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Staphylococcal Glycocalyx Activates Macrophage Prostaglandin E_2 and Interleukin 1 Production and Modulates Tumor Necrosis Factor Alpha and Nitric Oxide Production

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We have examined the effect of staphylococcal glycocalyces on the ability of murine peritoneal macrophages to produce prostaglandin E_2 (PGE_2) and the inflammatory cytokines interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF-α) and to generate nitric oxide. Glycocalyx partially purified under endotoxin-free conditions from defined liquid medium cultures of *Staphylococcus lugdunensis* or *Staphylococcus epidermidis* was a strong stimulator of PGE_2 and IL-1 production. The addition of 10 to 100 μg of glycocalyx per ml induced levels of IL-1 and PGE_2 production similar to that induced by 0.1 to 1 μg of *Escherichia coli* lipopolysaccharide (LPS) per ml. In contrast, glycocalyx induced ninefold less TNF-α and three- to fourfold less nitrite than LPS. A modulatory effect was suggested by the observation that the amount of TNF-α and nitrite generated remained constant whether the macrophages were stimulated with 10 or 100 μg of glycocalyx per ml. A selective modulation of macrophage activation was confirmed by the demonstration that costimulation of macrophages with both glycocalyx and LPS resulted in a reduction in TNF-α and nitrite generation relative to stimulation with LPS alone even though costimulation had no effect on PGE_2 production and increased IL-1 production. Involvement of PGE_2 in this modulatory effect was suggested by the ability of indomethacin to augment glycocalyx-stimulated TNF-α production and to reverse the inhibitory effect of glycocalyx on LPS induction of TNF-α production. However, the inability of indomethacin to reverse the inhibitory effect of glycocalyx on LPS-induced nitric oxide generation suggests that the selective modulation of macrophage function by glycocalyx may be more complex than increased sensitivity to PGE_2 feedback inhibition.

Bacteria can adhere to surfaces and form biofilms composed of bacterial cells and bacterial glycocalyx (15, 21, 35). This microcolonial form of growth has been observed in a variety of bacterial infections, including prosthesis-related colonization (15, 29) and osteomyelitis (23, 28, 30). Several research groups have hypothesized that the production of glycocalyx may be a major factor in the pathogenesis of foreign body infections (18, 22, 28, 34). Glycocalyx has been shown to protect bacteria from antibodies (2) and from phagocytosis (9, 19, 40, 44). Glycocalyx has been reported to inhibit normal phagocyte functions, such as movement along chemotactic gradients and oxidative burst (19, 32, 34, 40, 44), and to inhibit the proliferative response of peripheral blood mononuclear cells to the T-cell mitogen, phytohemagglutinin (16, 43).

In a recent study, we demonstrated that the glycocalyx from coagulase-negative staphylococci can activate monocyte prostaglandin E_2 (PGE_2) production and that it is this activity that in turn contributes to the inhibition of T-cell proliferation (43). PGE_2 plays a significant role in acute inflammatory responses (5). It appears somewhat paradoxical that glycocalyx could be of such apparent benefit to coagulase-negative staphylococci in the establishment of foreign body infections while stimulating monocytes to secrete proinflammatory products. However, PGE_2 is known to play a regulatory role in modulating the activities of lymphocytes and macrophages (24, 36, 41, 43). In the present study, we examined the ability of glycocalyces isolated from *Staphylococcus lugdunensis* and from *Staphylococcus epidermidis* to induce tumor necrosis factor alpha (TNF-α) and interleukin 1 (IL-1) production in macrophages as well as their ability to induce the production of reactive nitrogen intermediates (14, 17, 39).

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MATERIALS AND METHODS

Reagents. Bacterial lipopolysaccharide (LPS; from *Escherichia coli* O11:B4-W), N^G-monomethyl-L-arginine, and indomethacin were obtained from Sigma Chemical Co. (St. Louis, Mo.). Recombinant gamma interferon (IFN-γ) and TNF-α were obtained from Genzyme Corp. (Cambridge, Mass.). Chromogenic limulus kits, for quantitating endotoxin contamination, were obtained from BioWhittaker, Inc. (Walkersville, Md.). Enzyme-linked immunosorbent assay (ELISA) kits for quantitation of IL-1 and TNF-α were obtained from Genzyme Corp. Enzyme immunoassay kits for quantitating PGE_2 were obtained from Cayman Chemical Company (Ann Arbor, Mich.). Fetal bovine serum (prescreened for endotoxin levels of <0.05 ng/ml) was obtained from HyClone Laboratories, Inc. (Logan, Utah). The complete culture medium used for cell culture was RPMI 1640 with L-glutamine (HyClone Laboratories), supplemented to 5% with heat-inactivated (56°C for 1 h) fetal bovine serum, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (Gibco BRL Life Technologies, Grand Island, N.Y.), 50 μg of gentamicin per ml (Sigma Chemical Co.), and 50 μM 2-mercaptoethanol (Sigma Chemical Co.). All buffers, media, and reagents were monitored by chromogenic limulus assays to insure that endotoxin levels were below 50 pg/ml in the cell cultures.

Animals. Female BALB/cByJ mice were obtained from

TABLE 1. Characterization of bacterial glycocalyx preparations

Glycocalyx preparation	Organism	Amt (per 100 μg of glycocalyx) of:			
		Endotoxin (ng)	Carbohydrate (μg)	Protein (μg)	Phosphate (nmol)
G19-85	*S. epidermidis*	<0.1	19	67	120
G6-87	*S. lugdunensis*	<0.1	8.3	55	67
G2-89	*S. lugdunensis*	<0.1	11.9	62	65

Jackson Laboratories (Bar Harbor, Maine) and used for experimentation between the ages of 8 and 24 weeks.

Preparation of bacterial glycocalyx. The *Staphylococcus* species used in this study and their pathogenicity in humans and rodents have been previously described (43). The glycocalyx was isolated from bacteria grown in an endotoxin-free chemically defined liquid medium as previously described (22, 43). Endotoxin-free glass-distilled water and glassware were used throughout the preparation and isolation procedures. The results of biochemical analysis of the glycocalyx preparations used in the current study are displayed in Table 1. The total carbohydrate content was determined by the phenol-sulfuric acid assay of Dubois et al. (8). Total protein was estimated by micro-Lowry procedure with bovine serum albumin as the standard (26). Total phosphate was measured by the method of Ames with KH_2PO_4 as the standard (1). Endotoxin was quantitated by the chromogenic limulus assay.

Macrophage preparation and culture. Elicited peritoneal exudate cells were obtained by peritoneal lavage 3 days after intraperitoneal injection of 2 ml of 3% NIH thioglycolate broth (Difco Laboratories, Detroit, Mich.). The cells were washed three times with Dulbecco's phosphate-buffered saline (0.15 M, pH 7.3) supplemented to 2% with fetal bovine serum and resuspended in complete culture medium to 2×10^6/ml. The cells were dispensed in 200-μl aliquots to 96-well Microtest III plates (Falcon Plastics, Oxnard, Calif.) and incubated overnight to allow adherence of the macrophages. The nonadherent cells were removed by washing with complete culture medium three times. Complete medium containing the indicated experimental reagents was added, and after 8 or 48 h of culture, the supernatant fluid was collected for assay of TNF-α, IL-1α, PGE_2, and/or nitrite. In addition to ELISA for the presence of TNF-α, bioactivity of TNF-α in the supernatants was determined by the L929 bioassay, as previously described (7). Nitrite production was determined by a microplate colorimetric assay with Griess reagent, as previously described (6). Dilutions (2-fold, 20-fold, and 100-fold) of the supernatants were assayed to ascertain that optical density readings in the assays were within the linear range of the standard curves.

RESULTS

To examine the ability of staphylococcal glycocalyx to activate murine macrophages, adherent peritoneal macrophages were incubated with 10 to 100 μg of *S. lugdunensis* G2-89 glycocalyx per ml or 100 ng of LPS per ml for 8 or 24 h, after which the culture supernatants were assayed for TNF-α or PGE_2, respectively. PGE_2 production increased as the concentration of G2-89 glycocalyx increased from 10 to 100 μg/ml, and the quantity of PGE_2 produced by glycocalyx-stimulated macrophages was equivalent to that produced by LPS-stimulated macrophages (Fig. 1a). In contrast, although G2-89 glycocalyx did stimulate detectable TNF-α production (20 to 30 U/ml in the experiment whose results are displayed in Fig. 1b), the quantity of TNF-α secreted by glycocalyx-stimulated

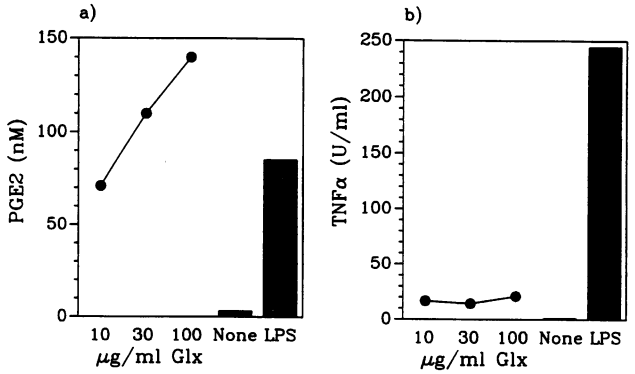

FIG. 1. Stimulation of PGE_2 and TNF-α production by glycocalyx. Peritoneal macrophages were incubated with the designated amount of G2-89 glycocalyx or with 100 ng of LPS per ml. Culture supernatants were collected at 8 or 24 h and assayed for TNF-α by bioassay or for PGE_2 by enzyme immunoassay, respectively. The arithmetic means of triplicate cultures obtained in one of four independent trials are presented.

macrophages was significantly (ninefold) lower than that secreted by LPS-stimulated macrophages. The quantity of TNF-α secreted by glycocalyx-stimulated macrophages did not increase as the concentration of glycocalyx increased from 10 to 100 μg/ml (Fig. 1b). Macrophages did not display a significant response to 1 μg or less of glycocalyx per ml (data not shown).

To determine if this poor stimulation of macrophage TNF-α production by glycocalyx was unique to the G2-89 preparation used in Fig. 1, another preparation of G2-89 glycocalyx as well as glycocalyx isolated from *S. lugdunensis* G6-87 and from *S. epidermidis* G19-85 was tested for its ability to induce TNF-α secretion in peritoneal macrophages. Culture supernatants of macrophages cultured for 8 h with any of the glycocalyx preparations contained significantly (>10-fold) less TNF-α than that of macrophages stimulated with LPS (Table 2). The addition of recombinant IFN-γ (rIFN-γ) to the cultures did result in a fourfold increase in TNF-α secretion by glycocalyx-stimulated macrophages (Table 2). However, even in the presence of rIFN-γ, TNF-α secretion by glycocalyx-stimulated macrophages was >7-fold less than that of LPS-stimulated macrophages. To determine if glycocalyx could be interfering with the TNF-α bioassay, 100 μg of glycocalyx per ml was added to a standard titration of recombinant TNF-α on L929 fibroblasts. Addition of glycocalyx directly into the TNF-α bioassay did not interfere with the cytotoxicity of recombinant

TABLE 2. Effect of rIFN-γ on glycocalyx stimulation of TNF-α production[a]

Stimulus	TNF-α (U/ml)	
	Alone	Plus IFN-γ
Medium	<0.1	0.24 ± 0.21
LPS	70 ± 7.7	87.4 ± 11.7
G2-89	1.6 ± 0.3	7.1 ± 1.4
G6-87	2.4 ± 0.4	10.6 ± 1.7
G19-85	3.4 ± 1.1	12.7 ± 1.5

[a] Macrophages were incubated for 8 h with 100 ng of LPS per ml or with 100 μg of the indicated preparation of glycocalyx per ml in the presence or absence of 50 U of rIFN-γ per ml. The supernatant fluid was collected and assayed for TNF activity in the L929 bioassay. The arithmetic means of triplicate cultures ± SD are presented.

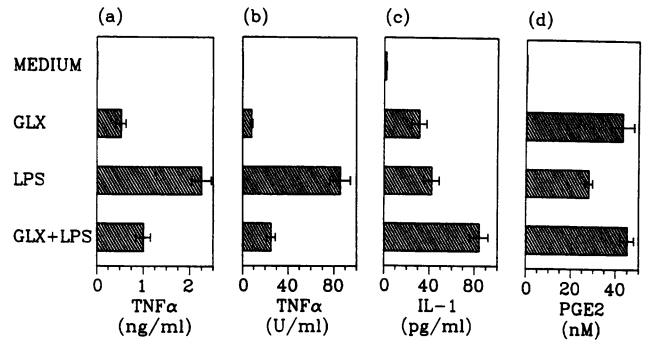

FIG. 2. Synergy and antagonism between glycocalyx and LPS in activation of macrophages. Peritoneal macrophages were incubated with 100 μg of G2-89 glycocalyx per ml, 100 ng of LPS per ml, or both. Supernatants were harvested at 8 h and assayed for IL-1 and TNF-α by ELISA. TNF-α was also assayed by bioassay (b). Supernatants harvested at 24 h were assayed for PGE_2. Arithmetic means of triplicate cultures ± standard deviations (SD) are presented. Data reported in panel b were obtained in an independent trial. Data reported in panels a, c, and d are from the same trial. Similar data were obtained in three independent trials.

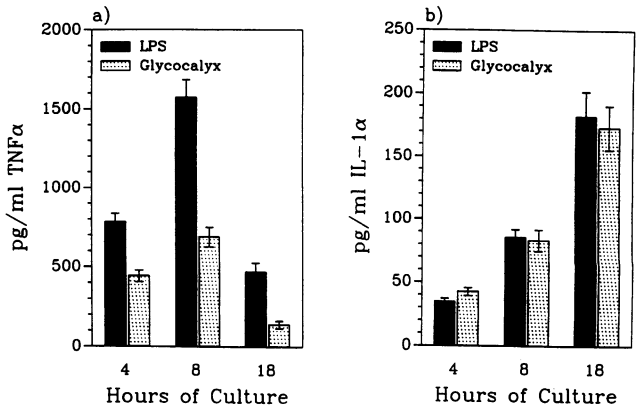

FIG. 3. Kinetics of TNF-α and IL-1 production by glycocalyx-stimulated macrophages. Peritoneal macrophages were incubated with 100 ng of LPS per ml or with 100 μg of glycocalyx per ml. Culture supernatants were harvested at 4, 8, or 18 h and assayed by ELISA for TNF-α or IL-1α. Data represent arithmetic means of triplicate cultures of macrophages ± SD.

TNF-α for L929 fibroblasts (data not shown). In addition, analysis of the supernatants for TNF-α by ELISA also indicated that the supernatants of glycocalyx-stimulated macrophages contained significantly less TNF-α than those of LPS-stimulated macrophages (Fig. 2).

To determine if the low production of TNF-α was due to an inhibitory effect of glycocalyx, macrophages were cultured with both glycocalyx and LPS. Supernatants of cultures stimulated with both G2-89 glycocalyx and LPS contained two- to threefold less TNF-α than supernatants of cultures stimulated with LPS alone, as determined both by bioassay and by ELISA (Fig. 2). In contrast, supernatants of macrophages stimulated with both glycocalyx and LPS contained PGE_2 levels equivalent to those of macrophages stimulated with glycocalyx alone. To examine the effect of glycocalyx on another inflammatory cytokine, IL-1, macrophages were incubated for 8 h with glycocalyx, LPS, or both, after which the culture supernatants were assayed for IL-1α by ELISA. No significant difference was detected in the amount of IL-1α secretion by macrophages stimulated by either glycocalyx or LPS (Fig. 2). However, in contrast to the inhibitory effect of glycocalyx on LPS-stimulated TNF-α production, the addition of both glycocalyx and LPS to macrophages resulted in increased secretion of IL-1α (Fig. 2).

To determine if the low TNF-α production by macrophages in response to glycocalyx represented a shift in the kinetics of cytokine production, macrophages were cultured with either LPS or glycocalyx for 4, 8, or 18 h and the culture supernatants were assayed by ELISA for TNF-α and IL-1α. TNF-α concentration was maximal at the 8-h time point for macrophages stimulated with either LPS or glycocalyx (Fig. 3a). At each time point, the TNF-α concentration was two- to threefold lower in cultures of glycocalyx-stimulated macrophages compared with LPS-stimulated macrophages. In contrast, no difference was detectable in kinetics or intensity of IL-1α production between glycocalyx- and LPS-stimulated macrophages (Fig. 3b).

Since PGE_2 can down-regulate TNF-α production (24, 37, 41), the involvement of glycocalyx-induced PGE_2 production in inhibition of TNF-α production was investigated. Macrophages were incubated for 8 h with either LPS or glycocalyx in the presence or absence of indomethacin. The addition of indomethacin at culture onset did result in a sixfold increase in TNF-α production by glycocalyx-stimulated macrophages and a threefold increase in TNF-α production by LPS-stimulated macrophages (Fig. 4). However, even in the presence of indomethacin, TNF-α production by glycocalyx-stimulated macrophages (123 U/ml) was twofold less than that of macrophages stimulated with LPS in the absence of indomethacin (244 U/ml) and sixfold less than that of macrophages stimulated with LPS in the presence of indomethacin (829 U/ml). Nonetheless, TNF-α production by macrophages stimulated with both glycocalyx and LPS in the presence of indomethacin was equivalent to TNF-α production by macrophages stimulated with LPS (Fig. 5). The presence of indomethacin in the cultures also increased the amount of IL-1α secreted by LPS-stimulated and glycocalyx-stimulated macrophages (Fig. 4). Indomethacin did not affect IL-1α secretion by macrophages stimulated with both glycocalyx and LPS.

TNF-α plays a significant role in activating macrophage antimicrobial effector functions such as nitric oxide generation (31, 33, 42). To determine if glycocalyx could induce nitric

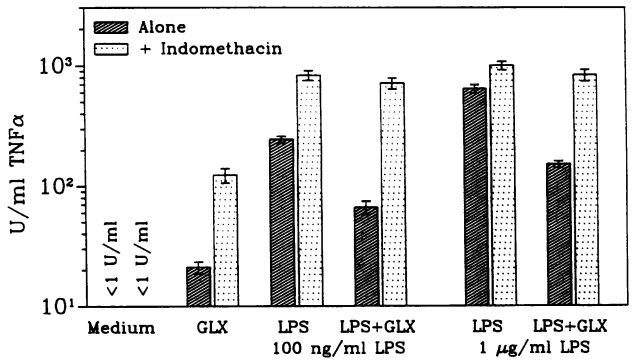

FIG. 4. Enhancement of TNF-α production by indomethacin. Peritoneal macrophages were incubated for 8 h with 100 μg of glycocalyx per ml, the designated concentration of LPS, or both, in the presence or absence of 10^{-6} M indomethacin. TNF-α activity was measured by bioassay. Data are presented as the arithmetic means of triplicate cultures ± SD.

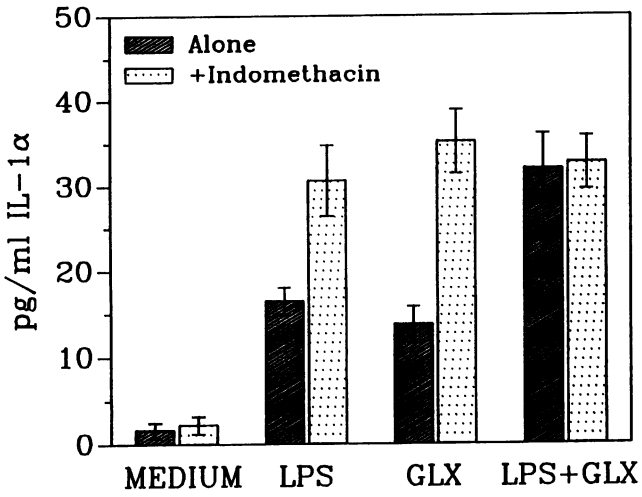

FIG. 5. Enhancement of IL-1α production by indomethacin. See legend to Fig. 4. IL-1α was assayed by ELISA. Data are presented as arithmetic means of triplicate cultures ± SD.

oxide generation, macrophages were cultured for 48 h with glycocalyx or LPS in the presence or absence of rIFN-γ, after which the culture supernatants were assayed for nitrite. The G2-89 glycocalyx did stimulate nitric oxide generation in the presence, but not in the absence, of rIFNγ (Fig. 6). The nitric oxide generation could be completely blocked by inclusion of N^G-monomethyl-L-arginine in the culture, confirming that it was derived from the arginine-dependent pathway (6). However, the quantity of nitrite detected (3 to 4 nmol per well) did not increase as the glycocalyx concentration increased from 10 to 100 μg/ml and remained three- to fourfold lower than the quantity of nitrite generated by macrophages stimulated with LPS plus rIFN-γ (13 nmol per well) (Fig. 6). Concentrations of glycocalyx of ≤1 μg/ml did not induce detectable nitrite production. To determine if this low nitrite response represented a shift in kinetics of nitrite generation, macrophages were stimulated with glycocalyx or LPS for 24, 48, or 72 h, after which the culture supernatants were assayed for nitrite. At each time point, macrophages stimulated with glycocalyx had produced approximately twofold less nitrite than macrophages stimulated with LPS (Fig. 7A).

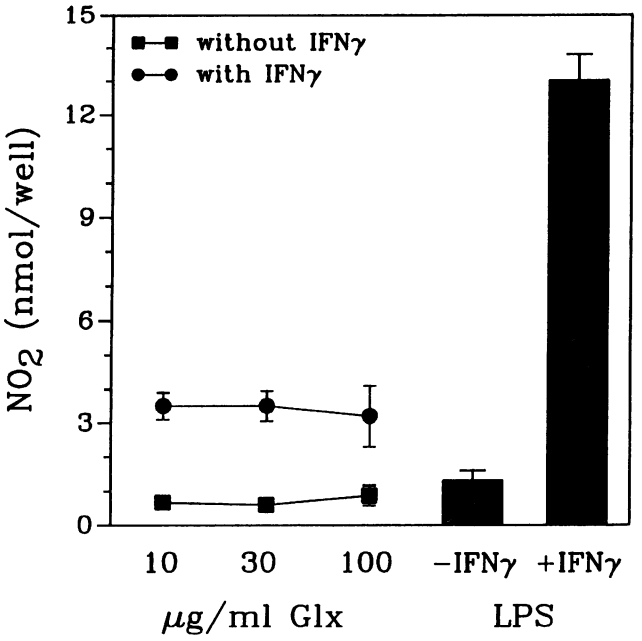

FIG. 6. Stimulation of nitric oxide generation by glycocalyx. Macrophages were cultured for 48 h with 10 to 100 μg of G2-89 glycocalyx per ml in the presence or absence of 50 U of rIFN-γ per ml or with 100 ng of LPS per ml in the presence or absence of 50 U of rIFN-γ per ml, after which the nitrite concentration in the culture supernatants was determined. Data are presented as arithmetic means of triplicate cultures ± SD. Macrophages cultured with rIFN-γ alone generated less than 1 nmol of NO_2 per well.

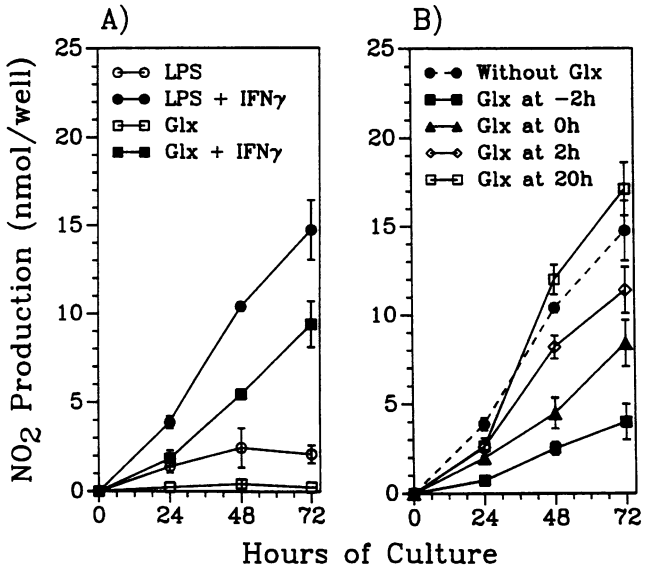

FIG. 7. Kinetics of glycocalyx-induced nitric oxide generation. (A) Macrophages were cultured with 100 ng of LPS per ml or 100 μg of glycocalyx per ml in the presence or absence of 100 U of rIFN-γ per ml. Culture supernatants were harvested at the indicated time points and assayed for nitrite. Data are presented as the arithmetic means of triplicate macrophage cultures ± SD. (B) Macrophages were cultured with 100 ng of LPS per ml plus 100 U of rIFN-γ per ml. Glycocalyx was added (100 μg/ml) 2 h before, simultaneously with, 2 h after, or 20 h after addition of LPS and rIFN-γ. Data are presented as the arithmetic means of triplicate macrophage cultures ± SD.

To determine if glycocalyx could inhibit LPS-induced nitric oxide generation, macrophages were incubated for 48 h with glycocalyx, LPS, or both in the presence of rIFN-γ. Macrophages incubated with both glycocalyx and LPS generated less nitrite than macrophages stimulated with LPS (Fig. 8). However, in contrast to its effect on TNF-α production, the addition of indomethacin did not restore nitrite generation in cultures of macrophages stimulated with both glycocalyx and LPS to levels of nitrite generation observed in cultures of macrophages stimulated with LPS without glycocalyx (Fig. 8). To determine if it was critical for inhibition that glycocalyx be added at culture onset or whether later additions could result in equal inhibition (e.g., by scavenging reactive nitrite intermediates), macrophages were stimulated with LPS plus IFN-γ either 2 h after addition of glycocalyx, simultaneously with addition of glycocalyx, or 2 h before addition of glycocalyx. Culture supernatants were assayed at 24, 48, or 72 h after culture initiation. Maximal inhibition of nitrite production was observed when glycocalyx was added 2 h before LPS plus

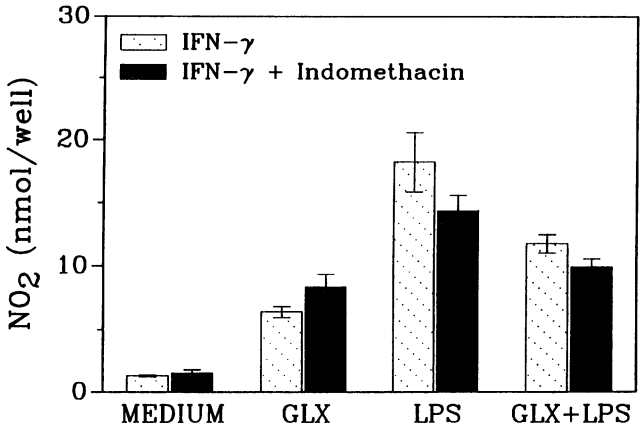

FIG. 8. Ineffectiveness of indomethacin in reversal of glycocalyx-mediated inhibition of LPS-induced nitric oxide generation. Macrophages were cultured for 48 h with 100 μg of G2-89 glycocalyx per ml, 100 ng of LPS per ml, or both, in the presence or absence of 10^{-6} M indomethacin. All cultures (including medium controls) contained 50 U of rIFN-γ per ml. Data are presented as arithmetic means of triplicate cultures ± SD.

IFN-γ (Fig. 7B). Addition of glycocalyx 2 h or later after LPS plus IFN-γ did not result in significant inhibition of LPS-induced nitrite generation.

DISCUSSION

Several investigators have now demonstrated a role for bacterial glycocalyx in the colonization of bone (23, 28, 30) and prosthetic devices (15, 27, 29). This role in bacterial colonization, coupled with the ability of glycocalyx to interfere with the phagocytosis-associated activities of neutrophils (2, 9, 19, 32, 40, 44), has implicated the glycocalyx as a pathogenic factor in coagulase-negative staphylococcus infections. In contrast to the apparent negative effects on neutrophil-mediated phagocytosis, two recent reports suggest that glycocalyx from *S. epidermidis* can activate macrophage PGE_2 and cytokine production (43, 45). The current study demonstrates that glycocalyx can activate macrophages but that the pattern of activities induced by glycocalyx differs from that induced by gram-negative bacterial endotoxin. Glycocalyx induced PGE_2 and IL-1 responses equivalent to that induced by LPS. However, glycocalyx was a poor inducer of TNF-α production and nitric oxide generation. Furthermore, although glycocalyx augmented LPS stimulation of macrophage IL-1 production, it inhibited LPS stimulation of macrophage TNF-α production and nitric oxide generation.

The weak TNF-α production by macrophages stimulated with glycocalyx was not a reflection of a shift in dose-response pattern. PGE_2 and IL-1 production increased as the concentration of glycocalyx increased from 10 to 100 μg/ml whereas macrophage production of TNF-α appeared to plateau at 10 μg of glycocalyx per ml. Concentrations of glycocalyx of ≤1 μg/ml did not stimulate detectable amounts of TNF-α or IL-1. The low TNF-α production was not due to a shift in kinetics of production of TNF-α since TNF-α concentrations in the culture supernatants were maximal at 8 h of culture with macrophages stimulated with glycocalyx as well as with those stimulated with LPS. The addition of glycocalyx directly to the TNF-α bioassay did not affect the standard recombinant TNF-α titration, thus precluding an inhibitory effect of glycocalyx on the TNF-α assay system. The low TNF-α production in response to glycocalyx was confirmed by ELISA, eliminating the possibility that the TNF-α was present in the culture supernatants but was biologically inactive. The inability to induce high levels of TNF-α production in macrophages was not idiosyncratic to one preparation of glycocalyx. Two separate glycocalyx preparations from the G2-89 isolate of *S. lugdunensis* and preparations from the G6-87 isolate of *S. lugdunensis* and from the G19-85 isolate of *S. epidermidis* all displayed the characteristic of strong stimulation of PGE_2 production and weak stimulation of TNF-α production in macrophages.

The weak stimulation of macrophage TNF-α production by glycocalyx appears to be due at least in part to PGE_2 feedback inhibition since indomethacin can enhance glycocalyx-stimulated macrophage TNF-α production and can completely reverse glycocalyx-mediated inhibition of LPS-induced TNF-α production. Glycocalyx is a potent stimulator of PGE_2 production by macrophages (Fig. 1), and PGE_2 has been demonstrated by several laboratories to inhibit TNF-α production by increasing intracellular cyclic AMP (11, 24, 41). Two other observations support the hypothesis that PGE_2 is at least partially responsible for the weak TNF-α production in response to glycocalyx. First, the addition of IFN-γ to glycocalyx-stimulated macrophages enhanced TNF-α production. The presence of IFN-γ has been hypothesized to reduce the sensitivity of macrophages to PGE_2 feedback inhibition (38). Secondly, glycocalyx was an effective inducer of IL-1α production and secretion. The induction of IL-1 is not as sensitive as that of TNF-α to PGE_2-mediated inhibition (11).

Although PGE_2 feedback inhibition does appear to play a role in modulating the response of macrophages to glycocalyx stimulation, the basis of the response of macrophages to glycocalyx is likely to be more complex. For example, LPS stimulation of macrophages results in production of PGE_2, IL-1, and TNF-α without the profound inhibition of TNF-α production observed upon glycocalyx stimulation of macrophages. The ability of glycocalyx to inhibit the response of macrophages to LPS (in the context of TNF-α production) and the ability of indomethacin to reverse that inhibition suggest that glycocalyx may induce increased sensitivity to PGE_2-mediated inhibition of TNF-α production. However, indomethacin does not enhance nitric oxide generation in glycocalyx-stimulated macrophages, nor does it reverse the glycocalyx-mediated inhibition of nitric oxide generation by LPS-stimulated macrophages. Therefore, modulatory effects of glycocalyx not involving PGE_2 feedback appear to be involved in glycocalyx-mediated regulation of nitric oxide generation. Glycocalyx does not appear to be scavenging reactive nitrogen intermediates since cultures of macrophages to which glycocalyx was added 2 h after LPS plus IFN-γ stimulation displayed concentrations of nitrite 70 h later that were almost equal to those generated by macrophages not treated with glycocalyx. The observation (Fig. 7B) that glycocalyx was most effective in inhibiting LPS-induced nitrite generation when added 2 h before LPS supports the hypothesis that early signaling pathways are a critical component of the inhibitory effect of glycocalyx.

It is not yet known what components of glycocalyx are responsible for the observed effects. Staphylococcal exotoxins have been shown to induce cytokine production and nitric oxide generation in macrophages (10, 13). However, *S. lugdunensis* G6-87 and G2-89 do not elaborate detectable amounts of these exoproteins (22). Lipoteichoic acid, at concentrations above 1 μg/ml, has been reported to induce macrophage oxidative burst, TNF-α production, and nitric oxide generation (4, 20, 25). If lipoteichoic acids were the main

macrophage stimulant in the glycocalyx preparations, they would have to represent 10% by weight of the glycocalyx, since 10 μg of glycocalyx per ml is stimulatory. The presence of some lipoteichoic acid is possible, given the phosphate levels in the glycocalyx preparations (Table 1). However, such a high lipoteichoic acid content (10% [wt/wt]) seems unlikely since the glycocalyx preparations were not derived from intact cells and since that level of lipoteichoic acid should be detected in the chromogenic limulus assay (12).

The glycocalyx component of most interest in the current study is that which inhibits antimicrobial activities of the immune system since it is these activities that would contribute to the pathogenesis of coagulase-negative staphylococcus infections. Glycocalyx from *S. lugdunensis* and *S. epidermidis* has been shown to inhibit oxidative burst, phagocytosis, and motility of neutrophils along chemotactic gradients (19, 32, 34, 40, 44). The current study demonstrates that glycocalyx can modulate two significant antimicrobial activities of macrophages—TNF-α production and nitric oxide generation (14, 17, 39). The down-regulation of TNF-α production is of particular importance since TNF-α is a major mediator of neutrophil adhesion, activation, and degranulation (3). The resolution of the mechanism of this inhibition awaits the molecular identification and characterization of the components of glycocalyx that modulate macrophage and neutrophil activities.

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