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Capsular Polysaccharide Types 5 and 8 of *Staphylococcus aureus* Bind Specifically to Human Epithelial (KB) Cells, Endothelial Cells, and Monocytes and Induce Release of Cytokines

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In order to examine the possible implication of capsular polysaccharide (CP) types 5 and 8 (CP5 and CP8) from *Staphylococcus aureus* in the pathological mechanism associated with staphylococcal infections, we tested the immunomodulatory effects of CP5 and CP8 on human epithelial KB cells, endothelial cells, and monocytes. Using biotinylated CP5 and CP8, we provide evidence that both CPs bind to KB cells, endothelial cells, and monocytes in a dose- and calcium-dependent manner through specific interactions. These results were confirmed by competition experiments using soluble cell extracts. Furthermore, we show that CPs bind to identical cell membrane receptors on all three types of human cells and that human normal serum contains a factor(s) which inhibits the binding of both CPs to human KB cells, endothelial cells, and monocytes. The ability of CP5 and CP8 to stimulate the production of cytokines by the human cells was then examined. CP5 and CP8 trigger KB cells to produce interleukin-8 (IL-8); endothelial cells to produce IL-8 and IL-6; and monocytes to produce IL-8, IL-6, IL-1β, and tumor necrosis factor alpha. The release of cytokines by all three types of cells is time dependent and dose dependent, and the tumor necrosis factor alpha production by monocytes is not affected by the addition of polymyxin B. We further confirm that human normal serum inhibits the immunomodulatory effects of both polysaccharides on each kind of cell. These results confirm that *S. aureus* CPs act as bacterial adhesins having immunomodulatory effects for human cells.

Several observations suggest that the interactions between epithelial cells and microorganisms are of importance in the initiation of microbial invasion of the host cells and tissues and in the progression of many bacterial infections. There is now evidence that inflammatory mediators play an important role in the pathology caused by bacterial infections. The production of the proinflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and IL-6, which have been associated with inflammatory diseases and septic shock, and the neutrophil chemoreactant IL-8 contributes to the host defense mechanisms in response to bacterial colonization or invasion and induces immunopathological disorders when these cytokines are secreted in excess. The production of IL-8 from epithelial cells can be expected to have a major impact in the neighboring microenvironment of the colonized tissues and to participate in the initiation of the inflammatory response. Epithelial cells from different anatomic locations are able to secrete TNF-α, IL-1β, IL-6, and IL-8 in response to various stimuli (1, 24, 29).

Many of the host responses to gram-negative bacteria could be attributed to lipopolysaccharide (LPS), which triggers monocytes to release inflammatory cytokines (20). Although the interactions of LPS with epithelial cells, endothelial cells, and monocytes are well documented, little is known about interactions of such cells with the components of gram-positive bacteria, e.g., *Staphylococcus aureus* used in this study.

S. aureus is considered an opportunistic bacterium and is isolated from the skin and nasopharynx of approximately 25% of healthy humans (31). Although *S. aureus* strains are noninvasive bacteria, they cause several diseases in humans by different pathogenic mechanisms. The most frequent and serious diseases include bacteremia in hospitalized and immunocompromised patients (5) and its complications such as endocarditis, septic arthritis, and osteomyelitis (30). In recent studies, increased secretion of TNF-α and IL-1β by monocytes subsequent to stimulation with *S. aureus* exotoxins (34), peptidoglycan (34), and membrane component (18) has been found. Approximately 90% of *S. aureus* isolates produce capsular polysaccharide (CP) (31). Although 11 capsular serotypes have been described (16), 80% of the human isolates belong to serotypes 5 and 8 (16), and there is now evidence that these CPs are virulence factors of *S. aureus*, causing bacteremia and associated diseases (6). However, the role played by the CPs in the pathological mechanism associated with staphylococcal infections remains unclear. It has been demonstrated that strains of type 5 or 8 resist opsonophagocytosis (15). Microcapsules elaborated by types 5 and 8 are extracellular uronic acids containing polysaccharides formed by a trisaccharide repeat unit, having identical monosaccharide compositions but differing in their linkages, and both CPs contain *N*-acetyl mannuronic acids which are *O* acetylated (C-3 for type 5 and C-4 for type 8) (22). Furthermore, recent studies have indicated that aside from LPS, various bacterial polysaccharides, including polyuronic acids (25), rhamnose-glucose polymers (4), and rhamnose-fucose polymers (33), trigger monocytes to release cytokines.

However, data concerning the release of cytokines into the microenvironment of the nasopharyngeal cavity after colonization with the opportunistic pathogen *S. aureus* are few.

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Therefore, to better understand the mechanisms of *S. aureus* pathogenicity, we have studied the interactions and immunomodulating activities of *S. aureus* CP type 5 (CP5) and CP8 with different cells. Since *S. aureus* types 5 and 8 can penetrate the bloodstream, epithelial and endothelial cells were selected and their interactions with CP5 and CP8 were compared with those of monocytes.

MATERIALS AND METHODS

Reagents. Materials were obtained from the following sources. Cell culture media (M 199 and RPMI 1640), HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), L-glutamine, penicillin, streptomycin, gentamicin, amphotericin B (Fungizone), and trypsin-EDTA solutions (0.5 g of trypsin per liter, 0.2 g of EDTA per liter, 0.85 g of NaCl per liter), and alkaline phosphatase-streptavidin were from Gibco BRL (Cergy-Pontoise, France); cell culture media had an endotoxin content which never exceeded 0.04 ng/ml as tested by a *Limulus* chromogenic assay. Polymyxin B, LPS from *Escherichia coli* O55:B5, biocytin, and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC) were from Sigma Chemical Co. (Saint-Quentin-Fallavier, France). DNase, RNase, and pronase were from Boehringer (Mannheim, Germany). Recombinant human IL-1β (rhIL-1β) was a gift from K. Vosbeck (Ciba-Geigy, Basel, Switzerland), and rhTNF-α was kindly given by L. Knoll (BASF, Ludwigshafen, Germany). A mouse monoclonal anti-*S. aureus* CP5 antibody was a gift from P. Sarradin (Institut National de la Recherche Agronomique, Tours, France); mouse monoclonal anti-rhTNF-α and anti-rhIL-1β antibodies were from Immunotech (Marseille, France). Biotinylated rabbit polyclonal anti-rhTNF-α and anti-rhIL-1β sera were prepared as previously described (4). The enzyme immunoassay kits for IL-6 and IL-8 were from Medgenix (Rungis, France); endotoxin-free human serum albumin, purified human fibronectin prepared according to the method of Ruoslahti et al. (28), and human normal serum (HNS) prepared from a pool of serum specimens from 13 to 15 healthy blood donors negative for hepatitis B virus and human immunodeficiency virus were from the Centre Regional de Transfusion Sanguine, Strasbourg, France. The serum was complement inactivated at 56°C for 30 min (HI-HNS).

Bacterial strains and purification of CP5 and CP8 from *S. aureus*. The purification of CPs was carried out by a method previously described (10), with some modifications. Briefly, *S. aureus* Reynolds (prototype 5) and Becker (prototype 8) were cultivated with shaking in liquid synthetic broth containing 1 g of glucose per liter for 48 h at 37°C (27). Cultures were then autoclaved at 120°C for 45 min. Cells were pelleted by centrifugation (3,000 × *g*, 30 min), and supernatants were retained. A second autoclaving-centrifugation cycle was performed under similar conditions. The supernatants were pooled and filtered through a 0.2-μm-pore-size membrane. This crude extract was ultrafiltered extensively at 4°C with a 30-kDa-cutoff hollow-fiber cartridge (P30) (Amicon, Epernon, France) and treated with 0.05 M $NaIO_4$ as described elsewhere (10). After a second ultrafiltration step, the P30 retentate was concentrated and lyophilized. The lyophilized fraction was resuspended (10 mg/ml) in 50 mM Tris-HCl–2 mM $MgSO_4$ (pH 7.5) buffer, treated with DNase and RNase (80 μg of each per ml) at 37°C for 4 h, and filtered through a 0.2-μm-pore-size membrane. Pronase was added (0.1 mg/ml), and the mixtures were incubated at 37°C for 3 h and filtered again through a 0.2-μm-pore-size membrane. A third ultrafiltration step was performed, and again P30 retentate was obtained. The quality of separation during this P30 diafiltration was controlled by high-performance liquid chromatography as described previously (10). Finally, the CPs purified in the P30 retentates were lyophilized (10). The purified polysaccharides were free of endotoxin (less than 0.03 ng/ml) as determined by a *Limulus* chromogenic test and contained less than 2% protein, 2% nucleic acids, and 0.01% teichoic acids. CP5 and CP8 contents were detected and measured during the purification procedures by competitive enzyme-linked immunosorbent assays (ELISAs) with rabbit polyclonal anti-CP5 and anti-CP8 sera as described previously (10).

EDAC was used for labelling CP5 or CP8 with biocytin according to the method described by van de Wiel et al. (35). Briefly, 2 mg of EDAC was added to 1 mg of CP5 or CP8 and 2.5 mg of biocytin in 0.5 ml of H_2O adjusted to pH 4.7. The reaction was allowed to proceed for 3 h at room temperature with gentle stirring at a constant pH (pH 4.7). The reaction was stopped by addition of ethanolamine (pH 10). After 3 h, the pH was adjusted to 7.4 and the biotinylated CPs were dialyzed against phosphate-buffered saline (PBS), pH 7.4, and stored at 4°C.

Preparation of cells and solubilization of membrane proteins. (i) KB cells. Cells of the human epithelioid carcinoma cell line KB were routinely grown in RPMI 1640 medium containing 2 mM L-glutamine, gentamycin (50 μg/ml), and 10% heat-inactivated fetal calf serum. Cells were cultured at 37°C in 50-ml culture flasks (Nunc, Roskilde, Denmark) in a humidified atmosphere of 5% CO_2 in air until they reached confluence. Cell monolayers were treated with fresh trypsin for 5 min at room temperature and centrifuged at 500 × *g* for 10 min, after which the cell pellet was suspended in fresh medium and 0.2 ml was added to each well of 96-well microtiter plates (Nunc) (10^5 cells per well) and allowed to grow until confluence (48 h), reaching a final density of $2.2 \times 10^5 \pm 0.2 \times 10^5$ cells per well.

(ii) Endothelial cells. Human endothelial cells (HSVEC) were collected from fragments of saphenous veins obtained during stripping (3) and cultured as previously described (17). The culture medium was M 199-RPMI 1640 (1:1) containing 10 mM HEPES, 2 mM L-glutamine, penicillin (100 IU/ml), streptomycin (100 μg/ml), amphotericin B (0.25 μg/ml), and 20% HI-HNS. The cells were frozen at the 2nd passage and used in experiments from the 3rd to the 10th passage. Cells were seeded at 3×10^3 cells per well in fibronectin-coated 96-well plates in culture medium and cultivated for 8 days until formation of confluent monolayers, reaching a final concentration of $1.5 \times 10^4 \pm 0.2 \times 10^4$ cells per well.

(iii) Monocytes. Mononuclear cells were isolated from blood of healthy donors by countercurrent elutriation (32). Under these conditions, more than 94% of the cells were monocytes as estimated by morphological analysis and histochemical staining. Monocytes (2×10^5 cells per well) were allowed to adhere to 96-well microtiter plates for 2 h.

Cell number and cell viability were examined by the MTT test as described elsewhere (23). Prior to each binding or activation experiment, the cells were intensively washed with serum-free RPMI 1640, incubated for 2 h in serum-free RPMI 1640 medium, and washed again three times to ensure maximal removal of serum components before addition of the appropriate stimuli diluted in serum-free RPMI 1640.

Monolayers of KB cells and endothelial cells grown in 50-ml culture flasks were harvested by repeated rinsing with ice-cold medium. Membrane proteins from pellets of approximately 10^7 KB cells, endothelial cells, or monocytes were extracted for 5 min on ice in 0.5 ml of PBS containing 1% Nonidet P-40 (NP-40) and antiproteases as previously described (9). Protein concentrations of samples were determined by using a dye reagent (Bio-Rad, Ivry sur Seine, France) according to the manufacturer's procedure.

Binding assays. Assays for binding of either biotinylated CP5 (Biot-CP5) or biotinylated CP8 (Biot-CP8) were carried out at 4°C in serum-free RPMI 1640. Microtiter plates coated with KB cells (2.2×10^5 cells per well), endothelial cells (2×10^4 cells per well), or monocytes (2×10^5 cells per well) were first incubated with 250 μl of RPMI 1640 containing 0.5% gelatin for 30 min. After being washed, the cells were incubated for 1 h with 200 μl of RPMI 1640 containing various concentrations of Biot-CP5 or Biot-CP8. After washing and fixation of the cells with methanol, bound CPs were detected by sequential incubations with alkaline phosphatase-streptavidin (1 h, 37°C) followed by enzyme substrate (1 h, 25°C) and A_{405} was read. Nonspecific binding was assayed in the presence of a 40-fold excess of unlabelled CPs. The level of specific binding was obtained by subtracting nonspecific binding from total binding. The results are expressed as amounts of specifically bound CPs for 10^5 cells. In parallel, Biot-CP binding was tested in the presence of various concentrations of HI-HNS. In competition assays, cells were incubated for 1 h with Biot-CPs in subsaturating concentrations (25 μg/ml) in the presence of various competitors. The results are expressed as percent inhibition of labelled CP binding: % inhibition = 100 − [(binding activity with competitor/binding activity without competitor) × 100].

Activation of cells. Human KB cells (2.2×10^5 cells per well), endothelial cells (1.5×10^4 cells per well), and monocytes (2×10^5 cells per well) were incubated at 37°C with 200 μl of RPMI 1640 supplemented with 2 mM L-glutamine, antibiotics, and 0.5% human serum albumin containing various amounts of either CP5 or CP8 for 0 to 24 h in the absence or presence of HI-HNS. In some experiments, polymyxin B (25 μg/ml) was added to confirm that the observed effects were not due to possible LPS contamination. After different incubation periods (0, 2, 6, 12, and 24 h), culture supernatants were harvested, centrifuged (3,000 × *g*, 10 min), and used to estimate TNF-α, IL-1β, IL-6, and IL-8 release. In some controls, cells were incubated with anti-CP5 monoclonal antibodies, and cells incubated with a single concentration of LPS (1 μg/ml) were used as positive controls.

Cytokine assays. Extracellular release of TNF-α and IL-1β was determined by a heterologous two-site sandwich ELISA as previously described (4). The readings were related to standard curves with rhTNF-α and rhIL-1β, and the sensitivity level was up to 100 pg. Extracellular release of IL-6 and IL-8 was measured by using ELISA kits for secreted IL-6 and IL-8 according to the manufacturer's instructions.

RESULTS

Specific binding of CP5 and CP8 to human cells. We first studied the binding of *S. aureus* CP5 and CP8 to three human cell systems, epithelial KB cells, endothelial cells, and monocytes.

(i) KB cells. KB cells were incubated for 1 h at 4°C with increasing amounts of either Biot-CP5 or Biot-CP8, ranging from 0 to 100 μg/ml, in the absence or presence of a 40-fold excess of unlabelled ligand to establish the specificity of binding in serum-free conditions. Incubation of KB cells with unlabelled ligand almost completely suppresses the binding of Biot-CP5 and Biot-CP8 to the receptors, and the remaining binding activity (ca. 10 to 20%) probably represents nonspe-

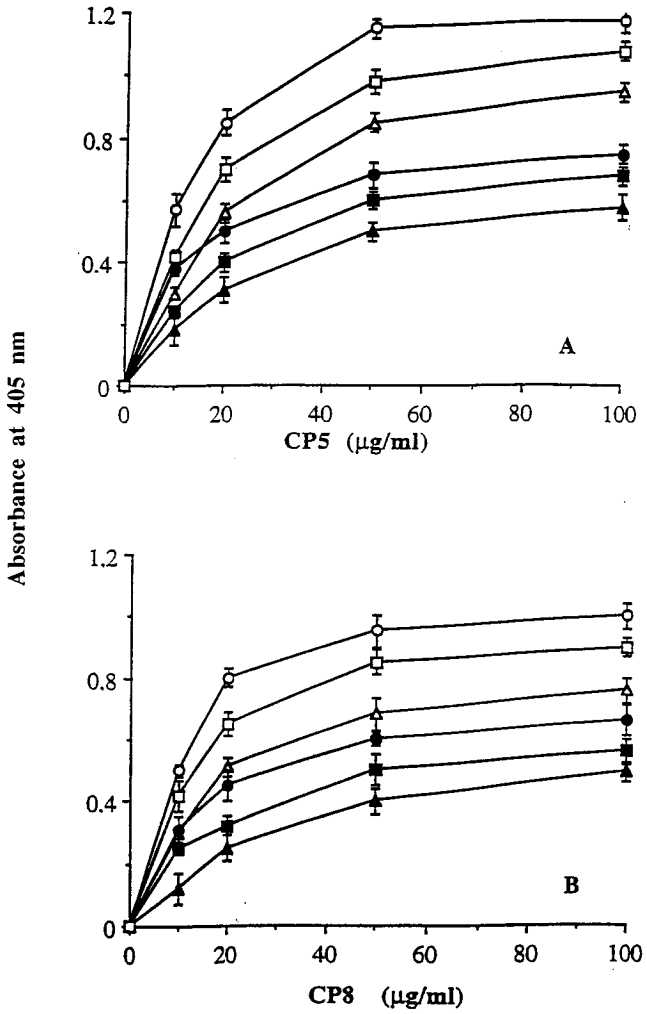

FIG. 1. Dose-response analysis of the specific binding of Biot-CP5 (A) and Biot-CP8 (B) to human KB cells (▲ and △), endothelial cells (● and ○), and monocytes (■ and □) in the absence (shaded symbols) or presence (unshaded symbols) of 20 mM $CaCl_2$. Specific binding was calculated for 10^5 cells as the difference between total binding in the absence of unlabelled CPs and nonspecific binding in the presence of unlabelled CPs at a 40-fold excess. Each datum point represents the mean of duplicate determinations ± the standard error (error bars) and is representative of three different experiments.

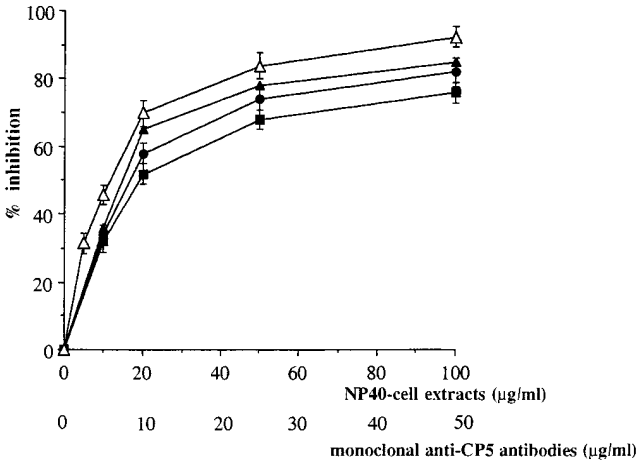

FIG. 2. Inhibition of binding of Biot-CP5 to KB cells (▲ and △), endothelial cells (●), and monocytes (■) by increasing amounts of soluble NP-40 extracts from KB cells (▲), monoclonal anti-CP5 antibodies (△), endothelial cells (●), and monocytes (■). The results are expressed as percent inhibition of CP binding without inhibitors and are arithmetic means ± standard errors (error bars) of triplicate determinations for three different experiments.

cific binding. KB cells exhibited specific and saturable binding of both CP5 (Fig. 1A) and CP8 (panel B), and saturation was achieved at 50 μg of polysaccharides per ml. The level of CP5 binding was slightly higher than that of CP8 binding at all concentrations. The addition of 20 mM $CaCl_2$ induced an increase of both CP5 and CP8 binding (Fig. 1A and B); therefore, all subsequent experiments were performed in the presence of 20 mM $CaCl_2$. To test whether CP5 and CP8 bind specifically to KB cells, we incubated plates coated with KB cells with either Biot-CP5 or Biot-CP8 at subsaturating concentrations (25 μg/ml) in the presence of variable amounts of KB cell NP-40 extract. The results show that NP-40 extract was able to inhibit the binding of CP5 (Fig. 2) or CP8 (data not shown) to plates coated with KB cells in a dose-dependent fashion. In control experiments, a 0.1% (vol/vol) solution of NP-40 had no effect on the binding of either CP5 or CP8 to KB cells. These results were confirmed by competition experiments using mouse monoclonal antibodies against CP5, which were able to inhibit the binding of CP5 to KB cells in a dose-dependent fashion; a maximal inhibiting activity of 90% was obtained with 50 μg of anti-CP5 monoclonal antibodies per ml (Fig. 2). To further characterize the receptor(s), we investigated the ability of CP8 to inhibit the binding of Biot-CP5 to KB cells. Unlabelled CP8 inhibited the binding of labelled CP5 to KB cells (Fig. 3A). As previous observations had shown that serum components could interfere with the binding of various polysaccharides to cells, it was of interest to test the effect of HI-HNS on the binding of Biot-CP5 to KB cells. The results shown in Fig. 3B indicate that HI-HNS substantially inhibited the binding of Biot-CP5 to KB cells. The maximal binding-inhibiting activity of 90% was obtained with 20% HNS, and this inhibition was effective for dilutions of HNS up to 1%. Identical results were obtained with CP8 (data not shown).

Taken together, these results demonstrate that (i) CP5 and CP8 bind specifically to the same KB cell surface receptor(s), (ii) CP binding is calcium dependent, and (iii) HNS contains a factor(s) which inhibits the binding of CPs to KB cells.

(ii) Endothelial cells. CP binding experiments were also performed with endothelial cells. The results show that Biot-CP5 (Fig. 1A) and Biot-CP8 (Fig. 1B) specifically bind to endothelial cells in a dose-dependent manner (nonspecific binding never exceeds 20%) and that calcium enhances specific binding of CP5 (Fig. 1A) and CP8 (Fig. 1B) to cells. These results are confirmed by competition studies, which showed that binding of CP5 (Fig. 2) to endothelial cells is inhibited by endothelial cell NP-40 extract and anti-CP5 monoclonal antibodies (data not shown); identical patterns of inhibition were observed with CP8 (data not shown). It was also found that unlabelled CP8 inhibited the binding of Biot-CP5 (Fig. 3A) to endothelial cells, confirming the fact that the two CPs bind to the same endothelial cell surface receptor(s). CP binding was also assessed in the presence of various dilutions of HI-HNS, and the results show that addition of 20% HI-HNS in sample mixtures almost completely inhibited the CP5 binding to endothelial cells (Fig. 3B), confirming the results obtained with KB epithelial cells.

(iii) Monocytes. CP5 and CP8 binding experiments were also performed with plates coated with monocytes. The results confirm that (i) CP5 and CP8 bind specifically to monocytes in a

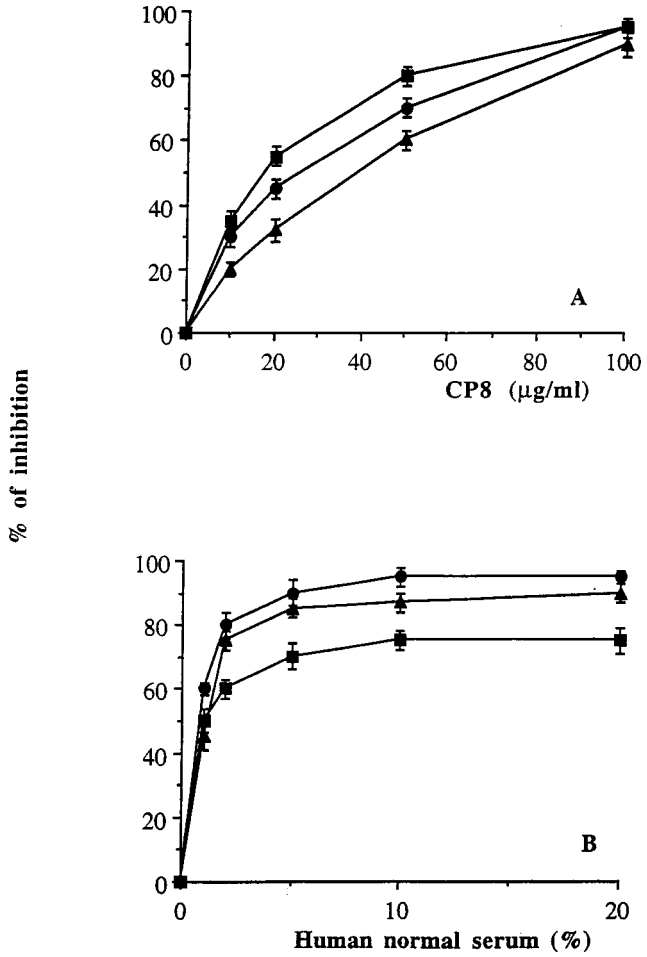

FIG. 3. CP8-mediated (A) and serum-mediated (B) inhibition of binding of Biot-CP5 to KB cells (▲), endothelial cells (●), and monocytes (■). The binding of CP5 observed in the presence of various amounts of either unlabelled CP8 (A) or HNS (B) is expressed as percent inhibition of CP5 binding measured in the absence of inhibitors. The results are expressed as the means ± standard errors (error bars) of triplicate determinations performed on three different cell preparations.

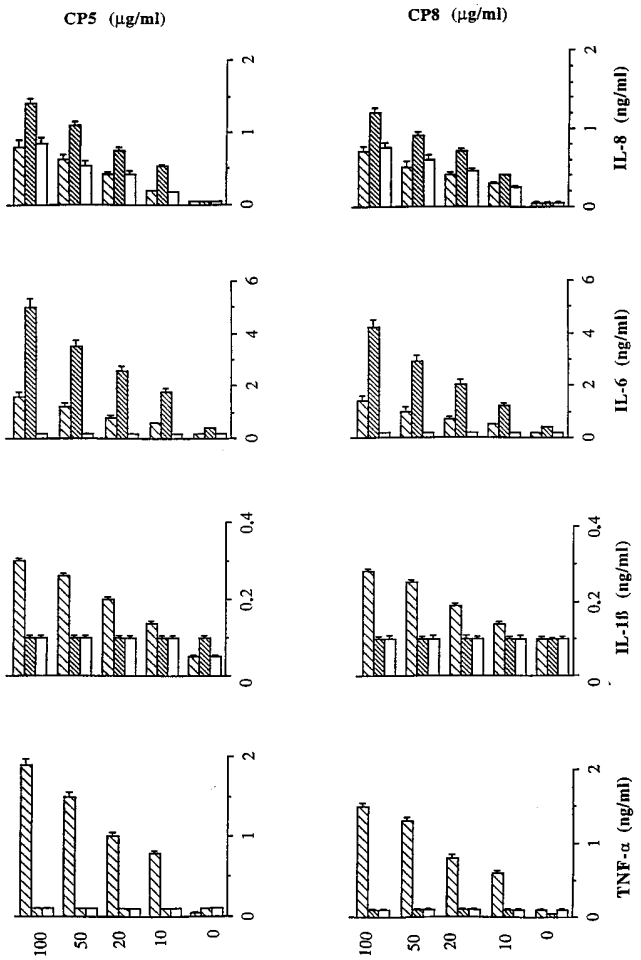

FIG. 4. Dose-dependent production of TNF-α, IL-1β, IL-6, and IL-8 by human epithelial KB cells (□), endothelial cells (□), and monocytes (□) stimulated for 24 h with increasing amounts of CP5 (left) or CP8 (right). Data are expressed as means of cytokine release by 10^5 cells ± standard deviations (error bars) for triplicate determinations, and the results are representative of four different experiments.

dose-dependent manner (Fig. 1A and B) (nonspecific binding was ca. 15 to 20%) and (ii) $CaCl_2$ enhances binding of both CPs (Fig. 1A and 1B). These results were confirmed by competitive studies using either monocyte NP-40 extract, anti-CP5 monoclonal antibodies, CP8, or HI-HNS. NP-40 extracts were able to inhibit the binding of both polysaccharides to monocytes (Fig. 2). Anti-CP5 monoclonal antibodies (data not shown), CP8 (Fig. 3A), and HI-HNS (Fig. 3B) inhibited the binding of CP5.

Cytokine release by human epithelial KB cells, endothelial cells, and monocytes after stimulation with CP5 and CP8. The specific binding of *S. aureus* CP5 and CP8 to the epithelial KB cells, the endothelial cells, and the monocytes led us to examine the ability of the two polysaccharides to stimulate the release of cytokines by these cells.

At time zero, the cells were incubated with several concentrations of either CP5 or CP8 diluted in RPMI 1640 or with RPMI 1640 alone for 24 h, and samples were taken at 0, 2, 6, 12, and 24 h. The TNF-α, IL-1β, IL-6, and IL-8 activities in the cell supernatants were measured by a two-site sandwich ELISA. The cytokine activity in the supernatants of cells maintained in medium without stimuli was used as a measure of constitutive secretion of cytokines by the cells.

(i) KB epithelial cells. In the absence of CP5 and CP8 stimuli, the KB cells released up to 100 pg of TNF-α and IL-1β per ml, 200 pg of IL-6 per ml, and 50 pg of IL-8 per ml. The addition of increasing amounts of either CP5 or CP8, ranging from 0 to 100 μg/ml, did not enhance the secretion of TNF-α, IL-1β, and IL-6 above the constitutive levels (Fig. 4). In contrast, IL-8 activity in the supernatants of KB cells stimulated with CP5 increased in a dose-dependent fashion above the constitutive level (Fig. 4) and was not affected by the addition of polymyxin B (25 μg/ml). The release of IL-8 was time dependent, the best secretion being obtained at 12 h with 50 μg of CP5 per ml, twofold higher than that in culture stimulated by LPS (1 μg/ml); and suppressed by incubation with anti-CP5 monoclonal antibodies. This IL-8 secretion profile was also obtained with CP8; however, the IL-8 levels were slightly lower than those obtained with CP5 (Fig. 4). To examine potential or suppressive effects of serum components on CP-mediated activation of KB cells, monolayers of KB cells were incubated with one concentration of either CP5 or CP8 and various dilutions of HNS. The results showed that HI-HNS inhibited

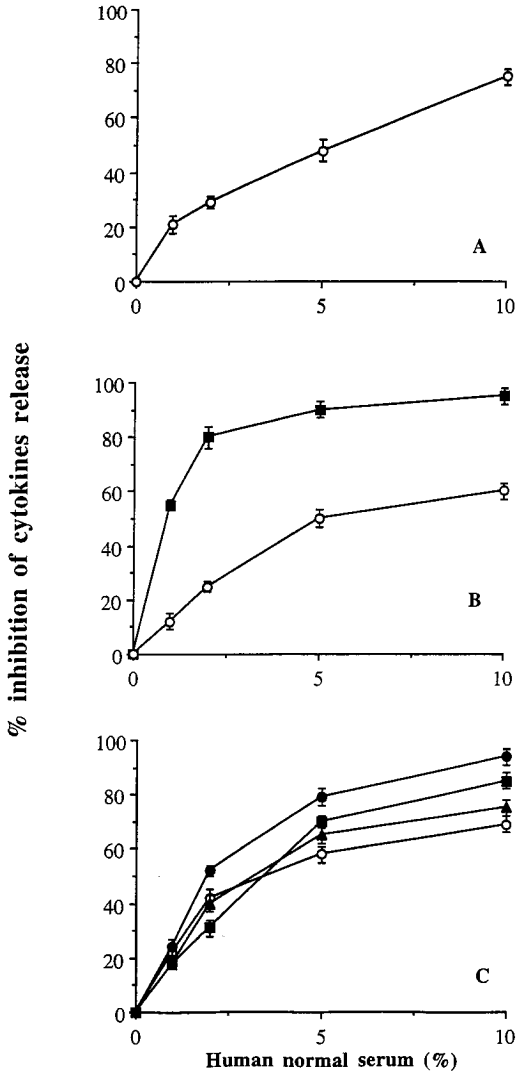

FIG. 5. Serum-mediated inhibition of TNF-α (●), IL-1β (▲), IL-6 (■), and IL-8 (○) release by KB cells (A), endothelial cells (B), and monocytes (C) stimulated with 50 μg of CP5 per ml. The results are expressed as percent inhibition of cytokine release without serum and are means ± standard errors (error bars) of triplicate determinations for three different experiments. Identical patterns of inhibition were observed with cells stimulated with CP8.

the CP5- and CP8-induced IL-8 production by KB cells (Fig. 5A), and maximal inhibitions of IL-8 release of 75 and 70%, respectively, were obtained with 10% HNS.

(ii) Endothelial cells. It was previously shown that both CP5 and CP8 trigger IL-8 production by KB cells. Therefore, we investigated the effect of these CPs on cytokine production by human endothelial cells. Endothelial cells constitutively released small amounts of TNF-α, IL-1β, IL-6, and IL-8, the four cytokines making up between 50 and 400 pg/ml as measured by ELISA. TNF-α and IL-1β were not generated from CP5- and CP-8-stimulated endothelial cells, whereas both polysaccharides were potent stimuli for secretion of IL-6 and IL-8. The patterns of IL-6 and IL-8 release were dose dependent, with maximal values of between 5 and 4.2 ng/ml for IL-6 and 1.4 and 1.2 ng/ml for IL-8, and the IL-6 and IL-8 levels reached a peak at 6 and 12 h, respectively. Furthermore, as noted previously for KB cells, CP5 was a more effective activator for endothelial cells than CP8 was. In addition, the overall IL-8 response to CPs, calculated at the same time and for the same cell concentration as for KB cells, was approximately two-fold higher for endothelial cells than for KB cells (Fig. 4). To test whether serum components inhibit endothelial cell response to CP5 or CP8, we compared the cell responses in the presence and in the absence of HI-HNS. As previously noted for KB cells, HI-HNS reduced in a dose-dependent fashion the release of IL-6 as well as the release of IL-8 in response to 50 μg of CP5 or CP8 per ml, and 95% or more inhibition of IL-6 secretion and 60% inhibition of IL-8 secretion were obtained with 10% HNS (Fig. 5B).

(iii) Monocytes. In the absence of stimuli, monocytes released small amounts of TNF-α, IL-1β, IL-6, and IL-8 (50 to 200 pg/ml). After stimulation with CP5 and CP8, the TNF-α, IL-1β, IL-6, and IL-8 activities in the supernatants from monocytes increased in a dose-dependent manner (Fig. 4). TNF-α, IL-1β, IL-6, and IL-8 levels peaked at 24, 12, 6, and 12 h, respectively, and the corresponding IL-6 and IL-8 levels calculated at the same time for identical cell concentrations were three- and twofold less for monocytes than for endothelial cells. Inasmuch as the above results suggested that HNS inhibited the binding and the stimulation of KB and endothelial cells, we investigated the ability of HI-HNS to interfere with CP5 and CP8 stimulation of monocytes. The results indicate that HNS was able to inhibit the TNF-α, IL-1β, IL-6, and IL-8 responses of monocytes to CP5 and CP8 in a dose-dependent fashion (Fig. 5C), and the patterns of HNS inhibition of cytokine release were similar to those observed for KB and endothelial cells.

DISCUSSION

Microcapsules are prevalent among *S. aureus* strains isolated from both commensal and pathogenic sources. However, the exact role played by these CPs in the pathogenesis of staphylococcal infections remains unclear. Recently, Lee et al. (19) reported that staphylococcal cells grown on solid support both in vivo and in vitro produced high levels of CPs, and Arbeit and Nelles (2) detected circulating CP8 in blood of animals with bacteremia and also in rats with subcutaneous staphylococcal infections. Therefore, we hypothesized that CPs could be an adherence factor and could contribute to the inflammatory response associated with staphylococcal colonization of host tissues. First we examined the binding of CPs to human epithelial and endothelial cells and monocytes, and second we investigated whether the binding of CPs activated cells to release cytokines.

This report demonstrates that purified CPs isolated from *S. aureus* prototypes 5 and 8 bind to human epithelial KB cells, endothelial cells, and monocytes, and we confirm that the cytokine-stimulatory activities of CP5 and CP8 resulted from ligand-receptor interactions. The specific binding of CP5 and CP8 to cells was demonstrated by direct binding assays using Biot-CP5 and Biot-CP8 and alkaline phosphatase-streptavidin and by competitive binding experiments using corresponding NP-40 extracts. With both methods, we showed that CP5 and CP8 bind to human epithelial KB cells, endothelial cells, and monocytes in a dose-dependent, saturable fashion in the absence of serum. The fact that CPs bind to cells in the absence of serum supports the concept that CPs are able to interact directly with the cell surface receptors. Furthermore, specific binding of the CPs to the three types of cells is enhanced by Ca^{2+} and reduced by serum components present in HI-HNS. There is also convincing evidence that CP5 and CP8 bind to the same receptor(s) present on the surface of each type of cell, since the binding of Biot-CP5 to the cells could be inhib-

ited by unlabelled CP8. However, at the present time there is no conclusive evidence that the CPs bind to surface receptors which are identical on all three cells. These results are in agreement with those of Hmama et al. (13), who showed that an acetylpolygalactosyl (APG) molecule isolated from *Klebsiella pneumoniae* binds to monocytes in the absence of serum. However, the interactions between APG and monocytes do not seem to be mediated by the polygalactose chain, indicating a lack of interaction between the *O*-polysaccharide antigen of APG and the monocyte cell surface receptor(s), contrary to the interactions of CPs with the cells. There is now evidence that LPS and other bacterial components, such as polysaccharides (4, 33), lipoteichoic acids (37), peptidoglycans (34), and various proteins (14, 26), bind to different cells, including epithelial cells, endothelial cells, monocytes/macrophages, and granulocytes, via different receptors under serum-free conditions. Furthermore, the binding of these molecules initiates the cellular response which results in the release of various cytokines and in the enhanced expression of adhesive surface molecules (36). We have shown that both CP5 and CP8 are potent stimulators of IL-8 production from KB epithelial cells, of IL-6 and IL-8 production from endothelial cells, and of TNF-α, IL-1β, IL-6, and IL-8 production from monocytes, as noted by others. These results confirm that (i) bacterial polysaccharides are powerful inducers of cytokine release and (ii) serum components are not essential to the binding of CPs to cells and to the formation of targeting signals leading to cytokine release. However, this is in contradiction with the work of Espevik et al. (8), which showed that β1-4-linked polyuronic acids and alginate from *Pseudomonas aeruginosa* induced monocytes to produce TNF-α only in the presence of serum. Furthermore, these authors showed that TNF-α release resulted from interactions of both polysaccharides with CD14 molecules and that LPS inhibited the binding of polyuronic acids to monocytes, and they concluded that there are common factors in serum which bind to polyuronic acids and to the polysaccharide part of the LPS molecule. We found that serum components inhibited the cellular response to CPs. An explanation could be that serum contains factors which neutralize the biological activities of CPs either by forming inactive complexes which are unable to bind to cellular receptors or by directing the complexes toward different receptors which are not involved in cell activation. The presence of natural anti-CP5 and anti-CP8 antibodies in HI-HNS was tested by ELISA, and the results showed that HI-HNS contains specific antibodies directed against both CP5 and CP8 (titers were between 32 and 64) (data not shown). Therefore, we cannot exclude the possibility that at least a part of the observed inhibitory activity of HI-HNS is due to anti-CP5 or anti-CP8 antibodies. Accordingly, Dentener et al. (7) showed that LPS binding protein (LBP) and bactericidal permeability-increasing protein (BPI) have dual activities against LPS and Malhotra et al. (21) reported that serum mannan binding protein (MBP) binds to different polysaccharides and directs them towards the monocyte C1q receptor without cytokine synthesis.

CP5 and CP8 were unable to induce TNF-α, IL-1β, and IL-6 production from KB epithelial cells, while both CPs triggered endothelial cells to release IL-6 and IL-8 and monocytes to release TNF-α, IL-1β, IL-6, and IL-8, suggesting that the epithelial cell response to CPs is different from the response of endothelial cells and monocytes and confirm previous observations which showed that epithelial cells have a more restricted cytokine response (12). The fact that epithelial cells secrete IL-8 after binding of CPs is consistent with the hypothesis that epithelial cells may be an important source of IL-8 produced in response to pharyngeal colonization of the host by *S. aureus*. These results suggest also that epithelial cells stimulated with bacterial components have the ability to recruit and to activate inflammatory and other cells by the release of IL-8. This confirms previous observations that epithelial cells participate in the cytokine network (11) and that they not only act as a mechanical barrier against bacterial infections but also are essential in initializing the cellular immune response against bacterial infections.

In conclusion, the present work demonstrates (i) that *S. aureus* CP5 and CP8 specifically bind to human cells, including epithelial and endothelial cells and monocytes, and could act as a bacterial adhesin for mammalian cells, (ii) that CP binding is enhanced by Ca^{2+}, (iii) that CPs bind either to identical cell membrane receptors on each type of human cell or to different receptors which sterically interfere with each other, (iv) that CPs exert immunomodulatory effects on all three types of cells, and (v) that serum inhibits the binding and immunomodulatory effects of both CPs, which could be putative components involved in the pathological mechanisms of the various diseases associated with *S. aureus*. These results reveal the existence of virulent staphylococcal components other than endotoxins and peptidoglycans. However, there are still questions to be answered. First, it is not clear which receptors are involved in both the binding and activation of cells, and the second question concerns the nature of the serum components which are involved in the inhibition of cell activation. Further work needs to be done to elucidate the differential cell activation processes mediated by *S. aureus* CP5 and CP8.

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