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Influence of serum on interaction of *Porphyromonas gingivalis* ATCC 33277 and *Aggregatibacter actinomycetemcomitans* Y4 with an epithelial cell line

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Background and Objective: The purpose of this study was to investigate the influence of serum on the interaction of periodontal pathogens with epithelial cells using an epithelial cell line (KB cells). This is important because serum is a key component of gingival crevicular fluid and may influence inflammatory responses in epithelial cells exposed to periodontal pathogens.

Material and Methods: Porphyromonas gingivalis ATCC 33277 and *Aggregatibacter actinomycetemcomitans* Y4 were co-cultured with KB cells either with or without the addition of up to 10% human serum or 50 mg/mL human serum albumin. The numbers of free-floating, adherent and intracellular bacteria were determined up to 18 h after exposure of the epithelial cells to the pathogens. Additionally, the concentrations of interleukin (IL)-6 and IL-8 produced by the epithelial cells in response to exposure to the bacteria were determined.

Results: Serum and human serum albumin reduced the number of internalized *A. actinomycetemcomitans* Y4 organisms in the epithelial cells, increased the levels of IL-6 and IL-8 in the supernatants of infected cells (those with internalized *A. actinomycetemcomitans*) and influenced non-infected epithelial cells. Increased IL-6 and IL-8 concentrations were also detected in the supernatants of KB cells infected with *P. gingivalis* ATCC 33277. Interleukin-6 and IL-8 were detectable after addition of serum, probably as a result of inhibition of the activity of *P. gingivalis* cysteine proteinases by serum.

Conclusion: Serum promotes the release of the cytokines IL-6 and IL-8 by epithelial cells. This mechanism is influenced by periodontal pathogens and may maintain clinical periodontal inflammation.

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Oral and gingival epithelial cells act as a barrier to prevent microbes from invading the periodontal tissues. It is now accepted that epithelial cells form an integral part of the innate immune system and play an active role in inflammatory responses (1). Epithelial cells are the major source of interleukin (IL)-8 (2), which can be detected in gingival crevicular fluid (3) and periodontal tissue biopsies (4). Interleukin-8 is considered one of the most important mediators for accumulation of neutrophils (5–7). Periodontitis patients have a higher level of IL-6 within gingival crevicular fluid (8). Interleukin-6 plays an important role in regulating the immune response to periodontal pathogens and is notably responsible for the differentiation of activated B cells into immunoglobulin-secreting plasma cells (9) as well as contributing to osteoclastogenesis (10).

Periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* can modulate the secretion of these proinflammatory cytokines (11,12). *A. actinomycetemcomitans* may induce the expression of the proinflammatory chemokine IL-8 by epithelial cells (13). *Porphyromonas gingivalis* is an asaccharolytic anaerobe with a high level of proteolytic activity, and produces arginine- and lysine-specific cysteine proteases referred to as gingipains (14,15), which are considered the most important virulence factors for this organism. Proteases of *P. gingivalis* degrade expressed IL-8 (16). In addition, both *A. actinomycetemcomitans* and *P. gingivalis* are able to adhere to and invade primary and transformed epithelial cells (5,6,17–20).

In vitro experiments which study the interaction of *P. gingivalis* and *A. actinomycetemcomitans* with epithelial cells normally use a starved cultivation of cells with a limited amount of serum. Nevertheless, gingival crevicular fluid contains up to 35% of the albumin found in human serum (21), so serum may influence the inflammatory responses in the periodontal tissues, particularly since gingival crevicular fluid flow increases as a result of inflammation (22).

The purpose of this study was to investigate the influence of serum and its main protein constituent, human serum albumin (HSA), on interactions of the periodontal pathogens *P. gingivalis* and *A. actinomycetemcomitans* with KB cells, an epithelial cell line. We studied the effects of serum and HSA on the numbers of adherent bacteria and internalized bacteria and the production of IL-6 and IL-8 by the epithelial cells.

Material and methods

The bacterial strains *P. gingivalis* ATCC 33277 and *A. actinomycetemcomitans* Y4 were investigated in this study. The strains were subcultivated on Schaedler agar enriched with 10% sheep blood and vitamin K for 16 h, harvested, washed twice with phosphate-buffered saline (PBS; Braun, Melsungen, Germany) and resuspended in medium 199 (Gibco Life Technologies Ltd, Paisley, UK) to an optical density of 0.1 at 660 nm, which is equivalent to 10^8 bacteria/mL. Unless indicated otherwise, *P. gingivalis* ATCC 33277 was cultured anaerobically, while *A. actinomycetemcomitans* Y4 was cultured with 5% CO_2.

The KB cells (epithelial cell line, HeLa derivative, ATCC CCL 17) were seeded into two 24-well tissue culture plates at a density of $\sim 10^5$ cells per well. The cells were grown to a just confluent monolayer in cell cultivation medium (EMEM; BioWhitacker, Verviers, Belgium) enriched with 10% fetal calf serum (Gibco Life Technologies Ltd). Then the cells were washed twice with PBS.

Human serum and HSA were purchased from Sigma (Sigma-Aldrich, Taufkirchen, Germany). Serum was used at concentrations of 1, 5 and 10% by volume. Human serum albumin was used at concentrations of 25 and 50 mg/mL. The concentrations reported previously in serum and gingival crevicular fluid were about 50 and 0–16 mg/mL, respectively (21).

Adhesion and invasion

An adhesion and invasion standard procedure was used, similar to methods previously described for *P. gingivalis* and/or *A. actinomycetemcomitans* (19,20,23–25). The bacterial suspension in medium 199 was mixed with EMEM and serum or HSA and each well was inoculated with 1 mL of this final suspension (about 20 bacteria per KB cell). Bacteria were co-cultured with KB cells at 37°C for 1, 6 and 18 h in air containing 5% CO_2. Medium 199 without bacteria served as a control for non-infected KB cells.

After co-cultivation of bacteria with KB cells for 1, 6 and 18 h, 750 µL of the bacterial suspension above the KB cells was carefully removed, mixed by pipetting, and 20 µL were subcultivated to determine the number (colony forming units) of free-floating bacteria. Then, the remaining suspension was centrifuged at 14,000 *g* for 10 min and the supernatants stored at −80°C until ELISA analysis of IL-6 and IL-8 concentrations.

Meanwhile, the epithelial monolayers were washed five times with PBS. The numbers of adherent and internalized *P. gingivalis* ATCC 33277 cells as well as *A. actinomycetemcomitans* Y4 cells were counted as colony forming units (cfu) from half of the wells after cell lysis. Cell cultivation medium (EMEM) supplemented with 10 µg/mL metronidazole was added to the *P. gingivalis* ATCC 33277 plate and 10 µg/mL gentamicin to the *A. actinomycetemcomitans* Y4 plate for 1.5 h to kill the extracellular adherent bacteria. In preliminary experiments, this antibiotic concentration and time of exposure had been proven to be sufficient to kill 99% of extracellular but not intracellular bacteria. After washing three times with PBS, the numbers of internalized bacteria were determined by cell lysis and subsequent cultivation. The number of adherent bacteria was calculated as the difference between the number of adherent plus internalized bacteria and the number of internalized bacteria.

Effect of serum on survival of bacteria without exposure to epithelial cells

To ascertain whether serum (via complement) affected the viability of the

bacterial strains, the numbers of colony forming units were determined after exposure to native and heat-inactivated serum. In a microtitre plate, mixtures of bacterial suspension (optical density at 640 nm = 0.1), EMEM and serum at the same concentrations that were used previously, diluted in PBS at a ratio 1:8:1 were pipetted into the wells. The serum had a final concentration of 1, 5 and 10%, and was either native serum or serum in which the complement had been inactivated at 56°C for 30 min. After incubation times of 1, 6 and 18 h with 5% CO_2 in air, the numbers of colony forming units were determined by subsequent cultivation on Schaedler agar plates (*P. gingivalis* ATCC 33277 anaerobically, *A. actinomycetemcomitans* with 5% CO_2 in air).

Cytokine assays

The concentrations of the cytokines IL-6 and IL-8 in supernatants of KB cells were determined using commercially available ELISA kits as described in the manufacturer's instructions (BioSource™; Invitrogen Corp., Carlsbad, CA, USA). The lowest detection level of the interleukin in each kit was ~2 pg/mL.

Extraction of RNA and RT-PCR

To further assess the impact of the experimental conditions on IL-6 and IL-8 production, PCR was undertaken to investigate the production of IL-6 and IL-8 mRNA. Therefore, mRNA was extracted for RT-PCR analysis from KB cells that had been exposed to 5 and 10% serum or 25 and 50 mg/mL has, as well as to *A. actinomycetemcomitans* Y4 or *P. gingivalis* ATCC 33277 each without and with 5% serum or 25 mg/mL HSA for 1.5 and 6 h. Total RNA from approximately 2×10^6 KB cells was purified using an RNeasy kit (Quiagen GmbH, Hilden, Germany), and cDNA was synthesized from 1 μg of total RNA using the Omniscript kit according to the manufacturer's instructions (BioSource™; Invitrogen Corp.). Oligonucleotide primers were used at a final concentration of 5 nmol. The oligonucleotide primers were as follows: IL-6 (10) 5′-AGC TCA GCT ATG AAC TCC TTC TC-3′ (forward), 5′-GTC TCC TCA TTG AAT CCA GAT TGG-3′ (reverse); IL-8 (26) 5′-CTT GGC AGC CTT CCT GAT TT-3′ (forward), 5′-CAG CCC TCT TCA AAA ACT TC-3′ (reverse); and β-actin (27) 5′-ATT GCC GAC AGG ATG CAG AA-3′ (forward), 5′-GCT GAT CCA CAT CTG CTG GAA-3′. The β-actin served as a control to ensure equal loading of cells.

The PCR amplification was carried out in a reaction volume of 25 μL, consisting of 2.5 μL cDNA and 22.5 μL of reaction mixture containing 2.5 μL 10× PCR buffer, 2.75 mM $MgCl_2$, 0.2 mM nucleotides, 0.5 μM of each primer, 10^{-4} SybrGreen, 1 U Taq polymerase (Fermantes Life Science, St Leon-Rot, Germany). The cycling conditions comprised an initial denaturation step at 95°C for 5 min, followed by 35 cycles at 95°C for 15 s, at 55°C for 20 s and at 72°C for 20 s. The specificity of the amplification was always assayed with the use of melting curves. Amplification, detection and data analysis were performed with the Rotorgene 2000 cycler system (Corbett Research, Sydney, NSW, Australia).

Determination of amidolytic activity

To validate the influence of gingipains on the measured IL-6 and IL-8 levels after exposure of the KB cells to *P. gingivalis* ATCC 33277, the arginine- and lysine-specific amidolytic activities of that strain were assayed at 37°C with 0.5 mM *N*-α-benzoyl-DL-arginine-*p*-nitroanilide (Sigma-Aldrich, Taufkirchen, Germany) or *N*-tosyl-glycyl-L-prolyl-L-lysine 4-nitroanilide acetate salt in 1.0 mM of 0.2 mL of 0.2 M Tris–HCl, 0.1 M NaCl, 5 mM $CaCl_2$ and 10 mM cysteine, pH 7.6 (27). For this purpose, serum at a final concentration of 10% was added to the bacterial suspension of this strain for 1 h at 37°C.

Statistical analysis

All experiments were performed at least in triplicate. Comparisons between test and control conditions were performed using Student's unpaired *t* test.

Results

Effect of human serum and human serum albumin on survival of bacteria

Without contact with KB cells, serum (both native and heat inactivated) did not influence the number of viable *A. actinomycetemcomitans* Y4. Furthermore, after 18 h the addition of serum promoted the survival of *P. gingivalis* ATCC 33277. Approximately 10 times more (1 log step) *P. gingivalis* bacteria were counted after addition of 2.5–10% inactivated serum or 10% native serum compared with control cultures, and when using native serum, a concentration-dependent effect was evident (Fig. 1).

The percentages of the free-floating viable bacteria in the KB cell assays decreased continuously over time (*P. gingivalis* ATCC 33277, 70% at 1 h, 40% at 6 h and 20% at 18 h after infection; and *A. actinomycetemcomitans* Y4, 55% at 1 h, 20% at 6 h and 10% at 18 h after infection). However, there was no influence of serum on the numbers of either *A. actinomycetemcomitans* Y4 or *P. gingivalis* ATCC 33277 (data not shown). The absolute number of adherent *A. actinomycetemcomitans* was enhanced by supplementation with 2.5% serum and 5% serum after 6 h. However, addition of serum significantly reduced the number of internalized bacteria in a concentration-dependent manner. When the cells were exposed to *P. gingivalis* ATCC 33277, all concentrations of serum increased the numbers of adherent bacteria compared with serum-free control cultures. Supplementation with 10% serum reduced the numbers of invasive *P. gingivalis* ATCC 33277 cells after 1 and 6 h of exposure; in addition, 5% serum showed the same effect after 6 h. The results of adherent and invasive bacteria after 1, 6 and 18 h co-cultivation with KB cells and exposure to serum are presented in Fig. 2.

Given that the most remarkable differences using serum were obtained after 6 h, only this time was chosen in experiments with HSA. The numbers of adherent and internalized *P. gingivalis* ATCC 33277 and

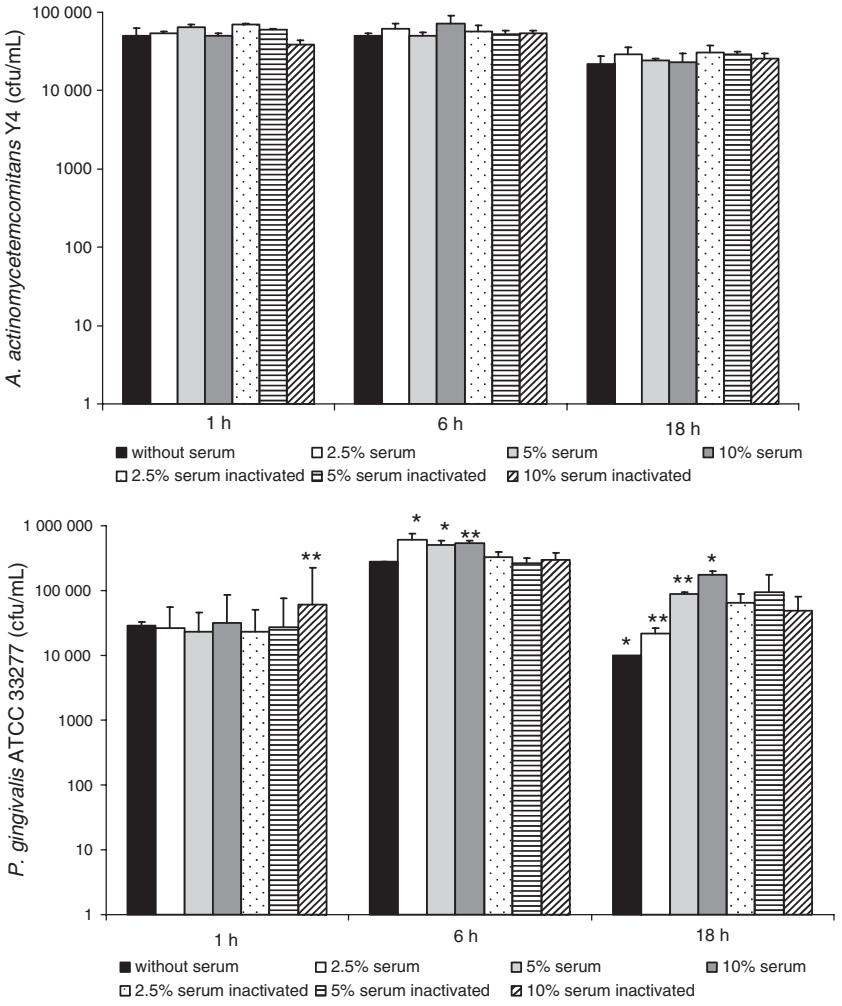

Fig. 1. Influence of serum on *Aggregatibacter actinomycetemcomitans* Y4 and *Porphyromonas gingivalis* ATCC 33277 cell counts per millilitre after an incubation time of 1, 6 and 18 h. Native serum and inactivated serum enhanced the growth of *P. gingivalis* ATCC 33277 (*$p < 0.05$, **$p < 0.01$ compared with numbers of colony forming units without addition of serum at the same time point).

A. actinomycetemcomitans Y4 cells decreased with increasing concentrations of human serum albumin. The effect was similar for both bacterial strains, in that the numbers of adherent viable bacteria were reduced by about 0.85 log steps and those of the internalized bacteria by about 0.50 log steps (Fig. 3).

Detection of IL-6 and IL-8

In general after 1 h, very low levels of IL-6 and IL-8 were detectable. The highest levels of IL-6 in the cell cultures were found 6 h after addition of serum, and then decreased at 18 h.

Addition of serum dramatically enhanced the release of IL-6 from non-infected cells. Thus, after addition of 5% serum, IL-6 levels in the supernatants above the KB cells were 72.6 ± 35 pg/mL, compared with 1.8 ± 2 pg/mL when serum was not added. *A. actinomycetemcomitans* Y4 also induced the release of IL-6 from KB cells. Supplementation with 10% serum enhanced the levels of IL-6 in the supernatants; nevertheless, the IL-6 concentration after addition of serum was higher from non-infected cells compared with those stimulated with *A. actinomycetemcomitans*. In general, almost no IL-6 was detectable in the supernatants from *P. gingivalis*-infected KB cells. In these cells, addition of serum led to a concentration-dependent increase of the measurable IL-6, the concentration being about one-third that of the non-infected cells after addition of 10% serum (Fig. 4).

Interleukin-8 was released from non-infected KB cells (337 ± 138 pg/mL after 18 h). This release was increased by *A. actinomycetemcomitans* Y4 (502 ± 52 pg/mL after 18 h), but in contrast, after infection with *P. gingivalis* ATCC 33277, no IL-8 was detectable. In all experiments, however, whether using non-infected cells or cells infected with one of the periodontal pathogens, serum enhanced the concentration of IL-8 in the supernatants compared with the serum-free environment. The concentration was up to 828 ± 108 pg/mL when considering the non-infected cells and up to 758 ± 98 pg/mL in the KB cells infected with *A. actinomycetemcomitans* Y4. The highest value after infection with *P. gingivalis* ATCC 33277 was 264 ± 86 pg/mL (10% serum, 6 h after infection; Fig. 4).

To ascertain whether the increased levels of IL-6 and IL-8 after infection with *P. gingivalis* ATCC 33277 and addition of serum resulted from an inhibitory effect of serum on activity of gingipains, the effect of serum on specific amidolytic activity was tested. Pre-incubation of *P. gingivalis* ATCC 33277 with 10% serum for 1 h reduced the arginine-specific amidolytic activity to 20% and the lysine-specific amidolytic activity to 39%.

Addition of human serum albumin led to a concentration-dependent increase in the levels of both IL-6 and IL-8 from non-infected cells and from cells treated with one of the bacterial strains. A clear influence of any of the two used bacterial strains was not seen after addition of human serum albumin (Fig. 5).

Interleukin-6 and IL-8 mRNA expression of KB cells

Before a protein (e.g. IL-6 or IL-8) is released into the environment, mRNA is transcripted from DNA. Expression of IL-6 and IL-8 mRNA in KB cells was about 10-fold higher after addition of 5 and 10% of human serum. In contrast, HSA only slightly (not significantly)

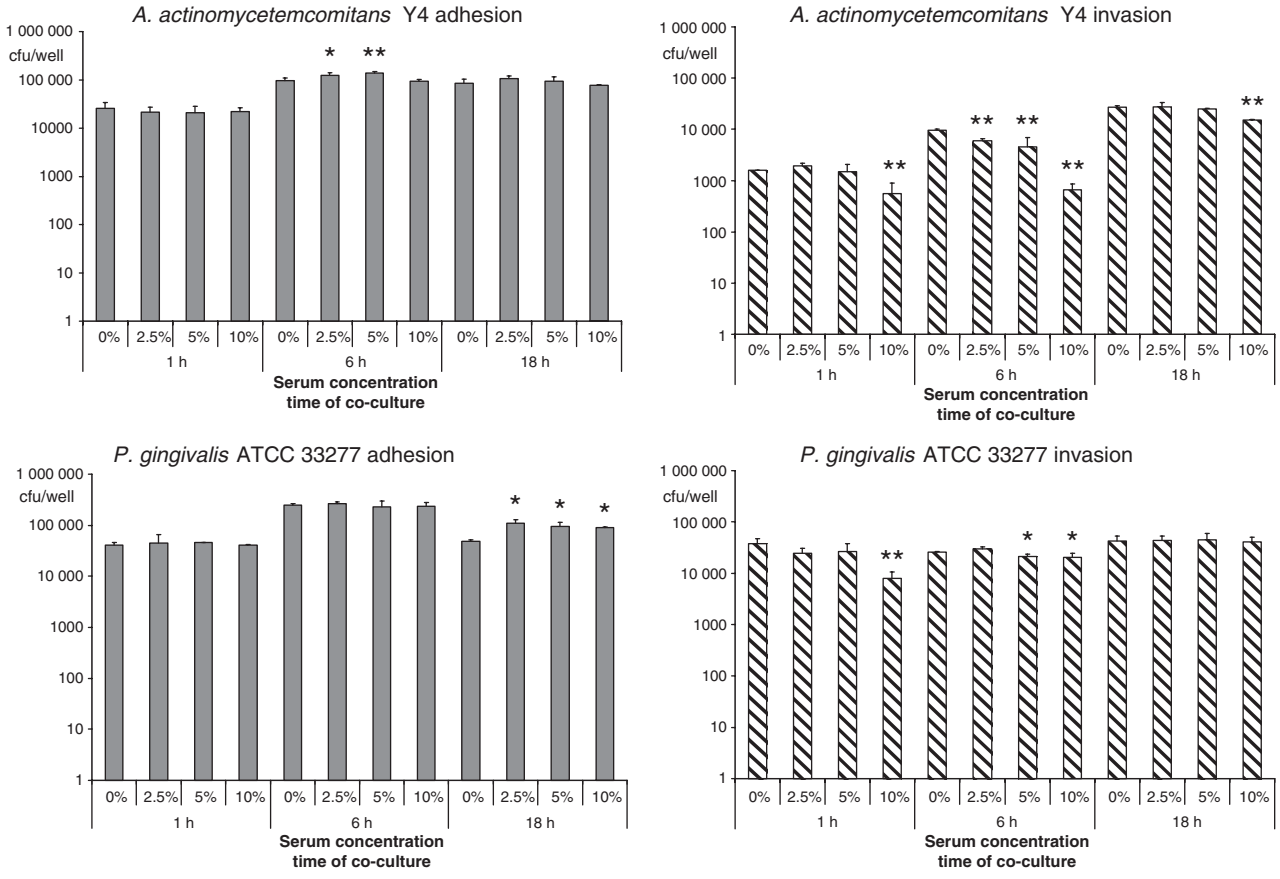

Fig. 2. Influence of serum on the numbers of adherent and invasive *Aggregatibacter actinomycetemcomitans* Y4 and *Porphyromonas gingivalis* ATCC 33277 cells 1, 6 and 18 h after co-cultivation with KB cells. Serum increased the number of adherent *A. actinomycetemcomitans* Y4 after 6 h of exposure, while the numbers of adherent *P. gingivalis* ATCC 33277 were enhanced after 18 h relative to control cultures to which serum was not added. Exposure to serum reduced the numbers of internalized or invaded bacteria from both strains (*$p < 0.05$, **$p < 0.01$, each compared with numbers of colony forming units without addition of serum at the same time point).

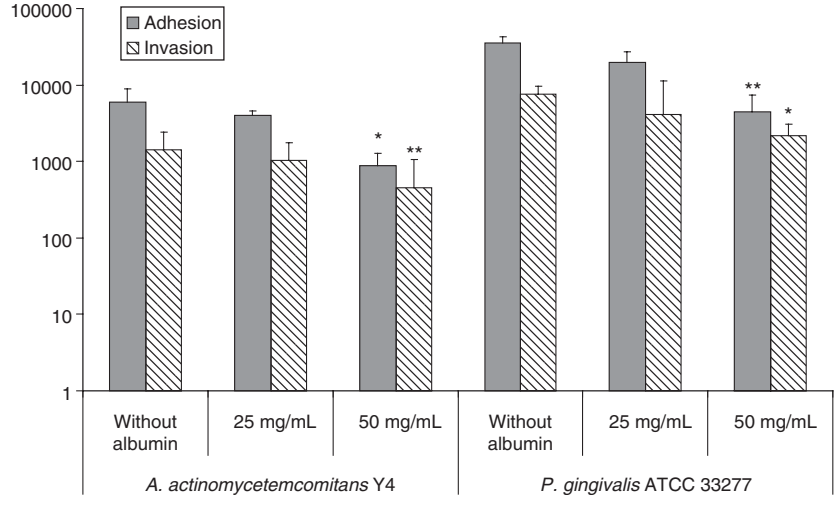

Fig. 3. Influence of human serum albumin on the numbers of adherent and invasive *Aggregatibacter actinomycetemcomitans* Y4 and *Porphyromonas gingivalis* ATCC 33277 cells 6 h after co-cultivation with KB cells. Human serum albumin reduced the numbers of adherent and internalized *P. gingivalis* ATCC 33277 and *A. actinomycetemcomitans* Y4 cells (*$p < 0.05$, **$p < 0.01$, each compared with numbers of colony forming units without addition of human serum albumin).

increased the mRNA IL-8 expression 1.5 h after infection (Fig. 6).

The mRNA expression was increased 10-fold 1.5 h after infection of KB cells with *A. actinomycetemcomitans* Y4. The addition of 5% serum led to a 100-fold increase in comparison with control cultures and to a 10-fold increase when compared with the strain without addition of serum. Human serum albumin did not further change the elevated level of mRNA expression after *A. actinomycetemcomitans* Y4 infection (Fig. 6).

After infection with *P. gingivalis* ATCC 33277 without other stimulus, the highest expression of IL-6 and IL-8 mRNA (both nearly 200-fold compared with control cultures) was measured. Serum in combination with *P. gingivalis* ATCC 33277 enhanced the mRNA expression of both cytokines 1.5 h after stimulation, and 6 h after stimulation

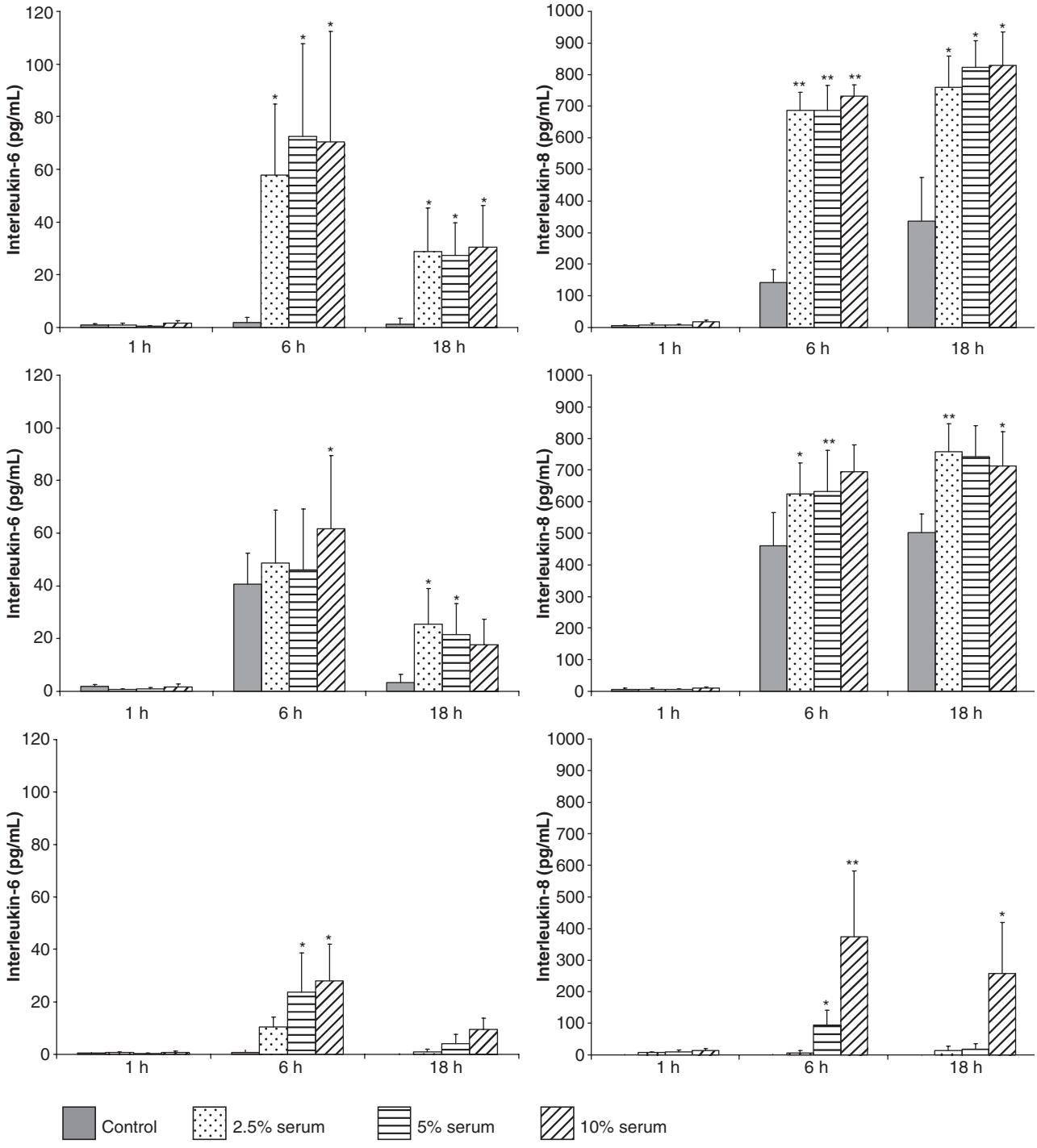

Fig. 4. Influence of serum on the release of IL-6 and IL-8 from non-infected KB cells (con) and from KB cells infected with *Aggregatibacter actinomycetemcomitans* Y4 or *Porphyromonas gingivalis* ATCC 33277 after an incubation time of 1, 6 and 18 h. Serum increased the levels of the cytokines in the cell supernatants, even after infection with *P. gingivalis* ATCC 33277 (*$p < 0.05$, **$p < 0.01$, each compared with the respective level without addition of serum at the same time point).

the mRNA expression levels were still higher than the control values but less than with the bacterial strain alone. Human serum albumin (25 mg) together with *P. gingivalis* ATCC 33277 increased the levels of expression compared with the non-infected control cells but not to the cells only infected with the *P. gingivalis* strain (Fig. 6).

Discussion

The periodontal epithelium is the first barrier that is encountered by periodontal pathogens. Epithelial cells

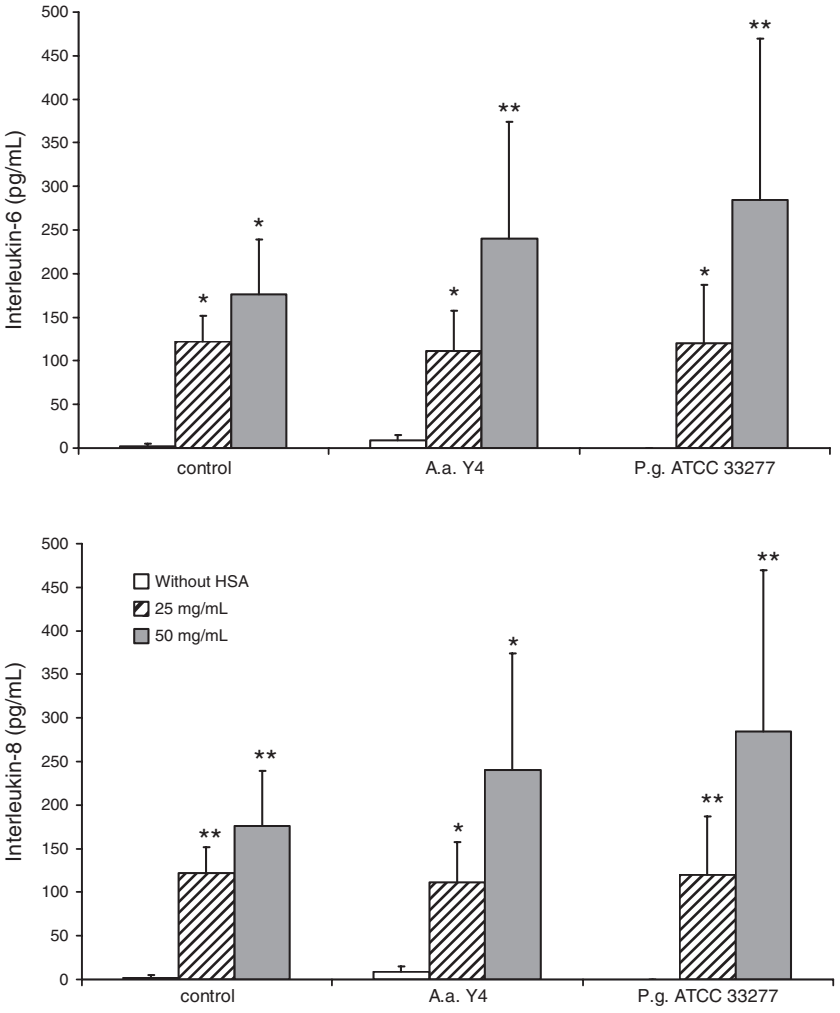

Fig. 5. Influence of human serum albumin (HSA) on the release of IL-6 and IL-8 from non-infected KB cells (control) and from KB cells infected with *Aggregatibacter actinomycetemcomitans* Y4 or *Porphyromonas gingivalis* ATCC 33277 after an incubation time of 6 h. Human serum albumin increased the level of IL-6 and IL-8 in the supernatants of the KB cells (*$p < 0.05$, **$p < 0.01$, each compared with the level of the cytokine without addition of HSA).

respond to these bacteria by producing cytokines that initiate inflammation (28). The gingival epithelium is also exposed to gingival crevicular fluid, which contains many components derived from serum (29). In this study, we investigated the influence of human serum and albumin on interactions of *P. gingivalis* ATCC 33277 and *A. actinomycetemcomitans* with KB cells, an epithelial cell line, which is an established model that has been used to investigate adherence and internalization of oral pathogens (19,20,30–32).

Data regarding the level of different proteins in gingival crevicular fluid are rare. By using strip methodology, Curtis *et al.* (33) determined that there is up to 46 μg/μL protein in periodontally healthy subjects. The major protein component of gingival crevicular fluid is HSA, followed by haemoglobin and immunoglobulins (34). The levels of proteins in gingival crevicular fluid have been reported to be generally lower than those in serum, and the concentrations did not correlate with the flow rate or the pocket depth (21). In serum, a low serum albumin concentration may be a risk predictor of periodontal disease progression among elderly non-smokers (35).

Without any contact with KB cells, serum did not influence the survival of *A. actinomycetemcomitans* Y4. However, serum increased the numbers of viable *P. gingivalis* ATCC 33277 cells. This is to be expected because *P. gingivalis* ATCC 33277 is able to degrade serum albumin as a nutrient source (36,37). Any clear influence of complement in killing of the strains used was not evident. Our results confirm those of others who reported no sensitivity amongst *Porphyromonas* strains (38) and *A. actinomycetemcomitans* (39).

There are few, if any, previously published data regarding any influence of serum or HSA on adhesion to and invasion of epithelial cells by periodontal pathogens. In our study, adherence of *A. actinomycetemcomitans* Y4 and *P. gingivalis* ATCC 33277 to the epithelial cells was slightly enhanced by serum, but not by HSA. This suggests that components of serum attached to the epithelial layer serve as bacterial receptors or activate receptors. Furthermore, our results showed an inhibitory effect of serum on invasion by periodontal pathogens into KB cells that might be associated with HSA. Interaction of bacterial fimbria with serum albumin, promoting adhesion and invasion, has been described for *Escherichia coli* (40) and *Legionella pneumophila* (41), but this mechanism does not appear to be a major component of binding of *P. gingivalis* fimbriae with saliva-coated hydroxyapatite (42) or of *A. actinomycetemcomitans* with epithelial cells (43). It can only be speculated that HSA attaches to receptors essential for invasion by the bacteria. Lamont *et al.* (44) described an inhibition of invasion of the ATCC 33277 strain of *P. gingivalis* into primary epithelial cells by serum.

Here, serum promoted the expression and release of IL-6 and IL-8. Human serum albumin was chosen for the experiments because it is the main protein in serum. Albumin is a strong stimulus for IL-8 expression in proximal tubular epithelial cells (45,46), and the rapid and intense induction of IL-8 caused by albumin was postulated as a reason for influx of inflammatory cells

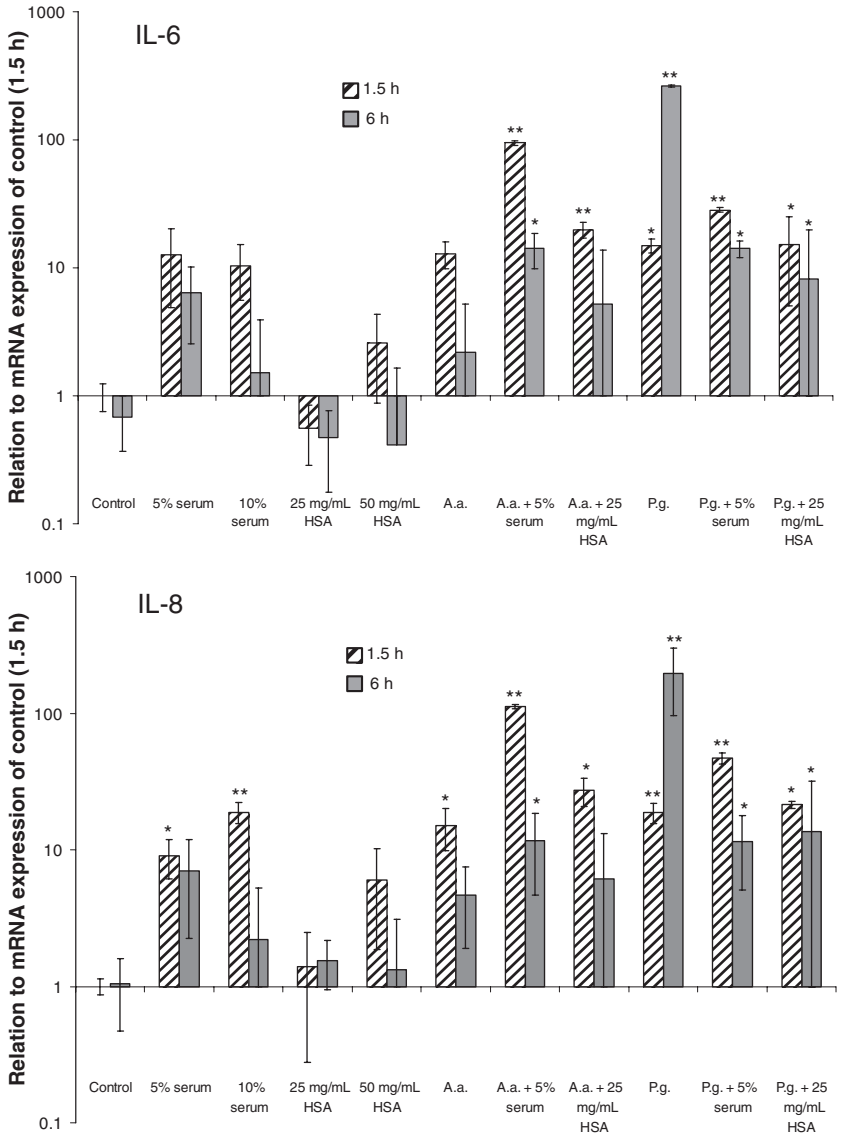

Fig. 6. Expression of IL-6 and IL-8 mRNA by KB cells after 1 and 6 h of exposure to serum or human serum albumin (HSA) as well as *Aggregatibacter actinomycetemcomitans* Y4 or *Porphyromonas gingivalis* ATCC 33277. Data represent fold change relative to control cultures (con; non-infected KB cells without serum and HSA 1.5 h after the beginning of the experiment). A combination of serum (5%) and *A. actinomycetemcomitans* Y4 resulted in maximal upregulation of IL-6 and IL-8 mRNA expression (*$p < 0.05$, **$p < 0.01$, each compared with control cultures).

into the tissues (45). Furthermore, increased serum albumin is related to changes in IL-6 release in patients receiving chronic dialysis therapy (47). Further support for serum albumin-induced interleukin release was provided by experiments with human adipocytes, which demonstrated increased secretion of IL-6, IL-8, IL-10 and tumour necrosis factor-α in the presence of serum albumin (48). These findings are confirmed by our observation that KB cells released IL-6 and IL-8 after exposure to HSA in a dose-dependent manner. In contrast, a stimulatory effect of serum albumin on mRNA expression was not shown. Maybe, mRNA expression occurs very rapidly, at a time point that was not measured. Taken together, the results indicate that albumin is not the only stimulating component in serum.

As previously described, infection with most *P. gingivalis* strains increases proinflammatory cytokine mRNA expression (49,50). However, without any addition of serum or HSA we did not measure IL-6 and IL-8 in the supernatants of KB cells infected with *P. gingivalis* ATCC 33277. Most bacterial strains retain gingipains on the cell surface (51). These enzymes, when associated with bacterial outer membrane blebs, instantly degrade IL-8 (16,52). Furthermore, IL-6 is rapidly and efficiently cleaved by Arg- and especially Lys-gingipains (15). An influence of lipopolysaccharide (LPS), normally a stimulant of cytokine expression, can be excluded because LPS does not react with gingival epithelial cells and KB cells (32). After addition of serum, IL-6 and IL-8 were detectable at a comparable low level. Soluble CD14 is present in serum and can transmit the response of LPS to cells (32). Furthermore, the suggestion of an inhibition of cysteine proteolytic activity by serum was confirmed by a decreased amidolytic activity of the strain *P. gingivalis* ATCC 33277 after addition of serum. Serum may interfere with gingipains, and the proteolytic activity of Lys-gingipain on the soluble receptor of IL-6 is abrogated by serum (54). Otherwise, *A. actinomycetemcomitans* may play an important role in amplifying the local immune response and initiating inflammatory reactions through release of IL-8 from gingival epithelial cells (28). Outer membrane protein 100 of *A. actinomycetemcomitans* Y4 induces IL-8 mRNA expression and, to a lesser extent, IL-6 mRNA expression. Interleukin-6 is also essential for adhesion and invasion of that strain (55). In our experiments, after infection with *A. actinomycetemcomitans* Y4, a 220-fold increase of IL-6 mRNA expression and a 15-fold increase of IL-8 mRNA expression were observed in comparison with non-infected cells. The mRNA expression was augmented by serum. A role for HSA in this process is not very clear as underlined in our experiments. Another possible molecule may be soluble CD14, which promotes the release of IL-8 from epithelial cells (56). Furthermore, it was shown that serum or *A. actinomycetemcomitans* augmented the release of proinflammatory

cytokines, but the level was below that of non-infected cells, which indicates that there are also some interactions of components of serum with *A. actinomycetemcomitans* Y4 which may be inhibitory.

To conclude, we identified that serum stimulates the release of the cytokines IL-6 and IL-8 by epithelial cells and influences the interaction of periodontal pathogens with host cells. This might contribute to inflammation of the periodontium. This finding is important and confirms that the influence of serum on interactions between periodontal bacteria and epithelial cells should not be neglected in *in vitro* assays.

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