Chemerin Regulates Crosstalk Between Adipocytes and Vascular Cells Through Nox

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See Editorial Commentary, pp 466–468

Abstract—Adipocytes produce adipokines, including chemerin, a chemoattractant that mediates effects through its ChemR23 receptor. Chemerin has been linked to endothelial dysfunction and vascular injury in pathological conditions, such as obesity, diabetes mellitus, and hypertension. Molecular mechanisms underlying this are elusive. Here we assessed whether chemerin through redox-sensitive signaling influences molecular processes associated with vascular growth, apoptosis, and inflammation. Human microvascular endothelial cells and vascular smooth muscle cells were stimulated with chemerin (50 ng/mL). Chemerin increased generation of reactive oxygen species and phosphorylation of mitogen-activated protein kinases, effects that were inhibited by ML171, GKT137831 (Nox inhibitors), and N-acetylcysteine (reactive oxygen species scavenger). Chemerin increased mRNA expression of proinflammatory mediators in vascular cells and increased monocyte-to-endothelial cell attachment. In human vascular smooth muscle cells, chemerin induced phosphorylation of mitogen-activated protein kinases and stimulated proliferation (increased proliferating cell nuclear antigen expression [proliferation marker] and BrdU incorporation [proliferation assay]). Chemerin decreased phosphatidylinositol 3-kinase/protein kinase B activation and increased TUNEL-positive human vascular smooth muscle cells. In human microvascular endothelial cells, chemerin reduced endothelial nitric oxide synthase activity and nitric oxide production. Adipocyte-conditioned medium from obese/diabetic mice (db/db), which have elevated chemerin levels, increased reactive oxygen species generation in vascular smooth muscle cells, whereas adipocyte-conditioned medium from control mice had no effect. Chemerin actions were blocked by CCX 832, a ChemR23 inhibitor. Our data demonstrate that chemerin, through Nox activation and redox-sensitive mitogen-activated protein kinases signaling, exerts proapoptotic, proinflammatory, and proliferative effects in human vascular cells. These findings elucidate some molecular mechanisms through chemerin, which is increased in obesity, whereby adipocytes may influence vascular function. We identify chemerin as a novel vasoactive adipokine, which may be important in obesity-related vascular injury. **(*Hypertension*. 2015;66:657-666. DOI: 10.1161/HYPERTENSIONAHA.115.05616.)** ● **Online Data Supplement**

Key Words: adipokines ■ diabetes mellitus ■ obesity ■ reactive oxygen species

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Adipose tissue is increasingly recognized as an important endocrine organ that secretes bioactive hormones and cytokines, called adipokines.[1–4] Adipokines may act as an autocrine/paracrine stimulus influencing adipose tissue function,[2] as well as energy homeostasis, glucose and lipid metabolism, food intake, inflammation, immunity, vascular function, and blood pressure.[5–7] In cardiovascular diseases, the production and release of proinflammatory adipokines is increased, whereas the production of anti-inflammatory adipokines is decreased. This dysregulation has been considered as a factor in the development of obesity-related vascular complications observed in type 2 diabetes mellitus.[4–6,8]

Chemerin, also known as retinoic acid receptor responder protein 2 or tazarotene-induced gene 2 protein, is highly expressed in placenta, liver, and white adipose tissue, but it is less expressed in other tissues, such as lung, brown adipose tissue, heart, ovary, kidney, skeletal muscle, and pancreas.[9–12] Chemerin is synthesized initially as preprochemerin, a 163 amino acid protein with an N-terminal signal sequence (20 aa) that is cleaved to the inactive 18-kDa precursor, prochemerin (Chem-163),[13] which subsequently undergoes proteolysis to form active chemerin.[14] Chemerin signals through CMKLR1 (chemokine-like receptor 1) or ChemR23 (chemerin receptor 23), expressed in macrophages, dendritic cells, adipocytes,[15]

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and vascular cells.[7] Chemerin also has affinity to 2 other receptors, G protein-coupled receptor 1 and chemokine receptor-like 2. Binding to chemokine receptor-like 2 does not seem to activate signaling pathways and may act as an inhibitor of chemerin-induced cellular responses.[16]

In mice fed high-fat diet, chemerin expression is upregulated in adipocytes,[17,18] and in db/db mice, a model of diabetes mellitus–associated obesity, levels of chemerin are increased in white adipose tissue, skeletal muscle, and liver. Chemerin reduces glucose uptake and increases glucose intolerance in vivo.[18,19] Levels of chemerin correlate positively with body mass index[10] and has been considered a biomarker of metabolic syndrome.[20] It is also linked to adipogenesis through activation of ChemR23.[17] This is evidenced by studies in ChemR23 knockout mice fed high-fat diets. Ernst et al showed that mice deficient in ChemR23 had altered adipocyte differentiation and reduced adiposity and body mass.[21] Rouger et al reported that ChemR23 knockout mice developed mature-onset obesity with no effect on adipocyte differentiation.[22] These contrasting results have been attributed to differences in the fat content of the diets.

Chemerin is also implicated in inflammation. Chemerin cleaves into different fragments with pro- or anti-inflammatory actions.[23–29] Plasma chemerin is increased in chronic inflammatory diseases[23–27] and levels of chemerin correlate with levels of the proinflammatory cytokines, such as tumor necrosis factor (TNF-α), interleukin (IL-6), and C reactive protein.[23,28] Chemerin, through ChemR23,[29] has also been shown to have anti-inflammatory effects in vascular cells through processes that involve protein kinase B (Akt) and endothelial nitric oxide synthase (eNOS) signaling and nitric oxide production.[29] The differential role of chemerin/ChemR23 in the control of inflammation depends on the site of inflammation and the class of proteases predominating in the microenvironment.[30]

Recent data demonstrate that in addition to regulating metabolic and inflammatory processes, chemerin may influence vascular function. Chemerin decreases nitric oxide (NO)–dependent cGMP signaling, thereby reducing vascular relaxation in rat aorta, an effect related to increased superoxide anion ($O_2^{-}\cdot$) generation.[31] In the vasculature, NADPH oxidases (Nox) are a major source of $O_2^{-}\cdot$ and play an important role in vascular damage and dysfunction during cardiovascular diseases. These data suggest that reactive oxygen species (ROS) and redox signaling may also contribute to chemerin vascular actions, but exact mechanisms are unclear.

We hypothesize that adipocyte-derived chemerin, through ROS, stimulates mitogenic and proinflammatory signaling pathways that influence cellular responses underlying vascular damage and remodeling. We were particularly interested in assessing whether aldosterone stimulates adipocyte-derived chemerin production because our previous studies identified an important role for aldosterone in adipocyte and vascular function.[32] Chemerin may act as an important link between adipose tissue and vessels.

Materials and Methods

See online-only Data Supplement for expanded Methods section.

Cell Culture

Human microvascular endothelial cells (HMEC) and human vascular smooth muscle cells (HVSMC) were studied. Cells were stimulated with chemerin for short (5, 15, 30, 60 minutes) and long (2, 8, 24 hours) periods. Chemerin was studied at a concentration of 50 ng/mL, representing the lower limit of normal in plasma in humans (50–200 ng/mL)[33,34] and accordingly has pathophysiological significance. In some studies, cells were preincubated with GKT137831 (Nox1/4 inhibitor), ML171 (Nox1 inhibitor), CCX832 (chemerin receptor antagonist), N-acetylcysteine (NAC; ROS scavenger), PD98059 (extracellular signal-related kinases [ERK1/2] inhibitor), and SP600125 (c-Jun N-terminal kinases [JNK] inhibitor).

Adipose Tissue–Conditioned Medium

SW872 were stimulated with aldosterone (100 nmol/L) for 24 hours to induce chemerin release, and medium was collected to obtain adipose tissue–conditioned medium (ACM). We previously showed that adipocytes are functionally responsive to aldosterone.[32] HVSMC were stimulated with ACM for 1 to 8 hours. Cells were preexposed to CCX832 and eplerenone (mineralocorticoid receptor blocker, 10 μmol/L) before ACM stimulation. VSMCs from control mice (C57BL6) were stimulated with ACM from db/+ (lean, nondiabetic) and db/db mice (obese, diabetic).

Chemerin Levels

Chemerin levels were measured by ELISA in medium from aldosterone-stimulated SW872 human adipocytes and ACM from db/+ and db/db mice.

Immunoblotting

Immunoblotting was used to examine phosphorylation of proliferating cell nuclear antigen and activation of signaling proteins: p38 mitogen-activated protein kinases (MAPK), ERK1/2, SAPK/JNK, eNOS, and phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt). In some cases, cells were pre-exposed (30 minutes) to CCX 832, ML171, GKT137831, or NAC 30. Results were normalized to β-actin or total PI3K, Akt, eNOS, ERK 1/2, p38MAP kinase, and SAPK/JNK for phospho-proteins.

Real-Time PCR

Quantitative real-time PCR (Applied Biosystems) was used to analyze mRNA expression. In some cases, chemerin-stimulated cells were pretreated for 30 minutes with PD98059 and SP600125.

Lucigenin-Enhanced Chemiluminescence

Lucigenin-derived chemiluminescence was used to determine NAD(P)H oxidase activity in total HMEC homogenates. The cells were exposed to CCX 832, ML171, GKT137831, or NAC 30 minutes before chemerin stimulation. The results are expressed as a fold change in arbitrary units per milligram of protein, as measured by the BCA assay.

Amplex Red Assay

Hydrogen peroxide (H_2O_2) measurements were made according to the manufacture's instruction using the horseradish peroxidase-linked Amplex Red fluorescence assay.

Nitric Oxide Production

Production of NO in response to chemerin was determined with the NO fluorescent probe diacetate 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate. Fluorescence intensity was adjusted to protein concentration and expressed as fluorescence emission per microgram of protein.

Caspase 3 Assay

HVSMC were treated with chemerin in DMEM containing 1% FBS for 1, 4, and 8 hours. The cells were pre-exposed to CCX 832 or NAC 30 minutes before adding chemerin. Caspase-3 activity in cell lysates was determined using a commercial kit.

Cell Death Detection (TUNEL Assay)

HVSMC were cultured in 48-well plate and stimulated with chemerin (50 ng/mL) for 8 hours. Cells were pretreated with CCX

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832 or NAC (30 minutes). Apoptosis was evaluated by TUNEL staining using In Situ Cell Death Detection Kit (Roche Diagnostics). Positive nuclei to TUNEL were counted and normalized by number of cells in each well.

BrdU Incorporation Assay

Cell proliferation was determined by BrdU Cell Proliferation Assay (Millipore). Cells were stimulated with chemerin in the absence or presence of CCX 832 or NAC. HVSMC cells were seeded in a 96-well plate and starved overnight before the experiment. The results were normalized as percent of control.

Monocyte Adhesion Assay

HMEC were stimulated with chemerin (24 hours) in the presence or absence of CCX 832 or NAC. Monocytes (2×10^5 cells/mL) were suspended in saline supplemented containing carboxyfluorescein succinimidyl ester, a fluorescent probe, and incubated for 20 minutes at 37°C. Labeled monocyte suspension was added and the number of adherent monocytes was determined.

Statistical Analysis

Mean values ± SEM were calculated for each experiment and statistical comparisons were made with 1-way ANOVA followed by Newman–Keuls test or 2-tailed Student's *t* test when appropriate. $P<0.05$ was considered statistically significant.

Results

Proinflammatory Responses of Chemerin Are Dependent on Nox–ROS-Induced MAPK Activation in HMEC

The potential pro-oxidant effects of chemerin were evaluated in HMEC. Chemerin increased $O_2^{-}\cdot$ production after 1 and 24 h of stimulation. These effects were mediated through Nox activation because chemerin effects were blocked by Nox inhibitors, ML171 and GKT137831 (Figure 1A; $P<0.05$). H_2O_2 levels were also increased after exposure of HMEC to chemerin (Figure S1A in the online-only Data Supplement). Because chemerin-induced ROS production was dependent on Nox activation, we evaluated Nox gene expression by chemerin. Nox 1 (Figure S1B) and Nox 4 (Figure S1C) mRNA levels were increased by chemerin after 1 and 24 h of stimulation ($P<0.05$). MAPK are redox-sensitive and classical downstream signaling pathways of ROS. Chemerin increased phosphorylation of SAPK/JNK (Figure 1B) and ERK1/2 (Figure 1C) in HMEC; an effect blocked by CCX832, ML171, and NAC ($P<0.05$).

MAPK activation regulates many cellular processes, including inflammation, and may be of importance in chemerin-induced proinflammatory effects. Chemerin, through its receptor ChemR23 and ROS generation, increased monocyte attachment to HMEC (Figure 1D and 1E, $P<0.05$). In addition, HMEC mRNA levels of monocyte chemoattractant protein-1 (MCP-1; Figure S2A), IL-8 (Figure S2B), TNF-α (Figure S2C), vascular cell adhesion molecule 1 (VCAM-1; Figure S2D), and intracellular cell adhesion molecule 1 (Figure S2E) were increased by chemerin ($P<0.05$). To assess the involvement of MAPK in chemerin-induced proinflammatory responses, we evaluated effects of ERK1/2 and JNK inhibitors, PD98059 and SP600125, respectively, on the regulation of mRNA levels of molecules important in monocyte attachment: (1) the chemokine MCP-1; (2) the cytokine TNF-α, and (3) the adhesion molecule VCAM-1. Inhibition of ERK1/2 and JNK blocked the increase in MCP-1 (Figure 1F), TNF-α (Figure 1G), and VCAM-1 (Figure 1H) gene expression induced by chemerin stimulation of HMEC.

Chemerin Decreases eNOS Activation and NO Production in HMEC

To verify a possible role for chemerin in endothelial dysfunction, HMEC exposed to chemerin were probed for eNOS activation and NO production. In HMEC, chemerin decreased phosphorylation of eNOS activation site (Ser^{1177}; Figure 2A; $P<0.05$), and increased the phosphorylation of eNOS inhibitory site (Thr^{495}; Figure 2B; $P<0.05$). Moreover, chemerin reduced NO production (Figure 2C; $P<0.05$), at the same time point when eNOS activity was reduced.

Chemerin Influences Cell Growth and Apoptosis Through Nox-Derived ROS Production in HVSMC

Chemerin increases $O_2^{-}\cdot$ (Figure 3A) and H_2O_2 (Figure S3A) in HVSMC ($P<0.05$). The increase in $O_2^{-}\cdot$ levels observed after chemerin stimulation was inhibited by CCX 832, ML171, and GKT 137831 (Figure 3A). ML171 effects were evident only at 1 hour, suggesting that Nox1 may be important during the acute phase of chemerin-induced ROS production. In parallel to an increase in ROS production, chemerin also increased gene expression of Nox 1 and Nox 4 in HVSMC (Figures S3B and S3C; $P<0.05$). Increased proproliferative, apoptotic, and inflammatory responses are important molecular mechanisms of vascular remodeling. Chemerin increased proliferating cell nuclear antigen protein levels (Figure 3B), a molecular marker of cell growth, and BrDU incorporation (proliferation assay; Figure 3C) in HVSMC ($P<0.05$). Apoptosis was measured by caspase-3 activation and DNA fragmentation (TUNEL assay). As demonstrated in Figure 3D and 3E, chemerin increased caspase-3 activity, followed by an increase in TUNEL-positive HVSMC. The proproliferative and apoptotic effects of chemerin were inhibited by CCX832 and NAC, but partially inhibited by ML171 and not inhibited by GKT137831. As for inflammation, we observed an increase in IL-6 (Figure S4A), MCP-1 (Figure S4B), VCAM-1 (Figure S4C), and intracellular cell adhesion molecule 1 (Figure S4D) induced by chemerin. Activation of the MAPK and PI3K pathways controls cell growth, inflammation, and survival. In HVSMC stimulated with chemerin, phosphorylation of ERK1/2 (Figure 4A), p38 MAPK (Figure 4B), and JNK (Figure 4C) was increased, whereas phosphorylation of PI3K (Figure 4D) and Akt (Figure 4E) was decreased compared with controls.

Adipocyte-Derived Chemerin Increases ROS and mRNA Expression of Proinflammatory Mediators

Chemerin may be an important factor involved in adipocyte-mediated regulation of VSMCs. To assess this, we exposed HVSMC to ACM in the absence and presence of CCX 832 and measured ROS production and molecular markers of inflammation. Human adipocytes were stimulated with aldosterone to induce chemerin production (Figure S5) and the conditioned medium (ACM) was collected for HVSMC

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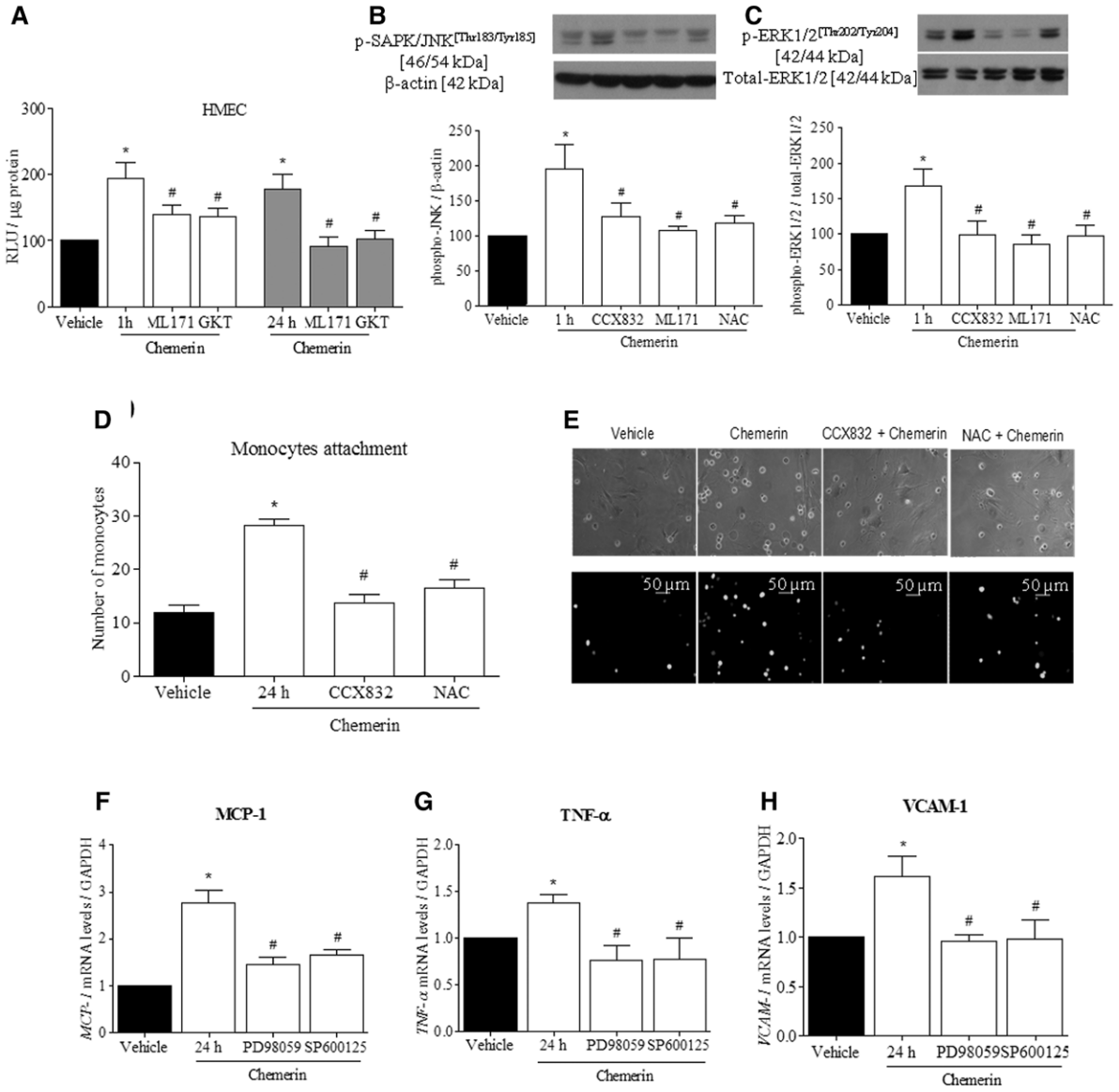

Figure 1. Chemerin increases reactive oxygen species (ROS) generation and inflammation in human microvascular endothelial cells (HMEC). **A**, $O_2^{\cdot -}$ generation in HMEC was measured by lucigenin, after stimulation with chemerin (50 ng/mL) for 1 (white bars) and 24 hours (gray bars). Lucigenin assay was performed in the presence or absence of ML171 and GKT 137831, added 30 minutes before stimulation with chemerin. Values were normalized by protein amount. **B** and **C**, Phosphorylation of c-Jun N-terminal kinases (JNK) and extracellular signal-related kinases (ERK) 1/2 was determined by immunoblotting. The inhibitors chemerin receptor antagonist (CCX) 832, ML171, or N-acetylcysteine (NAC) were added 30 minutes before chemerin. Values were normalized by expression of total ERK 1/2 and β-actin, respectively. **D** and **E**, Quantitative analysis and representative images of carboxyfluorescein succinimidyl ester (CFSE)–positive nuclei showing that chemerin enhances adhesion of THP-1 monocytes to HMEC. Values express the mean of the quantification of 3 images of each well. The inhibitors CCX 832 and NAC were added 30 minutes before chemerin (24 hours). Scale bar =50 µm. **F**, **G**, and **H**, Chemerin increases inflammatory markers in HMEC via ERK and JNK.Gene expression of monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor (TNF)-α, and vascular cell adhesion molecule 1 (VCAM-1) in HMEC was determined by real-time PCR. Values were normalized by gene expression of GAPDH. PD 98059 (ERK 1/2 inhibitor) and SP 600125 (JNK inhibitor) were added 30 minutes before stimulation with chemerin. Results represent the mean±SEM of 4 to 9 experiments. **P*<0.05 vs vehicle; #*P*<0.05 vs chemerin.

stimulation. ACM-containing chemerin augmented ROS generation (Figure 5A) and increased mRNA levels of IL-6 (Figure 5B), MCP-1 (Figure 5C), and VCAM-1 (Figure 5D). CCX 832 inhibited ACM-induced effects on IL-6 and VCAM-1, but not MCP-1 (Figure 5C), demonstrating differential pro-inflammatory responses through Chem R23.

As levels of chemerin increase with obesity, we sought to evaluate effects of ACM from adipocytes from lean/non diabetic (db/+) and obese/diabetic (db/db) mice. As observed in Figure 6A, chemerin levels were increased in ACM from db/db adipocytes, compared with db/+ adipocytes. Stimulation of VSMCs from control mice with db/db-derived ACM increased ROS production, which was blocked by CCX 832 (Figure 6B).

Discussion

Major findings from our study demonstrate an important role for ROS in chemerin signaling in human vascular cells. In

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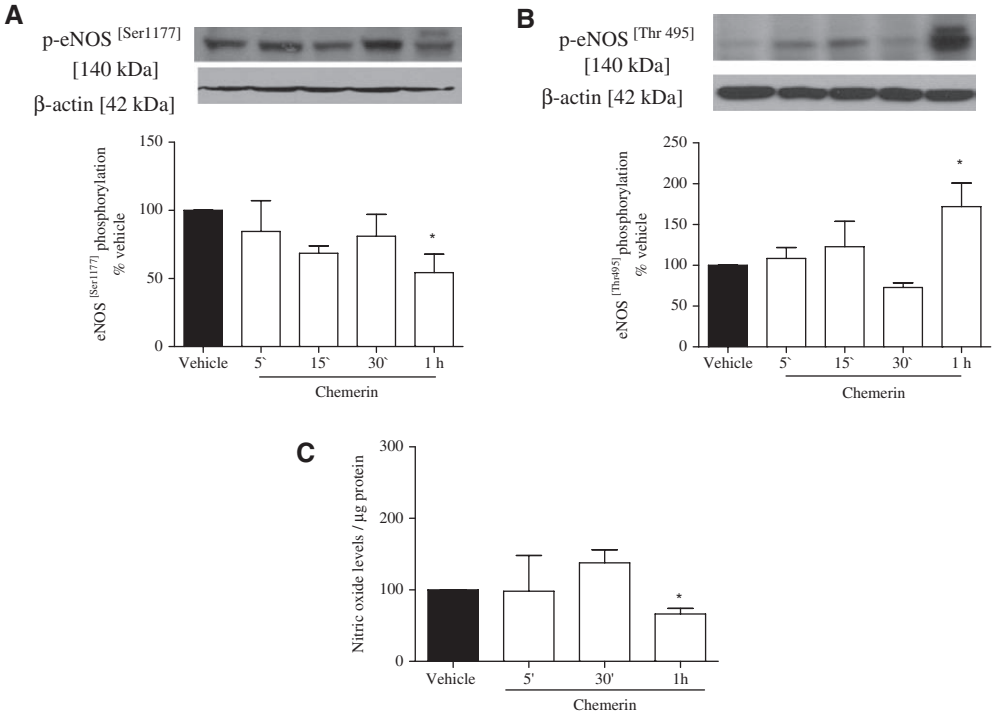

Figure 2. Chemerin decreases Nitric oxide (NO) signaling in human microvascular endothelial cells (HMEC). **A** and **B**, Phosphorylation of activation and inhibitory sites of endothelial nitric oxide synthase (eNOS) were determined by western blot in the presence of chemerin (50 ng/mL) at different time points. Values were normalized by expression of β-actin. **C**, NO production was evaluated by DAF2-DA fluorescence in HMEC, and values were normalized by protein. Bars represent the mean±SEM of 5 to 8 experiments. *P<0.05 vs vehicle.

particular, (1) chemerin stimulates Nox-derived ROS generation, which influences MAPK activation, leading to proinflammatory responses in endothelial cells; (2) chemerin, through redox-sensitive processes, induces proliferation and apoptosis in VSMCs; and (3) adipocyte-derived factors and adipocytes from obese/diabetic mice stimulate VSMC ROS production and inflammatory responses through chemerin receptors. Moreover, we found that chemerin downregulates eNOS and decreases NO production in endothelial cells, a molecular hallmark of endothelial dysfunction.

Endothelial dysfunction and vascular remodeling, because of aberrant growth/apoptosis, inflammation, and fibrosis, are characteristic features of cardio-metabolic diseases, where ROS play an important role.[35–38] Many of these processes are linked to adipose tissue through adipokines, adipocyte-derived bioactive factors that influence vascular function.[39] This may be particularly important in obesity where cardiovascular risk is increased, possibly due, in part, to increased production of injurious adipokines, such as chemerin.[10,28,40,41]

Chemerin, through its receptor ChemR23, regulate adipocyte differentiation[9,17] and insulin signaling in 3T3-L1 adipocytes, stimulates inflammatory responses,[42] and also acts as an endogenous vasoconstrictor.[7] These effects are all redox sensitive in the vasculature, where NADPH oxidases are major sources of ROS.[43,44] What has been unclear is whether chemerin influences vascular signaling and function through ROS. Using various strategies, we demonstrate that in human endothelial and vascular smooth muscle cells, chemerin acts as an injurious stimulus promoting apoptosis, inflammation, and proliferation, processes that are mediated via Nox-derived ROS. These effects may be of relevance in explaining molecular mechanisms, whereby adipocytes influence vascular function, especially in the context of obesity/adiposity, where chemerin production is increased and vascular dysfunction is amplified. Our findings are in line with previous studies that have demonstrated a proinflammatory function of chemerin.[13,33,34,45] It should be highlighted, however, that chemerin has also been found to have anti-inflammatory actions.[29,32] These differences may relate to the cell type studied, stimulus for chemerin production, and presence of proteases because chemerin is cleaved into pro- and anti-inflammatory fragments. Although we found Nox to be a major source of chemerin-induced ROS production in our experimental paradigm, we cannot exclude the possibility that other sources may also contribute, such as mitochondrial oxidases, which have been shown to be important in chemerin-stimulated aortic endothelial cells.[46]

Chemerin, via ERK1/2 activation, stimulates natural killer cells migration[47] and increases contraction.[48] Furthermore, chemerin leads to myoblast proliferation through activation of ERK1/2 and mTOR pathways.[49] Here we demonstrated that chemerin increases proliferation through ROS, although this seems to be independent of Nox 1 and 4, suggesting that other ROS sources may be important. Chemerin also induced apoptosis by inhibition of the PI3K-Akt pathway. Kaur and colleagues[50] reported that chemerin induces activation of signaling cascades relevant for angiogenesis and cell survival, such as MAPK and Akt in human endothelial cells. Because an imbalance of proliferation and apoptosis is an important feature of vascular remodeling, chemerin regulation of both biological responses may be of relevance in vascular diseases.

The increased levels of chemerin in obesity and its positive association with inflammation[51] establish a link between

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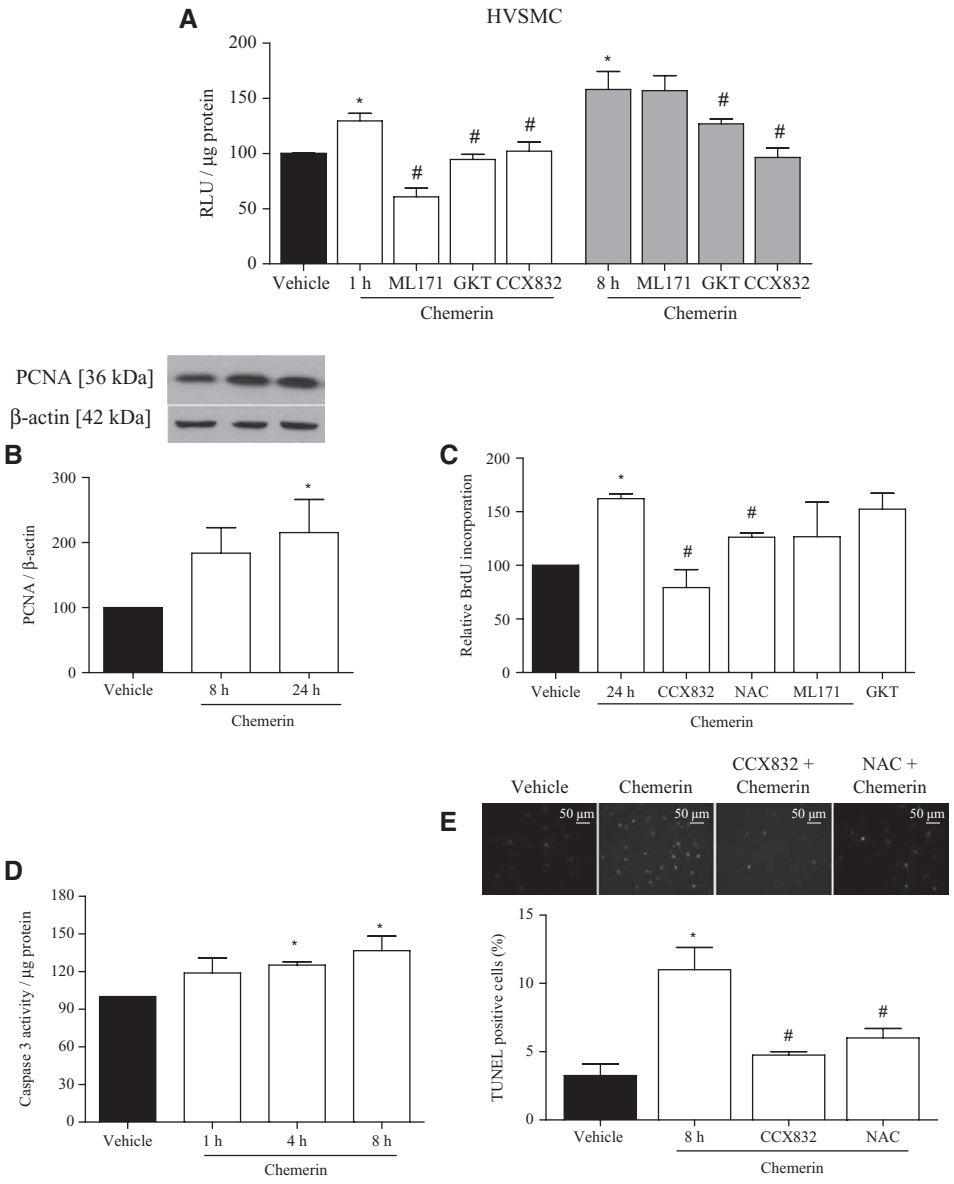

Figure 3. Chemerin increases reactive oxygen species (ROS) generation and induces proliferation and apoptosis in human vascular smooth muscle cells (HVSMC). **A**, $O_2^{\cdot-}$ generation and H_2O_2 levels in HVSMC were measured by lucigenin and Amplex red assay, respectively. Lucigenin assay was performed in the presence or absence of ML171, GKT 137831, or chemerin receptor antagonist (CCX) 832, added 30 minutes before chemerin (50 ng/mL) for 1 (white bars) and 8 hours (gray bars). **B**, Proliferating cell nuclear antigen (PCNA) expression was determined by western blot in the presence of chemerin for 8 and 24 hours. Values were normalized by expression of β-actin. **C**, BrdU assay was performed to evaluate proliferation induced by chemerin and values were normalized by vehicle group. CCX 832, N-acetylcysteine (NAC), ML171, and GKT 137831 were added 30 minutes before stimulation with chemerin (24 hours). Caspase 3 activity (**D**) and apoptosis (**E**) were determined by ELISA and TUNEL staining, respectively. ELISA values were normalized by protein amount. **F**, Quantitative analysis and representative fluorographs of TUNEL-positive nuclei in HVSMC. CCX 832 and NAC were added 30 minutes before stimulation with chemerin (8 hours). Scale bar =50 µm. Results represent the mean±SEM of 4 to 9 experiments. **P*<0.05 vs vehicle; #*P*<0.05 vs chemerin.

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chemerin and the risk of development of metabolic diseases. Importantly, our study demonstrates that chemerin influences the expression of inflammatory markers in human vascular cells and induces monocyte adhesion to endothelial cells in a ROS-dependent manner. The regulation of proinflammatory responses does not only contribute to the process of vascular injury and remodeling, but also may contribute to increasing chemerin itself and associated mechanisms. Kaur and colleagues[50] demonstrated that expression of ChemR23, which is responsible for the majority of chemerin harmful effects, is upregulated by proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6.

To better understand the biological significance of adipocyte-derived chemerin and vascular function, we studied effects of ACM, which contains chemerin, on vascular signaling and inflammatory responses. Similar to responses of exogenously added chemerin, ACM stimulated vascular ROS production and increased expression of proinflammatory

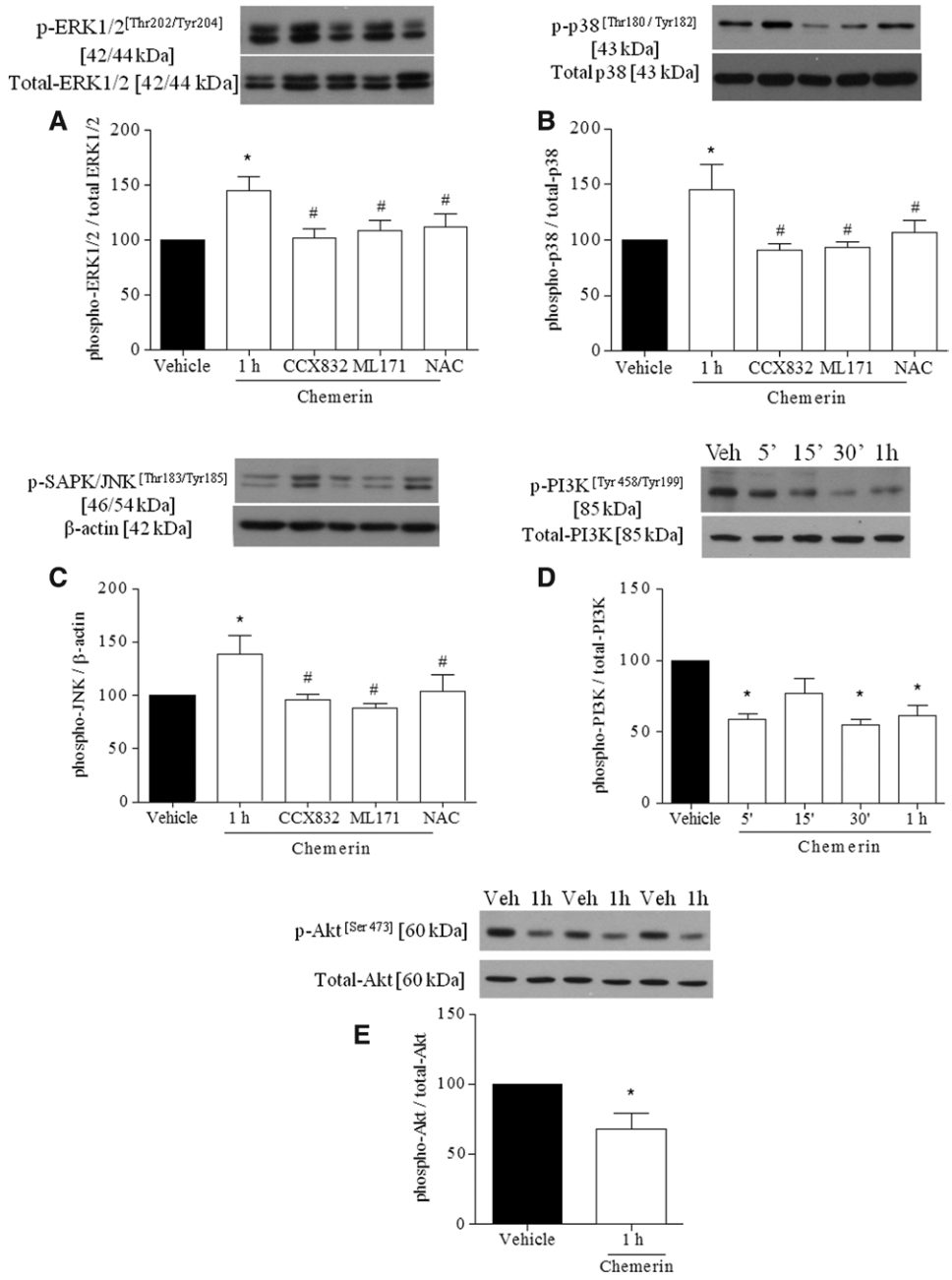

Figure 4. Chemerin increases phosphorylation of mitogen-activated protein kinases (MAPK) and decreases phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling in human vascular smooth muscle cells (HVSMC). Phosphorylation of extracellular signal-related kinases (ERK) 1/2 (**A**), p38MAPK (**B**), c-Jun N-terminal kinases (JNK; **C**), PI3K (**D**), and Akt (**E**) was determined by western blot. Cells were pretreated with chemerin receptor antagonist (CCX) 832, ML171, or N-acetylcysteine (NAC) 30 minutes before stimulation with chemerin (50 ng/mL). Values were normalized by expression of total protein or β-actin, respectively. Bars represent the mean±SEM of 5 to 6 experiments. **P*<0.05 vs vehicle; #*P*<0.05 vs chemerin.

markers, effects that were blocked by ChemR23 antagonism, suggesting chemerin-mediated actions. We extended our vascular studies to explore whether obesity, which is associated with increased chemerin production,[17,18] affects altered VSMC responses. Adipocytes from obese mice significantly increased VSMC oxidative stress and inflammation in a ChemR23-inhibitable manner. Our findings suggest that chemerin may be a functional mediator in the crosstalk between adipocytes and the vasculature, which in pathological conditions may promote endothelial dysfunction and vascular inflammation.

In conclusion, our study identifies chemerin as a new vasoactive adipokine that plays an important role in molecular and cellular processes associated with vascular injury and dysfunction. This may be especially important in conditions associated with increased adipocyte-derived chemerin, such as obesity, metabolic syndrome, and hypertension.

Perspectives

Adipokines, including chemerin, participate in many physiological processes implicated in cardiovascular complications

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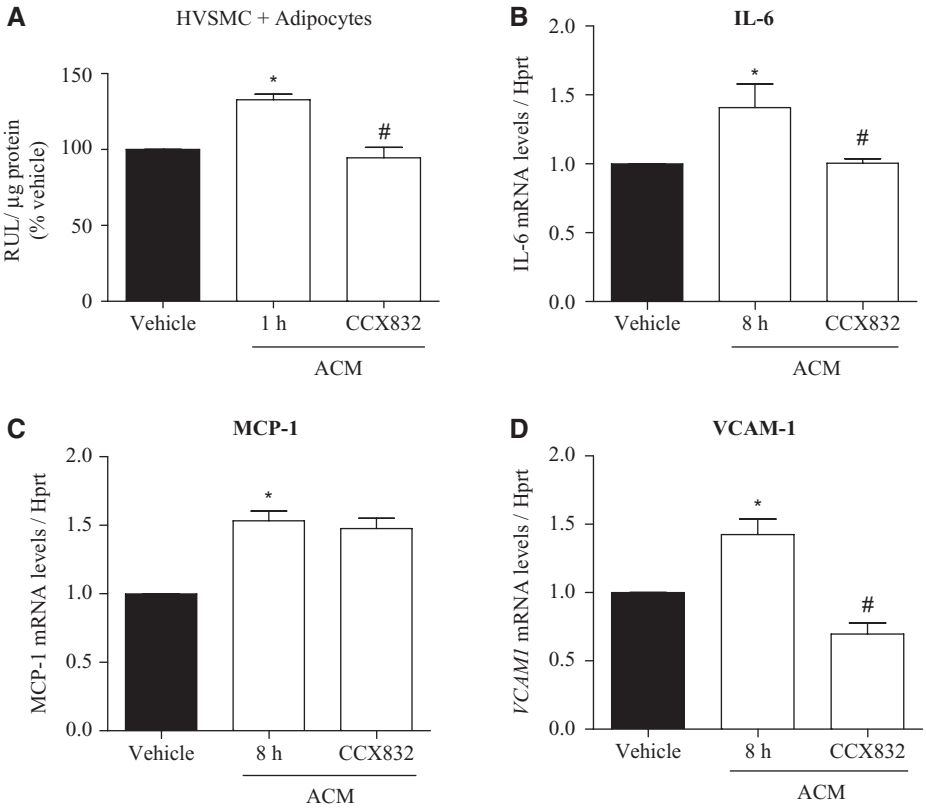

Figure 5. Adipocyte-derived chemerin increases reactive oxygen species (ROS) generation and inflammatory markers in human vascular smooth muscle cells (HVSMC). HVSMC were stimulated with adipocyte-conditioned medium (ACM). ROS generation was measured by lucigenin (**A**). Lucigenin assay (**A**) was performed in the presence or absence of chemerin receptor antagonist (CCX) 832 added 30 minutes before stimulation with ACM (1 hour), and values were normalized by protein amount. **B**, **C**, and **D**, Gene expression of interleukin (IL)-6, monocyte chemoattractant protein-1 (MCP-1), and vascular cell adhesion molecule 1 (VCAM-1) in HVSMC was determined by real-time PCR. Experiments were performed in the presence or absence of CCX 832 added 30 minutes before stimulation with ACM (8 hours). Values were normalized by gene expression of hypoxanthine guanine phosphoribosyltransferase (Hprt). Bars represent the mean±SEM of 6 experiments. **P*<0.05 vs vehicle; #*P*<0.05 vs ACM.

associated with obesity and metabolic syndrome. Current advances in the understanding of adipose tissue biology and its endocrine function have provided insights into mechanisms involved in adiposity-related cardiovascular diseases. Our results identify chemerin as a new vasoactive adipokine that plays an important role in molecular and cellular processes associated with vascular injury and dysfunction. Our data could have major significance in clinical medicine, particularly in the understanding of the role of adipocyte-derived factors in obesity-associated vascular dysfunction and may represent a new target in adiposity-related diseases.

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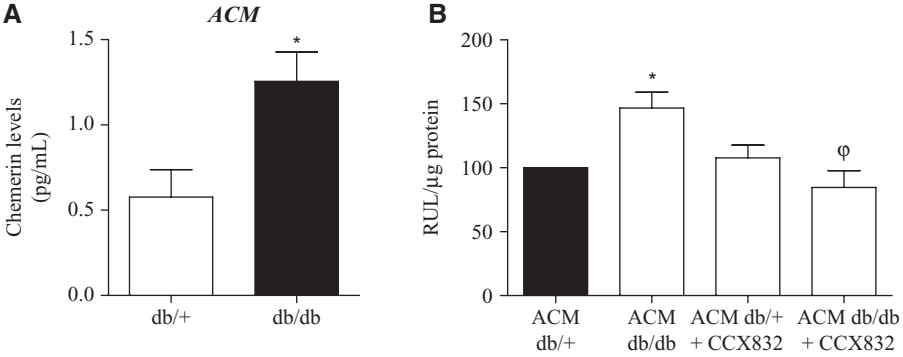

Figure 6. Adipocyte-derived chemerin increases reactive oxygen species (ROS) generation in vascular smooth muscle cell (VSMC) from mice. VSMC from C57BL6 mice were stimulated with adipocyte conditioned medium (ACM) from db/+ and db/db mice. Chemerin levels in ACM were measured by ELISA (**A**), and ROS generation was measured by lucigenin (**B**). Lucigenin assay (**B**) was performed in the presence or absence of chemerin receptor antagonist (CCX) 832 added 30 minutes before stimulation with ACM (1 hour). Values were normalized by protein amount. Bars represent the mean±SEM of 5 experiments. **P*<0.05 vs db/+; φ*P*<0.05 vs ACM db/db.

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Disclosures

None.

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Novelty and Significance

What Is New?

- This study demonstrates that chemerin plays an important role in molecular and cellular processes associated with vascular injury and dysfunction through reactive oxygen species generation.

What Is Relevant?

- Chemerin stimulates NADPH oxidases–derived reactive oxygen species generation, leads to proinflammatory responses in endothelial cells, and induces proliferation and apoptosis in vascular smooth muscle cells.
- Adipocyte-derived factors from obese/diabetic mice stimulate vascular smooth muscle cell reactive oxygen species production and inflammatory responses through chemerin receptor.
- Chemerin induces endothelial dysfunction by downregulation of endothelial nitric oxide synthase and decreasing NO production in endothelial cells.
- These data may contribute to a better understanding of cardiovascular complications in conditions associated with increased adipocyte-derived chemerin, such as in obesity, metabolic syndrome, and hypertension.

Summary

Chemerin is a new vasoactive adipokine that plays an important role in molecular and cellular processes associated with vascular injury and dysfunction through NADPH oxidases–derived reactive oxygen species generation and redox-sensitive MAPK signaling.

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