INFECTION AND IMMUNITY, Mar. 1997, p. 897–902 Vol. 65, No. 3
0019-9567/97/$04.00+0

Vitronectin-Binding Staphylococci Enhance Surface-Associated Complement Activation

FREDRIK LUNDBERG,[1] TOR LEA,[2] AND ÅSA LJUNGH[1]*

Department of Medical Microbiology, Lund University, Lund, Sweden,[1] and Institute of Immunology and Rheumatology, Oslo University, Oslo, Norway[2]

Received 30 August 1996/Returned for modification 27 September 1996/Accepted 11 December 1996

Coagulase-negative staphylococci are well recognized in medical device-associated infections. Complement activation is known to occur at the biomaterial surface, resulting in unspecific inflammation around the biomaterial. The human serum protein vitronectin (Vn), a potent inhibitor of complement activation by formation of an inactive terminal complement complex, adsorbs to biomaterial surfaces in contact with blood. In this report, we discuss the possibility that surface-immobilized Vn inhibits complement activation and the effect of Vn-binding staphylococci on complement activation on surfaces precoated with Vn. The extent of complement activation was measured with a rabbit anti-human C3c antibody and a mouse anti-human C9 antibody, raised against the neoepitope of C9. Our data show that Vn immobilized on a biomaterial surface retains its ability to inhibit complement activation. The additive complement activation-inhibitory effect of Vn on a heparinized surface is very small. In the presence of Vn-binding strain, *Staphylococcus hemolyticus* SM131, complement activation on a surface precoated with Vn occurred as it did in the absence of Vn precoating. For *S. epidermidis* 3380, which does not express binding of Vn, complement activation on a Vn-precoated surface was significantly decreased. The results could be repeated on heparinized surfaces. These data suggest that Vn adsorbed to a biomaterial surface may serve to protect against surface-associated complement activation. Furthermore, Vn-binding staphylococcal cells may enhance surface-associated complement activation by blocking the inhibitory effect of preadsorbed Vn.

Implantation of biomaterials for temporary and permanent use is common in modern medicine. A rare but serious complication of the use of biomaterials in contact with blood is hemolysis, leukopenia, and disrupted coagulation hemostasis (postperfusion syndrome), mediated by surface-associated complement activation (26, 40). Dialysis membranes, tubings for extracorporeal circulation, vascular grafts, heart valve prostheses, central venous catheters, and cell savers for autotransfusion are all examples of implants which come in contact with blood. Complement activation starts spontaneously with the conversion of C3 to inactivated C3 (iC3) (16). On an activating surface, the bound C3b forms the amplifying C3 convertase of the alternative pathway, C3bBb. Complement may also be activated at the air-blood interface in bubble oxygenators (32). The alternative pathway is mainly responsible for this surface-associated complement activation (22). Surface-bound complement factors, i.e., iC3 and C3b, bind to specific receptors on neutrophils, monocytes, and macrophages (3, 6, 8, 10). Cellular responses due to factor-receptor interaction may enhance the inflammatory process at the biomaterial surface (4).

The end product of complement activation is C5b-9, the terminal complement complex which attaches to cell membranes and induces lysis of the cell. Fluid-phase vitronectin (Vn) is able to inhibit the lysis by binding to either (i) the forming C5b-9 complex, thereby blocking tubular polymerization of C9, or (ii) the metastable site of C5b-7, preventing the membrane insertion. SC5b-9 (Vn bound to C5b-9) which lacks the tubular structure of the terminal complement complex is unable to induce lysis of the cell (33, 34). Vn has been detected on tridodecylmethylammonium chloride-heparinized catheters extirpated from the human bloodstream (44). Vascular endothelium is one of several types of tissue rich in Vn where it may serve a regulatory function (13).

Endpoint-attached (EPA) heparin added to polymers has been shown to give a stable and thromboresistant surface (15). Complement activation is thereby induced to a low level, which has been measured by using anti-C3c antibodies (14, 31). The balance between factors H and B, binding to surface-bound C3b, is important for the surface-associated complement activation. The low extent of activation on a heparinized surface is due to a 100-fold-greater affinity of bound C3b to factor H than to factor B (14, 19). Soluble heparin also reduces complement activation by inhibition of the binding site of C3b for factor B (14, 19). In addition, heparin is known to express binding sites for Vn (36).

The degree of surface-associated complement activation, through the alternative pathway, can be measured as levels of soluble C3a and C5a and on solid phase as levels of C3b. However, the measurement of C5b-9 activated complement product levels has proven to be far superior (21). Since native forms of complement can be passively adsorbed to biosurfaces without undergoing activation, monoclonal antibodies detecting activated forms of complement are required (21).

Coagulase-negative staphylococci (CoNS) are frequently isolated from biomaterial-associated infections (5). CoNS commonly express surface structures which bind immobilized Vn (18, 28, 29). The heparin-binding ability of CoNS shows great variability (27). Several strains bind immobilized heparin under stationary conditions but bind to a lesser extent under perfusion conditions (18).

For these purposes, we established a model in which the effects of surface-immobilized Vn with respect to complement activation can be studied. The purpose of this study was to investigate if surface-immobilized Vn could inhibit comple-

* Corresponding author. Mailing address: Department of Medical Microbiology, Sölvegatan 23, S-223 62 Lund, Sweden. Fax: 46-46-152564. E-mail: asa.ljungh@alinks.se.

ment activation. The surfaces used were unheparinized and EPA-heparinized polystyrene. Furthermore, we wished to know if the presence of Vn-binding staphylococci on these surfaces could block the inhibition of complement activation by immobilized Vn, thereby enhancing the complement activation on Vn-coated surfaces.

MATERIALS AND METHODS

Serum. Human serum was purchased from the blood bank, Lund University Hospital. Vn-depleted serum was obtained by affinity chromatography on a collagen-CNBr-activated Sepharose 4B column (Pharmacia Biotech, Stockholm, Sweden), mainly as described by Gebb et al. (9) and Hayman et al. (12). Collagen I was purchased from ICN Biomedicals Inc., Aurora, Ohio. The buffers used for depletion were as follows: for binding, 1 mM HCl; for washing, 0.06 M (phosphate-buffered saline [PBS]), pH 6.0; and for elution, 2 M PBS, pH 7.2. The depletion procedure, sodium phosphate from bleeding to storage (at −70°C), was completed in 1 day. The level of remaining Vn was determined as described by Rozalska and Ljungh (39).

Vn. Human Vn was purified from fresh urea-activated serum by affinity chromatography on a Sepharose 4B column and heparin-Sepharose 4B as described by Yatohgo et al. (43).

Antibodies. A rabbit polyclonal anti-human C3c antibody (DAKOPATTS, Immunoglobulins A/S, Glostrup, Denmark) that recognizes all forms of C3 containing the C3c fragment, i.e., C3, iC3, C3b, and C3c (24, 25), was used. The mouse monoclonal anti-human C9 antibody (clone aE11) recognizes a neoepitope on activated C9 (21). The anti-C9 and anti-C3c monoclonal antibodies were diluted 1:3,000 in PBS with 1% bovine serum albumin (BSA). Secondary rabbit anti-mouse immunoglobulin G and swine anti-rabbit immunoglobulin G antibody (DAKOPATTS, Immunoglobulins A/S), both horseradish peroxidase conjugated, were diluted 1:2,000.

Complement activation. For the various experiments, serum was diluted in either of the following three buffers: (i) Veronal-buffered saline (VBS), pH 7.5, with 0.10 mM Ca^{2+} and 0.5 mM Mg^{2+} for total complement activation; (ii) VBS with 10 mM MgEGTA and 2.5 mM $MgCl_2$, allowing only complement activation through the alternative pathway; and (iii) VBS with 10 mM EDTA, allowing no complement activation. Values obtained in assays using these buffers will be referred to as VBS, MgEGTA, and EDTA values, respectively. Full activation of the complement cascade was achieved by adding Zymosan A (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 1 mg per ml of serum (7).

Bacterial strains. *Staphylococcus hemolyticus* SM131 and *S. epidermidis* 3380, both coagulase-negative strains, were isolated from patients with osteomyelitis. Strain SM131 expresses binding of Vn, and strain 3380 does not (28, 29). Strains were cultured for 24 h at 37°C, harvested, washed twice in PBS, and adjusted to an optical density of 1.0 at 540 nm, corresponding to approximately 10^9 and 10^{10} cells/ml (strains SM131 and 3380, respectively). Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) was used as standard culture medium, permitting maximal expression of Vn-binding structures. As alternative culture media, tryptic soy broth (Difco) and blood agar (horse erythrocytes, 5% vol/vol) were used. Three hundred microliters of bacterial suspension (10^7 cells/ml) was treated with Vn (60 μg/ml) for 1 h at room temperature under gentle agitation. Centrifugation at 10,000 rpm for 5 min yielded a small pellet which was used for studies of Vn-treated bacteria.

Bacterial adhesion. Quantitation of the extent of bacterial adhesion was performed by bioluminescence (17) with a luminometer (LKB Wallac, Turku, Finland). Monitoring reagent was from BioThema, Stockholm, Sweden. Briefly, this method measures the amount of ATP of bacteria in the sample. The amount of ATP is determined in parallel in a known number of bacteria, and the number of bound bacteria can thereby be determined in the sample.

Enzyme-linked immunosorbent assay. Polystyrene 96-well microtiter plates (MaxiSorp F96; NUNC, Roskilde, Denmark) were used. Surface heparinization was done with EPA heparin as described by Larm et al. (15). Heparin was from Pharmacia & Upjohn, Stockholm, Sweden. Wells were coated with 100 μl of Vn (5 μg/ml) and left overnight at 4°C. The amount of Vn adsorbed to the wells was determined as described by Rozalska and Ljungh (39). Before use, the plates were rinsed three times in PBS with 0.05% Tween 20 and incubated with 150 μl of PBS containing 1% BSA for 1 h at 37°C. One hundred microliters of bacterial suspension was added to each well at a concentration of either 10^7 or 10^6 cells per ml and incubated for 1 h at 37°C under gentle agitation. Unbound bacteria were removed by PBS with 0.05% Tween 20 (three rinses). One hundred microliters of Vn-depleted serum, diluted 1:3 in either VBS, MgEGTA, or EDTA buffer, was incubated at 37°C for 1 h under gentle agitation. After washing, the wells were incubated with 100 μl of primary antibody for 1 h at 37°C under gentle agitation. Thereafter, the wells were washed three times as before and incubated with 150 μl of PBS containing 1% BSA for 1 h at 37°C to block nonspecific binding of secondary antibodies. Incubation with secondary antibodies was performed for 1 h at 37°C under gentle agitation. Bound secondary antibodies were detected with 100 μl of 1,2-phenylenediamine color reagent (Abbott Laboratories, Wiesbaden, Germany). The color reaction was stopped by adding 100 μl of 1 M H_2SO_4 after 5 min. The results were read at 492 nm in a Multiscan Plus 314 (Labsystems OY, Helsinki, Finland). The data presented (VBS and MgEGTA values) were obtained by subtraction of VBS and MgEGTA values by the corresponding EDTA values. The data presented are mean values. The abbreviation AU means arbitrary units and corresponds to the level of color reaction measured.

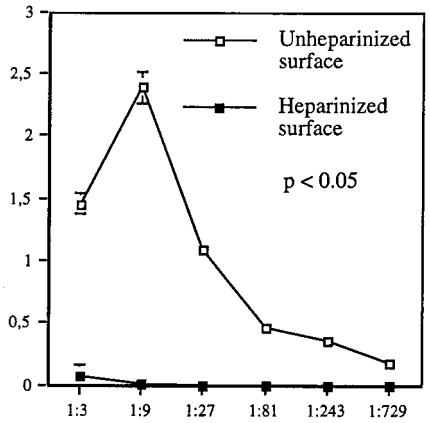

FIG. 1. Extent of complement activation by unheparinized (□) and heparinized (■) polystyrene surfaces. A significant difference was found between the two surfaces ($P < 0.05$). The level of activation, measured with an anti-C3c antibody, is indicated on the *y* axis as the level of absorbance at 492 nm. Three-step dilutions of Vn-depleted serum in MgEGTA buffer are represented on the *x* axis. Each experiment was carried out in triplicate.

Statistics. The *F* test was used prior to further statistical analyses. Unpaired two-tailed Student's *t* test and Wilcoxon's signed rank test were used when appropriate. A *P* value of <0.05 was considered to represent a significant difference.

RESULTS

Surface heparinization. Unheparinized and heparinized surfaces were incubated with Vn-depleted serum diluted in the complement buffers (VBS, MgEGTA, and EDTA buffers). Subsequent incubation with an anti-C3c antibody yielded a significant decrease in complement activation on heparinized compared to unheparinized surfaces ($P < 0.05$) (Fig. 1).

Immobilized Vn. Vn was immobilized on the two surfaces, 0.50 μg of Vn in each well. Of the 0.50 μg added, 0.37 ± 0.03 μg of Vn was detected in unheparinized wells and 0.21 ± 0.04 μg of Vn was detected in heparinized wells. The extent of complement activation of MgEGTA-diluted serum was determined in each of these wells with both anti-C3c and anti-C9 antibodies. Unheparinized surface without Vn showed a slightly higher level of activation (anti-C3c antibody), 0.11 AU, than the same surface with Vn, 0.037 AU (Fig. 2). No difference in complement activation was seen on the heparinized surface without Vn compared to with Vn (data not shown) irrespective of what antibody was used. The unheparinized surfaces without Vn showed a higher level of activation (0.38 AU) than the same surface with Vn (0.15 AU) ($P < 0.05$) (Fig. 3). This was measured with the anti-C9 antibody.

Vn-depleted serum. Though the serum used in these experiments was depleted from Vn by affinity chromatography, there was still some Vn in the second flowthrough serum. The extent of Vn was 36% of that before depletion. The levels of complement activation in these two sera, diluted in MgEGTA buffer, were determined as 0.42 AU before depletion and 1.8 AU after depletion (Fig. 4). The further influence of immobilized Vn on these two sera was determined on an unheparinized surface with Vn. The extent of complement activation in the Vn-depleted serum fell to 0.53 AU, and that of nondepleted serum remained at 0.60 AU (Fig. 4).

Staphylococcal cells and immobilized Vn. CoNS have been described to bind immobilized Vn (28). These experiments were carried out to analyze if staphylococcal cells were able to

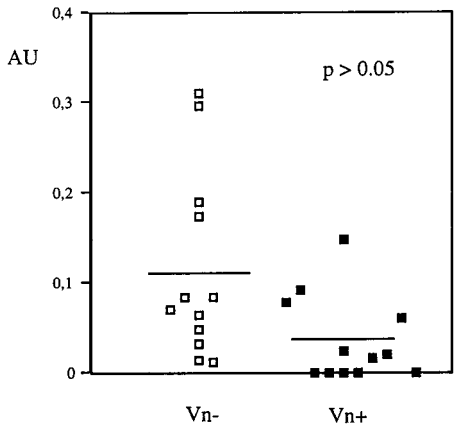

FIG. 2. Polystyrene surfaces with or without Vn activated complement in Vn-depleted serum. The serum was diluted in MgEGTA buffer to different extents. The extent of activation (*y* axis) was detected by using an anti-C3c antibody. Horizontal lines denote mean values of 12 determinations. Polystyrene without Vn (□) showed a higher level of activation (0.11 AU) than polystyrene with Vn (■) (0.037 AU).

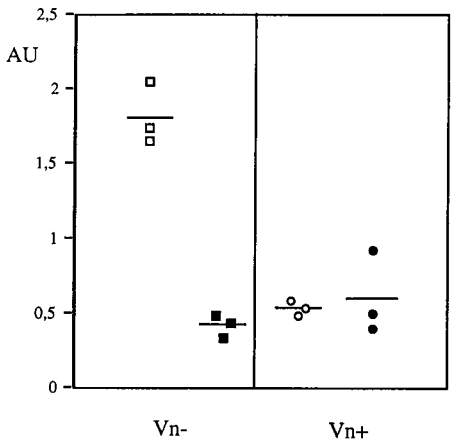

FIG. 4. Complement activation in Vn-depleted serum (□ and ○) compared to that in normal healthy serum (■ and ●). All sera were diluted 1:3 in MgEGTA buffer. The extent of complement activation decreased from 1.8 AU in Vn-depleted serum (□) to 0.42 AU in normal healthy serum (■). When polystyrene with immobilized Vn was used, the difference in complement activation between the two sera was not seen: Vn-depleted serum (○), mean of 0.53 AU; normal healthy serum (●), mean of 0.60 AU. Horizontal lines denote mean values of three determinations.

interfere with the effect of immobilized Vn on complement activation. Two different strains of CoNS were allowed to adhere to the surfaces: SM131, which binds Vn, and 3380, which does not. Thereafter, serum diluted in either of the complement buffers was added. The extent of complement activation was determined with the anti-C9 antibody.

The first experiment was carried out with strain SM131 on unheparinized surfaces that was either uncoated or coated with Vn. The bacterial inoculum was 10^7 cells/ml and obtained from a culture grown in Todd-Hewitt broth. The serum was diluted in MgEGTA buffer. As shown in Fig. 5a, there was no difference in complement activation between surfaces with Vn (0.57 AU) and without Vn (0.69 AU). In the second experiment, the same surfaces were incubated with strain 3380. The complement activation on surfaces with Vn was found to be significantly different from that on surfaces without (0.15 AU versus 0.69 AU, respectively; $P < 0.05$). Similar results were obtained when serum was diluted in VBS buffer: incubation with SM131 revealed no difference between surfaces with and without Vn (0.87 AU and 0.72 AU, respectively). However, incubation with strain 3380 resulted in significantly lower complement activation on Vn-coated surfaces compared to uncoated surfaces (0.35 AU versus 0.72 AU; $P < 0.05$).

We then investigated whether Vn coating would add to the complement-inhibitory properties of surface-adsorbed heparin. When strain SM131 was allowed to adhere to a heparinized surface that either contained or did not contain Vn, no difference in complement activation was found in serum diluted in VBS buffer (0.80 AU in the presence and 0.93 AU in the absence of Vn) (Fig. 5b). Incubation with strain 3380, however, resulted in significant differences: complement activation fell from 1.30 AU without Vn to 0.88 AU with Vn ($P < 0.05$) (Fig. 5b). When these experiments were repeated with serum diluted in MgEGTA buffer, SM131 activated complement to the same extent, irrespective of whether surfaces were coated with Vn or uncoated (1.40 AU versus 1.20 AU), while incubation with 3380 revealed a reduced complement activation in the presence of Vn compared to surfaces without Vn (1.20 AU versus 1.00 AU, respectively; $P < 0.05$). Decreasing the bacterial inoculum to 10^6 cells/ml did not alter the results (data not shown). Thus, these data corroborate our findings regarding the complement-inhibitory properties of immobilized Vn and furthermore argue that Vn-binding CoNS may interfere with or partly block this effect.

Effect of the levels of Vn-binding sites on CoNS. Culture conditions influence the levels of Vn-binding surface structures on the bacteria. Cultivation in Todd-Hewitt broth allows SM131 to express maximal numbers of Vn-binding sites, while cultivation in tryptic soy broth inhibits this expression. We wanted to relate our findings to the expression of Vn-binding structures on SM131; to this end, we carried out experiments in which bacteria cultivated in tryptic soy broth were investigated for complement-activating properties on Vn-coated and uncoated surfaces. Complement activation in serum diluted in MgEGTA buffer fell from 0.80 AU without Vn to 0.46 with Vn ($P < 0.05$) (Fig. 6). In serum diluted in VBS buffer, the complement activation was 1.30 without Vn and 0.96 with Vn ($P < 0.05$). The reduction could still be detected when the bacterial inoculum was reduced 10-fold (10^6 cells/ml). Thus, SM131 cultured in tryptic soy broth was no longer able to block the

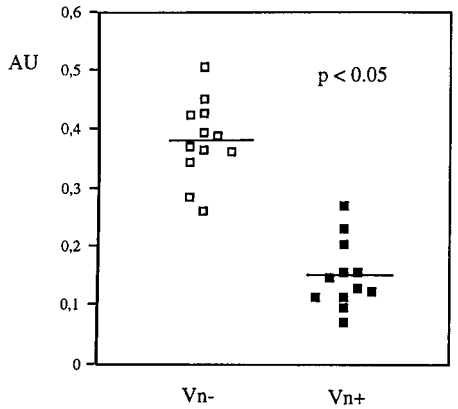

FIG. 3. Polystyrene surfaces with or without Vn activated complement in Vn-depleted serum. The serum was diluted in MgEGTA buffer to different extents. The level of activation (*y* axis) was detected with an anti-C9 antibody. Horizontal lines denote mean values of 12 determination. Polystyrene without Vn (□) showed a higher level of activation (0.38 AU) than polystyrene with Vn (■) (0.15 AU) ($P < 0.05$).

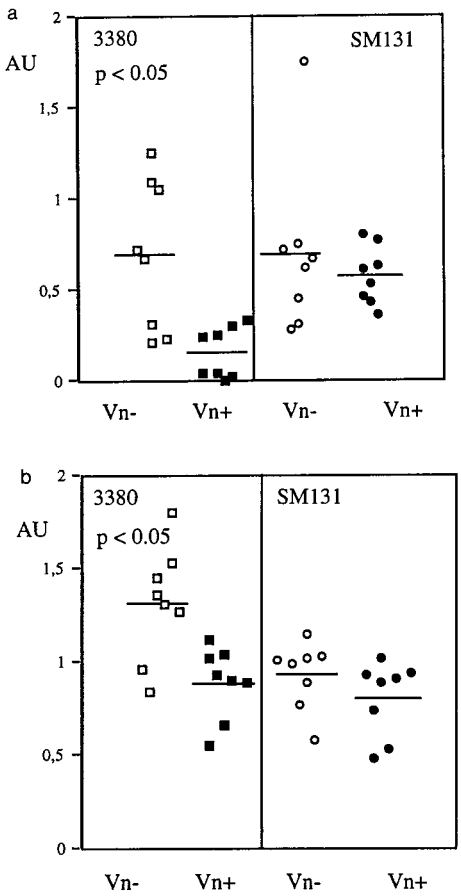

FIG. 5. (a) Unheparinized polystyrene with immobilized Vn was incubated with either Vn-binding strain SM131 or nonbinding strain 3380, both cultured in Todd-Hewitt broth. The surfaces were then incubated with Vn-depleted serum which was diluted 1:3 in MgEGTA buffer, and the level of complement activation was detected with an anti-C9 antibody. Results are expressed as median values represented by horizontal lines ($n = 8$). Strain 3380 with no Vn (□) and with Vn (■) yielded values of 0.69 and 0.15 AU ($P < 0.05$). Strain SM131 with no Vn (○) and with Vn (●) yielded values of 0.69 and 0.57 AU (no significant difference). (b) Heparinized polystyrene with immobilized Vn was incubated either Vn-binding strain SM131 or with nonbinding strain 3380, both cultured in Todd-Hewitt broth. The surfaces were then incubated with Vn-depleted serum diluted 1:3 in VBS buffer, and the level of complement activation was detected with an anti-C9 antibody. Results are expressed as median values represented by horizontal lines, ($n = 8$). Strain 3380 with no Vn (□) and with Vn (■) yielded values of 1.30 and 0.88 AU ($P < 0.05$). Strain SM131 with no Vn (○) and with Vn (●) yielded values of 0.93 and 0.80 AU (no significant difference).

effect of Vn, clearly demonstrating the significance of the suggested interaction.

Furthermore, when the bacteria were treated with soluble Vn, complement activation was decreased in comparison with untreated bacteria. The reduction after Vn treatment was almost 50% for both strain 3380 and strain SM131 (cultured in Todd-Hewitt broth), irrespective of whether serum was diluted in VBS or MgEGTA buffer.

Intrinsic complement activation of the two CoNS strains. It might be argued that different complement-activating properties of the two bacterial strains could influence our results. We therefore investigated this possibility by measuring intrinsic complement activation of the bacteria by using the anti-C9 antibody. SM131 activated complement to a slightly higher extent than strain 3380 when serum was diluted in MgEGTA buffer. However, if VBS buffer was used to dilute serum, both strains activated complement to approximately the same extent irrespective of culture conditions (data not shown). When

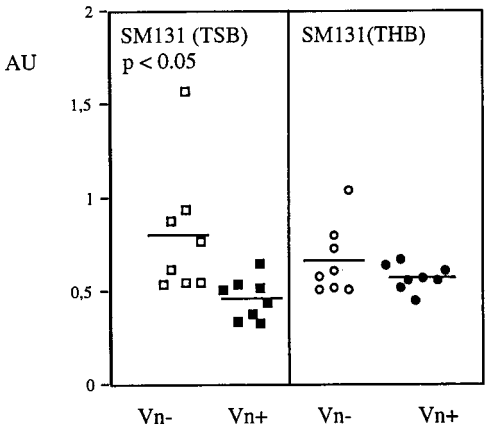

FIG. 6. Unheparinized polystyrene with immobilized Vn was incubated either with Vn-binding strain SM131 cultured in Todd-Hewitt broth (THB) or with nonbinding strain SM131 cultured in tryptic soy broth (TSB). The surfaces were then incubated with Vn-depleted serum diluted 1:3 in MgEGTA buffer, and the level of complement activation was detected with an anti-C9 antibody. Results are expressed as median values represented by horizontal lines ($n = 8$). Strain SM131 (cultured in tryptic soy broth) without (□) and with (■) Vn yielded values of 0.80 and 0.46 AU ($P < 0.05$). Strain SM131 (cultured in Todd-Hewitt broth) without (○) and with (●) Vn yielded values of 0.66 and 0.57 AU (no significant difference).

strain SM131 was cultured in tryptic soy broth, its complement-activating properties decreased compared to those of cells cultured in Todd-Hewitt broth.

Extent of bacterial adhesion. The strains adhered differently to unheparinized and heparinized surfaces and to surfaces with and without Vn (Table 1). The adhesion of strain 3380 to unheparinized surfaces with or without Vn was 4.9 or 4.7% of the total amount of added bacteria. The adhesion of strain SM131, cultured in Todd-Hewitt broth, was significantly ($P < 0.05$) greater to surfaces with Vn (20%) than to surfaces without Vn (15%). When strain SM131 was cultured in tryptic soy broth, its adhesion to surfaces with Vn was not significantly higher than that to surfaces without Vn (13 and 15%, respectively). The adhesion of the two strains to heparinized surfaces was close to zero, irrespective of culture conditions. Preadsorbed Vn did not enhance the adhesion of strain SM131 cultured in Todd-Hewitt broth to a heparinized surface.

DISCUSSION

Surface heparinization has been shown to decrease complement activation (20, 31, 32). This observation was confirmed in our test system as measured both with anti-C3c (Fig. 1) and anti-C9 (data not shown) antibodies. Soluble Vn also inhibits

TABLE 1. Adhesion of staphylococcal cells to unheparinized and heparinized polystyrene, with and without preadsorbed Vn

Strain	Adhesion (% of total amt of added ATP, mean ± SD [$n = 4$])			
	Unheparinized polystyrene		Heparinized polystyrene	
	Vn	No Vn	Vn	No Vn
3380[a]	4.7 ± 0.9	4.3 ± 0.9	0.050 ± 0.01	0.040 ± 0.04
SM131[a]	20 ± 0.7	15 ± 2.1[c]	0.15 ± 0.01	0.14 ± 0.01
SM131[b]	13 ± 1.1	15 ± 0.7[d]	0.12 ± 0.01	0.10 ± 0.04

[a] Todd-Hewitt broth.
[b] Tryptic soy broth.
[c] $P < 0.05$.
[d] No significance.

complement activation (33, 34). We wanted to see whether Vn immobilized on a polymer surface would retain its complement-inhibitory properties. Furthermore, we wanted to study the influence of Vn-binding staphylococci on the complement-inhibitory properties of Vn. For these purposes, we established a model in which complement activation in human serum could be studied.

In our test system, Vn purified from urea-treated human serum was used (43). Urea-treated Vn is denaturated protein (11, 45) with an impaired cell-spreading efficiency but an increased affinity for heparin (2). The complement-inhibitory effect is dependent on the heparin-binding domain of Vn (41). The complement inhibition of the immobilized Vn was studied better with the anti-C9 antibody (Fig. 3 and 4) than with the anti-C3c antibody (Fig. 2). Vn inhibits complement activation in two ways: by binding to the metastable membrane-binding site of C5b-7 (37) and by preventing the polymerization of C9 (34). It has been argued that the inhibition of polymerization of C9 might be particularly important in the vessel wall (35).

Slightly less Vn was found on the heparinized than on the unheparinized surface (0.37 μg versus 0.21 μg). Protein configuration may change upon adsorption to unheparinized surfaces (1a, 38). In our experiments, however, the complement-binding site of Vn was apparently not affected, at least not during the short interval between immobilization and experiments (1a). The level of complement activation on a heparinized surface was very low, and no further decrease by Vn could be measured. Heparin inhibits the complement cascade at the C3 level (14, 19). The effect of heparin could be detected with the anti-C3c antibody as earlier described by Nilsson et al. (24, 25). The anti-C9 antibody provided a better tool with which to study the effects of Vn on complement activation.

Three different buffers were used to dilute serum prior to the experiments: VBS and MgEGTA buffers, which permitted total and alternative pathway activation, respectively, and EDTA buffer, which allowed no complement activation. The results obtained with serum diluted in EDTA buffer represent passively adsorbed complement components. To differentiate between passive adsorption and activation-dependent binding, the values obtained in serum diluted in VBS and MgEGTA buffers were subtracted by the corresponding EDTA values.

The level of Vn in the Vn-depleted serum was determined to be 36% of that of the serum before affinity chromatography. This was a rather high level of Vn and might influence the level of complement activation to some extent. However, the complement activation in the Vn-free serum was approximately four times higher than that in the normal serum (Fig. 4). The amount of immobilized Vn on the unheparinized surface was shown to be sufficient to inhibit complement activation in the Vn-free serum (Fig. 4). The immobilized Vn did not influence the level of complement activation in normal serum. There was a small decrease in total complement activation after, compared to before, the depletion procedure. The affinity chromatography on collagen-CNBr-activated Sepharose, however, seems to be a mild depletion procedure.

These experiments were carried out to study if staphylococcal cells could interfere with the effect of immobilized Vn previously found in this study. CoNS have been shown to bind both soluble and immobilized Vn (28–30). In one study, 11 of 19 strains of CoNS from biomaterial-associated infections in the cerebral ventricles expressed binding of immobilized Vn (18). Complement activation has been suggested to play a role in the pathogenesis of atherosclerosis, and the finding of Vn in these lesions led the authors to emphasize Vn as a protective mechanism against uncontrolled complement activation (23). Depositions of Vn have been found on extirpated central venous catheters (44) and in a hydrocephalus shunt (18a).

In our study, colonization of an unheparinized surface with a Vn-binding strain, SM131, resulted in the same level of complement activation as in the absence of Vn. This finding was repeated on a heparinized surface with and without Vn, in spite of the low amount of Vn on that surface (Fig. 5b). Colonization of the surface by strain 3380, which did not express binding of Vn, was also tested. This strain did not interfere with the inhibitory effect of immobilized Vn. The complement activation on the surface with Vn was significantly less than that without Vn (Fig. 5a). The level of Vn-binding sites on the bacterial cell wall influenced the capacity of bacterial cells to interfere with the Vn-complement interaction. Decrease of Vn-binding sites by culture in tryptic soy broth was seen as a loss of interference with immobilized Vn (Fig. 6).

The level of adhesion of strain SM131 was increased on an unheparinized surface with Vn (Table 1). An enhanced bacterial adhesion on the surface could mask the inhibitory effect of Vn, by further stimulation of the complement system. However, the individual strains activated complement to different levels, which was consistent with earlier reports (42). The Vn-binding strain SM131 was indeed a slightly weaker activator of the alternative pathway of the complement system than the nonbinding strain 3380. The significance of the capacity to activate complement by each strain might be low, since the complement activator Zymosan A had been added to serum.

The blocking of the effect of immobilized Vn by Vn-binding bacterial cells could be by steric hindrance. The bacterial cells adhered to the Vn, which thereby should be hindered from interaction with complement proteins. This could indeed be the mechanism, since bacterial cells treated with soluble Vn could inhibit complement activation (data not shown). The bacteria were coated with Vn in a configuration still able to inhibit complement activation. This finding indicates that the blocking of complement inhibition by Vn-binding cells was more likely to depend on steric hindrance than on a change in protein configuration. SM131 has been shown to bind immobilized Vn, and 3380 has been shown not to bind Vn. However, both strains have been shown to bind soluble Vn (30), which might explain why complement activation was inhibited after incubation with Vn-treated 3380 cells.

All experiments were carried out with bacterial cells cultured in broth media. Bacterial cell wall protein-binding structures are more easily exposed when bacteria are cultured in broth than when they are cultured on agar plates (1). No decrease of complement activation on surfaces with Vn was seen after incubation with nonbinding strain 3380 cultured on agar plates.

In conclusion, Vn immobilized on unheparinized surfaces could inhibit complement activation. Strain 3380, which did not express binding of Vn, did not interfere with the effect of immobilized Vn. On a surface colonized with strain SM131, complement activation proceeded as in the absence of immobilized Vn. Thus, Vn-binding bacteria might disrupt the protective effect of Vn adsorbed to a foreign implant. Colonization of a biomaterial surface with a Vn-binding strain might contribute to an enhanced surface-associated complement activation and thereby induction of inflammation around the implant. Surface-associated complement activation has been held responsible for aseptic inflammation (22). Thus, its contribution to the pathogenesis of early biomaterial-associated infections would be important.

ACKOWLEDGMENTS

This study was supported by grants from the Swedish Board for Technical Development, Thelma Zoéga's Foundation, the Swedish Medical Association, the Royal Physiographic Society in Lund, the Faculty of Medicine of Lund University, Vera and Carl Michelsens Foundation in Helsingborg, and the Medical Research Council (grant B95-16X 11229).

We gratefully acknowledge stimulating discussions with and valuable advice from Ulf Nilsson. We thank Ibrahim Gouda for heparinization of polystyrene microtiter plates and Kerstin Grunnér for 1,2-phenylenediamine tablets.

REFERENCES

1. **Baldassari, L.** Personal communication.

1a. **Barbucci, R., and A. Magnani.** 1994. Conformation of human plasma proteins at polymer surfaces: the effectiveness of surfaces: heparinization. Biomaterials **15:**955–962.

2. **Barnes, D. W., J. E. Reing, and B. Amos.** 1985. Heparin-binding properties of human serum spreading factor. J. Biol. Chem. **260:**9117–9122.

3. **Becherer, J. D., J. Alsenz, C. Servis, B. L. Myones, and J. D. Lambris.** 1989. Cell surface proteins reacting with activated complement components. Complement Inflammation **6:**142–165.

4. **Berger, M., Broxup, and M. V. Sefton.** 1994. Using Elisa to evaluate complement activation by reference biomaterials. J. Mater. Sci. Mater. Med. **5:** 622–627.

5. **Clark, D. E., and T. A. Raffin.** 1990. Intravenous complications of indwelling long-term central venous catheters. Chest **97:**966–972.

6. **Dierich, M. P.** 1988. The receptors, p. 262–272. *In* K. Rother and G. O. Till (ed.), The complement system. Springer-Verlag, Berlin, Germany.

7. **Edens, R. E., R. J. Linhardt, C. S. Bell, and J. M. Weiler.** 1994. Heparin and derivatized heparin inhibit zymozan and cobra venom factor activation of complement in serum. Immunopharmacology **27:**145–153.

8. **Fearon, D. T., and W. W. Wong.** 1983. Complement ligand-receptor interactions that mediate biological responses. Annu. Rev. Immunol. **1:**243–271.

9. **Gebb, C., E. G. Hayman, E. Engvall, and E. Ruoslahti.** 1986. Interaction of vitronectin with collagen. J. Biol. Chem. **261:**16698–16703.

10. **Giese, M. J., B. J. Mondino, B. J. Glasgow, H. L. Sumner, S. A. Adamu, H. P. Halabi, and H. J. Chou.** 1994. Complement system and host defense against staphylococcal endophthalmitis. Invest. Ophthalmol. Visual Sci. **35:** 1026–1032.

11. **Hayashi, M., T. Akama, I. Kono, and H. Kashiwagi.** 1985. Activation of vitronectin (serum spreading factor) binding of heparin by denaturing agents. J. Biochem. **98:**1135–1138.

12. **Hayman, E. G., M. D. Pierschbacher, Y. öhgren, and E. Ruoslahti.** 1983. Serum spreading factor (vitronectin) is present at the cell surface and in tissues. Proc. Natl. Acad. Sci. USA **80:**4003–4007.

13. **Kanse, S. M., C. Kost, O. G. Wilhelm, P. A. Andreasen, and K. T. Preissner.** 1996. The urokinase receptor is a major vitronectin-binding protein on endothelial cells. Exp. Cell Res. **224:**344–353.

14. **Labarre, D. J.** 1990. Heparin-like polymer surfaces: control of coagulation and complement activation by insoluble functionalized polymers. Biomaterials **9:**30–35.

15. **Larm, O., L. Adolfsson, I. Gouda, A. Malmberg, and P. Olsson.** 1987. An improved method for covalent immobilization of heparin by end-point attachment. Thromb. Haemostasis **58:**84.

16. **Law, S. K. A., and K. B. M. Reid.** 1988. Complement. Coordinating ed., D. Male and D. Rickwood. IRL Press Limited, Oxford, England.

17. **Ludwicka, A., L. M. Switalski, A. Lundin, G. Pulverer, and T. Wadström.** 1985. Bioluminescence assay for measurement of bacterial attachment to polyethylene. J. Microbiol. Methods **4:**169–177.

18. **Lundberg, F., S. Schliamser, and Å. Ljungh.** Vitronectin may mediate staphylococcal adhesion to polymers exposed to perfusing human cerebrospinal fluid. J. Med. Microbiol., in press.

18a. **Lundberg, F., J. Tegenfeldt, L. Montelius, U. Ransjö, P. Appelgren, P. Siesjö, and Å. Ljungh.** Unpublished data.

19. **Meri, S., and M. K. Pangburn.** 1990. Discrimination between activators and nonactivators of the complement pathway of complement: regulation via a sialic acid/polyanion binding site on factor H. Proc. Natl. Acad. Sci. USA **87:** 3982–3986.

20. **Mollnes, T. E., J. Riesenfeld, P. Garred, E. Nordström, K. Hogåsen, E. Fosse, O. Götzse, and M. Harboe.** 1995. A new model for evaluation of biocompatibility: combined determination of neoepitopes in blood and on artificial surfaces demonstrates reduced complement activation by immobilization of heparin. Artif. Org. **19:**909–917.

21. **Mollnes, T. E., V. Videm, J. Riesenfeld, P. Garred, J. L. Svennevid, E. Fosse, K. Hogasen, and M. Harboe.** 1991. Complement activation and bioincompatibility. The terminal complement complex for evaluation and surface modification with heparin for improvements of biomaterials. Clin. Exp. Immunol. **1:**21–26.

22. **Mondino, B. J., S. Nagata, and M. M. Glovsky.** 1985. Activation of the alternative complement pathway by intraocular lenses. Invest. Ophthalmol. Visual Sci. **26:**905–908.

23. **Niculescu, F., H. G. Rus, D. Porutiu, V. Ghiurca, and R. Vlaicu.** 1989. Immunoelectron-microscopic localization of S-protein/vitronectin in human atherosclerotic wall. Atherosclerosis **78:**197–203.

24. **Nilsson, U. R., K. E. Storm, H. Elwing, and B. Nilsson.** 1993. Conformational epitopes of C3 reflecting its mode of binding to an artificial polymer surface. Mol. Immunol. **30:**211–219.

25. **Nilsson-Ekdahl, K., B. Nilsson, M. Pekna, and U. R. Nilsson.** 1992. Generation of iC3 at the interface between blood and gas. Scand. J. Immunol. **35:**85–91.

26. **Nydegger, U. E., R. Rieben, and B. Aeschbacher.** 1990. Biocompatibility of apheresis harness. Transfus. Sci. **11:**43–54.

27. **Paulsson, M., I. Gouda, O. Larm, and Å. Ljungh.** 1994. Adherence of coagulase-negative staphylococci to heparin and other glycosaminoglycans immobilized on polymer surfaces. J. Biomed. Mater. Res. **28:**311–317.

28. **Paulsson, M., O. D. Liang, F. Ascencio, and T. Wadström.** 1992. Vitronectin-binding surface proteins of *Staphylococcus aureus*. Zentrabl. Bakteriol. **277:**54–56.

29. **Paulsson, M., Å. Ljungh, and T. Wadström.** 1992. Rapid identification of fibronectin, vitronectin, laminin and collagen cell surface binding proteins on coagulase-negative staphylococci by particle agglutination assays. J. Clin. Microbiol. **30:**2006–2012.

30. **Paulsson, M., and T. Wadström.** 1990. Vitronectin and type-I collagen binding by *Staphylococcus aureus* and coagulase-negative staphylococci by particle agglutination assays. J. Clin. Microbiol. **30:**2006–2012.

31. **Pekna, M., R. Larsson, B. Formgren, U. R. Nilsson, and B. Nilsson.** 1993. Complement activation by polymethylmethacrylate minimized by end-point heparin attachment. Biomaterials **14:**189–192.

32. **Pekna, M., L. Nilsson, K. Nilsson-Ekdahl, U. R. Nilsson, and B. Nilsson.** 1993. Evidence for the generation of iC3 during cardiopulmonary bypass as the result of blood-gas interaction. Clin. Exp. Immunol. **91:**404–409.

33. **Podack, E. R., and H. J. Müller-Eberhard.** 1979. Isolation of human S-protein, an inhibitor of the membrane attack complex of complement. J. Biol. Chem. **254:**9908–9914.

34. **Podack, E. R., K. T. Preissner, and H. J. Müller-Eberhard.** 1984. Inhibition of C9 polymerization within the SC5b-9 complex of complement by S-protein. APMIS Sect. C Suppl. **92:**89–96.

35. **Preissner, K. T.** 1991. Structure and biological role of vitronectin. Annu. Rev. Cell Biol. **7:**275–310.

36. **Preissner, K. T., and G. Müller-Berghauser.** 1985. S-protein modulates the heparin-catalyzed inhibition of thrombin by antithrombin. III. Evidence for a direct interaction of S-protein with heparin. Eur. J. Biochem. **156:**645–649.

37. **Preissner, K. T., E. R. Podack, and H. J. Müller-Eberhard.** 1985. The membrane attack complex of complement: relation of C7 to the metastable membrane binding site of the intermediate complex C5b-7. J. Immunol. **135:**445–451.

38. **Rapoza, R., and T. A. Horbett.** 1990. Postadsorptive transitions in fibrinogen: influence of polymer properties. J. Biomed. Mater. Res. **11124:**1263–1287.

39. **Rozalska, B., and Å. Ljungh.** 1995. Biomaterial-associated staphylococcal peritoneal infections in a neutropaenic mouse model. FEMS Microbiol. Lett. **11:**307–320.

40. **Salama, A., D. Hugo, R. Heinrich, R. Höge, R. Müller, V. Kiefel, C. Mueller-Eckhardt, and S. Bhakdi.** 1988. Deposition of terminal C5b-9 complement complexes on erythrocytes and leucocytes during cardiopulmonary bypass. N. Engl. J. Med. **7:**408–414.

41. **Tschopp, J., D. Masson, S. Schafer, M. Peitsch, and K. T. Preissner.** 1988. The heparin binding domain of S-protein/vitronectin binds to complement components C7, C8, and C9 and perforin from cytolytic T-cells and inhibits their lytic activities. Biochemistry **31:**4103–4109.

42. **van Wamel, W. J., A. C. Fluit, T. Wadström, H. van Dijk, J. Verhoef, and M. J. E. C. Vandenbroucke-Grauls.** 1995. Phenotypic characterization of epidemic versus sporadic strains of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **33:**1769–1774.

43. **Yatohgo, T., M. Izumi, H. Kashiwagi, and M. Hayashi.** 1988. Novel purification of vitronectin from human plasma by heparin affinity chromatography. Cell Struct. Funct. **13:**281–292.

44. **Yu, J., M. Nordman-Montelius, M. Paulsson, I. Gouda, O. Larm, L. Montelius, and Å. Ljungh.** 1994. Adhesion of coagulase-negative staphylococci and adsorption of plasma proteins to heparinized polymer surfaces. Biomaterials **15:**805–814.

45. **Zhuang, P., H. Li, J. G. Williams, N. V. Wagner, D. Seiffert, and C. B. Peterson.** 1996. Characterization of the denaturation and renaturation of human plasma vitronectin. J. Biol. Chem. **271:**14333–14343.

Editor: V. A. Fischetti