ORIGINAL ARTICLES

Induction of chemokine expression by adiponectin *in vitro* is isoform dependent

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Adiponectin is reported to have both proinflammatory and anti-inflammatory effects. Because adiponectin circulates in isoforms of various sizes and some responses to adiponectin are isoform dependent, it was postulated that the proinflammatory effects of adiponectin may be isoform specific. To test this theory, peripheral blood mononuclear cells (PBMCs), microvascular endothelial cells (MVECs), and human glomerular mesangial cells (HMCs) were treated with high-molecular-weight (HMW) or low-molecular-weight (LMW) recombinant human adiponectin, and chemokine production was measured. The PBMCs were isolated from healthy volunteers by density gradient centrifugation of ethylenediaminetetraacetic acid (EDTA) anticoagulated whole blood through endotoxin-free Ficoll (General Electric Healthcare Bio-Sciences, Uppsala, Sweden). The MVECs were of dermal origin, and the HMCs were isolated from kidneys not suitable for transplantation. Overnight (16 h) incubation with HMW adiponectin (0.01–1 μg/mL for PBMCs; 5–20 μg/mL for MVECs and HMCs) induced a dose-dependent increase in production of monocyte chemoattractant protein-1 and interleukin-8 by PBMCs and MVECs, but it had no effect on HMC chemokine production (n = 3–5). LMW adiponectin at the same concentrations did not induce chemokine production in any of the cell types tested, and it did not block cytokine-induced chemokine production by PBMCs or MVECs (n = 3–5). These *in vitro* data suggested that the HMW adiponectin isoform is proinflammatory. To examine the possibility of a relationship between HMW adiponectin and inflammation *in vivo*, the urine of patients with systemic lupus erythematosus (SLE) and kidney involvement, which was shown previously to contain immunoreactive adiponectin, was examined for the presence of specific adiponectin isoforms by nondenaturing gel electrophoresis. HMW adiponectin was found in the urine of patients with active lupus nephritis. Therefore, HMW adiponectin may contribute to the renal inflammation of SLE. (Translational Research 2009;154:18–26)

Abbreviations: AICAR = 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AMPK = AMP-kinase; EGM = endothelial growth media; ELISA = enzyme-linked immunosorbent assay; HMC = human mesangial cells; HMW = high molecular weight; IL-1β = interleukin-1β; LMW = low molecular weight; MCP-1 = monocyte chemoattractant protein-1; mRNA = messenger RNA; MVEC = microvascular endothelial cell; NF-κB = nuclear factor-kappa B; PBMC = peripheral blood mononuclear cell; RPMI = Roswell Park Memorial Institute; RT-PCR = reverse transcription-polymerase chain reaction; SLE = systemic lupus erythematosus; TNFα = tumor necrosis factor alpha

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AT A GLANCE COMMENTARY

Background

Adiponectin may have a beneficial role in metabolic syndrome and cardiovascular disease, possibly through anti-inflammatory actions. However, adiponectin may also be proinflammatory and drive inflammation in certain autoimmune diseases. We previously showed that adiponectin upregulates proinflammatory chemokine expression, and this current article examines whether this effect is caused by a specific isoform.

Translational significance

In this article, we show that high-molecular-weight (HMW) adiponectin is the proinflammatory isoform. The potential relationship to clinical disease is illustrated by our data on the appearance of HMW adiponectin in the urine of patients who have active lupus nephritis. HMW adiponectin may contribute to ongoing kidney inflammation in lupus by inducing chemokine production by interstitial renal monocytes, which thereby promotes additional infiltration of inflammatory cells into the kidneys.

Adiponectin is a 30-kDa adipocyte-derived cytokine (adipokine) that has been implicated in the regulation of metabolic disorders such as obesity and diabetes mellitus.[1-7] Plasma adiponectin circulates at concentrations of 3–20 μg/mL as homotrimers, hexamers, and high-molecular-weight (HMW) structures.[8,9] These isoforms are stable *in vivo* and do not interconvert.[10] The metabolic effects of adiponectin seem to be mediated by 2 specific receptors, AdipoR1 and R2.[11-14] R1 signals through the activation (ie, phosphorylation) of AMP-activated protein kinase (AMPK).[15]

The physiologic effects of adiponectin may be isoform dependent.[9,16] For example, hexamers and HMW isoforms can activate the transcription factor nuclear factor-kappa B (NF-κB), but trimers cannot.[9,16] Conversely, in skeletal muscle, trimers, but not higher order structures, induce AMPK.[16]

While performing an analysis of the urine proteome in patients with systemic lupus erythematosus (SLE) and glomerulonephritis, we identified adiponectin as one of the most abundant cytokines present in the urine of patients with active SLE nephritis.[17] Plasma adiponectin levels were also significantly higher in patients with SLE nephritis than healthy individuals or patients with nonrenal SLE.[17] A preliminary longitudinal analysis of patient flare cycles showed that urine adiponectin increased significantly at renal but not nonrenal flare.

Initially, these puzzling lupus data were difficult to reconcile with the known metabolic effects of adiponectin. However, in addition to metabolic regulation, adiponectin was shown recently to modulate inflammation; an effect that could be relevant to an inflammatory disease such as SLE. Initial studies suggested that adiponectin is anti-inflammatory because it blocked proinflammatory cytokine production, decreased leukocyte adherence, and increased production of anti-inflammatory cytokines.[18-21] Several of these studies used bacterially produced recombinant adiponectin that is not representative of the multimeric isoforms found in the human circulation.[22] In contrast, using human recombinant adiponectin prepared in mammalian cells, we and others have shown that adiponectin may actually be proinflammatory. It can induce the production of the proinflammatory chemokines and cytokines monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), IL-1β, tumor necrosis factor alpha (TNFα), and IL-6 *in vitro* in various tissues.[23-28] Interestingly, adiponectin itself can be induced in several cell types by cytokines known to be expressed in SLE nephritis, such as interferon-γ and TNFα, which suggests that production is not restricted to adipose tissue.[29-32]

Because circulating adiponectin is composed of different sized isoforms, and evidence suggests that at least some of its effects are isoform specific,[16,33,34] we postulated that the proinflammatory effects of adiponectin are size dependent. Specifically, we postulated that the HMW isoforms, but not low-molecular-weight (LMW) adiponectin, induce the expression of the proinflammatory chemokines MCP-1 and IL-8. This hypothesis seemed reasonable, given the fact that HMW, but not LMW, adiponectin can active NF-κB, and both MCP-1 and IL-8 are NF-κB dependent.[35] The current study was undertaken to test this hypothesis. Furthermore, because MCP-1 and IL-8 are involved in the pathogenesis of kidney injury during lupus nephritis,[36] we sought *in vivo* evidence that proinflammatory, HMW adiponectin was present in the kidneys of patients with active SLE nephritis.

METHODS

Cell culture and treatments. Human peripheral blood mononuclear cells (PBMCs) were isolated from healthy volunteers by density gradient centrifugation of ethylenediaminetetraacetic acid anticoagulated whole blood through endotoxin-free Ficoll-Paque Plus (Amersham, Arlington Heights, Ill). PBMCs (5×10^6) were plated in Roswell Park Memorial Institute (RPMI) 1640 overnight to allow the monocytes to adhere. After this incubation, the cultures were washed 3 times with fresh