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Interleukin-8 Production by Human Mesothelial Cells after Direct Stimulation with Staphylococci

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Mesothelial cells (MC) are able to produce interleukin-8 (IL-8) after stimulation with IL-1β or tumor necrosis factor alpha. The aim of our study was to investigate whether MC are able to produce IL-8 after direct stimulation with clinically relevant bacteria. We observed a significant IL-8 response by the MC which were directly stimulated with viable staphylococci.

Bacterial peritonitis, caused mainly by *Staphylococcus epidermidis* or *Staphylococcus aureus* (23), is still an important complication in continuous ambulatory peritoneal dialysis and causes a high level of morbidity (6, 15). Most studies emphasize the role of peritoneal macrophages in the local peritoneal antibacterial defense mechanism. These macrophages are considered to be important in the prevention of bacterial outgrowth in the peritoneal cavity, since they have bactericidal capacity (8). However, the low number of peritoneal macrophages does not correspond with the high incidence of peritonitis (4), whereas an inverse correlation between numbers of mesothelial cells (MC) in peritoneal dialysis effluent and incidence of peritonitis has been demonstrated (2, 5). Furthermore, neutrophils are more potent in the killing of bacteria (3).

Moreover, recent studies have shown that cultured peritoneal MC are able to produce several cytokines, such as interleukin-6 (IL-6) and IL-8, after incubation with the proinflammatory cytokines IL-1β and tumor necrosis factor alpha (TNFα) (3, 10, 13, 20, 21). IL-8 is especially important in the local situation, because it is a strong chemoattractant for neutrophils and also has activating properties on these cells (1, 17, 25). In a bacterial invasion, an influx of neutrophils from the circulatory system is essential to eliminate the microorganisms, as outlined above. The aim of this study was to investigate whether direct stimulation of MC with clinically relevant bacteria induces an IL-8 response.

MC were isolated from samples of human omenta, obtained during elective surgery, and then cultured as described previously (18, 24). All experiments were performed with confluent monolayers of MC in culture passage 1. To determine the purity of the MC, a small number of cells were prepared for ultrastructural determination by previously described methods (14).

The bacteria used in the experiments were isolated from peritoneal dialysis effluent of continuous ambulatory peritoneal dialysis patients suffering from peritonitis. Four different strains of *S. aureus*, four different strains of *S. epidermidis*, and also four different strains of *Escherichia coli* were used. Hemolysin production by these bacteria was determined by culturing the said bacteria on blood-agar plates and measuring the zone of hemolysis. Bacteria were inactivated by incubating them for 30 min at 60°C. Bacterial-culture supernatants were obtained by culturing the bacteria for 18 h in RPMI 1640 medium (Gibco, Uxbridge, United Kingdom) at 37°C. Bacteria were separated from the medium by centrifugation at 15,000 × *g*. As positive controls, physiological concentrations of recombinant human TNFα (10 ng/ml; Genzyme, Cambridge, Mass.) and IL-1β (1 ng/ml; British Bio-technology Products Ltd., Abingdon, Oxon, United Kingdom) were used.

MC monolayers, cultured on 96-well culture plates, were incubated for 24 h with living bacteria ($n = 7$), heat-inactivated bacteria ($n = 3$), bacterial-culture supernatants ($n = 3$), and the positive controls ($n = 7$), all diluted in RPMI 1640. RPMI 1640, without any additions, was used as a negative control to determine the background level. The inoculum of bacteria was set at 10^5 CFU/100 μl. To several of the wells containing the living bacteria, and the positive controls, blocking antibodies against IL-1 and TNFα (Genzyme) were added, in optimal blocking concentrations, to examine whether the bacterial effect was mediated by these macrophage-derived proinflammatory cytokines.

IL-8 was measured by an IL-8-specific sandwich enzyme-linked immunosorbent assay (CLB, Amsterdam, The Netherlands) (11).

All data are expressed as means with the standard error of the mean. The Student's *t* test was performed to determine whether differences were significant.

IL-8 production after stimulation with bacteria. After stimulation with all viable *S. aureus*, the IL-8 production of all MC monolayers ($n = 7$) increased significantly above the background level ($P < 0.01$) (Fig. 1a). Three of the four strains (for epid4, $P = 0.053$) of viable *S. epidermidis* induced an IL-8 response ($P < 0.04$) (Fig. 1b), whereas none of the *E. coli* strains induced a response of IL-8 production above the background level (Fig. 1c). IL-8 peaks reached by stimulation with viable bacteria were considerably lower than those reached by stimulation with either IL-1β or TNFα (Fig. 1d). The IL-8 response induced by bacteria could not be blocked with monoclonal antibodies against IL-1 or TNFα (Table 1), whereas the response induced by the positive controls could be blocked entirely. Heat-inactivated bacteria or bacterial-culture supernatants did not induce an IL-8 response (Table 2). No hemolysis zone was found on any of the bacterial cultures on the blood-agar plates. Purity of the MC cultures was confirmed by ultrastructural morphology (Fig. 2) and the characteristic appearance in culture (16).

The present study clearly shows that IL-8 production by

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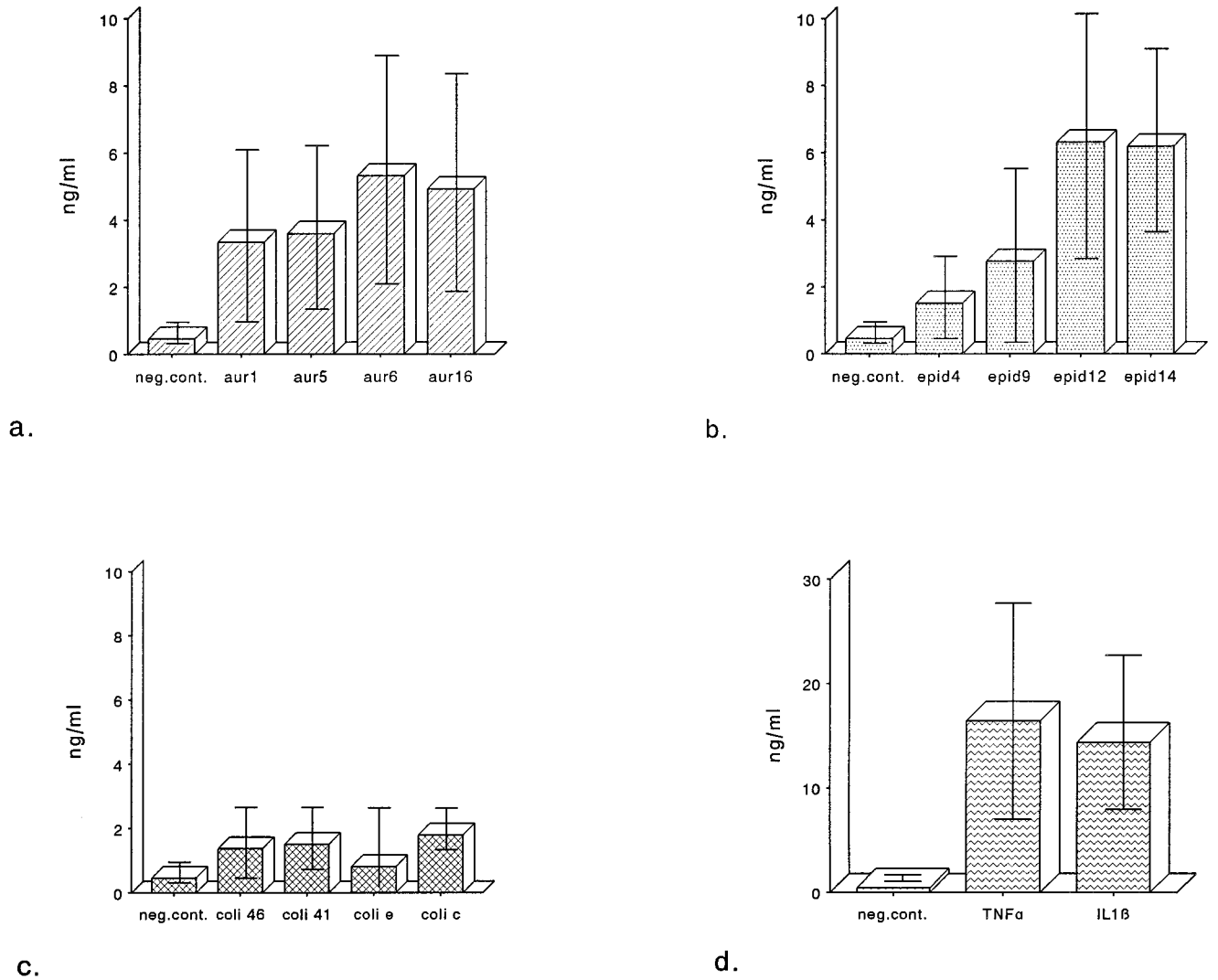

FIG. 1. IL-8 production by MC after incubation with four different strains of *S. aureus*, aur1, aur5, aur6, and aur16 (a); four different strains of *S. epidermidis*, epid4, epid9, epid12, and epid14 (b); four different strains of *E. coli*, coli 46, coli 41, coli e, and coli c (c); and the proinflammatory cytokines TNFα (10 ng/ml) and IL-1α (1 ng/ml) as positive controls (d). Error bars indicate standard errors of the mean. neg. cont., negative control. $n = 7$.

direct stimulation of MC with viable staphylococci is possible. This phenomenon of direct stimulation of human cells with bacteria has been described in an earlier study of gingival fibroblasts stimulated by *Bacteroides* spp. (19). The interbacterial variation in IL-8 induction, shown in the present study, might explain why some organisms, such as gram-negative bacteria, induce a much more severe peritonitis than, for instance, staphylococci (7). All patients from whom the staphylococci were isolated developed only a mild peritonitis, whereas patients from whom *E. coli* was isolated usually suffered severe peritonitis, usually caused by more than one species of gram-negative bacterium (7). IL-8 is probably an essential cytokine in the regulation of neutrophil influx. The level of IL-8 production after stimulation with either IL-1β or TNFα is considerably higher than the level of production after direct stimulation with living bacteria. This might be explained by the fact

TABLE 1. IL-8 production by MC after direct stimulation with bacteria in combination with anti-TNFα and anti-IL-1β antibodies

Antibody	Amt of IL-8 produced (ng/ml)[a] with strain:							
	aur.1	aur.5	aur.6	aur.16	epid.4	epid.9	epid.12	epid.14
None	2.56 ± 1.59	2.54 ± 1.35	2.47 ± 2.15	2.70 ± 3.60	1.77 ± 1.18	5.27 ± 4.79	5.94 ± 5.95	7.71 ± 6.98
Anti-TNFα	2.36 ± 1.20	3.77 ± 2.73	2.75 ± 2.49	3.18 ± 1.17	1.89 ± 1.40	2.39 ± 1.67	6.68 ± 4.37	8.19 ± 6.58
Anti-IL-1	2.06 ± 2.44	2.35 ± 1.88	2.93 ± 3.63	1.95 ± 0.72	1.97 ± 2.05	4.06 ± 3.15	5.14 ± 3.89	5.64 ± 3.41
Anti-TNFα + anti-IL-1	3.54 ± 3.37	1.99 ± 0.87	2.71 ± 3.06	5.14 ± 5.86	1.80 ± 1.26	4.72 ± 4.67	6.20 ± 4.04	7.38 ± 7.08

[a] Results are expressed as means and standard errors of the mean ($n = 3$). The result for the negative control was 1.30 ± 1.27 ng/ml.

TABLE 2. IL-8 production by MC after direct stimulation with living bacteria and heat-inactivated bacteria

Bacterial prepn	Amt of IL-8 produced (ng/ml)[a] with strain:							
	aur.1	aur.5	aur.6	aur.16	epid.4	epid.9	epid.12	epid.14
Living	1.93 ± 2.07	1.93 ± 1.89	5.97 ± 5.15	3.47 ± 2.05	0.84 ± 0.19	1.75 ± 0.50	7.81 ± 4.75	6.78 ± 0.11
Heat inactivated	0.29 ± 0.43	0.35 ± 0.40	0.45 ± 0.33	0.32 ± 0.32	1.34 ± 1.43	1.21 ± 0.70	0.44 ± 0.56	0.59 ± 0.69
Culture supernatant	0.21 ± 0.36	0.61 ± 0.57	0.40 ± 0.40	0.47 ± 0.81	0.29 ± 0.29	0.47 ± 0.69	0.32 ± 0.56	0.60 ± 1.04

[a] Results are expressed as means and standard errors of the mean ($n = 3$). The result for the negative control was 0.47 ± 0.30 ng/ml.

that in the latter case, viability is diminished by the invading bacteria, although 80% are still alive. In contrast, viability in the stimulation experiments with the proinflammatory cytokines remains the same. Furthermore, the cytokine production of the remaining 80% could be influenced in a negative way by the invading bacteria (intracellular CFU of all staphylococci, ca. 1×10^7 CFU/5×10^4 cells). Another possible explanation of this difference in production might be the fact that the staphylococci stimulate IL-8 production not by receptor-mediated endocytosis, but rather by direct activation of p38 kinase. It has been shown that nonspecific stimuli, such as heat or hyperosmolar solutions, can induce an IL-8 response by the phosphorylation of p38 kinase (12). Possibly, the mechanism of activation of the IL-8 synthesis by invading bacteria is based on a similar principle.

Stimulation with *E. coli* did not induce an IL-8 response. The fact that the majority of MC died when incubated with *E. coli* and no IL-8 response could be induced excludes the possibility that the measured IL-8 response after incubation with the staphylococci is due to the release of intracellular stored IL-8.

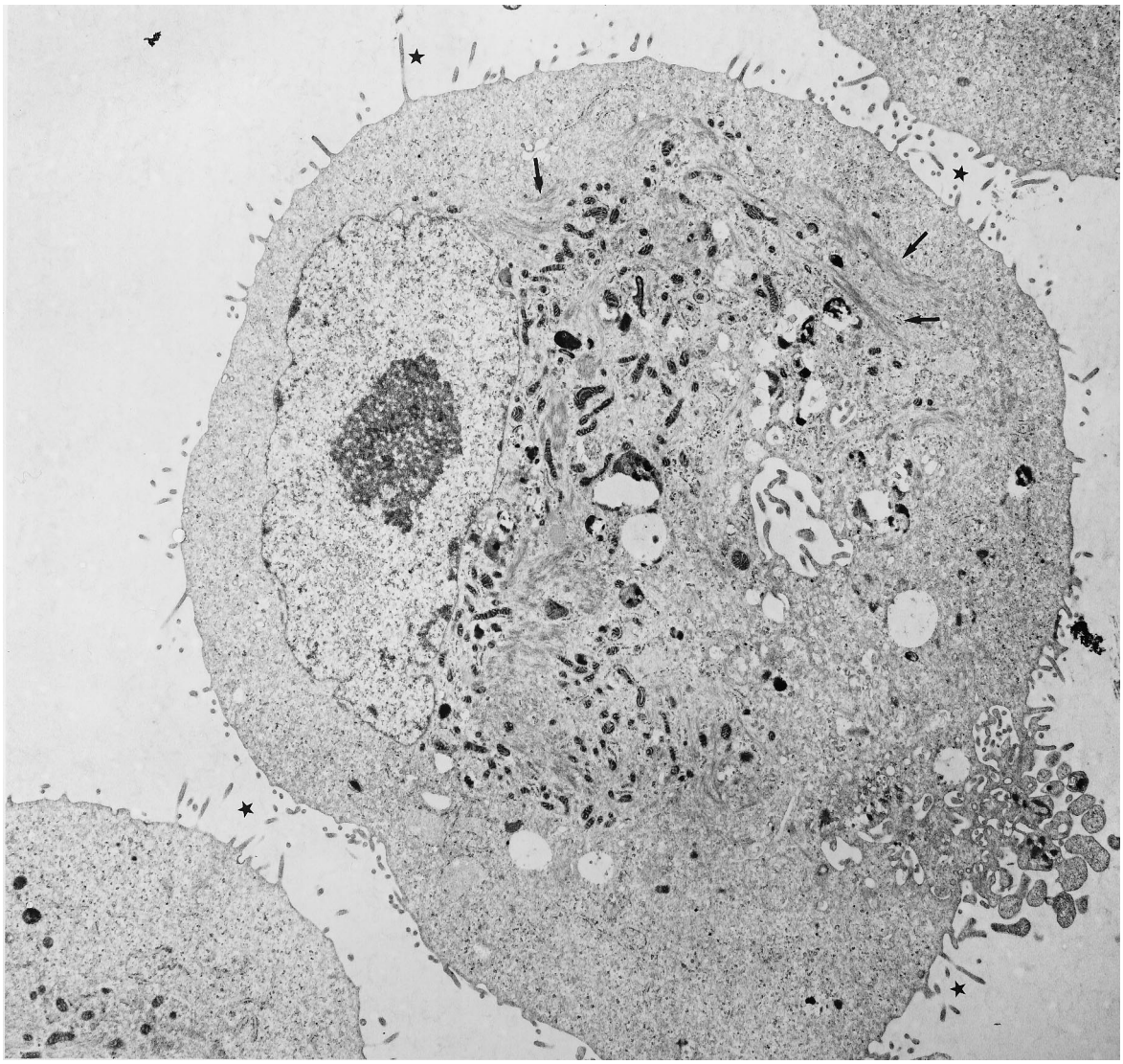

FIG. 2. Electron micrograph showing MC with the characteristic microvilli (★) and cytokeratin filaments (→). Magnification, ×5,400.

Bacterial culture supernatant did not induce an IL-8 response (Table 2). This demonstrates that the IL-8 response by living bacteria is not induced by a bacterial exoproduct. The massive MC death could nor be explained by the presence of an exoproduct either, since the MC viability remained 100% after incubation with the bacterial-culture supernatant; nor could hemolysin be held responsible, because all bacteria were negative for this product. The fact that heat-inactivated bacteria (Table 2) or formaldehyde-inactivated bacteria (data not shown) were unable to induce an IL-8 response indicates that the component that is responsible for the induction is probably a cell wall component that is altered by heat or formaldehyde. This component has not yet been identified.

These results, together with the knowledge that MC can produce other substances, such as phospholipids and prostaglandins (9, 22), show that these stromal cells are important in the direct antibacterial defense mechanism by their production of proinflammatory cytokines, such as IL-8. It has been demonstrated that in the dialysis effluent of patients suffering from peritonitis, all these substances are elevated in comparison with the steady-state levels (26, 27). These cytokines may be of importance in the regulation of the influx of leukocytes from the circulatory system into the peritoneal cavity. It is known that a wide range of cell types, including peritoneal macrophages, are able to produce IL-8. However, since MC are by far the most numerous cells in the peritoneal cavity, their capability to produce cytokines may be an important mechanism to induce an antibacterial reaction.

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