Interleukin-19 (IL-19) Induces Heme Oxygenase-1 (HO-1) Expression and Decreases Reactive Oxygen Species in Human Vascular Smooth Muscle Cells*

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Heme oxygenase-1 (HO-1) has potent anti-inflammatory activity and recognized vascular protective effects. We have recently described the expression and vascular protective effects of an anti-inflammatory interleukin (IL-19), in vascular smooth muscle cells (VSMC) and injured arteries. The objective of this study was to link the anti-inflammatory effects of IL-19 with HO-1 expression in resident vascular cells. IL-19 induced HO-1 mRNA and protein in cultured human VSMC, as assayed by quantitative RT-PCR, immunoblot, and ELISA. IL-19 does not induce HO-1 mRNA or protein in human endothelial cells. IL-19 activates STAT3 in VSMC, and IL-19-induced HO-1 expression is significantly reduced by transfection of VSMC with STAT3 siRNA or mutation of the consensus STAT binding site in the HO-1 promoter. IL-19 treatment can significantly reduce ROS-induced apoptosis, as assayed by Annexin V flow cytometry. IL-19 significantly reduced ROS concentrations in cultured VSMC. The IL-19-induced reduction in ROS concentration is attenuated when HO-1 is reduced by siRNA, indicating that the IL-19-driven decrease in ROS is mediated by HO-1 expression. IL-19 reduces vascular ROS in vivo in mice treated with TNFα. This points to IL-19 as a potential therapeutic for vascular inflammatory diseases and a link for two previously unassociated protective processes: Th2 cytokine-induced anti-inflammation and ROS reduction.

Many vascular diseases are inflammatory in nature, and the deleterious effects of pro-inflammatory cytokines on resident vascular cells (vascular smooth muscle cells, VSMC) and endothelial cells, EC) pathophysiology are well characterized (1, 2). It is recognized that VSMC are capable of synthesizing many pro-inflammatory immune modulators (3, 4). The potential for autocrine anti-inflammatory, or protective effects of Th2 interleukins on VSMC pathophysiology have been particularly ignored, as almost all studies addressing the role of Th2 interleukins in vascular disease have focused on immune cells. Consequently, a gap in our knowledge exists concerning potential protective mechanisms of Th2 interleukins on vascular cells. Heme oxygenase-1 (HO-1) is involved in playing a major role in heme breakdown but also has recognized potent anti-inflammatory and anti-apoptotic effects (5, 6). HO-1 protects against vascular inflammation by multiple mechanisms, including: decreasing monocyte arterial transmigration induced by oxidized LDL, decreasing VSMC proliferation, and acting as a potent anti-oxidant (5, 6). VSMC proliferation and increase in ROS are essential steps for development of restenosis, and HO-1 attenuates both processes (5–7). Accumulation of monocyte/macrophage in the vascular wall and atherosclerotic lesion generates ROS, enhancing oxidation of LDL to oxidized LDL. HO-1 co-localizes with oxidized lipids in early stages of human atherosclerotic lesions, and foam cell and SMC in advanced lesions (6, 8). HO-1 can be transcriptionally induced as an anti-inflammatory protective protein by many types of vascular stressors, such as inflammation, oxidized LDL, and hypoxia (9, 10). Because HO-1 expression is induced by oxidized LDL and other oxidative stressors, its expression is suggested to be an autocrine-protective mechanism (6, 11, 12). Despite these data, nothing has been reported on induction of HO-1 in resident vascular cells (EC and VSMC) by any anti-inflammatory cytokine or Th2 interleukin.

We previously reported that interleukin-19 (IL-19) is expressed in injured, but not naïve arteries, and in stimulated, but not quiescent VSMC (13). This was novel and unexpected because IL-19 expression was previously thought to be restricted to immune cells (14). IL-19 is considered to be an anti-inflammatory interleukin because in T-lymphocytes it...
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promotes the Th2 (regulatory), rather than the Th1 (T helper) response (15, 16). It is known that IL-19 can down-regulate Th1-like adaptive immune responses in T-cells, but the molecular mechanisms of these effects, in any cell type is yet uncharacterized. We reported that IL-19 had protective effects on cultured human VSMC, and IL-19 adenoviral gene transfer significantly reduced neointimal hyperplasia and proliferation of intimal VSMC in balloon angioplasty-injured rat carotid arteries (13, 17). We have recently reported that IL-19 decreases VSMC migration and activation of cytoskeletal remodeling proteins (18). Interestingly, IL-19 has converse effects on EC, where it is mitogenic, chemotactic, and angiogenic (19). In VSMC, IL-19 treatment reduces the mRNA stability and protein abundance of pro-inflammatory transcripts (17). These are the only reports describing IL-19 expression and protective function in vascular cells and vascular biology.

Induction of HO-1 in EC and VSMC by any anti-inflammatory cytokine or Th2 interleukin has not been reported. It would seem reasonable to speculate that anti-inflammatory interleukins could induce this important protein, but a molecular link between two important protective mechanisms, anti-inflammation, and reduction of ROS, remains uncharacterized. In this report we test the hypothesis that the anti-inflammatory interleukin IL-19 can induce HO-1 mRNA and protein expression, and that through HO-1 expression, IL-19 can have multiple protective effects in cultured VSMC and vascular cells in vivo.

MATERIALS AND METHODS

Cells and Culture—Primary human coronary artery EC and VSMC were obtained as cryopreserved secondary culture from Cascade Corporation (Portland, OR) and subcultured in growth medium as described previously 17,19). Cells from passage 3–5 were used in the described studies. Recombinant IL-19 (100 ng/ml for all studies), and PDGF-AB (40 ng/ml) was prepared from R&D, Inc.

Western Blotting and ELISA—Briefly, cell extracts were prepared as described and lysates frozen until use (17). Membranes were incubated with a 1:3000–5000 dilution of primary antibody, and a 1:5000 dilution of secondary antibody. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and STAT3 antibody were from Neo Markers, Inc, HO-1 from Santa Cruz Biotechnology, Nrf2 antibody from Epitomics, Inc. Reactive proteins were visualized using enhanced chemiluminescence (Amersham Biosciences) according to the manufacturer’s instructions. Relative intensity of bands were normalized to total protein and quantitated by scanning image analysis using the Image J densitometry program. ELISA for HO-1 quantitation was purchased from Enzo Life Sciences, Inc, and performed according to the manufacturer’s instructions.

RNA Extraction and Quantitative RT-PCR—RNA was isolated and reverse transcribed into cDNA as we have described, and target genes amplified using an Eppendorf Realplex4 Mastercycler (17). Multiple mRNAs (Ct values) were quantitated simultaneously by the software. The following primer pairs were used: GAPDH Forward: 5’-ATC TTC TTT TGC GTC GCC AG-3’, Reverse: 5’-ATA CGA CCA AAT CCG TTG ACT C-3’, HO-1 Forward: 5’-ATG ACA CCA AGG ACC AGA GC-3’, Reverse: 5’-GTG TAA GGA CCC ATC GGA GA-3’. Transfection and siRNA Knockdown—Gene silencing was performed using ON-TARGET plus SMARTpool STAT3 siRNA (20 nM) and HO-1 siRNA (10 nM) purchased from Dharmacon, Inc. SMARTpool siRNA contains a mixture of four siRNAs which target different regions of the target mRNA. Transfection of VSMC was performed using the Human AoSMC NucleofectorTM Kit (Amaza, Inc) following the manufacturer's instructions. siRNA and transfection was as described (17). Two days after transfection, VSMC were challenged with IL-19 and used for Western blotting or ROS determination.

Luciferase Reporter Constructs—The following vector constructs containing the 4 kb region of the human HO-1 promoter phHOLuc (−4000) fused to a firefly luciferase vector, pGL3-basic (Promega, Madison, WI) (20) and the potential signal transducer and activator of transcription (STAT) 3 site located at −2361 to −2369-deleted construct were used for luciferase reporter assay (HO-1ASTAT). The STAT3 site deleted luciferase reporter vector (phHOLuc (−1401) was constructed by restriction digestion of the phHOLuc (−4000) with EcoRI (−1401) and Nhel (in MCS region of the vector) followed by re-ligation and transformation of JM109. Plasmid DNAs were prepared using PureYield plasmid maxiprep system (Promega). Transfection of VSMC was performed using the Human AoSMC NucleofectorTM Kit (Amaza, Inc) following the manufacturer’s instructions as we have described (17). Cells were lysed in 1× cell lysis buffer (Promega) and extracts were prepared for luciferase reporter assay (21). Protein concentration in lysates was determined using BCA reagent (Pierce). Luciferase activity was assessed using luciferase assay system (Promega) and relative light unit was measured using an FB12 Luminometer (Zylux). Data are representative for at least three independent experiments and are expressed as RLU/μg of protein.

Flow Measurement of ROS—2’7’-Dichlorofluorescin diacetate (DCFH-DA) is permeable to the cellular membrane and once inside the cell is rapidly hydrolyzed by cellular esterases to non-fluorescent DCFH. Oxidation of DCFH by H2O2 or other ROS produces the fluorescent indicator DCF(22). For cultured VSMC, ROS production was measured by flow cytometry using 2’7’-Dichlorofluorescin diacetate (DCFH-DA, Sigma). HVSMSM were grown to 70% confluence in 10% serum-enriched media 231, serum starved in 0.1% serum-containing media for 72 h and stimulated with IL-19 (100 ng/ml) for 4, 8, and 16 h. Then attached cells were incubated in the dark with DCFH-DA (10 μM) for 10 min on 37 °C. After staining PDGF was added for 15 min and incubated on 37 °C. After the incubation cells were washed 2× with PBS, trypsinized (250 μl), and subjected to flow cytometry analysis (FACS Calibur, Becton Dickinson). The mean of DCF fluorescence intensity was obtained from 10,000 events. Cells were excited at 488 nm, and DCF fluorescence was read on FL1 (530 ± 15) in log scale (22, 12).

For in vivo measurement of ROS, The oxidative fluorescent dye dihydroethidium (24, 25) was used to evaluate in situ production of superoxide from aortic tissue. WT mice were intra-venously injected with a bolus of saline or TNFα (2 μg/25 g).
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A. IL-19 (h) HO-1

B. IL-19 (h) HO-1 protein expression

C. IL-19 (h) HO-1 mRNA abundance

D. HO-1 concentration

Following 1 h of exposure, aortic tissue was removed, embedded in OCT and frozen at ~80 °C. Aortas were serial cryosectioned (30 μm), placed on microscope slides, and then equilibrated for 30 min at 37 °C in Krebs-HEPES buffer. Fresh buffer containing DHE (2 μmol/l) was topically applied to each tissue section and incubated for 30 min in a light-protected, humidified chamber at 37 °C. Oxidized DHE was detected by fluorescence microscopy (Nikon TE2000; excitation 488 nm, emission 610 nm).

Apoptosis Assay—ROS-mediated apoptosis was induced by treating VSMC for 1 h in HBSS containing 50 mM H2O2 in 100 mM/l ferrous sulfate as described (26). The reaction was stopped by removal of the HBSS containing H2O2/ferrous sulfate. Some samples were treated with IL-19 4 h prior to addition of the H2O2/ferrous sulfate. For longer term apoptosis, VSMCs were grown to 60% confluence and then incubated in 0.01% serum for 120 h days to induce apoptosis (26) and some treated with 100 ng/ml IL-19 every 24 h. For the estimation of apoptotic cells, Alexa fluorescein isothiocyanate (FITC)-conjugated Annexin V (BD Biosciences) was determined using flow cytometric analysis together with propidium iodide (PI) dead cell counterstain according to the manufacturer’s recommendations as described (19).

Statistical Analysis—Results are expressed as mean ± S.E. Differences between groups were evaluated with the use of ANOVA, with the Newman-Keuls method applied to evaluate differences between individual mean values and by paired Student’s t tests where appropriate, respectively. Differences were considered significant at a level of p < 0.05.

RESULTS

IL-19 Can Induce HO-1 Protein and mRNA Expression in Primary Human VSMC—We tested the hypothesis that IL-19 could induce expression of HO-1. Primary human VSMC were serum starved in 0.1% FCS for 72 h, then stimulated with 100 ng/ml IL-19. HO-1 protein expression was assayed by Western blot, and Fig. 1A shows that IL-19 can induce a rapid and transient induction of HO-1, with protein levels peaking 4 h after stimulation. HO-1 mRNA abundance was assayed by quantitative RT-PCR, and Fig. 1C shows similar expression kinetics. Interestingly, IL-19 was not able to induce HO-1 mRNA or protein expression in human coronary artery EC. HO-1 protein concentrations in IL-19-stimulated VSMC were quantitated by commercially available ELISA. HO-1 concentrations rapidly increase from 9.0 pg/mg protein in unstimulated VSMC to 46 pg/mg protein in IL-19-stimulated VSMC. HO-1 concentrations in IL-19-stimulated EC ranged from 2.8 pg/mg protein in unstimulated EC, to 7.5 pg/g protein in IL-19-treated EC (Fig. 1D). We focused on VSMC for the remainder of this study. Together, these indicate that IL-19 can induce expression of HO-1 mRNA and protein in human VSMC.

IL-19 Induces HO-1 Expression in a STAT3-dependent Mechanism—Nrf2 is a key transcription factor, which regulates HO-1 transcriptions (27). IL-19 did not induce Nrf2 expression, phosphorylation, or nuclear translocation (data not shown). However, one of the earliest biochemical events in IL-19 stimulation of VSMC is activation of STAT3 (13). To determine the role of STAT3 activation in IL-19-driven HO-1 expression, two experiments were performed. First, STAT3 expression was reduced by transfection of VSMC with STAT3 siRNA. VSMC were then serum starved in 0.1% FCS for 72 h, then stimulated with 100 ng/ml IL-19 for various times. HO-1 protein expression was assayed by Western blot, and Fig. 2A shows that IL-19-driven HO-1 expression is at least in part STAT3 dependent. In the second experiment, a luciferase reporter gene driven by the full-length HO-1 promoter (20) was transfected into human VSMC, which were then stimulated with IL-19 for 30 min. Fig. 2B shows that IL-19 can activate the HO-1 promoter with a 5-fold induction (27.5 ± 1.9 versus 131.5 ± 17.9 units for unstimulated versus IL-19 stimulated, p < 0.01). We then mutated the promoter to eliminate the STAT3 binding site
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(HO-1 ΔSTAT) (21), transfected VCMC with this construct, and stimulated with IL-19. Fig. 2B shows that IL-19-stimulated activation of the HO-1 promoter is significantly reduced (131.5 ± 2.5 versus 46.4 ± 2.5 units p < 0.01) when the STAT3 site is deleted. However, IL-19 still significantly activated the HO-1 promoter even when the STAT3 site is deleted (26.8 ± 4.6 versus 46.4 ± 25 for unstimulated versus IL-19 stimulated HO-1 ΔSTAT, p < 0.01). Together, this suggests that in VSMC, IL-19-induced HO-1 expression is partially mediated by STAT3 activation.

IL-19 Reduces Intracellular Reactive Oxygen Species Concentration—An increase in ROS is an essential step for development of atherosclerosis and other vascular pathologies, and HO-1 has been demonstrated to attenuate ROS accumulation (5–7). We tested if IL-19 could decrease ROS in VSMC. VSMC were serum starved in 0.1% FCS for 72 h and pretreated with IL-19 for 4, 8, and 16, and 24 h, then stimulated with 10 ng/ml PDGF for 10 min to generate intracellular ROS (12). ROS concentration was measured by flow cytometry using DCFH-DA (22). IL-19 can significantly decrease ROS concentration in a rapid and transient manner, by 52 ± 2.8% at 4, and 22.4 ± 1.2% at 8 h stimulation (p < 0.01 and 0.05). B, induction of HO-1 protein expression is maintained with chronic IL-19 stimulation. IL-19 was added to VSMC four times (every 6 h for 24 h) and IL-19 protein expression determined by Western blot. C, IL-19-induced decrease in intracellular ROS is sustained when VSMC are treated with IL-19 four times (every 6 h for 24 h). ROS concentration was measured by flow cytometry using DCFH-DA (p < 0.01).
IL-19-driven HO-1 expression, we reduced HO-1 expression by transfection of VSMC with HO-1 siRNA. VSMC were then stimulated with IL-19 for various times, then stimulated with PDGF to generate intracellular ROS (12). Western blot verifies that HO-1 protein expression was effectively reduced with transfection with specific siRNA (Fig. 4A). We then determined if ROS concentrations were reduced in these HO-1 siRNA-transfected cells even when treated with IL-19. Fig. 4B shows ROS is transiently but significantly decreased 35.1 ± 1.9% by 4 h pretreatment with IL-19 in VSMC in which HO-1 is reduced by siRNA (304.1 ± 7.8 versus 466.7 ± 8.6 for siRNA and scrambled siRNA control, p < 0.001). ROS reduction at 24 h is significant, but reduced compared with 4 h, which does correlate with the observed transient HO-1 expression (Fig. 1). This indicates that in VSMC, the IL-19-driven reduction in ROS is mediated at least in part by HO-1 expression.

**IL-19 Protects Cultured VSMC from Apoptosis**—Oxidative stress induced by ROS can cause apoptotic death in VSMC (26). One effect of HO-1 on VSMC is protection from apoptosis (5, 6). We tested the hypothesis that IL-19 could also protect VSMC from oxidative stress-induced apoptosis. For this experiment, primary human VSMC were pretreated 4 h or not with IL-19, then treated with H2O2 in ferrous sulfate to induce oxidative stress and apoptosis (26). Apoptosis was assayed by Annexin V flow cytometry. Fig. 5A shows that IL-19 could significantly reduce apoptosis by 44% (35.9 ± 1.1 versus 19.9 ± 3.0% for control and IL-19-treated, respectively, p < 0.001). This suggests that in serum-starved VSMC, IL-19 has anti-apoptotic effects.

Cell viability and apoptosis over long periods of time are important cellular outcomes in progression of vascular wall remodeling (1, 2). For this experiment, primary human VSMC were serum starved in 0.01% fetal calf serum for 120 h to induce apoptosis, in the presence and absence of IL-19 (26). Apoptosis was assayed by Annexin V flow cytometry. Fig. 5B shows that IL-19 could significantly reduce apoptosis by 25% (39.9 ± 0.97 versus 30.2 ± 1.2% for control and IL-19-treated, respectively, p < 0.001). This suggests that in serum-starved VSMC, IL-19 has anti-apoptotic effects.

**IL-19 Reduces Arterial ROS in Vivo**—TNFα can induce robust and rapid generation of ROS in vascular cells (25). We tested the ability of IL-19 to reduce ROS in vascular tissue in vivo. Wild-type C57B6 mice were pretreated for 4 h with either PBS or 10 ng/g of IL-19, then injected intraperitoneally with 2 μg/25g TNFα for 1 h to induce ROS. Coronary artery were removed, sectioned, and ROS abundance in frozen sections was assayed by DHA (26). DHA is directly oxidized by O2 into EtBr, which intercalates with DNA, and when excited at 488 nm, will emit red. Fig. 6 shows representative images from three differ-
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FIGURE 6. IL-19 reduces arterial ROS in vivo. In situ detection of superoxide in mouse carotid artery. Wild-type C57B6 mice were pretreated for 4 h with either PBS or 10 ng/g of IL-19, then injected intraperitoneally with 2 ng/25 g TNFα for 1 h to induce ROS. Coronary artery were removed and ROS abundance in frozen sections was assayed by DHA (24, 25). At identical laser and microscope capture settings, fluorescence in PBS and TNFα only aorta is markedly increased compared with IL-19-pretreated mice. Fluorescent photomicrographs of sections are representative of multiple sections from three different mice.

ent mice and demonstrate robust induction of ROS in coronary arteries from TNFα-treated and PBS-pretreated mice. IL-19 pretreatment reduced ROS abundance in medial VSMC. Together, this suggests that IL-19 pretreatment can reduce TNFα-induced arterial ROS abundance in vivo.

DISCUSSION

Both anti-inflammatory compounds and HO-1 have demonstrated vascular protective effects. This work implicates IL-19 as a potential link between these two powerful and protective systems. It has been shown that IL-10 can induce HO-1 in monocyte/macrophages, and that much of the anti-inflammatory effects of IL-10 are mediated by HO-1 in these cells (28). Induction of HO-1 expression in resident vascular cells by any Th2 interleukin has not been described, and the first major finding of this report is that IL-19 can induce HO-1 mRNA and protein in VSMC. It was quite interesting that we were unable to detect HO-1 expression in EC. There is precedence for differential cell-type induction of HO-1. For example, IL-6 could induce HO-1 in murine macrophage, but not in EC, leading the authors to conclude that induction of HO-1 was dependent on cell type. There also are demonstrated cell-specific differences in HO-1 activity in EC compared with VSMC. In SMC, up-regulation of HO-1 decreased cell cycle progression whereas in EC, HO-1 induction increased cell cycle progression (29–31). Likewise, inhibition of HO-1 in SMC increased cell proliferation, whereas in EC proliferation decreased. Correspondingly, we have shown that IL-19 is anti-migratory for VSMC, yet angiogenic for EC (13, 19). Because IL-19 does not induce HO-1 expression in EC, this implies that IL-19 angiogenic effects in EC are not mediated by HO-1.

HO-1 is induced mainly at the transcriptional level by pro-inflammatory mediators such as IL-6, oxidative stress, hypoxia, and some growth factors such as PDGF (32). The promoter HO-1 region contains consensus binding sites for numerous transcription factors, including STAT proteins (33). Nrf2 has been implicated as the most important of these. We were not able to detect Nrf2 activation by IL-19 in VSMC (data not shown). Phosphorylated STATs dimerize and then translocate into the nucleus where they recognize specific DNA sequences or other transcription factors and influence gene transcription (34). STAT3 activation is one of the earliest events in IL-19 stimulation of VSMC (13), and reduction of STAT3 by siRNA knockdown did significantly reduce IL-19 induction of HO-1. We were not able to completely knock down STAT3 with siRNA without compromising cell viability, and could not completely inhibit HO-1 expression. To determine the role of STAT3 in IL-19-driven HO-1 expression we utilized a luciferase construct driven by the native HO-1 promoter, and a promoter lacking the STAT consensus site. Deletion of the STAT element did not completely abolish HO-1 promoter activation. IL-19 reliance on STAT3 for HO-1 expression is in contrast to a report showing that IL-10 induction of HO-1 in mouse macrophage was STAT3 independent (28). This may provide one mechanism for the earlier mentioned cell-specific differences in HO-1 expression. While we can conclude that the STAT element is very important in IL-19-driven HO-1 expression, we cannot unequivocally conclude that HO-1 expression is solely dependent on STAT3. These results are novel in that at least in VSMC, IL-19 can induce HO-1 expression in an Nrf2-independent mechanism, which is a second major finding of the present study.

HO-1 is known to be up-regulated by oxidized LDL, its expression is increased in human atherosclerotic lesions, and its induction is cytoprotective in atherosclerosis and occlusive vascular diseases (5–8). HO-1 co-localizes foam cell and SMC in advanced lesions (6, 8), and acts as a potent anti-oxidant (5, 6). There is limited literature concerning reduction of cellular ROS by anti-inflammatory cytokines in VSMC. Consequently, it was important to determine if IL-19 could decrease ROS abundance in VSMC. PDGF is a potent inducer of cellular ROS, and a third major finding of this study is that IL-19 could rapidly and transiently decrease PDGF-induced ROS in cultured VSMC with the same kinetics as IL-19 induction of HO-1 protein. This decrease in ROS was due at least in part to HO-1

FIGURE 6. IL-19 reduces arterial ROS in vivo. In situ detection of superoxide in mouse carotid artery. Wild-type C57B6 mice were pretreated for 4 h with either PBS or 10 ng/g of IL-19, then injected intraperitoneally with 2 ng/25 g TNFα for 1 h to induce ROS. Coronary artery were removed and ROS abundance in frozen sections was assayed by DHA (24, 25). At identical laser and microscope capture settings, fluorescence in PBS and TNFα only aorta is markedly increased compared with IL-19-pretreated mice. Fluorescent photomicrographs of sections are representative of multiple sections from three different mice.
expression, as knockdown of HO-1 by specific siRNA significantly decreased IL-19-mediated reduction in ROS by 35%. Importantly, though significant, the siRNA effectiveness was reduced at 24 h, a time which IL-19-induced HO-1 expression is also reduced. In in vivo situations the vasculature would be exposed to IL-19 over a much longer time. VSMC were treated with three consecutive treatments of IL-19 for 24 h. Chronic IL-19 exposure sustained HO-1 expression and a concomitant sustained decrease in ROS for 24 h. This is important considering that many vascular disorders such as atherosclerosis and restenosis are chronic inflammatory situations where resident vascular cells would be exposed to long term IL-19 stimulation. The kinetics of ROS reduction corresponds with IL-19-mediated HO-1 expression in both experiments and strongly suggests that IL-19 reduction of ROS is mediated at least in major part by HO-1. This is in contrast to a report suggesting that in murine macrophage, IL-19 increases ROS to a small degree. It was not specified in this manuscript if this small increase was statistically significant (23). Again, this may be due to the observed cell type-specific differences in HO-1 expression in cultured cells.

HO-1 is a recognized cytoprotective agent, and several studies have shown that HO-1 over expression blocks apoptosis (7, 35–37). Vascular smooth muscle cells from HO−/− mice demonstrate increased apoptosis in response to oxidative stress (7). In VSMC, one major cause of apoptosis is ROS (38, 39), which prompted us to investigate if IL-19 could protect VSMC from oxidative stress induced apoptosis. Apoptosis is an important component of vessel wall remodeling in many chronic vascular diseases. IL-19 treatment over a 5 day span improved cell viability in serum-starved VSMC. Consequently, a fourth important finding from this study is that IL-19 can significantly reduce apoptosis in VSMC in situations when the apoptotic stimuli is acute from ROS-induced, or prolonged from serum-deprivation. IL-19 protection from apoptosis in VSMC has important implications for many vascular diseases, including atherosclerosis and restenosis (1, 2).

To extend this finding to in vivo scenario, mice were injected with TNFα to induce vascular accumulation of ROS (25). When these mice received TNFα 4 h after receiving 10 ng/g IL-19, vascular ROS was decreased. 4 h IL-19 pretreatment was more effective than 24 h pretreatment, which again, is similar to the kinetics of IL-19 induction in cultured VSMC. This is analogous to a similar study in which mice were treated with IL-10 3 h prior to injection with LPS and were found to express increased amounts of HO-1 in peritoneal macrophages (28).

The major novel finding of this report is that IL-19, a Th2 anti-inflammatory interleukin, can induce expression of HO-1 in vascular smooth muscle cell, leading to increased VSMC survival and a decrease in ROS concentration in cultured cells and in vivo. We remain the only laboratory to investigate IL-19 effects on vascular cells, and as such very little is reported concerning the effects of this interleukin on vascular pathophysiology. We have previously shown that IL-19 has protective and anti-inflammatory effects on cultured VSMC, and anti-restenotic effects in angioplasty-injured rat carotid arteries. In a recent report, we demonstrate that IL-19 decreases mRNA stability in VSMC through an HuR-dependent mechanism. However, because of multiple protective effects mediated by HO-1 and its induction by IL-19, this report extends that work and implies additional mechanisms of atheroprotection by IL-19 in addition to HuR modulation. This is novel and potentially links two important protective mechanisms; anti-inflammation with HO-1 protection.

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