INTERLEUKIN 10 ANTIOXIDANT EFFECT DECREASES LEUKOCYTES/ENDOTHELIAL INTERACTION INDUCED BY TUMOR NECROSIS FACTOR α

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ABSTRACT—Little is known about the endothelial mechanisms involved in the anti-inflammatory effects of interleukin 10 (IL-10). The goal of this study was to evaluate the effects of IL-10 on endothelial oxidative stress and endothelial inflammation induced by tumor necrosis factor α (TNF-α). Production of reactive oxygen species (ROS) in perfused human umbilical vein endothelial cells (HUVECs) was studied by fluorescent microscopy using dichlorodihydrofluorescein diacetate. Tumor necrosis factor α (1 ng/mL) was added to the perfusion medium in the absence and presence of IL-10 (1 ng/mL). The role of phosphatidylinositol 3-kinase (PI3-kinase) was assessed using wortmannin and LY 294002 (inhibitors of PI3-kinase). Specific inhibition of p110α and p110γ/δ PI3-kinase subunits was studied using A66 and TG100–115. As well, levels of ceramide and intercellular adhesion molecule 1 (ICAM-1) expression were measured. Finally, the effect of IL-10 on TNF-α-induced leukocyte/endothelium interaction was examined using an ex vivo perfused vessel model. Interleukin 10 significantly reduced dichlorodihydrofluorescein diacetate fluorescence induced by TNF-α in HUVECs (12.5% ± 3.2% vs. 111.7% ± 21.6% at 60 min). Pretreatment by LY2940002 or wortmannin restored ROS production induced by TNF-α in the presence of IL-10. In HUVECs treated by TNF-α + IL-10, inhibition of p110α PI3-kinase subunit significantly increased ROS production, whereas p110γ/δ inhibition did not have a significant effect. Pretreatment with IL-10 significantly decreased TNF-α-induced increased levels of ceramide (TNF-α vs. TNF-α + IL-10: 6.278 ± 1.013 vs. 1.440 ± 130 pmol/mg prot), as well as ICAM-1 expression and leukocyte adhesion (TNF-α vs. TNF-α + IL-10: 26.8 ± 2.6 vs. 6.7 ± 0.4 adherent leukocytes/field at 15 min). Interleukin 10 decreases the level of inflammation induced by TNF-α in endothelial cells by reducing the TNF-α-induced ROS production, ICAM-1 expression, and leukocyte adhesion to the endothelium. The antioxidant effect of IL-10 is mediated through PI3-kinase and is paralleled by a decrease in ceramide synthesis induced by TNF-α.

KEYWORDS—Endothelial cells, leukocytes, inflammation, cytokines, reactive oxygen species, oxidative stress

INTRODUCTION

Impairment of the microcirculation has been shown to be tightly correlated with the prognosis of patients with septic shock, and as such, the endothelium, the major cellular makeup of the microcirculation, is a potential therapeutic target (1). An increase in oxidative stress linked to the severity of patients is now widely acknowledged as a feature of septic shock (2). Reactive oxygen species (ROS) play a key role in endothelial activation and dysfunction. These are involved in several critical cellular inflammatory responses including the expression of adhesion molecules and leukocyte-endothelial interaction. We recently demonstrated that plasma from septic shock patients increases in vitro endothelial ROS production, leading to an increase in cell death (3, 4). Controlling ROS production with antioxidant therapy has been postulated, but the clinical results remain controversial (5, 6), and despite promising data, systemic inhibition of the inflammatory response has failed to demonstrate efficacy in clinical trials for the treatment of septic shock (7). This may be explained by the fact that these global interventions reduce the innate immune response as well as the inflammatory response and therefore compromise the host against pathogen defenses.

Interleukin 10 (IL-10) is an anti-inflammatory cytokine with global effects on cytokine release, adhesion molecule expression, and cell death (8). IL-10 has been reported to decrease endothelial dysfunction in vessels stimulated by different proinflammatory agents such as angiotensin II (9, 10), endothelin 1 (11), and hypoxia/reoxygenation (12), and it is thought that some of this is mediated by an increase in endothelial nitric oxide synthase (eNOS) expression or an inhibition of the ERK1/2 (13) pathway. The downstream event is postulated to be a decrease in ROS production (11, 14), but the intracellular pathways involved have not been delineated. IL-10 has been shown to decrease oxidative stress in macrophages, epithelial cells, and endothelial cells (15–17) and can modulate ceramide synthesis induced by tumor necrosis factor α (TNF-α) via

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phosphatidylinositol 3-kinase (PI3-kinase) in astrocytes (18). These findings are consistent with studies showing that ceramide is a key mediator of TNF-α–induced inflammation in macrophages and neurons (19, 20).

Despite these promising anti-inflammatory properties, IL-10 does not show any protective effect in septic shock models using live bacteria (21), despite that positive effects in acute inflammatory models have been observed (22, 23).

In an attempt to better understand why IL-10 failed to demonstrate a positive effect in bacteria models of septic shock, we explore whether IL-10 decreases TNF-α–induced endothelial inflammation and activation in a functional model of leukocyte to endothelium adhesion. As well, the cellular mechanism by which this occurs was explored.

**MATERIALS AND METHODS**

Human TNF-α and IL-10 were used in all the human endothelial cell experiments, and mouse IL-10 and TNF-α were used for the *ex vivo* perfused mouse vessel experiments (Sigma Chemical Co, St Louis, Mo).

**Perfused endothelial cells**

Human umbilical vein endothelial cells (HUVECs) were obtained from PromoCellR Laboratories (Heidelberg, Germany) as cryopreserved cells. Assessment of cellular ROS levels and cell mortality—As previously described, cellular ROS production and cell mortality were assessed using perfused naive endothelial cells exposed to the various protocols described below (4, 24). Cellular ROS generation was measured using 2,7-dichlorofluorescein (DCFH; Molecular Probes Europe BV, Leiden, the Netherlands). Results are reported as a percentage in elevation of fluorescence using the following formula: [(DCFH fluorescence at T120) – DCFH fluorescence at T60] / DCFH fluorescence at T60] × 100 [25]. Cell viability was confirmed using propidium iodide (PI; Molecular Probes Europe BV) fluorescent probe. At the end of each experiment, cells were lysed with Triton X100, to provide an estimate of total fluorescence when all cells are permeable. The mortality rate induced by plasma was then calculated using the following formula: [(PI fluorescence at Tx100) – PI fluorescence at T60] / (PI fluorescence at T100 – PI fluorescence at T120)] × 100.

Experimental protocol—The effect of IL-10 on endothelial ROS production during TNF-α exposure. Tumor necrosis factor α dose of 1 ng/mL was used as previously published (24). An equivalent dose of IL-10 was used as reported by Kaur and coworkers (25). Cells were perfused with IL-10 at 30 min before TNF-α exposure. After 1 h of exposure to TNF-α, perfusion was continued with Krebs-Henseleit buffer, and the cells observed for a further hour. Fluorescent images were obtained every 10 min and analyzed to assess ROS production. Cell viability was also assessed every 10 min. Tumor necrosis factor α and IL-10 were purchased from Sigma Chemical Co. For each group, the experiment was repeated six times.

The role of PI3-kinase in mediating the IL-10 antioxidant effect during TNF-α exposure was studied using specific inhibitors to elucidate the role of PI3-kinase in the intracellular signaling pathway involved in the IL-10 modulation of oxidative stress induced by TNF-α.

(1) Global inhibition of PI3-kinase:

- LY-294002 (Sigma Chemical Co), a specific inhibitor of PI3-kinase, which blocks the binding site of ATP to the enzyme; the concentration of LY-294002 used in the perfusate was 20 μM (18).
- Wortmannin (Sigma Chemical Co), a fungal metabolite that is an irreversible inhibitor of PI3-kinase catalytic activity, used at a concentration in the perfusate of 300 nM (18).
- LY-294002 and wortmannin were added during 5 min before IL-10 perfusion.

(2) Specific inhibition of p110α and p110γ/δ PI3-kinase subunits:

- A66 (Selleckchem, Houston, Tex) is a highly specific and selective inhibitor of p110α PI3-kinase subunit; A66 is more than a 100-fold less active against the other class I PI3-kinase isoforms and had no inhibitory activity against 200 protein kinases when tested at 10 μmol (26).
- TG100-115 (Selleckchem) inhibits p110γ/δ PI3-kinase subunits, whereas both PI3Kα and PI3Kβ subunits are relatively unaffected (27).

A66 and TG100-115 were used at a concentration of 10 μmol and were added at the same time than TNF-α.

**Intercellular adhesion molecule 1 expression in endothelial cells**

Human umbilical vein endothelial cells were cultured in 6-well plates. At confluence, they were starved for 1 h and then cultured for 4 h in Krebs-Henseleit solution supplemented with TNF-α (1 ng/mL), TNF-α + IL-10 (1 ng/mL each), or IL-10 (1 ng/mL). In the TNF-α + IL-10, IL-10 was added 30 min before TNF-α. Total RNA was extracted from the cell samples using Trizol reagent (Invitrogen, Carlsbad, Calif). After deoxyribonuclease I treatment (Promega, Madison, Wis), 1 mg of total RNA was reverse-transcribed into cDNA using M-MLV reverse transcriptase (Promega). Real-time polymerase chain reaction was carried out using 5 ng of cDNA as template, using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, Calif) on an ABI Prism 7500 Sequence Detection System to determine mRNA levels for intercellular adhesion molecule 1 (ICAM-1) and 18S genes (the internal housekeeping genes). Polymerase chain reaction results were normalized to 18S. For each group, the experiment was repeated three times.
Assessment of ceramide levels in endothelial cells

Cells were cultured in 75-cm² flasks. At confluence, cells were starved for 1 h and then exposed for 4 h to Krebs-Henseleit buffer alone or with added TNF-α (1 ng/mL), TNF-α + IL-10 (1 ng/mL) each) in which IL-10 was added 30 min before TNF-α, or IL-10 (1 ng/mL). After 4 h, cells were manually peeled from the flask and centrifuged at 1,500 revolutions/min, followed by two washes with phosphate-buffered saline. The dry pellet was sonicated in phosphate-buffered saline, and proteins extracted. The lipids associated with 20 μg of protein were extracted into chloroform/methanol (2:1; 20 volumes) after the addition of the internal standard (ceramide 17:0). Analysis was performed by electrospray ionization–tandem mass spectrometry using a PE Sciex API 4000 Q/Trap mass spectrometer with a turbo ion spray source and an Analyst 1.5 data system (Applied Biosystem, California, USA). Quantification of individual lipid species (Cer 16:0; Cer 18:0, Cer 20:0, Cer 22:0, Cer 24:1, Cer 24:0) was performed using scheduled multiple-reaction monitoring in positive ion mode. For each group, the experiment was repeated six times.

Assessment of leukocyte adhesion to endothelial cells

The appropriate ethics approval was obtained from the AMREF Animal Ethics Committee. Aortae were isolated from C57BL/6 mice aged from 8 to 10 weeks old. Vessels were mounted in a vessel chamber primed with Krebs buffer and maintained at physiological pH by infusing carbogen gas through the buffer at 37°C. Human whole blood from healthy donors, anticoagulated with heparin, was labeled with DiIC18 (1:1,000)(Invitrogen) and perfused through the aorta at 0.1 mL/min for 15 min using a syringe pump. Images were obtained with a Zeiss discovery V20 fluorescence microscope fitted with a 24× lens and recorded using a Hamamatsu Orca 2 ER camera coupled to AxioVision software (Zeiss Imaging, Oberkochen, Germany) (28). Images were acquired every 5 min in two different segments of the aorta over a period of 15 min. Results are reported as number of adherent leukocytes per field. For each group, the experiment was repeated six times. The experimental protocol is described Figure 1B.

Presentation of results and statistical analyses

Data were analyzed using a two-way analysis of variance for repeated measures with a between-factor (i.e., treatment) and a within-factor (i.e., time) to compare changes in fluorescence and leukocyte/endothelial interaction and a one-way analysis of variance for the other experiments. Differences between groups were studied using Bonferroni post hoc tests. The statistical significance was determined at the 0.05 level. Values are reported as mean ± SEM. Graphs are represented by histograms or by box and whiskers (min-max).

RESULTS

Effect of IL-10 on oxidative stress induced by TNF-α

Tumor necrosis factor α (1 ng/mL) induced a significant increase in DCFH fluorescence compared with controls (TNF-α: 157.3% ± 48.5% vs. control: 26.2% ± 20.2%; after 1 h; P < 0.001) as soon as TNF-α was added to the perfusate. Interleukin 10 (1 ng/mL), on its own, had no effect on ROS production. When added 30 min before TNF-α, IL-10 significantly reduced the TNF-α-induced increase in the level of ROS (Fig. 2A; TNF-α: 157.3% ± 48.5% vs. TNF-α + IL-10: 23.9% ± 12%; after 1 h; P < 0.001). When added 10 min after the beginning of exposure to TNF-α, IL-10 significantly decreased TNF-α-induced DCFH fluorescence (Fig. 2B; TNF-α: 100.4% ± 13.19% vs. TNF-α + IL-10: 17.43% ± 2.39%).

No statistical differences were observed between the control, IL-10, and TNF-α + IL-10 groups. As previously reported (24), TNF-α at this concentration did not induce cell death. No change in cell viability was observed in any of the groups studied (data not shown). Neither PI3-kinase inhibitor induced cell death at the concentrations used.

The role of PI3-kinase in the IL-10 antioxidant effect

In the presence of IL-10 (1 ng/mL), the PI3-kinase inhibitors LY-294002 (20nM) (Fig. 3A; LY + TNF-α + IL-10: 120% ± 10% vs. control: 80% ± 10%) and wortmannin (Fig. 3B; TNF-α + wortmannin: 110% ± 10% vs. control: 80% ± 10%) did not reduce the IL-10 antioxidant effect. However, in the presence of TNF-α alone, the PI3-kinase inhibitors LY-294002 (20nM) (Fig. 3A; TNF-α: 150% ± 10% vs. control: 100% ± 10%) and wortmannin (Fig. 3B; TNF-α + wortmannin: 120% ± 10% vs. control: 100% ± 10%) significantly increased DCFH fluorescence compared with TNF-α alone.
139% ± 35.23% vs. TNF-α + IL-10: 20.156% ± 6.0% after 1 h; P < 0.01) and wortmannin (300 nm/mL) (Fig. 3B; wortmannin + TNF-α + IL-10: 86% ± 9.975% vs. TNF-α + IL-10: 7.33% ± 2.06% after 1 h; P < 0.05) restored the increase in DCFH fluorescence levels induced by TNF-α (1 ng/mL). No significant difference was observed between the LY + TNF-α + IL-10 or wortmannin + TNF-α + IL-10 and the TNF-α groups. Neither LY-294002 nor wortmannin alone induced a significant increase in DCFH fluorescence.

In the presence of TNF-α + IL-10, specific inhibition of p100 α subunit by A66 induced a significant increase in DCFH fluorescence (Fig. 4A; A66 + TNF-α + IL-10: 43.5% ± 4.7% vs. TNF-α + IL-10: 3.95% ± 0.77% after 1 h; P < 0.05). The A66 + TNF-α + IL-10 increase in DCFH fluorescence was significantly lower than the increase induced by TNF-α (Fig. 4A; TNF-α: 82.1% ± 9.6% vs. A66 + TNF-α + IL-10: 43.5% ± 4.7% after 1 h; P < 0.01). A66 alone did not have any further effect on DCFH fluorescence compared with TNF-α + IL-10.

In the presence of TNF-α + IL-10, specific inhibition of p110 γ, δ subunits by TG100-115 did not significantly increase DCFH fluorescence (Fig. 4B; TG100-115 + TNF-α + IL-10: 30.15% ± 3.8% vs. TNF-α + IL-10: 3.97% ± 0.788%, not statistically significant). TG100-115 alone induced a significant increase in DCFH fluorescence compared with TNF-α + IL-10 (TG100-115: 36.23% ± 7.723% after 1 h). No differences were found between TG100-115 + TNF-α + IL-10 and TG100-115 alone.

**Effect of IL-10 on ceramide levels following TNF-α stimulation**

Tumor necrosis factor α significantly increased total ceramide levels (Fig. 5; TNF-α: 6,278 ± 1,013 pmol/mg of protein vs. control: 4,261 ± 686 pmol/mg of protein; P < 0.05). This effect was significantly decreased in the presence of IL-10 (TNF-α + IL-10: 1,440 ± 129 vs. TNF-α: 6,278 ± 1,013 pmol/mg of protein; P < 0.001). Total ceramide levels were significantly lower in the IL-10 group compared with the control group (P < 0.05). No significant differences were found between cells treated with IL-10 and TNF-α + IL-10 (P > 0.05).

**Effect of IL-10 on ICAM-1 expression following TNF-α stimulation**

A significant increase in ICAM-1 gene expression was observed when cells were exposed to TNF-α for 4 h, compared with control cells (Fig. 6; TNF-α: 267.7 ± 70.8 vs. control: 2,170 ± 1,632 fold; P < 0.001).

Interleukin 10 (1 ng/mL) significantly blunted the increase in TNF-α-induced ICAM-1 expression (TNF-α: 267.7 ± 70.82 vs. TNF-α + IL-10: 130.6 ± 30.96; P < 0.05). No differences were observed between the control, TNF-α + IL-10, and IL-10 groups (P > 0.05).

**Effect of IL-10 on TNF-α–induced leukocyte adhesion to the endothelium**

Tumor necrosis factor α induced a significant increase in leukocyte adhesion to the endothelium compared with control group (Fig. 7; TNF-α: 31.2 ± 5 vs. control: 8.16 ± 2 adherent leukocytes/field; P = 0.0005).

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**Fig. 4.** Specific inhibition of p110 α PI3-kinase subunit with A66 (A, n = 4/group) increased significantly DCFH fluorescence during exposure to TNF-α compared with TNF-α + IL-10 (*P < 0.05, **P < 0.01; fluorescence levels are expressed in arbitrary units [AU]). Specific inhibition of p110 γ/δ PI3-kinase subunits with TG100-115 (B, n = 4/group) did not significantly increase DCFH fluorescence compared with TNF-α + IL-10.
Interleukin 10 significantly reduced the TNF-α–induced leukocyte adhesion (at 15 min: TNF-α: 31.2 ± 5 adherent leukocytes/field vs. TNF-α + IL-10: 7.5 ± 4.5; \( P < 0.001 \)). No differences were observed between the control and IL-10 groups (\( P < 0.05 \)).

**DISCUSSION**

In the present study, we demonstrate that IL-10 decreases the level of inflammation induced by TNF-α in endothelial cells by reducing the TNF-α–induced ROS production, ICAM-1 expression, and leukocyte adhesion to the endothelium. The antioxidant effect of IL-10 is mediated through PI3-kinase and is paralleled by a decrease in ceramide synthesis induced by TNF-α.

Thus, we describe, for the first time, the cellular mechanism by which IL-10 inhibits the pro-oxidant and downstream effects of TNF-α in endothelial cells. We show that the antioxidant effect of IL-10 is PI3-kinase dependent and further that it is associated with decreased ceramide concentration. We propose that, in the TNF-α–activated endothelial cell, IL-10 activates PI3-kinase to inhibit the production of ceramide, thus reducing ROS production. These results provide new insight into the mechanisms involved in the control of TNF-α–provoked endothelial inflammation and confirm findings previously reported by Corda et al. (24), showing that TNF-α increases mitochondrial ROS production in endothelial cells by involving ceramide.

Using the PI3-kinase inhibitors LY 294002 and wortmannin, the current study demonstrates that the antioxidant effect of IL-10 was PI3-kinase dependent in endothelial cells. Our findings are consistent with those of Pahan and colleagues (18) for astrocytes, where IL-10 was demonstrated to reduce the increase in ceramide concentration mediated by TNF-α via PI3-kinase, but are in contrast with data for monocytes from Crawley and colleagues (29), who reported that IL-10–associated PI3-kinase was involved in cell proliferation but not in IL-10 anti-inflammatory effects. It is also of note that the different subunits of PI3-kinase appear to have different effects such that selective inhibition of the PI3-kinase γ/δ subunit has a protective effect on ischemia-reperfusion, whereas inhibition of PI-3K α/β significantly impacts on endothelial cell proliferation (27, 29). In our study, specific inhibition of p110α subunit by A66 significantly increased ROS production in HUVECs treated by TNF-α + IL-10. The antioxidant effect of IL-10 appears to involve p110α PI3-kinase subunit. Specific inhibition of p110 γ/δ significantly increased ROS production in HUVECs by itself. Therefore, we could not conclude on the role of p110 γ/δ in IL-10 antioxidant effect.

In the current study, IL-10 was also demonstrated to decrease ceramide production induced by TNF-α. Ceramides are sphingolipids that can be formed during the metabolism of sphingomyelin by sphingomyelinase. They serve as second messengers via ceramide-activated protein phosphatase and kinase, leading to the arrest of cell growth or apoptosis. Tumor necrosis factor α, lipopolysaccharide, and ischemia-reperfusion are all known to induce ceramide production (24, 30), but this is the first report, in endothelial cells, that IL-10 decreases ceramide production through a possible PI3-kinase involvement (18, 31). In our study, IL-10 alone decreased ceramide levels in nonstimulated endothelial cells. As an analog of ceramides has been reported to induce IL-10 production (32, 33), the effect seen in our study may reflect the retro control of IL-10 on ceramide baseline production.

Recent studies demonstrate that IL-10 protected vessel reactivity countering different inflammatory stimulus (10, 12). Zemse and colleagues (9) show that IL-10 abrogates endothelial-dependent relaxation impairment induced by TNF-α in mouse aorta. This appeared to involve eNOS as IL-10 was shown to restore phosphorylated eNOS levels within the vessel. The demonstration in the current study that IL-10 decreases ROS production induced by TNF-α may explain how IL-10 restores phosphorylated eNOS expression because ROS are involved in the uncoupling of eNOS.

In addition to the antioxidant effect of IL-10, the current studies demonstrated that IL-10 decreases the level of inflammation induced by TNF-α in endothelial cells by reducing the TNF-α–induced ICAM-1 expression and leukocyte adhesion to the endothelium.

Interleukin 10 failed to show a protective effect in an animal septic shock model (21) despite promising results in acute inflammatory models (23) (e.g., lipopolysaccharide). This is consistent with the findings of Osuchowski and colleagues’ (34) study, where a murine model of septic shock was reported to demonstrate an increase in IL-10 at the very early phase of septic shock (within 6 h), before the onset of the TNF-α peak, which correlated to an increase in death rate (i.e., the higher the IL-10 levels, the worse the outcome). This was substantiated by Gogos and colleagues (35), who reported that an increase in IL-10 concentration on admission and an increase in IL-10/TNF-α ratio were predictive of a worse outcome in septic shock patients. From this and based on our current findings that the recruitment of leukocytes to the endothelium is suppressed by IL-10, we postulate that this may in part contribute to the deleterious effect observed during the early phase of an innate immune response when leukocyte recruitment is pivotal to the fight against infection.

We would note several limitations to the data we present here. The use of mouse blood may have been a more scientifically rigorous approach but would require a large number of animals. Using human whole blood allowed us to reduce the number of mice in this study. As reported in this study and previously published studies, the use of human blood does not activate immune responses in the short time frame studied.
The temporal relationship between incubation of cells with IL-10 and TNF-α needs to be studied in more detail, using different durations of TNF-α exposure. Future data should test inhibition/activation of the different molecules and enzymes involved in the interaction of TNF-α and IL-10 in a more translational setting.

CONCLUSIONS

We report for the first time that the antioxidant effect of IL-10 decreases endothelial cell inflammation induced by TNF-α. These findings may be relevant, explaining the deleterious effect of IL-10 during septic shock and the positive effect in inflammatory models.

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REFERENCES