Adiponectin Specifically Increased Tissue Inhibitor of Metalloproteinase-1 Through Interleukin-10 Expression in Human Macrophages

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Background—Vascular inflammation and subsequent matrix degradation play an important role in the development of atherosclerosis. We previously reported that adiponectin, an adipose-specific plasma protein, accumulated to the injured artery and attenuated vascular inflammatory response. Clinically, high plasma adiponectin level was associated with low cardiovascular event rate in patients with chronic renal failure. The present study was designed to elucidate the effects of adiponectin on matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) in human monocyte-derived macrophages.

Methods and Results—Human monocyte-derived macrophages were incubated with the physiological concentrations of human recombinant adiponectin for the time indicated. Adiponectin treatment dose-dependently increased TIMP-1 mRNA levels without affecting MMP-9 mRNA levels. Adiponectin also augmented TIMP-1 secretion into the media, whereas MMP-9 secretion and activity were unchanged. Time course experiments indicated that TIMP-1 mRNA levels started to increase at 24 hours of adiponectin treatment and were significantly elevated at 48 hours. Adiponectin significantly increased interleukin-10 (IL-10) mRNA expression at the transcriptional level within 6 hours and significantly increased IL-10 protein secretion within 24 hours. Cotreatment of adiponectin with anti–IL-10 monoclonal antibody completely abolished adiponectin-induced TIMP-1 mRNA expression.

Conclusions—Adiponectin selectively increased TIMP-1 expression in human monocyte-derived macrophages through IL-10 induction. This study identified, for the first time, the adiponectin/IL-10 interaction against vascular inflammation. (Circulation. 2004;109:2046-2049.)

Key Words: proteins ■ glycoproteins ■ metalloproteinases ■ interleukins ■ inflammation

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dantly present. This study was designed to elucidate the effects of adiponectin on expression of MMPs and TIMPs in human monocyte-derived macrophages (HMMs).

Methods

Materials

Mononuclear cells and recombinant human adiponectin were prepared as previously reported. We used recombinant human interleukin-10 (IL-10) (PeproTech, Inc.), monoclonal anti-human IL-10 antibody (Genzyme Technne), and mouse IgG (Sigma). TIMP-1, MMP-9, and IL-10 concentrations of media were measured with enzyme-linked immunosorbent assay (ELISA) kits (Biotrac).

Gelatinolytic Zymography

Analysis of MMP-9 activity was performed by zymography according to the manufacturer’s protocol (TEFCO).

Northern Blot Analysis

Total RNA was prepared by RNA-Isolation kit (GIBCO) and treated with DNase I, then electrophoresed and transferred to a nylon membrane as previously described. The membranes were hybridized with human TIMP-1 and MMP-9 cDNA probes labeled with \( ^{32}P \) dCTP.

Reverse Transcription–Polymerase Chain Reaction

cDNA was produced with the use of the ThermoScript reverse transcription–polymerase chain reaction (RT-PCR) system (Invitrogen). Real-time PCR was performed as previously described. Primers were 5'-CCTGTTGTCGCTGGCTGA-3' and 5'-CATAACGCTGGTATAAGGTGGTCTG-3' for human TIMP-1, 5'-GCTACACCTCAGACTGGACAG-3' and 5'-TGCCGGATG-CCATTACAC-3' for human TIMP-9, 5'-CTTGCTGGAGGACTTTAGGGTT-3' and 5'-GGAGTGTCACATGCCCTTG-3' for human IL-10, and 5'-CAATGACCCCTTCATTGACCTC-3' and 5'-AGCATGCCCCCATCTGT-3' for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The mRNA levels of target genes were divided by those of GAPDH, a standard control gene, and normalized.

Cell Transfection and Measurement of Luciferase Activity

Human IL-10 promoter fragment (from -1044) was subcloned into the luciferase reporter vector with pGL3-Basic (Promega). For the transfection study, a human monocytic cell line (THP-1 cells; Riken Gene Bank) was used. Equivalent transcriprional efficacy was confirmed by cotransfecting the Renilla luciferase control vector, pRL-TK (Promega). THP-1 cells were transfected by the DEAE-dextran sulfate method, as previously reported. After transfection, the cells were incubated with RPMI-1640 supplemented with 10% fetal calf serum for 18 hours and then treated with or without 30 \( \mu \)g/mL adiponectin for 6 hours. Luciferase activity was measured with a dual luciferase assay kit (Promega) and a luminometer.

Statistical Analysis

Data are presented as mean±SD. Differences were analyzed by Student unpaired \( t \) test. Between-group comparison of means was performed by ANOVA, followed by \( t \) tests. A level of \( P<0.05 \) was accepted as statistically significant.

Results

Effect of Adiponectin on MMP and TIMP Expression

Adiponectin treatment at 30 \( \mu \)g/mL for 48 hours significantly increased TIMP-1 mRNA levels in HMMs, whereas MMP-9 mRNA levels were unchanged, as shown by Northern blot analysis (Figure 1A). MMP-1, -2, and -3 and TIMP-2 expression levels were low and were unchanged with adiponectin treatment in HMMs (data not shown). We next investigated the TIMP-1 and MMP-9 protein levels secreted into the media. Adiponectin also augmented secreted levels of TIMP-1 without affecting secreted levels of MMP-9 (Figure 1B). Zymogram study revealed that adiponectin had no effect on MMP-9 activity (Figure 1C). The quantitative RT-PCR revealed that adiponectin dose-dependently increased TIMP-1 mRNA levels without affecting MMP-9 mRNA levels (Figure 1D). Time course experiments indicated that TIMP-1 mRNA levels started to increase at 24 hours of incubation with adiponectin and were significantly elevated at 48 hours of treatment. The MMP-9 mRNA levels were not changed over this period (Figure 1E).

Adiponectin Increased TIMP-1 Expressions via Upregulating IL-10 Expression

We next investigated the effect of adiponectin on IL-10 because the antiinflammatory cytokine was reported to in-
Adiponectin rapidly upregulated IL-10 and subsequently increased TIMP-1 levels in HMMs. This effect of adiponectin is specific to HMMs among vascular component cells. It is generally accepted that MMPs and TIMPs play a crucial role in arteriosclerosis and plaque disruptions. The balance between MMPs and TIMPs determines the actual metalloproteinase activities and controls the extracellular matrix degradation. We focused on MMP-9 and TIMP-1 because they have been dominantly secreted from HMMs. In this study, adiponectin selectively increased the expression of TIMP-1 in both mRNA and protein levels, whereas the mRNA, protein levels, and activities of MMP-9 were not changed in HMMs.

We have reported that adiponectin suppressed stimulated vascular cellular response in vitro, and overexpression of adiponectin with recombinant adenovirus suppressed the development of atherosclerosis in apolipoprotein E–deficient mice. However, adiponectin-inducible molecules have not been identified. In the present study we found that adiponectin selectively upregulated TIMP-1 expression in HMMs. TIMP-1 mRNA levels started to increase at 24 hours of incubation with adiponectin, suggesting that adiponectin-stimulated TIMP-1 induction was an indirect effect. Because IL-10 was reported to increase TIMP-1 expression without changing MMP-9 expression in HMMs, we next focused on IL-10.

Adiponectin has a variety of anti-inflammatory functions against atherosclerosis. Therefore, we hypothesized that adiponectin may modulate the inflammatory response through a multifunctional paracrine factor, IL-10. Adiponectin increased IL-10 mRNA expression within 6 hours. This effect preceded TIMP-1 mRNA expression, anti–IL-10 monoclonal antibody completely blocked adiponectin-induced TIMP-1 mRNA expression. Moreover, the promoter activity of human IL-10 was significantly increased by adiponectin treatment. These data suggest that adiponectin-induced IL-10 production is at least partly due to the enhanced IL-10 transcription in HMMs. Clinically, both hypoadiponectinemia and low IL-10 plasma concentration are independently reported to be associated with acute coronary syndrome. These findings suggest the importance of the adiponectin/IL-10 interaction against vascular inflammation in vivo, although further study will be necessary to elucidate the precise mechanism in vivo.

In conclusion, adiponectin selectively increased TIMP-1 expression in HMMs through IL-10 induction. The adiponectin/IL-10 interaction will provide important information for understanding the pathogenesis of atherosclerosis.

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