 1.5 hr before centrifuging at 750 × *g* for 3 min. Tubes were then tapped and observed for evidence of agglutination. A final reading was made after overnight incubation at 4 C.

Antisera from rabbits immunized with cells of strains S and H were tested, before and after adsorption, with trypsinized, homologous cells for ability to agglutinate latex particles which had been treated with extracts derived from homologous cells and cells of 10 other strains (Table 1). Extract-latex agglutinations were also performed with S and H antisera which had been adsorbed with cells of a CF-negative mutant (Table 2).

Fibrinogen-cell clumping inhibition tests. Antiserum, in a series of twofold dilutions beginning with a 1:10 dilution, was mixed with an equal volume (0.5 ml) of a cell suspension of the same density as that used in determining the CF activity. Tubes were held at room temperature for 2 hr without shaking. No agglutination was observed at the end of this time. A 0.1-ml amount of 2.0% fibrinogen solution, an amount well in excess of that required to clump any test strain, was then added to each tube. The tubes were shaken for 5 min and were read immediately thereafter. Three controls tested cells in saline, cells in fibrinogen solution, and cells in antiserum. The clumping inhibiting titer was recorded as the highest dilution of antiserum which inhibited clumping of the cells in the presence of fibrinogen.

Antisera produced in response to cells of four CF-positive strains (S, H, Hvl, and J17) and to cells of four CF-negative strains (Sv, Hv2, K6, and K93) were tested against homologous cells and the CF-positive cells of nine other strains (Table 3). The CF-inhibiting capacity of S, H, Hvl, and J17 antisera was also determined after these sera had been adsorbed with untreated and trypsin-treated, CF-positive cells, as well

TABLE 3. *Inhibition of fibrinogen-cell clumping by CF-positive strain S antiserum before and after adsorption with trypsinized or CF-negative cells*

Antiserum	CF activity	CF-positive strains tested[a]									
		S	H	Hvl[b]	J17	Wood	604	JR	EM	VQ	VA
Unadsorbed		1:320	1:160	1:320	1:320	1:320	1:320	1:320	1:640	1:640	1:640
Adsorbed with strain:											
S	+	0	0	0	0	0	0	0	0	0	1:40
H	+	0	0	0	0	0	0	0	0	1:20	0
Hvl[b]	+	0	0	0	0	0	0	0	0	1:20	0
J17	+	0	0	0	0	0	0	0	1:40	0	0
S-trypsin[c]	−	1:640	1:640	1:640	1:320	1:640	1:320	1:320	1:640	1:160	1:640
H-trypsin	−	1:320	1:320	1:640	1:320	1:640	1:320	1:640	1:640	1:160	1:640
Sv[d]	−	0	0	0	0	0	0	1:160	1:160	1:180	1:160
Sv-trypsin	−	1:160	1:320	1:320	1:640	1:160	1:160	1:320	1:640	1:160	1:640
Hv2[b]	−	1:160	1:160	1:320	1:320	1:320	1:160	1:640	1:320	1:320	1:320

[a] Agglutination titers of S antiserum for test strains were: S, 1:5,120; H, 1:5,120; Hvl, 1:1,280; J17, 1:40; Wood, 0; 604, 1:20,480; JR, 1:80; EM, 1:2,560; VQ, 1:160; VA, 1:80.

[b] Coagulase-negative, CF-positive strain Hvl and coagulase-negative, CF-negative strain Hv2 are mutants of strain H.

[c] Trypsin-treated cells.

[d] Coagulase-positive, CF-negative strain Sv is a mutant of strain S.

as with cells of the CF-negative mutants Sv and Hv2. Sera from rabbits injected with extracts of three CF-positive strains (S, H, and J17) and two CF-negative strains (Sv and Hv2) were similarly tested for CF-inhibiting antibodies.

RESULTS

Fibrinogen-cell clumping activity. The concentration of fibrinogen required to clump the cells of strains used in fibrinogen-cell clumping inhibition tests were as follows: strain 604, 0.0001%; S, 0.003%; H, 0.003%; Hvl, 0.015%; J17, 0.015%; Wood, 0.03%; JR, 0.03%; VA, 0.03%; EM, 0.07%; and VQ, 0.06%. Cells of CF-positive strains E35 and HA, which, together with certain of those listed above, served as sources of extracts, clumped in 0.03 and 0.06% fibrinogen, respectively.

Agglutination titers of antisera. Homologous cell agglutination titers of sera used in extract-latex agglutination tests and in fibrinogen-cell clumping inhibition tests were as follows: S, 1:10,240; H, 1:20,480; Hvl, 1:20,480; J17, 1:20,480; Hv2, 1:640; Sv, 1:640; K6, 1:160; and K93, 1:1,280. Although none was remarkably high, the contrast between titers of antisera from rabbits immunized with CF-positive strains (S, H, Hvl, and J17) and those of the CF-negative antisera (Hv2, Sv, K6, K93) is noteworthy. Relatively low titers were characteristic of CF-negative antisera obtained from different rabbits. S, H, and Hvl antisera each agglutinated S, H, and Hvl cells at about the same titers (1:10,240 to 1:20,480), whereas with J17 cells, these sera gave titers in the range of 1:320 to 1:1,280. Agglutination titers obtained with CF-positive antisera and cells used in clumping inhibition tests showed that the test cells included those of serologically distinguishable strains (Table 3). After adsorption with trypsin-treated, homologous cells, the CF-positive antisera agglutinated untreated homologous cells at relatively low titers (S, 1:1,280; H, 1:640; Hvl, 1:640; J17, 1:2,560).

Precipitating antibodies in sera from rabbits injected with extracts of CF-positive cells have not been demonstrated. None of the sera of rabbits receiving extracts of CF-positive strains S, H, and J17 and CF-negative strains Sv and Hv2 agglutinated cells from which the extract was obtained. Serum from one rabbit injected with H extract agglutinated J17 cells at 1:20 titer and S cells at 1:40 titer. All other cross-agglutination tests were negative.

Staphyloccal extract. Phenol-ether extracts of *S. aureus* cells were transparent, nonviscous, and yellow in color, as described by Kato and Omori (9) for the P-extract of the Newman strain. Before dialysis, extracts of 11 strains (Table 1) all had the same appearance, except that they varied in color, depending on the pigmentation of the strain. After dialysis, extracts of all strains had the following properties: clear and colorless; neutral in reaction; not precipitated at *p*H 3 nor by boiling for 10 min; Molisch-negative; biuret-positive; positive reaction with the Folin-Ciocalteau reagent, with CF-positive strains having a higher content of the reactive substance than CF-negative strains, according to quantitative determinations.

Extract-latex agglutinations. Results of extract-latex agglutination tests are shown in Tables 1 and 2. In Table 1, two different antisera, prepared against cells of the C-positive, CF-positive S and H strains, are compared before and after they were adsorbed with homologous, trypsin-treated cells. In repeated experiments, latex particles coated with extracts derived from homologous CF-positive cells and from either the CF-positive or CF-negative cells of 10 other strains were agglutinated by unadsorbed S antiserum, and, with the single exception of strain FR, by unadsorbed H antiserum. When extracts of the five CF-positive strains were similarly reacted with H antiserum after it had been adsorbed with trypsinized H cells, agglutination also occurred. Likewise, after adsorption with trypsinized S cells, S antiserum agglutinated latex which had been treated with extracts of all CF-positive strains tested. On the other hand, although extracts of CF-negative strains gave positive reactions with both S and H unadsorbed antisera, no agglutination was observed with an extract of any of the CF-negative strains and either S or H antiserum after it had been adsorbed with homologous, trypsinized cells.

Table 2 shows the results of reacting latex which had been treated with extracts of strains S, H, and their mutants (Sv, Hvl and Hv2) with S and H antisera before and after each serum had been adsorbed with homologous (CF-positive) cells and with cells of a CF-negative mutant (Sv or Hv2). Agglutination reactions of antiserum which had been adsorbed with a CF-negative mutant (Table 2) can be compared with those of the same antiserum after adsorption with trypsinized cells (Table 1). In either case, adsorption failed to remove the ability of the CF-positive antiserum to agglutinate latex which had been coated with a CF-positive extract. Adsorbed S antiserum, whether adsorbed with homologous, trypsinized cells or with CF-negative mutant cells, gave the same results when reacted with extracts of the CF-negative mutants, i.e., no agglutination occurred. After adsorption with CF-negative mutant (Hv2) cells, H antiserum no

longer agglutinated latex treated with Hv2 extract, but it did agglutinate latex treated with extract of the Sv strain Adsorption of antisera with homologous (CF-positive) cells eliminated the reaction whether the extract was derived from a CF-positive or a CF-negative strain, regardless of the coagulase property of the strain.

Fibrinogen-cell clumping inhibition tests. Unadsorbed antisera prepared against the CF-positive cells of strains S, H, Hvl, and J17 each inhibited the CF activity of homologous cells at titers of 1:320 or higher. Cross-reactions were observed with each antiserum and the nine other strains tested. After adsorption with homologous cells, S, H, and J17 antisera no longer inhibited the fibrinogen-clumping reaction of these cells, whereas the inhibition titer of Hvl antiserum for Hvl cells was reduced from 1:640 to 1:20. Adsorption of each antiserum with either homologous or heterologous, trypsin-treated, CF-positive cells failed to eliminate CF activity; inhibition titers were generally in the same range as those obtained with unadsorbed antisera. Cross-reactions between cells of different strains and each antiserum persisted after adsorption of the serum with trypsinized cells. When H and Hvl antisera were adsorbed with the CF-negative cells of mutant strain Hv2 and were tested with H and Hvl cells, there was no significant decrease in their inhibition titers. Titers obtained with Hv2-adsorbed H and Hvl antisera and cells of the nine other strains were also the same as titers of the unadsorbed sera or differed by no more than one tube dilution. The effect of using cells of the CF-negative Sv strain as the adsorbent, however, differed with the antiserum and the strain of cells tested. Furthermore, results with antisera adsorbed with different batches of Sv cells were not always consistent. One regular finding was that Sv cells removed the inhibiting action of S antiserum for S cells and cells of most other strains. Similarly, after J17 antiserum was adsorbed with Sv cells, inhibition by the serum was reduced to zero or low titer for all strains. Conversely, Sv-adsorbed H and Hvl antisera retained much, if not all, of the inhibiting capacity of unadsorbed H and Hvl antisera for seven strains, but little or none for three strains (604, EM, and VQ). After adsorption with trypsinized Sv cells, each of the four antisera inhibited the reaction of all strains at titers approaching or matching those of the unadsorbed antisera. Table 3 presents typical results, in this case, inhibition titers obtained with the CF-positive cells of 10 different strains and S antiserum before and after adsorption with trypsinized or CF-negative mutant cells. Except for the behavior of SV-adsorbed antisera described above, results with H, Hvl, and J17 antisera were substantially the same as those reported for S antiserum. Repeated tests gave similar inhibition titers.

Antisera prepared against the CF-negative cells of strains Sv, K6, K93, and Hv2 did not inhibit the fibrinogen-cell clumping reaction of any of the 10 CF-positive strains tested. Sera from rabbits injected with extracts of CF-positive strains S, H, and J17 also failed to inhibit the clumping reaction.

DISCUSSION

Kato and Omori (6) studied two coagulase-negative variants of the Newman strain of *S. aureus*, one of which (D_2) was bound coagulase-positive and the other (D_3), bound coagulase-negative. They concluded that staphylococcal cells contain two different fibrinogen-adsorbing substances: a substance obtained by phenol extraction of bound coagulase-positive cells only, and a substance associated with bound coagulase-negative cells and trypsin-treated, bound coagulase-positive cells. Crude phenol extracts of D_2 cells adsorbed agglutinin, clumping inhibiting antibody, and fibrinogen. Acetone fractionation of the phenol extract resulted in a precipitate which adsorbed antibodies, but not fibrinogen, and a supernatant fluid which adsorbed fibrinogen. The nondialyzable, precipitated fraction lost its fibrinogen-adsorbing capacity when treated with trypsin, whereas the dialyzable, fibrinogen-adsorbing fraction was resistant to trypsin. Both acetone fractions gave negative results when tested for precipitating antibodies in D_2 and D_3 antisera.

In the work presented here, phenol extracts were not subjected to acetone fractionation; instead, test material was the nondialyzable portion of the phenol extract. To date, no satisfactory direct method for testing the ability of extract-coated particles to clump in fibrinogen solutions has been achieved. Latex and erythrocytes tend to clump spontaneously in the presence of fibrinogen. Attempts to coat CF-negative staphylococci with extracts of CF-positive cells have not succeeded. Addition of different batches of extracts directly to fibrinogen solutions gave erratic results. Extracts of the coagulase-positive, CF-positive cells of strains S, H, and J17 generally produced a precipitate, whereas extract of Hvl, a coagulase-negative, CF-positive mutant of strain H only occasionally gave this reaction. However, in no instance was fibrinogen precipitation observed with extracts of the CF-negative cells of coagulase-positive Sv and coagulase-negative Hv2 strains.

Antisera prepared against CF-positive cells gave much higher agglutinin titers with intact

cells than did antisera from animals immunized with CF-negative cells. Latex agglutinations showed that extracts of both the CF-negative and CF-positive strains contained antigen which was shared by the CF-positive cells of both S and H strains (Table 1). Adsorption of S and H antisera with trypsinized homologous cells removed antibodies responsible for agglutination of CF-negative extract-latex, but not antibodies which agglutinated CF-positive extract-latex. When adsorbed with untreated homologous (CF-positive) cells, neither antiserum reacted with extracts of CF-positive cells or CF-negative cells. Results obtained with antisera which were adsorbed with CF-negative mutant cells differed somewhat from those obtained after adsorption of the antisera with homologous trypsinized cells (Table 2). S antiserum adsorbed with cells of the CF-negative mutant Sv behaved the same as S antiserum adsorbed with trypsinized S cells; the adsorption removed antibodies for components of the Sv extract, but not of the S extract. Similarly, adsorption of H antiserum either with trypsinized H cells or with CF-negative mutant Hv2 cells abolished the reaction with Hv2 extract, but not with extracts of the CF-positive H and Hvl cells. However, Hv2 cell adsorption of H antiserum did not remove agglutinins for the CF-negative Sv cells, and readsorption of the serum with Hv2 cells did not change this result. Apparently, the extract of Sv cells contains antigen which is also present in H, but not in Hv2, extract. Since Sv and Hv2 are CF-negative, this reaction cannot be attributed to antigen associated with CF activity. The extract-latex studies contribute no information as to whether CF is a participant in the agglutination reaction, nor even if substance responsible for CF activity is present in extracts of CF-positive cells. Nonetheless, the extract-latex agglutinations indicate that extracts of CF-positive strains contain antigens which are not present in extracts of CF-negative strains, and that at least one antigen is shared by the CF-positive strains tested.

Results of fibrinogen-cell clumping inhibition tests confirm reports (4, 6) that the substance responsible for the clumping of staphylococci in the presence of fibrinogen is antigenic. Cross-reactivity of four antisera with 10 CF-positive strains indicates that the antigen is similar in various strains. Inhibition of the clumping reaction was completely eliminated in almost all instances when CF-positive antisera were adsorbed with CF-positive cells, but not with the same cells after they had lost CF activity due to trypsin treatment or with cells of the coagulase-negative, CF-negative Hv2 mutant. In those rare instances in which CF-positive cell-adsorbed antisera retained some inhibiting effect, titers were greatly decreased (from 1:640 to 1:20 or 1:40). Removal of CF-inhibiting antibodies from antisera by cells of the coagulase-positive, CF-negative mutant Sv may be due to the presence of CF-positive cells in Sv populations. Fibrinogen-soft agar cultures (1) show that, in some cultures, as many as 10% of strain Sv cells are CF-positive. This explanation cannot account for the different effects of Sv adsorption on the CF-inhibition titers of the four antisera. The erratic behavior of Sv-adsorbed antisera toward different strains could mean that more than one antigen is associated with CF activity of the test strains. Yet each unadsorbed antiserum gave approximately the same clumping inhibition titer with the 10 strains, and adsorption of each serum with untreated CF-positive cells of strains S, H, Hvl, or J17 had similar effects on all strains. Furthermore, sera from different rabbits immunized with cells of strains Sv failed to inhibit the clumping reaction of any CF-positive strain, as was also true of sera from animals injected with cells of CF-negative strains K6, K93, and Hv2.

The question arises as to whether CF-inhibiting antibody also functions as agglutinin. Unadsorbed S, H, and Hvl antisera agglutinated the CF-positive cells of strains S, H, and Hvl at about the same relatively high titer, but not those of strain J17. Nevertheless, the CF activity of strain J17, as well as of other serologically distinguishable strains, was inhibited by each antiserum before and after adsorption with trypsinized cells of strains S, H, Hvl, and J17. Agglutinating and CF-inhibiting antibodies for S, H, Hvl, and J17 cells were removed from each antiserum by adsorption with untreated, homologous cells. After adsorption of CF-positive antisera with trypsinized, homologous cells, each serum still agglutinated these cells, although, compared with the agglutination titers of unadsorbed sera, those obtained after trypsinized cell adsorption were significantly reduced (from 1:10,240 or more to 1:2,560 or less). In the extract-latex studies, adsorption of S and H antisera with trypsinized S or H cells had little or no effect on the agglutination of CF-positive extract-latex, whereas, in every case, the same treatment of the sera eliminated the agglutination reaction of CF-negative extract-latex. These results suggest that the antigens participating in CF-positive extract-latex agglutinations are CF-associated.

Duthie (4) reported that serum from rabbits immunized with an extract prepared from supernatant fluids of broth cultures of bound coagulase-positive staphylococci contained specific antibodies which inhibited the clumping reaction. Kato and Omori (6) made no mention of attempts

to immunize animals with their extract. In the present study, sera from animals receiving extracts of CF-positive cells gave negative results in cell agglutination, extract-latex agglutination, CF inhibition, and agar-gel precipitin tests. From these results, it would seem that the extracts contained insufficient CF or that CF has little or no immunizing capacity.

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