Interleukin-10 controls the protective effects of circulating microparticles from patients with septic shock on tissue-engineered vascular media

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Abstract
During sepsis, inflammation can be orchestrated by the interaction between circulating and vascular cells that, under activation, release MPs (microparticles). Previously, we reported that increased circulating MPs in patients with sepsis play a pivotal role in ex vivo vascular function suggesting that they are protective against vascular hyporeactivity. The present study was designed to investigate the effects of MPs from patients with sepsis on the contractile response of TEVM (tissue-engineered vascular media). TEVM that were composed only of a media layer were produced by tissue engineering from human arterial SMCs (smooth muscle cells) isolated from umbilical cords. TEVM was incubated with MPs isolated from whole blood of 16 patients with sepsis. TEVM were incubated for 24 h with MPs and used for the study of vascular contraction, direct measurements of NO and O₂⁻ (superoxide anion) production by EPR and quantification of mRNA cytokine expression. MPs from patients with sepsis increased contraction induced by histamine in TEVM. This effect was not associated with inflammation, neither linked to the activation of NF-κB (nuclear factor κ B) pathway nor to the increase in iNOS (inducible NO synthase) and COX (cyclo-oxygenase)-2 expression. In contrast, mRNA expression of IL (interleukin)-10 was enhanced. Then, we investigated the effect of IL-10 on vascular hyporeactivity induced by LPS (lipopolysaccharide). Although IL-10 treatment did not modify the contractile response in TEVM by itself, this interleukin restored contraction in LPS-treated TEVM. In addition, IL-10 treatment both prevented vascular hyporeactivity induced by LPS injection in mice and improved survival of LPS-injected mice. These findings show an association between the capacity of MPs from patients with sepsis to restore vascular hyporeactivity induced by LPS and their ability to increase IL-10 in the tissue-engineered blood vessel model.

Key words: inflammation, microparticle, nitric oxide, oxidative stress, sepsis, shock

INTRODUCTION
During severe sepsis, the cardiovasculary system adopts a high cardiac output/low peripheral resistance haemodynamic profile whose vascular component includes arterial dilatation [1,2]. The increased cardiac output and peripheral dilatation may improve tissue perfusion and probably contributes to protection against multivisceral injury caused by sepsis. However, a prolonged or...
excessive fall in peripheral resistance may also cause progressive hypotension refractory to catecholamines, and contribute to life-threatening cardiovascular failure [3].

Previously, we reported that circulating levels of MPs (microparticles), membrane vesicles with procoagulant and pro-inflammatory properties (for reviews see [4,5]), are elevated in patients with septic shock compared with subjects without sepsis [6]. Evidence of the physiological relevance of MPs from patients with sepsis (referred to subsequently as septic MPs) in the regulation of vascular contraction has been provided [6]. Surprisingly, septic MPs enhance, they do not reduce, the sensitivity of contraction in response to 5-HT (5-hydroxytryptamine). This effect of MPs is linked neither to increased nitrosative nor oxidative stress, but is associated both with a mechanism sensitive to TXA₂ (thromboxane A₂) receptor antagonism and increased TXA₂ production. Thus septic MPs may be protective in countering the fall in peripheral resistance and progressive hypotension during severe sepsis under the experimental conditions used [6]. These data confirm our previous studies, showing that contractile responses of small omental arteries from patients with sepsis are not significantly changed ex vivo despite the fact that they are removed from patients whose peripheral resistance and BP (blood pressure) are dramatically reduced [7]. A hyper-reactivity to agonists has been shown in these arteries, which compensates for the increased production of NO, in conjunction with vasodilatory products from COX (cyclo-oxygenase) metabolites.

However, the cellular process and the mechanisms involved in the effect of MPs are not completely understood. Moreover, the effect of septic MPs has been assessed ex vivo on mice aorta [6], but not on vessels from human origin [8].

It is well accepted that the media of a blood vessel is responsible for the control of vasomotor tone by contracting and relaxing in response to different hormonal factors released such as histamine and endothelin. However, the study of vascular reactivity of media from native human vessels must take into account the limited availability and intrinsic variability associated with individuals and therapies applied to patients from whom the samples are taken. Interestingly, we have developed, using the self-assembly approach, a human tissue-engineered blood vessel produced in vitro composed of the three tunicae of native blood vessels [9]. More simplified vascular constructs comprising solely one tunica, TEVM (tissue-engineered vascular media) or TEVA (tissue-engineered vascular adventitia) can be produced from human SMCs (smooth muscle cells) or fibroblasts respectively. These tissue-engineered human vascular constructs possess characteristic properties of native blood vessels such as agonist reactivity (bradykinin, endothelin, histamine and UTP) [9] and structure. Thus they allow the study of different pharmacological responses on human vascular constructs. Recently, we demonstrated that apoptotic T-lymphocyte MPs affect NO, O₂⁻ (superoxide anions), NF-κB (nuclear factor κB) and COX-2 in order to maintain the contractile response of human tissue-engineered vascular constructs to histamine [8].

The aim of the present study was to investigate the effects of circulating septic MPs on the regulation of vascular tone in response to histamine using TEVM. We took advantage of the self-assembly method to produce human TEVM from vascular SMCs of human umbilical artery. The cellular mechanism involved was further analysed with respect to nitrosative and oxidative stresses and the release of different cytokines.

**MATERIALS AND METHODS**

**Patients and MP preparation**

The present study was approved by the Ethics Committee of the Société de Réanimation de Langue Française. Septic shock was defined according to standard criteria of the American College of Chest Physicians/Society of Critical Care Medicine/European Society of Intensive Care Medicine/American College of Chest Physicians/Surgical Infection Society [9]. Baseline characteristics of patients with septic shock (n = 16) are shown in Table 1. Patients with pre-existent chronic inflammatory disease, diabetes mellitus, obesity [BMI (body mass index)] > 35] and cancer or leuko-neutropenia were not included. Some patients were under statin treatment and three were taking anticoagulant medication. During hospitalization, all patients had heparin treatment. In addition, the sources of sepsis are shown in Table 1.

Peripheral blood (20 ml) from patients with sepsis was collected during the early phase of septic shock (10 ± 4 h after enrolment in the intensive care unit) from the arterial line and was processed for assay within 2 h. Samples were centrifuged for 10 min at 170 g, and the plasma supernatant was then harvested and centrifuged for 20 min at 1500 g to obtain PFP (platelet-free
flow cytometry, as described previously [6], the remaining PFP was subjected to three series of centrifugations at 21,000 g for 45 min each, in order to eliminate plasma and to pellet MPs for in vitro studies, and the supernatant was replaced with 0.9% (w/v) saline. Finally, MP pellets were resuspended in 200 μl of 0.9% saline and stored at 4°C until subsequent use. Levels of endotoxin were assessed in all MP preparations with the Limulus amebocyte lysate kit QCL-1000 (Lonza) and were found to be below the lower detection limit of the kit (<0.1 endotoxin units/ml). Membrane MP subpopulations were discriminated in PFP according to the expression of membrane-specific antigens, and samples were analysed by flow cytometry using a Cytomics PFP according to the expression of membrane-specific antigens, and samples were analysed by flow cytometry, as described previously [6].

**Tissue culture**

Human arterial SMCs were isolated by the explant method of Ross as described previously [7] from umbilical cords obtained following informed consent of the mother. Briefly, arteries from umbilical cords were slit longitudinally and the endothelium was removed by scrubbing with gauze. Strips of the media were dissected, cut in small sections and placed in a gelatin-coated and pre-wetted Petri dish to allow their attachment to the plastic. Explants were cultured in 3:1 (v/v) DMEM (Dulbecco’s modified Eagle’s medium)/Ham’s F12 (Invitrogen), 20% (v/v) FBS (fetal bovine serum) (HyClone) and antibiotics (100 units/ml penicillin and 25 μg/ml gentamicin). After 2 weeks of culture, SMCs migrated from the explants and proliferated. At 2 weeks later, the cells were trypsinized and plated at a density of 10⁴ viable cells/cm² in tissue culture flasks and maintained at 37°C in a humidified atmosphere (8% CO₂).

**Production of tissue-engineered vascular constructs**

TEVM was produced using the tissue engineering method described previously [10,11]. Briefly, human artery SMCs were cultured in 3:1 (v/v) DMEM/Ham’s F12 supplemented with 30% (v/v) FBS, 20 μg/ml endothelial cell growth supplement (Calbiochem), antibiotics and 50 μg/ml sodium ascorbate (Sigma–Aldrich) to promote extracellular matrix assembly. After 10–15 days of culture, cells formed thick living tissue sheets, comprising cells embedded in the extracellular matrix they secreted. These sheets can be peeled off from the culture flask, using fine forceps, to be rolled. TEVM construct was obtained by wrapping a tissue sheet around a tubular support (diameter of 4.5 mm) resulting in a four-layer-thick media construct containing contractile SMCs. This construct was maturated for 21 days in 3:1 (v/v) DMEM/Ham’s F12 supplemented with 10% (v/v) FBS (FetalClone II; HyClone), antibiotics and 50 μg/ml sodium ascorbate.

**TEVM treatment with MPs and contraction experiments**

After 21 days of maturation, TEVM were cut in two, while remaining on the tubular support, and each half was incubated with either medium [3:1 (v/v) DMEM/Ham’s F12 supplemented with 10% (v/v) FBS and antibiotics] or the same medium plus MPs at the circulating levels of MPs detected in the blood of each patient (range, 1417–48190 MPs/μl of plasma), as described previously [6,12]. It should be noted that, independently of the circulating level, all septic MPs displayed hyper-reactivity in aortic rings [6]. After 24 h of incubation at 37°C in a humidified atmosphere (8% CO₂), each half were cut into 4-mm-long rings and processed for assays as described below.

Rings of TEVM were removed from their respective tubular support used for culture and rinsed in physiological salt solution (Krebs solution; 119 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 1.2 mM MgSO₄, 2.5 mM CaCl₂ and 10 mM glucose). Rings were separately mounted in isolated organ baths containing Krebs solution maintained at 37°C and gassed with a mixture of 95% O₂/5% CO₂ (pH 7.4). They were set up between two L-shaped wires for isometric force measurements (Radjoti; Harvard Apparatus). After being mounted, each ring was equilibrated for 30 min before being passively stretched with a preload of 500 mg. During the next 60 min, each tissue was rinsed three times and tension was readjusted until a stable tension of 500 mg was observed. TEVM were challenged with increasing concentrations of histamine (Sigma–Aldrich) from 10 nM to 100 μM in the absence or presence of either the iNOS (inducible NO synthase) inhibitor compound 1400W (100 μM; Calbiochem) or the COX-2 inhibitor NS398 (1 μM; Calbiochem) alone or in combination.

In another set of experiments, TEVM was incubated in presence or absence of 10 μg/ml LPS (lipopolysaccharide; Sigma–Aldrich). To assess the effect of human recombinant IL (interleukin)-10, the same experimental conditions were conducted in the presence of IL-10 (300 ng/ml; Peprotech) alone or in combination with LPS. After 24 h of incubation at 37°C in a humidified atmosphere (8% CO₂), rings were obtained for measurements of vascular reactivity as described above.

**Mouse model of LPS-induced endotoxic shock and contraction experiments**

LPS (from Escherichia coli 055:B5; Sigma–Aldrich) was administered at a dose of 150 mg/kg of body mass i.p. (intraperitoneal) Control mice received equivalent volume of vehicle (0.9% NaCl solution). In the other two groups, each mouse was administered (i.p.) 1 μg of recombinant IL-10 (Peprotech) alone or 1 h prior to LPS treatment.

Segments of aortic rings (2 mm) with functional endothelium were mounted on a myograph filled with Krebs solution, 4 h after treating the mice with saline, LPS, IL-10 or combination of IL-10 and LPS. The integrity of the endothelium was studied by using acetylcholine (1 μM) in the in aortas precontracted with U46619 (0.1 μM; Sigma–Aldrich). Concentration–response curves were constructed by cumulative application of 5-HT (3 nM–10 μM; Sigma Aldrich) to vessels with functional endothelium.

**NO spin-trapping and EPR studies**

NO content was assayed after formation of EPR-detectable Fe(II)-NO(DETC)₂ [Fe(II)-NO (diethyldithiocarbamate)₂] in rings of TEVM. Samples were placed in 24-well clusters filled with 250 μl of Krebs solution and then treated with 250 μl of colloid
Fe(DETC)₂ (0.5 mM; Sigma–Aldrich) and incubated at 37°C for 1 h. After the incubation, the samples were rapidly frozen and kept in liquid N₂ until EPR measurements. These studies were performed using a table-top x-band spectrometer miniscope (Magnettech). Recording were made at 77 K using a Dewar flask. Instrument settings were 10 mW of microwave power, 1 mT of amplitude modulation, 100 kHz of modulation frequency, 60 s of sweep time and five scans. After EPR measurements, the rings were dried and weighed. The relative [Fe(II)-NO(DETC)₂] concentrations were determined by dividing the third-component amplitude of the three-line EPR signal by the mass of the dried sample [6, 8, 12].

O₂ spin-trapping
Rings of TEVM were allowed to equilibrate in deferoxamine-chelated Kreb’s/Hepes solution (pH 7.4) containing 500 μM CMH (1-hydroxy-3-methoxycarbonyl 2,2,5,5-tetramethylpyrroloildin; Noxygen), 25 μM deferoxamine (Sigma–Aldrich) and 5 μM DETC (Sigma–Aldrich) under constant temperature (37°C) for 60 min. The reaction was stopped by placing samples on ice. They were frozen in liquid N₂ and analysed in a Dewar flask by EPR as described previously [6, 12].

Western blot analysis
Western blots were performed as described previously [6, 12]. Protein extracts were prepared by homogenization of rings of TEVM in a lysis buffer [50 mM Tris/HCl, pH 7.4, 250 mM NaCl, 8 mM MgCl₂, 1 mM PMSF, 5 mM EDTA, 0.5 mM EGTA, 2 mM NaVO₃, 10 μg/ml each of aprotinin, leupeptin and pepstatin and 1% Triton X-100]. Samples were centrifuged at 14 000 g for 10 min and the supernatants were transferred to microcentrifuge tubes and maintained in an ice-bath for immediate use. Protein concentration was determined using the Bradford method. Approximately 70 μg of total protein from supernatant fractions were separated on SDS/PAGE (8% gel) and transferred on to nitrocellulose membranes to be probed with antibodies against iNOS, nNOS (neuronal NO synthase), COX-1, COX-2 (all from BD Transduction Laboratories), NF-κB, IκBα, pIκBα, IRAK1 (IL-1-receptor-associated kinase 1), IKKα, IKKβ, IL-10, IL-1β, IL-1α, IL-31, IL-6, IL-8, IFNβ (interferon β), IRAK1 (IL-1-receptor-associated kinase 1), IL-1β, MCP (monocyte chemoattractant protein), MIP (macrophage inflammatory protein) 1α, MIP3α, MIP3β, SDF1 (stromal-cell-derived factor 1) and TNFα (tumour necrosis factor α) related to inflammation by qRT–PCR (quantitative reverse transcriptase–PCR). Control TEVM expressed NF-κB, iNOS, COX-2 and β-actin (Sigma–Aldrich).

qRT–PCR (quantitative reverse transcriptase–PCR)
Rings of TEVM were frozen in liquid N₂ and used to investigate the mRNA expression of 24 transcripts [CCR (CC chemokine receptor) 3, CCR5, CCR6, CXCR (CX chemokine receptor) 3, CXCR4, gp130, ICAM-1 (intercellular adhesion molecule-1), IKK (IκB kinase) α, IKKβ, IL-10, IL-17, IL-1α, IL-31, IL-6, IL-8, IFNβ (interferon β), IRAK1 (IL-1-receptor-associated kinase 1), IL-1β, MCP (monocyte chemoattractant protein), MIP (macrophage inflammatory protein) 1α, MIP3α, MPB3β, SDF1 (stromal-cell-derived factor 1) and TNFα (tumour necrosis factor α)] related to inflammation by qRT–PCR. qRT–PCR analyses were carried out by Service Commun de Cytométrie et d’Analyses Nucléotidiques from Angers University, using a Chromo 4™ (Bio-Rad) and SYBR Green detection. Primers were designed using Primer3 software (http://frodo.wi.mit.edu/primer3) [13]. Quantifications were performed using the ΔCt method and the relative gene expression levels were normalized using the geometric mean of three housekeeping genes, as described previously [14].

Data analysis
Results are means ± S.E.M., and n represents the number of rings or samples. Statistical analyses were performed as appropriate by unpaired Student’s t test or two-way ANOVA for repeated measures with a subsequent Bonferroni post-hoc test. P < 0.05 was considered to be statistically significant.

RESULTS

MPs increased the contraction in response to histamine in TEVM
Histamine induced a concentration-dependent contraction in TEVM treated or not with MPs. Interestingly the contraction induced by histamine was significantly increased in TEVM treated with septic MPs (Figure 1A).

TEVM treated or not with MPs exhibited an EPR feature of signals derived from NO-Fe(DETC)₂ following incubation with Fe(DETC)₂ (results not shown). As illustrated in Figure 1(B), MPs significantly decreased NO content in TEVM compared with control. Measurement of O₂⁻ production showed that MPs significantly reduced its production in TEVM compared with control (Figure 1C). These results suggest that septic MPs enhanced histamine-induced contraction in TEVM and that this effect is associated with a decrease in both NO and O₂⁻ productions.

Next, we tested the hypothesis of whether the ability of septic MPs to increase histamine-induced contraction was mediated by the down-regulation of inflammatory pathways involving NF-κB, pIκBα, iNOS or COX-2. Control TEVM expressed NF-κB, pIκBα, iNOS and COX-2 (Figures 2A–2C and 2E). Treatment of TEVM with septic MPs did not modify the expression of NF-κB, iNOS, COX-2 or pIκBα. Besides, septic MPs did not affect the expression of nNOS (Figure 2D), but they significantly increased the expression of COX-1 (Figure 2F). Taken together, the ability of septic MPs in potentiating the response to histamine was not linked to vascular inflammation associated with increased NF-κB, iκBα phosphorylation, iNOS or COX-2 expression. In contrast, the expression of COX-1 was increased in MP-treated TEVM.

MPs enhance IL-10 mRNA expression in TEVM
The possible regulation of tissue expression and release of inflammatory mediators by TEVM treated with septic MPs was evaluated further. Most of the inflammatory mediators were not affected by septic MPs including IL-1α, IL-1β, IL-6, IKKβ and ICAM-1 as assessed by qRT–PCR (results not shown). Surprisingly among the 24 inflammatory-related transcripts tested (see the Materials and methods section), septic MPs drastically increased (∼4000-fold) the expression of IL-10 mRNA in TEVM when compared with the control untreated TEVM (Figure 3).

Therefore we tested the effect of IL-10 on the regulation of contraction in TEVM. First, we reproduced an in vitro experimental model of endotoxic shock in TEVM using LPS treatment. As expected, LPS treatment significantly reduced the contraction
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**Figure 1** Treatment with MPs increased the reactivity of TEVM to histamine

(A) Concentration-response curves of TEVM incubated for 24 h in the absence (CTL) or presence of MPs. Production of NO (B) and O$_2^-$ (C) in TEVM in the absence (CTL) or in the presence of MPs. *P < 0.05 and ***P < 0.001. Values are expressed as means ± S.E.M. for 5–8 vessels.

**Figure 2** Treatment with MPs increased COX-1 expression

Western blot analysis of protein expression in TEVM after MP treatments. Whole protein extract was separated by SDS/PAGE (8% gels), transferred to nitrocellulose and probed using specific antibodies raised against NF-$\kappa$B p65 (A), pIkB (B), iNOS (C), nNOS (D), COX-2 (E) and COX-1 (F). Band intensities were normalized on β-actin and presented in arbitrary units (A.U.). *P < 0.05. Values are expressed as means ± S.E.M. for 6–10 vessels.

Induced by histamine in TEVM when compared with the control, highlighting LPS-induced vascular hyporeactivity in this preparation (Figure 4A). In control TEVM, IL-10 treatment alone did not significantly modify the response to histamine (Figure 4B). Interestingly, IL-10 significantly enhanced histamine-induced contraction in LPS-treated TEVM towards that of control vessels (Figure 4C).

Taken together, these findings show an association between the ability of septic MPs to enhance both the contraction in response to histamine and the production of IL-10 in TEVM, and the capacity of this cytokine to correct vascular hyporeactivity induced by LPS. Besides, LPS significantly increased NO production in TEVM that was prevented upon IL-10 treatment (Figure 5A). LPS treatment did not induce a statistically significant increase of O$_2^-$ in TEVM compared with control (Figure 5B). Under these experimental conditions, O$_2^-$ levels decreased in TEVM treated with both LPS and IL-10 compared with control. IL-10 had a significant effect on NO but had no effect on O$_2^-$ in LPS-treated TEVM.

**IL-10 treatment protects mice from LPS-induced shock**

We assessed the effect of LPS injection on survival of mice either alone or in combination with IL-10 (1 μg/mouse). Mice were...
subjected to i.p. injection of LPS (150 mg/kg of body mass) and survival was assessed during the following 36 h. All LPS-treated mice died 30 h after of LPS injection; however, 50% of IL-10 plus LPS-injected mice survived at the same time (Figure 6A). Interestingly, IL-10 alone had no effect on survival.

IL-10 treatment prevented vascular hyporeactivity induced by LPS injection in mice

Finally, it was important to test whether IL-10 prevents LPS-induced vascular hyporeactivity. Mice were systemically injected with saline, IL-10, LPS or the combination of IL-10 and LPS, then aortic rings from treated animals were mounted on a myograph and mechanical activity in response to the vasoconstrictor agonist 5-HT was recorded. Vascular reactivity in response to 5-HT was decreased in vessels from LPS-treated mice compared with those taken from mice treated with saline or with IL-10 alone (Figures 6B and 6C). Interestingly, IL-10 completely prevented the vascular hyporeactivity induced by LPS (Figure 6D).

DISCUSSION

The main results of the present study were that septic MPs were able to increase contraction induced by histamine and reduce NO and $O_2^-$ production in TEVM. These effects were not associated with the activation of the NF-κB pathway, or changes in nNOS, iNOS or COX-2 expression. Interestingly, septic MPs enhanced COX-1 expression.

One of the most important findings was the ability of septic MPs to dramatically increase the expression of IL-10 mRNA considering that recombinant IL-10 itself prevented the vascular hyporeactivity induced by LPS in TEVM. These effects were linked to the capacity of IL-10 to reduce nitrosative stress in this model. Taken together, these results strengthened both the relevance of septic MPs in the regulation of vascular contraction in human TEVM and the fact that these MPs enhanced the contraction in response to histamine. Furthermore, they highlight an association between the ability of septic MPs to increase IL-10 expression and vascular reactivity.
from TEVM and the capacity of IL-10 to normalize the vascular hyporeactivity induced by LPS in TEVM. Pathophysiological relevance of these data is provided by the ability of IL-10 both to prevent vascular hyporeactivity induced by LPS injection into mice and the improvement of survival upon LPS treatment.

In the present study, our expertise in the production of TEVM, exclusively composed of human cells embedded in the extracellular matrix they secreted, was used to perform pharmacological studies. We have already demonstrated that TEVM display fundamental histological, functional and many pharmacological characteristics of the human artery from which they were originally isolated [15,16]. Moreover, the use of TEVA, a media or the combination of both lead to better understandings of the effects of MPs from apoptotic T-lymphocytes in the modulation of vascular tone [8]. In the present study, only TEVM was used as it is accepted that the media of a blood vessel is responsible for the control of vasomotor tone by contracting and relaxing in response to the release of different hormonal factors such as histamine.

We previously found an increase in the number of circulating septic MPs and also in the capacity of these MPs to enhance the sensitivity of contraction in response to 5-HT without affecting endothelium-dependent vasodilatation on mouse aorta [6], indicating that MPs are not involved in the reduction of the vasomotor tone. Thus septic MPs might participate as a protective mechanism counteracting vascular hyporeactivity in this disease. Results obtained in the present study further support this conclusion. Actually, MPs isolated from patients exhibiting the similar baseline characteristics of patients with sepsis used in our previous study [6] also increased the contraction induced by histamine in human TEVM. These findings not only confirm the validity of TEVM as a model to study the effect of septic MPs on human media, but they also strengthened the fact that septic MPs are probably the biological vector that regulate contraction in an identical manner to that observed in arteries taken from patients with septic shock.

In the present study, we have shown that septic MPs reduced oxidative stress in TEVM. In our previous study performed ex vivo on mouse aorta, septic MPs did not affect O$_2^-$ production although they significantly reduced the expression of the NOX-4 subunit of NADPH oxidase [6]. Both studies are in accordance with the fact that septic MPs might not be responsible for the increase in oxidative stress in the vessel wall during sepsis. Possible explanations for the different results obtained in TEVM and mouse aorta could be due to species specific (human against mice), and/or specific to the origin of vessels (umbilical arteries against aorta); however, we cannot distinguish among these possibilities. Nevertheless, only the media (TEVM) was used compared with aorta, which is composed of the three tunics: adventitia, media and intima. This is of importance inasmuch as we previously highlighted an interaction between engineered tissues composed of TEVA and TEVM. Indeed, the ability of T-lymphocyte MPs to regulate O$_2^-$ production is different depending on the constructs used, TEVA, TEVM or both [8].

Sepsis, like other inflammatory conditions, results in the synthesis of inflammatory mediators during the acute phase, including NO from iNOS and COX-2 metabolites under the activation of NF-κB. Such an effect might lead to intense vasodilatation, and subsequent potential hypotension such as that observed in severe sepsis. Previously, Mortaza et al. [17] have shown that injection of MPs from rats with sepsis induces iNOS and oxidative stress in aorta and heart of rats receiving MPs. However, we have shown that injection of septic MP into mice does not affect either aortic NF-κB, iNOS and COX-2 expression or NO production [6]. Consistent with these results, treatment of TEVM with septic MPs did not modify NF-κB, IκB phosphorylation, iNOS or COX-2 expression. Under these experimental conditions, septic MPs reduced NO content in TEVM. Moreover, pharmacological blockade of either iNOS with compound W1400 or COX-2 with NS-398, alone or in combination, did not modify the capacity of septic MPs to enhance the contraction in response to histamine in TEVM (results not shown). Taken together, these findings demonstrate once again that septic MPs are not the biological vectors that trigger vascular inflammation linked to nitrosative stress and increased COX-2 metabolites during severe sepsis.

However, one has to take into account that septic MPs increased COX-1 expression in TEVM. In our previous study, septic MPs increased sensitivity to vasoconstrictor as a consequence of increased TXA$_2$ production from COX-1 [6], although no change in COX-1 expression was observed. Thus it might be possible that increased TXA$_2$ also occurs in TEVM treated with septic MPs. Further studies are needed to determine whether the increase in COX-1 expression is associated with enhanced release of TXA$_2$ in TEVM.

Finally, we analysed the expression of different genes in septic MP-treated TEVM by qRT-PCR. Surprisingly, among
the 37 transcripts tested, septic MPs increased IL-10 mRNA expression by 4000-fold in TEVM compared with untreated TEVM control. Indeed, IL-10 is known to be an anti-inflammatory cytokine. Previous studies suggest that IL-10 limits the increase in O$_2^-$ and protects against endothelial dysfunction following LPS treatment in vivo or during diabetes [18–20]. In several animal models of sepsis, neutralization of IL-10 results in exaggerated pro-inflammatory cytokine expression and death, while administration of recombinant IL-10 confers significant therapeutic protection [21–23]. In the present study, as expected, LPS induced hyporeactivity in TEVM. Interestingly, IL-10 treatment of TEVM completely restored vascular hyporeactivity induced by LPS and improved survival in LPS-induced mortality in mice. The mechanisms associated with the prevention of vascular hyporeactivity induced by LPS in the presence of IL-10 were studied further in terms of NO production. Indeed, it is accepted that LPS induces vascular hyporeactivity by increasing iNOS expression and NO production [24]. In the present study, increased NO production in TEVM by LPS was completely prevented by IL-10 treatment. Furthermore, IL-10 abrogated oxidative stress induced by LPS. Taken together, these findings support the view that septic MPs are able to restore normal reactivity following administration of LPS in vivo and provide evidence that IL-10 might be the mediator implicated in the protective effects of septic MPs on vasomotor function during sepsis.

Results from our recent study and the present data strengthen the hypothesis that septic MPs might be the vector able to counteract the increased production of NO and vasodilator COX metabolites observed in vessels from patients with septic shock through both their anti-inflammatory effects and increase of vasoconstrictor metabolites. However, the link between the ability of septic MPs to increase IL-10 production (the present study) and TXA$_2$ release (our former study) [6] needs to be validated. Nevertheless, the results from both TEVM and mice aorta reinforce the fact that septic MPs may rather be protective against vascular hyporeactivity in order to maintain a tonic pressor response in the early phase of septic shock. Finally, they also confirm the usefulness of the TEVM model for a better understanding of the effect of septic MPs on human media in order to unravel their mechanism of action.

Limitations of the study

We have used TEVM that are exclusively composed of human SMCs embedded in the extracellular matrix they secreted. Although these engineered vessels have been largely used to perform pharmacological studies [10,11,15,16], they are not representative of the whole vessel. It should to be noted that both LPS-treated TEVM and aortic rings from mice treated with LPS display the same hyporeactivity and, in both experimental conditions, IL-10 was able to restore contraction. Thus it is plausible to postulate that IL-10 acts on SMCs rather than other cell types of the vascular wall vessel. Nevertheless, one can advance the hypothesis that IL-10 release might participate in the vascular effects of septic MPs.

CLINICAL PERSPECTIVES

- Septic MPs may be protective in counteracting the fall in peripheral resistance and progressive hypotension during severe sepsis under the experimental conditions used.
- These results may also explain the fact that increased levels of MPs may predict a more favourable outcome in severe sepsis in terms of mortality rate and organ dysfunction.
- More interestingly, these findings bring the experimental basis in support of the observation that administration of recombinant IL-10 confers significant therapeutic protection to limit the increase in oxidative stress, inflammation and vascular dysfunction in patients with sepsis.

AUTHOR CONTRIBUTION

Hadj Mostefai, Jean-Michel Bourget, Maria Martinez and Daniela Leonetti performed the experiments and analysed the data. Ferhat Meziani, Alain Mercat and Pierre Asfar collected the clinical data. Maria Martinez, Pierre Asfar, Lucie Germain and Ramaroson Andriantsitohaina wrote the paper. Lucie Germain and Ramaroson Andriantsitohaina designed the study.

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