Interleukin 22 mitigates endothelial glycocalyx shedding after lipopolysaccharide injury

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BACKGROUND: The endothelial glycocalyx (EG) on the luminal surface of endothelial cells contributes to the permeability barrier of vessels and prevents activation of the coagulation cascade. Endothelial glycocalyx damage, which occurs in the shock state, results in endotheliopathy. Interleukin (IL)-22 is a cytokine with both proinflammatory and anti-inflammatory properties, and how IL-22 affects the EG has not been studied. We hypothesized that IL-22:Fc, a recombinant fusion protein with human IL-22 and the Fc portion of human immunoglobulin G1 (which extends the protein half-life), would not affect EG shedding in endothelium after injury.

METHODS: Human umbilical vein endothelial cells (HUVECs) were exposed to 1 μg/mL lipopolysaccharide (LPS). Lipopolysaccharide-injured cells (n = 284) were compared with HUVECs with LPS injury plus 0.375 μg/mL of IL-22:Fc treatment (n = 293) for 12 hours. These two cohorts were compared with control HUVECs (n = 286) and HUVECs exposed to IL-22:Fc alone (n = 269). Cells were fixed and stained with fluorescein isothiocyanate-labeled wheat germ agglutinin to quantify EG. Total RNA was collected, and select messenger RNAs were quantified by real-time quantitative polymerase chain reaction (RT-qPCR) using SYBR green fluorescence.

RESULTS: Exposure of HUVECs to LPS resulted in degradation of the EG compared with control (5.86 vs. 6.09 arbitrary unit [AU], p = 0.01). Interleukin-22:Fc alone also resulted in degradation of EG (5.08 vs. 6.09 AU, p = 0.01). Treatment with IL-22:Fc after LPS injury resulted in less degradation of EG compared to LPS injury alone (5.86 vs. 5.08 AU, p = 0.002). Expression of the IL-22Rα receptor was not different for IL-22:Fc treated compared with LPS injury only (0.69 vs. 0.86 relative expression, p = 0.10). Treatment with IL-22:Fc after LPS injury resulted in less matrix metalloproteinase 2 (0.79 vs. 1.70 relative expression, p = 0.005) and matrix metalloproteinase 14 (0.94 vs. 2.04 relative expression, p = 0.02).

CONCLUSIONS: Interleukin-22:Fc alone induces EG degradation. However, IL-22:Fc treatment after LPS injury appears to mitigate EG degradation. This protective effect appears to be mediated via reduced expression of metalloproteinases. (J Trauma Acute Care Surg. 2021;90: 337–345. Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.)

KEY WORDS: Sepsis; endothelial glycocalyx; endotheliopathy; glycocalyx degradation; interleukin 22.

Septic shock is a clinical syndrome with mortality rates as high as 56%. At the endothelial level, sepsis is associated with microthrombus and endothelial dysfunction that leads to altered microhemodynamics and malperfusion of vital organs. The endothelial glycocalyx (EG) is a glycoprotein matrix on the luminal side of endothelial cells with anticoagulant and anti-inflammatory properties. The glycocalyx plays a key role in maintaining the transvascular exchange of fluids and solutes. The glycocalyx is in a constant state of turnover, with continuous degradation by sheddases, and synthesis of the glycocalyx layer. In addition, it plays an important role in the coagulation cascade. A variety of insults, such as shock, trauma, ischemia-reperfusion, and infection, can cause damage to the glycocalyx, resulting in increased vessel permeability, perivascular inflammation, and dysfunction of leukocyte and platelet adhesion. Our previous work has demonstrated that hypoxia-reoxygenation alone is sufficient to produce glycocalyx shedding. Sepsis leads to abundant degradation of the EG, causing altered endothelial permeability, hypovolemia, hypoaalbuminemia, and edema.

Interleukin (IL) 22 is a cytokine with anti-inflammatory properties in several different organ systems. This feature makes IL-22 an attractive therapeutic target for trauma patients, in which severe infection and sepsis are leading causes of death. Our previous work has shown that IL-22 plays an integral role in repair of epithelial cells in the injured lung and helps maintain epithelial cell integrity in an influenza model. Interleukin 22 has antimicrobial effects in the lung and helps clear bacterial, viral, and fungal infection. However, the way in which IL-22 affects endothelial cells and specifically the EG has not been described.

Lipopolysaccharide (LPS) activation of endothelial cells causes EG degradation and shedding with a resultant increase in vascular permeability. Interleukin-22:Fc (f-652) (Evive Biotech, Shanghai, China) is a recombinant fusion protein with human IL-22 and the Fc portion of human immunoglobulin G2, which extends the serum half-life of the protein. The half-life of IL-22 is 2 hours, while the half-life of the fusion protein in vivo is 3.02 days. In this study, we set out to determine whether IL-22 affects the EG of LPS activated human umbilical vein endothelial cells (HUVECs). We hypothesized that IL-22:Fc (f-652) would decrease EG shedding after LPS injury in HUVECs.
MATERIALS AND METHODS

HUVEC Culture

Human umbilical vein endothelial cells were purchased from the American Type Culture Collection. Cells were initially grown in 2% gelatin-coated 10-cm plastic dishes using M200 medium supplemented with low serum growth supplement and penicillin/streptomycin in a cell culture incubator at 37°C with 5% CO₂ atmosphere as previously described. Cells were passaged by digestion in 0.25% trypsin in Hanks’ Balanced Salt Solution after reaching 80% confluence. Cells were used for experiments between passages 1 and 3. For glycocalyx quantification, HUVECs were plated in 48-well plastic cell culture plates coated with 2% gelatin, at a confluence of approximately 80%. M200–low serum growth supplement–penicillin/streptomycin was supplemented with 1% bovine serum albumin (BSA) to support glycocalyx growth. Cells were cultured for 24 hours to allow glycocalyx development before LPS exposure.

Experimental Design

To investigate the effects of f-652 on the EG, cultured HUVECs were exposed to either untreated media (n = 269), 1 μg/mL of LPS (n = 284), 1 μg/mL of LPS and 0.375 μg/mL of IL-22:Fc (n = 293), or 0.375 μg/mL of IL-22:Fc alone (n = 269) for a total of 24 hours. Dose of IL-22:Fc was determined based on previous studies demonstrating its beneficial effects. Sample sizes were determined based on previous studies demonstrating degree of glycocalyx degradation after exposing HUVECs to injury.

Glycocalyx Quantification

Glycocalyx staining of HUVECs was carried out by established techniques. After completion of the LPS exposure ± f-652, cells were fixed by addition of concentrated formaldehyde solution directly to the culture medium to yield a final formaldehyde concentration of 3.5%. After 10 minutes of fixation, cells were washed with phosphate-buffered saline (PBS) supplemented with 1% BSA. Cells were then stained with 23 μg/mL wheat germ agglutinin (WGA) and 23 μg/mL 4′,6-diamidino-2-phenylindole in PBS with 1% BSA for 20 minutes at room temperature in the dark. Staining was performed for this short period to ensure no penetration of the wheat germ agglutinin into the cytoplasm, confounding results with nonsurface layer staining. Cells were then washed twice with 1% BSA in PBS and covered with Fluoro-Gel mounting medium (Electron Microscopy Sciences, Hatfield, PA). Glycocalyx and nuclei (4′,6-diamidino-2-phenylindole) were imaged on an EVOS fluorescence microscope (Thermofisher, Waltham, MA) under identical conditions. Three images were taken of each condition, with approximately 100 cells per image. ImageJ software (National Institutes of Health, Bethesda, MD) was used to quantify glycocalyx fluorescence intensity overlaying the nuclei of each visible cell.

Measuring the IL-22Ra1 Receptor With Immunofluorescence

Human umbilical vein endothelial cells were fixed in 3.5% formaldehyde in PBS for 10 minutes. Cells were then blocked in 1% BSA in PBS for 1 hour. Cells were then incubated overnight in primary antibody for IL-22Ra1 (Invitrogen, Carlsbad, CA) diluted 1:100 in 1% BSA in PBS. Cells were then washed with PBS three times. Cells were incubated with secondary antibody, goat antimouse Alexa Fluor 488 (1:500, A28175; Invitrogen) diluted 1:500 in 1% BSA in PBS along with 0.1 μg/mL of 4′,6-diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, MO) for 1 hour, followed by three washes in PBS. Cells were then cover slipped with Fluoro-Gel mounting medium and imaged on an EVOS fluorescence microscope (Thermofisher, Waltham, MA). Fluorescence intensity was quantified using ImageJ.

SDS-PAGE Western Blots for Total STAT3 and Phosphorylated STAT3

Human umbilical vein endothelial cells were lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5 M ethylenediamine tetraacetic acid [EDTA], 1% Triton X-100, and Halt protease inhibitor cocktail [Thermofisher, Waltham, MA]). Proteins were quantified using Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA), and 20 to 50 μg of protein was separated by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (SDS-PAGE) on a 4% to 12% gradient acrylamide gel run at 100 V. Proteins were then transferred to 0.45-μm polyvinylidene difluoride (PVDF) membrane at 30 V for 2 hours. Membranes were blocked in Tris-buffered saline (137 mM NaCl, 20 mM Tris base), 0.1% Tween 20, and 5% BSA (blocking solution) for 1 hour, followed by overnight incubation with primary antibody diluted in Tris-buffered saline, 0.1% Tween 20, and 3% BSA, and 1-hour incubation with horseradish peroxidase-conjugated secondary antibody diluted at 1:5,000. The primary antibody used for signal transducer and activator of transcription 3 (STAT3) was rabbit monoclonal antibody 30835S (Cell Signaling Technology, Danvers, MA), and the primary antibody for phosphorylated STAT3 was rabbit monoclonal antibody 9145 (Cell Signaling Technology, Danvers, MA). Immunoreactive protein was detected using ECL (GE Healthcare, Boston, MA) imaged on a Bio-Rad ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA).

Real-Time Quantitative Reverse-Transcription Polymerase Chain Reaction

RNA was isolated with Trizol (Invitrogen) and used as a template for reverse transcriptase (iScript RT Supermix; Bio-Rad, Hercules, CA). Messenger RNAs (mRNAs) were quantified by real-time polymerase chain reaction with IQ SYBR Green Supermix (Bio-Rad, Hercules, CA) and normalized against PPIA mRNA as the internal control gene. Relative changes in expression were calculated using the ΔΔCt method as established in prior studies. Primer sequences are listed in Supplementary Table 1 (http://links.lww.com/TA/B845).

Measuring Actin Disruptions With Immunofluorescent

Human umbilical vein endothelial cells were grown to confluence as described above. Lipopolysaccharide and IL-22Fc were administered as specified previously for the various conditions. Twenty-four hours after treatment, cells were fixed for 10 minutes in 3.5% paraformaldehyde and permeabilized and blocked for 1 hour in 0.1% Triton X-100 in 1% BSA in PBS. Cells were incubated overnight at 4°C with primary antibody, anti–vascular endothelial cadherin (VE-CAD) antibody (CD144), clone BV9 (1:100, MABT129; Sigma, St. Louis, MO). Then, three serial washes were performed in PBS, followed by a

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60-minute incubation with secondary Alexa-fluor 488 antibody (1:500, A32723; ThermoFisher, Waltham, MA), Alexa-fluor 555 phalloidin (1:500, A34055; ThermoFisher, Waltham, MA), and DAPI. The coverslips were mounted by using Fluoro-Gel as for glycocalyx experiments. All images were taken with an Olympus fluorescent microscope (Olympus Corporation, Center Valley, PA). The number of actin disruptions was counted in five random images from three replicate wells of HUVECs.

**Statistical Analysis and Power Analysis**

Glycocalyx staining intensity and RNA levels are presented as means ± SDs. A p value of <0.05 was considered significant for all tests. For comparisons of more than two groups, one-way analysis of variance was first performed, and if \( p < 0.05 \), pairwise comparisons were performed using a one-tailed Student’s t test. All figures show mean with error bars that demonstrate SD.

Based on previous data showing a difference of approximately 8% and SD of 33% of the mean value in injured HUVECs, a power analysis and sample size determination were performed. With a significance level (\( \alpha \)) of 0.05 and power (1 - \( \beta \)) value of 0.8 that was calculated, a sample number of 252 was required to achieve the 0.8 power value.

**RESULTS**

**Glycocalyx Shedding**

A comparison of glycocalyx intensity is shown in Figure 1. A comparison of all four groups showed that glycocalyx intensity was different (\( p = 0.002 \)). When compared with control, LPS exposure led to decreased glycocalyx intensity (6.09 ± 5.95 vs. 5.10 ± 2.85 arbitrary unit [AU], \( p = 0.01 \)). However, exposure to LPS and IL-22:Fc did not result in decreased glycocalyx intensity as compared with control (6.09 ± 5.95 vs. 5.86 ± 3.07 AU, \( p = 0.38 \)). Human umbilical vein endothelial cells’ exposure to IL-22:Fc alone resulted in decreased glycocalyx intensity (6.09 ± 5.95 vs. 5.08 ± 2.38 AU, \( p = 0.01 \)). Glycocalyx intensity was less in HUVECs exposed to LPS alone as compared with LPS with IL-22:Fc (5.10 ± 2.85 vs. 5.86 ± 3.07, \( p = 0.001 \)). Representative images of fluorescent microscopy are shown for all four groups in Figure 1.

**IL-22Ra1 Receptor and STAT3 Signaling**

To determine the role of the IL-22 receptor, we examined the expression of the IL-22Ra1 receptor. As shown in Figure 2A, exposure to LPS (0.86 ± 0.27), IL-22:Fc alone (0.94 ± 0.19), or coexposure to both LPS and IL-22:Fc (0.69 ± 0.14) did not result in a difference in the IL-22Ra1 receptor expression as compared with control (1.00 ± 0.11; \( p = 0.07 \)). Representative images after immunofluorescence staining for IL-22Ra1 receptors and DAPI (Fig. 2B) are shown along with imaging for no primary antibody control staining (Fig. 2C). Figure 2B and C demonstrates that IL-22Ra1 receptors (green fluorescence) are present on the surface of endothelial cells.

The IL-22Ra1 receptor is known to be a potent stimulator of the canonical signaling pathway of phosphorylation and activation of STAT3. To confirm that IL-22:Fc is signaling through the IL-22Ra1 receptor on HUVECs, we measured the ratio of phosphorylated STAT3 to total STAT3 in control HUVECs compared with HUVECs exposed to IL-22:Fc alone (Fig. 3A). The ratio of phosphorylated STAT3 to total STAT3 in the IL-22:Fc treated (293.6 ± 74.9) is significantly higher in the IL-22:Fc treated as compared with control (100.0 ± 0.0; \( p = 0.01 \)). A representative image of an SDS-PAGE Western blot quantifying phosphorylated STAT3 and total STAT3 is shown in Figure 3B.

![Figure 1. A comparison of glycocalyx staining intensity is shown in control HUVECs, the LPS exposed, the LPS and IL-22:Fc exposed, and the IL-22:Fc only exposed. Representative images of all four groups are shown. Cotreatment of HUVECs with IL-22:Fc results in protection of the glycocalyx. Treatment with IL-22:Fc alone results in less glycocalyx intensity. Green fluorescence indicates glycocalyx. All data are means ± SDs.](image-url)
Altogether, these data demonstrate that the IL-22 receptor is present and functional on endothelial cells.

**Metalloproteinases**

To determine if IL-22 was inducing glycocalyx shedding, we examined the expression of matrix metalloproteinases (MMPs) because they have been implicated in glycocalyx degradation. Treatment of HUVECs with LPS or LPS and IL-22:Fc did not change expression of MMP-1 (Supplemental Digital Content, Supplementary Fig. 1A, http://links.lww.com/TA/B841). Matrix metalloproteinase 2 (p = 0.03) and MMP-14 (p = 0.02) expressions were significantly different as measured by one-way analysis of variance. Pairwise comparisons of MMP-2 and MMP-14 expression are shown in Figure 4A and B. For MMP-2, exposure of HUVECs to LPS and IL-22:Fc (0.79 ± 0.33) resulted in lower relative expression as compared with LPS only (1.70 ± 0.63; p = 0.005). For MMP-14, HUVECs exposed to LPS only (2.04 ± 0.99) had higher levels of MMP-2 as compared with controls (1.08 ± 0.42; p = 0.04). In addition, exposure of HUVECs to LPS and IL-22:Fc (0.94 ± 0.42) resulted in lower relative expression of MMP-14 as compared with LPS only (2.04 ± 0.99; p = 0.02). Disintegrin and metalloproteinase domain-containing protein 15 (ADAM15) expression was not different (p = 0.11) as shown in Figure 4C.

**Pro-Glycocalyx Agents**

Tissue inhibitor of metalloproteinase (TIMP) 1 and TIMP2 are natural inhibitors of matrix metalloproteinases, while exostosin-1 and exostosin-2 help build the EG through the biosynthesis of heparin sulfate, a key component of the EG. To determine how IL-22 was affecting the synthesis of the EG layer, we examined these four pro-glycocalyx agents. As seen in Figure 5A to C, when comparing the various exposure groups, TIMP2 (p = 0.14), exostosin-1 (p = 0.17), and exostosin-2 (p = 0.08) expressions were not different. Tissue inhibitor of metalloproteinase 1 was not different with the various HUVEC exposures (p = 0.11; Supplemental Digital Content, Supplementary Fig. 1B, http://links.lww.com/TA/B841).

**Syndecan and VE-CAD Levels**

Because syndecans are a major component of the EG, we measured mRNA levels in the HUVECs. Syndecan-1 (p = 0.43), syndecan-2 (p = 0.24), syndecan-3 (p = 0.07), and syndecan-4 (p = 0.07) expression levels were not different with the various HUVEC exposures (Supplemental Digital Content, Supplementary Fig. 2A–D, http://links.lww.com/TA/B842).

Vascular endothelial cadherin expression by endothelial cells has shown to be increased in response to endothelial injury; therefore, we examined expression of VE-CAD. Vascular endothelial cadherin mRNA levels were different in the four various exposure groups (p = 0.03). Vascular endothelial cadherin expression was higher in HUVECs exposed to LPS only (1.96 ± 1.02 relative expression [RE]) as compared with control (1.06 ± 0.37 RE; p = 0.048). Lipopolysaccharide only treated (1.96 ± 1.02 RE) had higher RNA levels than LPS and IL-22:Fc coexposed HUVECs (0.81 ± 0.49; p = 0.02).

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**Figure 2.** There is no difference in IL-22Ra1 expression in the various HUVECs conditions. (A), A comparison of IL-22Ra1 relative expression in all four groups of HUVECs is shown. To demonstrate that IL-22Ra1 receptors are present on the surface of endothelial cells, images from immunofluorescent microscopy show (B) IL-22Ra1 receptors (green), DAPI (blue), and (C) no primary antibody control staining and DAPI (blue) alone. All data are means ± SDs.

**Figure 3.** Treatment of HUVECs with IL-22:Fc results in activation of the canonical signaling pathway of phosphorylation and activation of STAT3. (A), A comparison of phosphorylated STAT3/total STAT3 ratio in control HUVECs and IL-22:Fc-treated HUVECs. (B), SDS–polyacrylamide gel electrophoresis Western blot quantifying phosphorylated STAT3 and total STAT3. All data are means ± SDs.
To determine the extent of endothelial damage, we examined the number of actin disruptions in HUVECs as shown in Supplementary Fig. 3 (http://links.lww.com/TA/B843). The number of VE-CAD disruptions in the four different exposure groups was significantly different ($p = 0.001$). Supplementary Figure 3D (http://links.lww.com/TA/B843) demonstrates that there are less VE-CAD disruptions in the IL-22:Fc and LPS group. Representative images are shown in Supplementary Figure 3A to D (http://links.lww.com/TA/B844). The VE-CAD disruptions (white arrow) and actin stress fibers (black arrow) are shown in a representative image of LPS-injured HUVECs in Supplementary Figure 3B (http://links.lww.com/TA/B844). Supplementary Figure 3E (http://links.lww.com/TA/B843) shows pairwise comparisons of the number of actin disruptions in the various HUVEC exposure groups.

**TLR4 Signaling Pathway**

Lipopolysaccharide exerts its effects through Toll-like receptor 4 (TLR4); therefore, we next investigated whether IL-22:Fc affects expression of the TLR4 signaling pathway components. Toll-like receptor 4 mRNA was not significantly different in all comparisons ($p = 0.30$; Fig. 6A). Myeloid differentiated primary response 88 (MYD88) RNA expression was different in the four different exposures ($p = 0.049$). Pairwise comparisons of MYD88 are shown in Figure 6B. Myeloid differentiated primary response 88 expression was lower in LPS and IL-22:Fc coexposed HUVECs (0.72 ± 0.25 RE) as compared with LPS only (1.48 ± 0.79 RE; $p = 0.03$). Toll–interleukin-1 receptor domain containing adapter protein mRNA expression ($0 = 0.11$) and interleukin-1 receptor associated kinase 4 mRNA expression ($p = 0.24$) were not significantly different with the various HUVECs exposures as shown in Figure 6C and D. Several other mediators of the TLR4 signaling pathway, including TIR-domain–containing adapter–inducing interferon-β adapter molecule, tumor necrosis factor receptor–associated factor 6, interleukin-1 receptor–associated kinase 1, and TIR-domain–containing adapter–inducing interferon-β, were not different and are shown in Supplemental Figure 4A to D (http://links.lww.com/TA/B844).

**DISCUSSION**

Interleukin 22 has regenerative and anti-inflammatory properties in multiple organ systems, making it an attractive potential therapeutic for severe infection and sepsis. Endothelial dysfunction and alteration of microvascular blood flow are a well-known sequela of septic shock. During sepsis, the glycocalyx is degraded via several inflammatory mechanisms, including sheddases such as metalloproteinases, heparanases, and hyaluronidases. This contributes to the vascular hyperpermeability, microvascular thrombosis, and enhanced leukocyte adhesion. In this study, we attempt to determine how IL-22 affects the EG after LPS injury and to determine if f-652 may have therapeutic benefit in severe infection.

Our study found that f-652 has a protective effect on the EG. Interestingly, treating the EG with IL-22:Fc alone led to decreased EG intensity; however, coexposure with LPS and IL-22:Fc

![Figure 4](http://links.lww.com/TA/B843)

**Figure 4.** Treatment with IL-22:Fc results in less expression of MMP-2, MMP-14, and ADAM15. Relative expression of (A) MMP-2, (B) MMP-9, and (C) MMP-14 mRNA levels in control, LPS-exposed, LPS- and IL-22:Fc-exposed, and IL-22:Fc-only–exposed HUVECs. All data are means ± SDs.

![Figure 5](http://links.lww.com/TA/B844)

**Figure 5.** Relative expression of (A) TIMP-1, (B) TIMP-2, (C) exostosin-1, and (D) exostosin-2 mRNA levels in control, LPS-exposed, LPS- and IL-22:Fc-exposed, and IL-22:Fc-only–exposed HUVECs. All data are means ± SDs.
preserved the EG layer with respect to control. Interleukin-22:Fc has been shown to have anti-inflammatory and antimicrobial properties.\textsuperscript{17,19,32,33} Preservation of the EG may play a role in these beneficial properties. Furthermore, this finding supports the need to examine IL-22 as a potential therapeutic target in severe infection and septic shock where endotheliopathy leads to deleterious changes.

The IL-22Ra1 receptor is present on epithelial cells of the skin, intestines, and lung and helps maintain epithelial integrity.\textsuperscript{16} Our study demonstrates that it is also expressed on endothelial cells. Furthermore, we confirmed that IL-22Ra1 activation on endothelial cells induces the canonical signaling pathway of phosphorylation and activation of STAT3. This may explain the protective effects of IL-22 on the EG as prior studies have shown that IL-22Ra1 enhances repair of epithelial cells through the STAT3 activation pathway.\textsuperscript{34,35} The IL-22Ra1 receptor is normally expressed in the airways. However, its expression in the lung parenchyma and alveoli does not occur until after injury.\textsuperscript{15} This phenomenon was not seen in the endothelial cells because the IL-22Ra1 was found in control HUVECs and not increased after LPS injury. This finding is similar to prior studies showing that IL-22Ra1 receptor induction is mediated through Toll-like receptor 3.\textsuperscript{36} Further studies are needed to examine this finding.

Matrix metalloproteinases are upregulated in various models of LPS injury.\textsuperscript{37,38} In addition, MMPs play a key role in degradation of the EG.\textsuperscript{39,40} Disintegrin and metalloproteinase domain-containing protein 15 (ADAM15) in particular has been shown to play a key role in glycocalyx degradation after LPS injury.\textsuperscript{28} Interestingly, we did not find any difference in ADAM15 expression with IL-22:Fc treatment. However, we did find that IL-22:Fc resulted in less expression of MMP-2 and MMP-14. This effect was not seen with exposure to IL-22:Fc alone but was present with coexposure of LPS and IL-22:Fc. The mechanism by which this downregulation of metalloproteinases occurs is not clear. This was not found to be due to increased expression of TIMP1 or TIMP2 because TIMP1 and TIMP2 levels were not changed with LPS and IL-22:Fc coexposure. Tissue inhibitor of metalloproteinase 1 and TIMP2 are natural inhibitors of MMPs and inhibit the shedding of EG by metalloproteinases. Prior work has shown that IL-22 can actually increase various MMP levels in skin and the digestive tract.\textsuperscript{41-43} The relationship between IL-22 and metalloproteinase expression at the endothelial level needs further investigation.

Exostosin-1 and exostosin-2 are enzymes responsible for the biosynthesis of heparin sulfate, a key component of the EG. Exostosin-1 and exostosin-2 promote rebuilding of the EG after degradation and as part of the natural EG turnover process.\textsuperscript{44,45}
We found that neither exostosin-1 nor exostosin-2 upregulation can explain the IL-22:Fc-mediated protection from EG degradation. In fact, expression of both exostosin-1 and exostosin-2 was not different in HUVECs exposed to both LPS and IL-22:Fc. Because there was no degradation of the EG when exposed to LPS and IL-22:Fc, there was no increase in expression of exostosin-1 and exostosin-2 as a compensatory measure. This further strengthens the argument that IL-22:Fc mitigation of EG degradation is mediated by downregulation of MMPs.

Syndecan mRNA levels were found to be no different in LPS-only-exposed HUVECs, when compared with the LPS and IL-22:Fc exposed. Syndecans are an integral component of the EG. Degradation of the EG by sheddases after LPS exposure may result in increased expression of syndecan mRNA to rebuild the EG.46 We found that VE-CAD expression was higher in LPS exposed with respect to control and HUVECs exposed to both LPS and IL-22:Fc. While VE-CAD helps promote cell junctions, endothelial VE-CAD mRNA expression is known to increase in response to endothelial cell injury by LPS injury.47,48 The higher levels of VE-CAD expression seen in our study by the LPS-injured HUVECs are reflective of the more severe injury in the LPS exposed. We confirmed this by showing that there were more VE-CAD disruptions in the LPS injured along with the presence of stress fibers (Supplemental Digital Content, Supplementary Fig. 3B, http://links.lww.com/TA/B843). Lower expression of VE-CAD along with less VE-CAD disruptions in the HUVECs receiving both LPS and IL-22:Fc concomitantly are reflective of the mitigated damage to the EG.49

While IL-22:Fc coexposure with LPS did not decrease TLR4 expression, it did downregulate a mediator of this proinflammatory pathway. The MYD88 is a key mediator in the TLR4 pathway that was decreased in the presence of LPS and IL-22:Fc. Multiple studies have shown that TLR4 activation leads to increased expression of IL-22 in epithelial cells of multiple organ systems.50–54 However, the decrease in expression of MYD88 in the presence of IL-22 observed in our study is a novel finding. Decrease in downstream mediators of the TLR4 pathway can occur by numerous mechanisms. Decreased expression of MYD88 leads to reduced inflammation. Some downregulators of MYD88 include IL-10 and SMAD6, among others.55,56 Further studies are needed to determine how MYD88 expression is decreased by IL-22:Fc in the absence of changes seen in TLR4 expression. The TLR4 activation pathway is known to increase MMP expression, and downregulation of this pathway57,58 may explain the decrease in MMP-2 and MMP-9 that was observed in the present study. Furthermore, this finding highlights the potential for IL-22:Fc to be a novel therapeutic in severe infection.

While IL-22:Fc alone was harmful to the EG, this was not due to increased expression of various sheddases. Syndecan levels were not found to be different with IL-22:Fc alone. This suggests that IL-22:Fc may blunt the biosynthesis of the EG layer without affecting sheddases. The EG is in a constant state of metabolic turnover, with EG biosynthesis and shedding occurring in a dynamic fashion.6 In the absence of injury, IL-22:Fc may reduce the biosynthesis of the EG resulting in decreased glyocalyx intensity upon staining. Further studies are needed to elucidate which patient populations and disease states may benefit from IL-22:Fc and where IL-22:Fc may be harmful.

This study was not without limitations. Dosage of IL-22:Fc given to HUVECs was calculated based on in vivo studies.16 IL-22:Fc can have proinflammatory or anti-inflammatory effects based on organ tissue or disease process.59 Whether varying doses of IL-22:Fc may have proinflammatory effects in HUVECs was not determined in this study. In addition, IL-22:Fc was given concomitantly with LPS injury. This was done as a proof of concept experiment to determine if IL-22:Fc may have potential therapeutic benefit in severe infection. Further studies are needed to determine whether IL-22:Fc given after injury has a therapeutic effect. In addition, in vivo studies will help determine whether IL-22:Fc may be therapeutic in the trauma population. Finally, there are regional differences in endothelial responses to LPS based on which tissue or organ system is being affected. The use of HUVECs for our experiments will not account for these regional differences in endothelium.

In conclusion, this study demonstrates that f-652 alone induces EG degradation. However, in the presence of LPS injury, f-652 appears to mitigate EG degradation. Interleukin-22Ra1 receptors are present on endothelial cells and signal through the phosphorylated-STAT3 pathway. The protective effect of f-652 to the EG appears to be mediated via metalloproteinases and downregulation of the TLR4 pathway via MYD88. These findings suggest a potential therapeutic effect of f-652 in the endotheliopathy that occurs in severe infection.

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