Monocyte CD147 is induced by advanced glycation end products and high glucose concentration: possible role in diabetic complications

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1 Discipline of Medicine and Bosch Institute, University of Sydney, Sydney; 2 Department of Endocrinology, Royal Prince Alfred Hospital, Sydney; 3 Diabetes Centre, Royal Prince Alfred Hospital, Sydney; and 4 Liver Cell Biology, Centenary Institute and the A. W. Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital, Sydney, New South Wales, Australia

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Bao W, Min D, Twigg SM, Shackel NA, Warner FJ, Yue DK, McLennan SV. Monocyte CD147 is induced by advanced glycation end products and high glucose concentration: possible role in diabetic complications. Am J Physiol Cell Physiol 299: C1212–C1219, 2010. First published September 1, 2010; doi:10.1152/ajpcell.00228.2010.—CD147 is a highly glycosylated transmembrane protein that is known to play a role in regulation of many protein families. It has the unique ability to maintain functional activity in both the membrane bound state and in the soluble form. CD147 is known to play a role in regulation of matrix metalloproteinase (MMP) expression, but whether its expression is affected by the diabetic milieu is not known, and its role in regulation of monocyte MMPs in this environment has not been investigated. Therefore, in this study we investigated the effect of advanced glycation end products (AGEs) and high glucose (HG; 25 mM), on monocyte CD147 expression. Culture of THP-1 monocytes in the presence of AGEs or HG significantly increased CD147 at the gene and protein level. THP-1 cell results were confirmed using freshly isolated monocytes from human volunteers. The effect of AGEs and HG on CD147 expression was also mimicked by addition of proinflammatory cytokines. Addition of AGEs or HG also increased expression of monocyte MMP-1 and MMP-9 but not MMP-2. This increase in MMPs was significantly attenuated by inhibition of CD147 using either a small interfering RNA or an anti-CD147 antibody. Inhibition of NF-κB or addition of antibodies to either TNF-α or the receptor for AGE (RAGE) each significantly prevented in a dose-dependent manner the induction of CD147 gene and protein by AGE and also decreased MMP-1 and MMP-9. This novel result shows that AGES can induce monocyte CD147 expression, an effect mediated by inflammatory pathways and RAGE. Because MMPs play a role in monocyte migration, inhibition of their regulator CD147 may assist in the prevention of diabetic complications, particularly those where monocyte infiltration is an early initiating event.

The expression of the MMPs, in particular the collagenase MMP-1 and the gelatinases, MMP-2 and -9, is known to be increased when monocytes are differentiated to form macrophages (14, 24). Furthermore, the MMP increase during the differentiation process is in part regulated by CD147, also known as extracellular matrix metalloproteinase inducer (EMMPRIN) or basigin (28). CD147 was originally described in tumor cells (2, 29), and its expression is induced in pathological conditions that involve dysregulation of proteolysis and tissue degradation, including atherosclerosis and liver fibrosis (21, 27, 29). It is a highly glycosylated 58-kDa transmembrane protein that in humans is expressed as four differentially spliced isoforms (called basingins 1–4) (1). Each of the CD147 isoforms is variably glycosylated, and this affects its molecular weight and its function (1, 10, 11). CD147 has many functions, but its role in the induction of MMPs is the most well characterized. In vitro, in tumor cells and macrophages, homophilic CD147-CD147 interactions activate signaling pathways and induce MMP expression (27). In vivo, increased CD147 expression has been associated with increased MMP expression and activities in atherosclerotic plaques (21, 27, 28) and more recently, increased expression of CD147 on circulating leukocytes correlated with increased coronary artery disease (18). Other in vitro studies have shown that proatherogenic stimuli such as low-density lipoproteins (LDL) also stimulate monocyte CD147 and MMP expression (20, 21).

Although the mechanism is not clear, monocytes tend to be activated by the diabetic milieu to increase migration (15), and exposure to high glucose concentration has been shown to induce monocyte MMP expression and activities (3). Prolonged exposure to hyperglycemia also leads to the formation of advanced glycation end products (AGEs). These products are involved in the tissue damage associated with diabetic complications and aging. They act by binding to a cell surface receptor called RAGE to induce oxidative stress and proinflammatory responses. Whether AGES and high glucose concentration can affect monocyte CD147 to induce monocyte expression of MMPs, in particular MMP-1, -2, and -9, is not known and was investigated in this study. The mechanism of AGES to induce CD147 and the role of CD147 in the regulation of monocyte MMPs were also examined.

MATERIALS AND METHODS

Cell Culture Studies

The human monocyte cell line THP-1 (American Type Culture Collection, Manassas, VA) cells were maintained in RPMI media containing...
### Table 1. Primer sequences

<table>
<thead>
<tr>
<th>Primer Sequence</th>
<th>Forward (5'→3')</th>
<th>Reverse (5'→3')</th>
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<tr>
<td>β-Actin</td>
<td>GAATTCTGGGCGAGGCTGCCGCTTCCAGCT</td>
<td>AAGCTTTTTCGTGGATSCCAGACT</td>
</tr>
<tr>
<td>28S RNA</td>
<td>TGGAAAATCCGGGGGAGAA</td>
<td>ACCTGTCCCAAGACATCCAG</td>
</tr>
<tr>
<td>MMP-1</td>
<td>CTGGTTACGGGACAGAATGCTT</td>
<td>TTGGACCTACACGATACATTT</td>
</tr>
<tr>
<td>MMP-2</td>
<td>TGGATTGAGAAAAAGGAGAGTG</td>
<td>GCCGGAGGGAGGAGAGAGT</td>
</tr>
<tr>
<td>MMP-9</td>
<td>CTGCCCACGGGAGAGGCTCTAC</td>
<td>GGTGTAAGGGGAGAGGAT</td>
</tr>
<tr>
<td>Basigin-2</td>
<td>GGCGTTAGGATGCTGAGGAC</td>
<td>GGGAGGAAGGAGGAGGTA</td>
</tr>
<tr>
<td>Basigin-3/-4</td>
<td>CGCGTTAGGATGCTGAGGAC</td>
<td>GGGAGGAAGGAGGAGGTA</td>
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MMP, matrix metalloproteinase.

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**Fig. 1.** Effect of advanced glycation end products (AGEs)-bovine serum albumin (BSA), carboxymethyl lysine (CML)-BSA, and glucose on expression of CD147 by THP-1 monocytes. A–C: representative agarose gels show that AGE-BSA (100 μg/ml; A), CML-BSA (2 μg/ml; B), and high glucose concentration (HG; 25 mM glucose; C) all increase gene expression of CD147 isoforms (basigin-2, -3, and -4). THP-1 cells grown in normal glucose concentration (NG; 5 mM glucose) were used as control. β-Actin was used for a loading control. D: results from three individual experiments shown graphically. E: AGE-BSA, CML-BSA, and HG concentration increase soluble CD147. F: AGE-BSA also increased human peripheral blood monocyte CD147 levels. *P < 0.05, **P < 0.01 significantly different from NG control values analyzed by ANOVA.
11 mM glucose and 10% fetal bovine serum (FBS, Invitrogen), at 37°C in 95% air and 5% CO2. For experiments the THP-1 cells were seeded at a density of 0.5 × 10^6/ml in RPMI 1640 medium in normal glucose (NG; 5 mM) in the presence or absence of AGEs from glycated albumin (AGE-BSA; 0.1–100 μg/ml) prepared as described below. In parallel experiments, THP-1 cells were incubated in medium containing high glucose (HG; 25 mM) and serum-free RPMI containing either 1.0 mg/ml bovine serum albumin (BSA, Sigma, St. Louis, MO) or carboxymethyl lysine-bovine serum albumin (CML-BSA; 2 μg/ml, Circulex). To investigate the effect of proinflammatory cytokines on CD147 expression, THP-1 cells were seeded as above before addition of TNF-α (10 ng/ml; Promega G5241), transforming growth factor (TGF)-β1 (1 ng/ml; R&D 240-B), IL-6 (10 ng/ml; Sigma I1395), or PMA (5 ng/ml; Sigma).

After 24 or 48 h the cells and media were collected and centrifuged at 1,000 rpm for 5 min. The media were removed and stored at −20°C for analysis of CD147 and MMP-1 by ELISA, as well as MMP-2 and -9 by gelatin zymography. The cells were washed twice with PBS before either extraction of RNA for measurement of the expression of CD147 isoforms by reverse transcription-PCR (RT-PCR) and MMP gene expression by quantitative real-time PCR or lysis and storage at −80°C for study of CD147 protein by Western immunoblot.

For some studies, human monocytes were isolated from 50 ml of whole blood using an OptiPrep Density Gradient medium (Sigma). By this technique we routinely isolate 1.0–5 × 10^6 cells from 50 ml of whole blood, and the monocyte purity as assessed by flow cytometry analysis and morphology was >90%. The protocol was approved by the Sydney South West Area Health Service Human Research Ethics Committee.

**Preparation of AGE-BSA**

To prepare AGE-BSA, endotoxin-free BSA (Sigma) was dissolved in PBS to 100 mg/ml and incubated at 37°C in the presence or absence of 500 mM d-ribose for 10 days, as described previously (13). The degree of glycation in each solution was assessed by measurement of fluorescence at an excitation wavelength of 370 nm and emission of 440 nm (Spectrofluorimeter model L3000, Hitachi), and the CML content was determined by competitive ELISA (Cycllex, Nagana, Japan), using synthetic Nε-CML as the standard. Incubation of albumin in ribose increased fluorescence by approximately fourfold (AGE-BSA: 98.3 ± 26.3 vs. control albumin: 48.2 ± 12.5) and the CML content by ninefold (AGE-BSA: 9.2 ± 1.4 pmol CML/mg BSA), and in the control albumin, fluorescence was undetectable. The endotoxin levels of the albumin was <1.25 endotoxin units/ml (E-TOXATE, Sigma).

**Measurement of CD147 Expression**

**RT-PCR for measurement of CD147 isoforms.** The effect of glucose, AGE-BSA, and CML-BSA on the expression of CD147 isoforms was measured by RT-PCR as described by Belton et al. (1). Briefly, RNA was extracted with Tri-reagent (Sigma), and the concentration and purity were determined using the NanoDrop 1000 (Thermo Scientific, Wilmington, DE). Total RNA (1 μg) was reverse transcribed to cDNA using Superscript III Reverse Transcriptase (Invitrogen) and was amplified using Redtaq (Sigma) in 20 cycles for CD147 isoform-2 (basigin-2) and 25 cycles for CD147 isoforms 3 and 4 (i.e., basigin-3 and -4). The PCR products were then run on agarose gels (1%), the gels were photographed, and the band intensities were determined using Phoretix Image Analysis Program (Nonlinear Dynamics). Experiments were performed at least three times independently, and results were corrected for the housekeeper gene β-actin.

**Western immunoblot for measurement of cell-associated CD147.** The expression of CD147 in cells was analyzed using Western blot analysis. Whole cells were homogenized in RIPA buffer. Cellular protein was then quantified using the Bio-Rad DC protein assay (Bio-Rad, Hercules, CA), and cell lysates containing 30 μg protein were loaded and run under reducing conditions on 12.5% SDS-PAGE gels (Bio-Rad). Proteins were transferred to Hybond-ECL membrane (Amersham-Pharmacia), and the membranes were blocked (5% skim milk) and washed before overnight incubation with anti-human CD147 (R&D Systems). The membranes were then washed with Tris-buffered saline buffer containing Tween 20 (0.05%) and incubated with peroxidase-conjugated secondary antibody (1:5,000, Sigma). The bands were visualized by chemiluminescence using ECL reagent (Amerham). Band intensities were quantified after digitizing using Phoretix Image Analysis software.

**ELISA for measurement of soluble CD147.** Media CD147 levels were determined by ELISA as previously described (23). Briefly, 96-well plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with anti-CD147 antibody (250 ng/well, R&D Systems). Wells were washed with PBS-T (PBS containing 0.05% Tween 20), and the nonspecific binding sites were blocked by incubation in blocking buffer (3% BSA in PBS) for 1 h at 37°C. Standards or media samples (50 μl per well) were added to each well and incubated (2 h at 37°C) before addition of biotin-conjugated anti-CD147 antibodies (50 μl, 1:5,000; RDI-CD147, Research Diagnostics). After incubation (2 h at 37°C), horseradish-peroxidase-conjugated streptavidin diluted in PBS (50 μl, 1:1,000) was added to each well and incubated (45 min at 37°C). The wells were then washed with PBS-T before the addition of 1-Step Ultra TMB-ELISA substrate solution (50 μl, Pierce). The reaction was stopped by addition of 2M H2SO4 (50 μl), and the absorbance was measured at 450 nm with additional measurement at 650 nm. The absorbance was measured at 450 nm with additional measurement at

![Fig. 2. Effect of proinflammatory cytokines and AGE-BSA on CD147 concentration in cells and in the media. As shown in the representative Western blot (inset) and quantitatively, proinflammatory cytokines and AGE-BSA increase cell-associated CD147 protein (A) and soluble CD147 concentration (B). For the Western blot analysis, GAPDH was used as loading control. The band intensities were quantitated by densitometry, and the results for the glycosylated form (58 kDa) and the nonglycosylated form (27 kDa), respectively, are expressed as a percentage of control (NG) values. TGF-β, transforming growth factor-β. Results are expressed as means ± SD. **P < 0.01, significantly different from NG control values, ANOVA.](http://ajpcell.physiology.org/)
Effect of glucose concentration and AGEs on matrix metalloproteinase mRNA levels

Table 2. Effect of glucose concentration and AGEs on matrix metalloproteinase mRNA levels

<table>
<thead>
<tr>
<th></th>
<th>NG (5 mM)</th>
<th>HG (25 mM)</th>
<th>AGE-BSA (100 μg/ml)</th>
<th>CML-BSA (2 μg/ml)</th>
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<tr>
<td>MMP-1</td>
<td>1</td>
<td>1.89 ± 0.18*</td>
<td>9.31 ± 3.31*</td>
<td>2.93 ± 0.45*</td>
</tr>
<tr>
<td>MMP-2</td>
<td>1</td>
<td>0.95 ± 0.12</td>
<td>0.87 ± 0.32</td>
<td>1.18 ± 0.21</td>
</tr>
<tr>
<td>MMP-9</td>
<td>1</td>
<td>2.28 ± 0.30*</td>
<td>7.20 ± 1.19*</td>
<td>2.03 ± 0.15*</td>
</tr>
</tbody>
</table>

Values are means ± SD expressed as fold increase from control. AGEs, advanced glycation end products; NG, normal glucose; HG, high glucose; BSA, bovine serum albumin; CML, carboxymethyl lysine. *P < 0.01, significantly different from NG (t-test).

Investigation of the Mechanism of Induction of CD147 by AGE-BSA

To determine whether the induction of CD147 by AGE-BSA was mediated via activation of inflammatory pathways, we first confirmed that AGE-BSA induced NF-κB family members using the TransAM NF-κB Transcription Factor Assay Kit (Active Motif, Carlsbad, CA). The effect of inhibition of NF-κB, TNF-α, or activation of the AGE receptor RAGE on monocyte expression of CD147 was then investigated. For each of these studies, cells were pretreated with either the NF-κB inhibitor BAY 11 (0.15 and 0.3 μM) or the antibodies to TNF-α (2.5, 5.0, 10 μg/ml, R&D Systems) or RAGE (10–20 μg/ml, R&D Systems) for 1 h before the addition of AGE-BSA (100 μg/ml). Cells incubated in the absence of inhibitor, antibody, or in the presence of either DMSO or mouse IgG (10 and 20 μg/ml, respectively) were used as control. For all studies the cells and media were collected after 24 h for later studies.

Role of CD147 in Regulation of MMP Expression and Activities

The role of the increased CD147 in induction of monocyte MMPs was examined by overexpression and inhibition of monocyte CD147 expression. To induce expression of CD147, the adeno-associated virus (AAV) Helper-Free system (Stratagene, La Jolla, CA) was used to produce recombinant AAV particles containing CD147 expression cassettes. For these studies, CD147 was excised from the PCMV-SPORT6 vector (Open Biosystems, Huntsville, AL) and inserted into the pAAV-IRES-hGFP vector. THP-1 cells were plated at 5 × 10^5 cells/ml in 2 ml of DMEM containing 10% FBS in six-well plates, 24 h before transfection. The plasmids (recombinant pAAV expression plasmid, pAAV-RC, and pAAV-Helper, each 2 μg DNA) were mixed with 0.3 mM CaCl_2 and 2× Hanks’ balanced salt solution (280 mM NaCl, 1.5 mM Na_2HPO_4, and 50 mM HEPES) and incubated with the cells. The medium was changed to serum-free DMEM containing 0.1% BSA 6 h posttransfection, and the cells and medium were harvested after an additional 72 h.

The role of CD147 in the AGE-BSA-induced increase in MMPs was then examined by inhibition of CD147 expression using a siRNA to CD147, and in a parallel experiment, the effect of inhibition of CD147 using a neutralizing antibody was also investigated. The following siRNA oligonucleotides each at 50 nM [sense r(GAUCACUGACUCUGAGGACA)dTdT, antisense r(GUCCUCAGACUCUGAUUCA)dTdT] and scramble control siRNA [sense r(UCCAAGGCAAUCGUAGA)dTdT, antisense r(UCAUCCGAUGCGUUGGArFdTdT)] designed by Qiagen (Valencia, CA) were transfected into THP-1 cells (12.5 × 10^4 cells/ml–1) using Lipofectamine RNAiMAX (Invitrogen) as follows. After incubation for 24 h at 37°C, the media were changed to media containing AGE-BSA (100 μg/ml) and 24 h later, the medium and cells were collected and stored at −80°C for analysis of CD147 and MMPs.

Measurement of Matrix Metalloproteinases

The expression and activity of MMPs were determined at the gene and protein level. To measure MMP gene expression, THP-1 cells were cultured and RNA was extracted, quantified, and reverse tran-
scribed as before. The mRNA levels of the various MMPs and 28S ribosomal RNA as the housekeeper gene were measured by quantitative real-time PCR using specific primers (Table 1) and Platinum Quantitative PCR Supermix-UDG (Invitrogen) using the SYBR fluorophore (Invitrogen). The cycle conditions were as follows: 50°C for 2 min and 95°C for 5 min, followed by 40 cycles of 95°C for 10 s, 54°C for 15 s, and 72°C for 20 s. To control for variations in initial RNA concentration, the values for all samples were corrected for the housekeeper gene 28S, and the results are expressed as a percent change from untreated control cells using the delta-delta method. Each sample was analyzed in triplicate in at least two separate experiments.

Gelatin zymography was used to measure MMP-2 and -9 activity as previously described (16). Briefly, the media (10 μl) were mixed with sample loading buffer and loaded onto polyacrylamide gels (8%) containing 2-methoxy-2,4-diphenyl-3(2H)-furanone (MDPF)-labeled gelatin (10%). After renaturation and equilibration the gels were incubated overnight at 37°C. Band intensities were determined using Phoretix, and results are expressed as percent change from NG. MMP-1 levels were determined by ELISA (R&D Diagnostics) according to the manufacturers’ instructions.

### Statistical Methods

All results are from at least three independent experiments and are expressed as means ± SD. Data sets were analyzed using either t-test or, where appropriate, a one-way ANOVA. Post hoc analysis was performed using Bonferroni’s test for multiple comparisons, and significance was accepted at $P < 0.05$.

### RESULTS

#### The Effect of AGEs and HG on Monocyte CD147 Expression

Monocytes express three predominant CD147 isoforms (known as basigin-2, -3, and -4). As shown in Fig. 1, A–D, addition of HG, AGE-BSA, or CML-BSA significantly increased the expression of all three CD147 isoforms. Similar to the mRNA levels, AGE proteins and HG significantly increased (both $P < 0.05$) the media levels of CD147 (Fig. 1E). A similar effect was seen when isolated human monocytes were treated with AGE-BSA. In these cell media, CD147 concentration increased by 1.6 ± 0.1-fold ($P < 0.05$) compared with untreated controls (Fig. 1F).

#### The Role of Proinflammatory Pathways in Induction of CD147 Expression by AGE-BSA

HG concentration and AGEs can increase expression of proinflammatory cytokines in a variety of cell types (7, 25). AGE-BSA increased monocyte TGF-β1 and TNF-α by 1.9-fold and 5.5-fold, respectively. We next examined the effect of proinflammatory cytokines on THP-1 monocyte CD147 expression. Cells treated with phenyl methyl mercuric acetate (PMA; 1 × 10^{-7} M) were used as positive controls. Results are shown in the representative immunoblot and quantitatively in Fig. 2A. Treatment with either TNF-α or TGF-β slightly increased the nonglycosylated 27-kDa form of CD147 but had differing effects on the higher-molecular-weight (58-kDa form) glycosylated form. The media concentration of CD147 was increased by TNF-α and TGF-β but not IL-6 (Fig. 2B). By Western blot analysis the media from these treatments contained a single band of CD147 at ~58-kDa (not shown).

We next examined whether the effect of AGE-BSA on CD147 was mediated by induction of NF-κB. As shown in Fig. 3A, addition of AGE-BSA increased the expression of the p50, p65, RelB, and cRel subunits of NF-κB. This induction of CD147 was abolished by inhibition of the NF-κB with BAY 11 (Fig. 3B). Inhibition of TNF-α (Fig. 3C) or binding of the AGE-BSA to RAGE (Fig. 3D) using either anti-TNF-α or anti-RAGE antibodies produced similar effects.

![Fig. 4. Effect of induction of CD147 on THP-1 monocyte matrix metalloproteinase (MMP) production](http://ajpcell.physiology.org/)

Fig. 4. Effect of induction of CD147 on THP-1 monocyte matrix metalloproteinase (MMP) production. As shown by the representative gel as well as graphically, overexpression of CD147 in THP-1 monocytes increased the expression of all three CD147 isoforms (A) and increased the activity of pro- and active MMP-9 but not MMP-2 (B). As shown in C, overexpression of CD147 increased media MMP-9 and MMP-1. Results are expressed as a percentage of untransfected THP-1 control cells. **$P < 0.01$ significantly different from untransfected THP-1 control cells.
The Role of CD147 in the HG and AGE-Induced Increase in THP-1 Expression of MMP-1, -2, and -9

In addition to CD147, the MMPs including MMP-2 and -9 can be induced by proinflammatory cytokines and by growth factors (6). In monocytes, addition of TNF-α and IL-6 induced MMP-9 while TGF-β induced MMP-2 (Supplemental Fig. S1; Supplemental Material for this article is available online at the Journal website). We next examined whether HG and AGEs affected monocyte MMPs and the role of CD147 in their induction. HG, AGE-BSA, or CML-BSA all increased the gene and protein levels of MMP-9 and MMP-1 (Table 2 and Supplemental Fig. S2) but had no effect on MMP-2. As shown, the effect of HG was of a lesser magnitude than that seen for AGE-BSA or CML-BSA. A similar pattern was seen for human monocytes (results not shown).

The role of CD147 in the AGE-BSA induction of MMPs in the THP-1 monocytes was next investigated. As shown in Fig. 4A, overexpression of CD147 increased the expression of all three isoforms of CD147 (by 1.5–2.5 fold) and increased the pro- and active forms of MMP-9 and MMP-1 but had no effect on MMP-2 (Fig. 4, B and C). The media concentration of CD147 was also significantly increased (18.69 ± 2.92 ng/ml) when compared with either untransfected cells (2.12 ± 0.22 ng/ml) or cells transfected with empty vector (1.94 ± 0.07 ng/ml). Inhibition of expression of CD147 using a siRNA significantly inhibited the induction of CD147 by AGE-BSA by 70% (Fig. 5A, \(P < 0.05\)) and the increase in MMP-9 protein, by 50%, \(P < 0.05\) (Fig. 5B). Preincubation of THP-1 cells with an anti-CD147 antibody before the addition of AGE-BSA also prevented the induction of MMP-9 and MMP-1 in a dose-dependent fashion (Fig. 5, C and D, respectively), although it was less effective than the siRNA. Treatment of THP-1 monocytes with the CD147 siRNA or the anti-CD147 antibody had no effect on MMP-2.

![Fig. 5](http://ajpcell.physiology.org/)

Fig. 5. The effect of inhibition of CD147 activity on the AGE-BSA-induced increase in THP-1 monocyte MMP levels. THP-1 monocyte CD147 expression was inhibited using either small interfering (si)RNA or an anti-CD147 antibody, and the effect on MMP activities was examined. A: inhibition of CD147 expression using siRNA prevented the AGE-BSA-induced increase in soluble CD147. Scramble siRNA (siCtr) and Lipofectamine (vehicle) were used as internal and vehicle controls, respectively. B: as shown both by the representative gel and graphically, inhibition of CD147 by siRNA decreased MMP-9. C and D: addition of an anti-CD147 antibody attenuated the AGE-BSA-induced increase in MMP-9 (C) and MMP-1 (D). \(*P < 0.05\), \(**P < 0.01\), significantly different from NG control. \#P < 0.01, significantly different from AGE-BSA-treated cells.
We had previously shown that antibodies to TNF-α or RAGE inhibited CD147 expression. We next investigated the effect of inhibition of TNF-α or RAGE on induction of MMP-9 by AGE-BSA. As shown, addition of either the anti-TNF-α antibody (Fig. 6A) or the anti-RAGE antibody (Fig. 6B) significantly prevented the induction of MMP-9 by AGE-BSA.

DISCUSSION

CD147 has been shown to promote the invasion and migration of tumor cells by induction of MMP activities (4), and increased expression of CD147 has been reported when monocytes are differentiated to macrophages (12). However, there are few data regarding the effect of factors altered in a diabetic environment on monocyte CD147 expression. Here we report that AGEs and high glucose can induce CD147 in monocytes. Because there was no change in monocyte morphology or attachment, the induction of CD147 did not appear to be associated with differentiation of the monocytes to macrophages (data not shown). Furthermore, high glucose and AGEs increased expression of the CD147 isoforms (basigin-2, -3, and -4) as well as cell membrane and soluble CD147 levels. These effects were inhibited by inhibition of NF-κB and TNF-α as well as RAGE. The ability of these inhibitors to prevent the induction of CD147 by AGEs suggests a role for this pathway in regulation of CD147 expression. Finally, we show by inhibition and overexpression studies that this effect of AGEs to increase expression and secretion of CD147 is causally related to increased monocyte expression and activities of MMP-9 and -1.

Induction of MMP expression and activity by CD147 is regulated by isoform and glycosylation pattern. For example, it has been reported that cell surface basigin-2 can bind to soluble CD147 by a homophilic CD147-CD147 interaction. The bound ligand is then internalized to interact with basigin-3 and induce MMP expression (1). In our studies, high glucose and AGEs both increased the expression of basigin-2 and basigin-3 as well as the level of soluble CD147. AGEs also increased basigin-4, but the role of this isoform in induction of MMPs is not known. Another factor considered to be important for the induction of MMPs by CD147 is its level of glycosylation (4, 8). Our results show that AGE proteins increased the cellular concentration of the highly glycosylated 58-kDa form as well as the smaller 27-kDa glycoform. This pattern of changes was also seen when the effects of proinflammatory cytokines on monocyte CD147 were examined. TNF-α and the growth factor TGF-β both increased the production of CD147 glycoforms, but to varying levels. Addition of most of the proinflammatory cytokines also increased media levels of CD147.

AGEs and high glucose can induce the expression of proinflammatory cytokines (17, 19, 22) and by this mechanism directly increase expression and activities of MMPs, in particular MMP-9. For this reason we investigated the mechanism of the AGE effect on CD147 expression. Whether the induction of CD147 was responsible for the increase in MMP activities was also examined. Inhibition of NF-κB activation either with the antibody to TNF-α or BAY 11, an inhibitor of TNF-α-inducible phosphorylation of IκBα as well as the anti-RAGE antibody, all decreased the media concentrations of CD147. Furthermore, inhibition of CD147 by each of these mechanisms also partially prevented the AGE-induced increase in MMP-9 and MMP-1. Our studies also showed that addition of TGF-β could induce CD147 protein and glycosylation. Investigation of its effects on monocyte MMP levels showed an induction of MMP-2. Because no effect of AGE or CD147 on monocyte MMP-2 was observed, the possible interaction between TGF-β and TNF-α to induce CD147 was not further studied. Together these results confirm a role for inflammatory pathways in induction of monocyte CD147 by AGEs and also the role of CD147 in induction of MMP expression and activities.

There is increasing evidence for an important role for CD147 in the regulation of monocytes/macrophage MMP expression. CD147 is quantitatively upregulated in vivo in patients with acute myocardial infarction and is normalized with therapy (21). In vitro, proatherogenic stimuli, such as
oxidized LDL, increase monocyte CD147 expression (28), and increased CD147 is associated with increased monocyte migration (26). The present study shows for the first time that the diabetic milieu can increase monocyte CD147 expression. This increase in CD147 can induce monocyte MMPs and may lead to increased migration of monocytes from the circulation to the tissue. Regulation of CD147 expression may provide another mechanism for the transcriptional regulation of MMPs and suggest its potential as a possible therapeutic target.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES