Receptor for advanced glycation end-products (RAGE) modulates neutrophil adhesion and migration on glycooxidated extracellular matrix

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AGEs (advanced glycation end-products) accumulate in collagen molecules during uraemia and diabetes, two diseases associated with high susceptibility to bacterial infection. Because neutrophils bind to collagen during their locomotion in extravascular tissue towards the infected area we investigated whether glycoxidation of collagen (AGE-collagen) alters neutrophil migration. Type I collagen extracted from rat tail tendons was used for in vitro glycoxidation (AGE-collagen). Neutrophils were obtained from peripheral blood of healthy adult volunteers and were used for the in vitro study of adhesion and migration on AGE- or control collagen. Glycoxidation of collagen increased adhesion of neutrophils to collagen surfaces. Neutrophil adhesion to AGE-collagen was inhibited by a rabbit anti-RAGE (receptor for AGEs) antibody and by PI3K (phosphoinositide 3-kinase-3) inhibitors. No effect was observed with ERK (extracellular-signal-regulated kinase) or p38 MAPK (mitogen-activated protein kinase) inhibitors. AGE-collagen was able to: (i) induce PI3K activation in neutrophils, and (ii) inhibit chemotaxis and chemokinesis of chemoattractant-stimulated neutrophils. Finally, we found that blocking RAGE with anti-RAGE antibodies or inhibiting PI3K with PI3K inhibitors restored fMLP (N-formylmethionyl-leucyl-phenylalanine)-induced neutrophil migration on AGE-collagen. These results show that RAGE and PI3K modulate adhesion and migration rate of neutrophils on AGE-collagen. Modulation of adhesiveness may account for the change in neutrophil migration rate on AGE-collagen. As neutrophils rely on their ability to move to perform their function as the first line of defence against bacterial invasion, glycoxidation of collagen may contribute to the suppression of normal host defence in patients with diabetes and uraemia.

Key words: advanced glycosylated end-product (AGE), collagen, defence, neutrophil, phosphoinositide 3-kinase (PI3K), receptor for advanced glycosylated end-products (RAGE).

INTRODUCTION

Patients with diabetes and uraemia have an increased susceptibility to bacterial infection, suggesting that professional phagocytes such as neutrophils are functionally impaired [1,2]. However, the function of neutrophils isolated from these patients and tested in vitro is not greatly defective. Thus it is conceivable that the in vivo environment plays a major role in the dysfunction of neutrophils.

Diabetes and chronic uraemia are characterized by the accelerated generation of AGEs (advanced glycation end-products) [3,4]. AGEs are formed on proteins and peptides by non-enzymatic glycoxidation [5,6]. Glucose, a carbonyl compound present at millimolar concentrations in blood, reacts non-enzymatically with amino groups of proteins to form a Schiff base, which undergoes the Amadori rearrangement. A complex series of poorly understood oxidative reactions converts the Amadori product into various AGEs. Glycoxidation products may induce protein cross-linking, affect protein function and interact with cell-surface receptors, such as RAGE (receptor for AGEs) [7]. AGE accumulation appears to contribute to the pathophysiology of uraemic and diabetic complications [8,9]. Because AGEs are irreversible chemical modifications of proteins, they accumulate with time in long-lived proteins such as collagen. The half-life of skin collagen, for example, has been calculated to be 15 years [10]. Collagen proteins are therefore a major source of AGE accumulation. Several studies have demonstrated high concentrations of the glycoxidation products N-(carboxymethyl)lysine and pentosidine in collagen (AGE-collagen) from patients with uraemia and diabetes [3,11].

Neutrophils rely on their ability to move to perform their function as the first line of defence against bacterial invasion. In response to inflammatory signals, neutrophils quickly leave the blood stream by crossing vascular endothelium and progress across the extravascular space towards the infected area through regulated and reversible interactions of cell adhesion molecules with extracellular matrix components [12]. During migration, neutrophils undergo various modifications which enable them to respond in an adapted way to sequential stimuli and which prime them for their final mission, i.e. the killing of infectious microorganisms. Neutrophils bind to collagen, a major component of the extracellular matrix, during their locomotion in extravascular tissue. Adhesion molecules of the β2 and β1 integrin families have been shown to mediate neutrophil interaction with collagen [13,14].

Because collagen is glycoxidated during uraemia and diabetes, two diseases associated with high susceptibility to bacterial infection, and because neutrophil functions have been shown to
be modulated by AGE-modified proteins [15–22], in the present study we investigated whether AGE-collagen alters neutrophil migration.

**EXPERIMENTAL**

**Preparation of AGE-collagen**

Type I collagen extracted from rat tail tendons, as described previously [13], was used for in vitro glycoxidation. Incubation with 1 M D-glucose (in 50 mM sodium phosphate buffer, pH 7.4) was made under sterile conditions. Control collagen was subjected to the same conditions except that D-glucose was omitted. After incubation for 10 days, preparations were extensively dialysed against PBS/18 mM acetic acid until complete solubilization, lyophilized and conserved at −80 °C. Presence of AGEs was determined using spectrofluorimetry. Briefly, control collagen and AGE-collagen (2 mg/ml) were dissolved in 18 mM acetic acid. As fluorescence is a property of AGEs, samples were submitted to spectrofluorimetry (excitation 340 nm/emission 415 nm) as described previously [18]. Mean fluorescence was 212.25 ± 82.46 units for control collagen and 604.51 ± 57.5 units for AGE-collagen (P < 0.05). Preparations were tested for presence of LPS (lipopolysaccharide), and contained < 0.5 endotoxin unit/ml (Limulus Test; Bio-Whittaker).

**Isolation of human neutrophils**

Peripheral blood was obtained from healthy adult volunteers, following informed consent, by venipuncture into 0.1 ml of EDTA anticoagulant. Neutrophils were prepared as described previously [23] by centrifugation on polymorph Prep (Nycodenz) and suspended at 5 × 106 cells/ml in HBSS (Hanks balanced salt solution) solution without Ca2+ and Mg2+ (Gibco). All procedures were performed with endotoxin-free solutions, and all experiments were completed within 3 h of blood collection. When indicated, cells were first pre-incubated with buffer alone, various inhibitors (10 μM wortmannin, 50 μM LY294002, 40 μM PD98059 and 10 μM SB203580) or antibodies [70 μg/ml rabbit anti-human RAGE) antibody and 70 μg/ml irrelevant rabbit antibody (Dakocytomation)] for 30 min at room temperature (20–21°C). Inhibitors were purchased from Sigma. Optimal concentrations for wortmannin and SB203580 were determined according to previous publications [24,25]. A dose–response curve was performed to detect the optimal dose of PD98059: partial inhibition was detected at 10 μM and complete inhibition at 40 μM. The latter concentration was then employed in all the experiments. Where indicated, F(ab′)2 fragments of the immune IgG to RAGE or non-immune control IgG were used. F(ab′)2 fragments were prepared by pepsin digestion, and optimal concentrations were determined as described previously [19].

**Adhesion assays**

Strong adhesion (spreading) of neutrophils to control collagen or AGE-collagen was tested. 96-well plates were pre-coated with control collagen or AGE-collagen, and allowed to adhere for 30 min at 37 °C. The medium was then discarded and cells were subjected to lysis, at 4 °C, in buffer containing 10 mM Tris/HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 10% (w/v) glycerol, 0.02% sodium azide, 10% (w/v) Brij, 0.1 M orthovanadate and protease inhibitors (Sigma). Immunoprecipitation of PI3K (phosphoinositide 3-kinase) from lysates was done using a phosphotyrosine monoclonal antibody (P-Tyr-100; New England Biolabs). The precipitate, containing PI3K, was done using a phosphotyrosine monoclonal antibody (P-Tyr-100; New England Biolabs). The precipitate, containing PI3K, was

**In vitro kinase assay**

Neutrophils (10 × 106 cells/well) were placed in six-well plates pre-coated with control collagen or AGE-collagen, and allowed to adhere for 30 min at 37 °C. The medium was then discarded and cells were subjected to lysis, at 4 °C, in buffer containing 10 mM Tris/HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 10% (w/v) glycerol, 0.02% sodium azide, 10% (w/v) Brij, 0.1 M orthovanadate and protease inhibitors (Sigma). Immunoprecipitation of PI3K (phosphoinositide 3-kinase) from lysates was done using a phosphotyrosine monoclonal antibody (P-Tyr-100; New England Biolabs). The precipitate, containing PI3K, was used for in vitro formation of radiolabelled PIP3 (phosphatidylinositol 3,4,5-trisphosphate) in the presence of micella (phosphatidylinositol and phosphatidylerine), 2.5 μM ATP and [γ-32P]. The reaction was performed at 37 °C for 15 min and stopped by the addition of 100 μl of 1M HCl and 350 μl of chloroform/methanol (1:1, v/v), followed by centrifugation for 2 min at 3000 g. After two successive washes with 200 μl of methanol/HCl (1:1, v/v), 110 μl was taken from the lower phase of each tube, corresponding to the radiolabelled PIP3, formed in vitro. This sample was then submitted to chromatography on a silica plaque. Radioactivity was revealed by autoradiography of the silica plaque.

**Morphological examination of neutrophils**

Neutrophils were allowed to adhere for 30 min at 37 °C to coverslip chambers coated with control collagen or AGE-collagen (with or without stimulation with 10−8 M fMLP). Cells were then fixed with 4% (v/v) paraformaldehyde for 15 min at room temperature, and permeabilized with 0.05% Triton X-100 for 1 min, before staining with 0.33 μM fluorescein/phalloidin (Molecular Probes) for 45 min at room temperature. Images of each condition were acquired on an inverted fluorescence microscope. Cellular area, a measure of cell spreading, was determined by manually tracing the cell perimeter of the digital image. Determinations were made for 20 cells for each condition.

**Under-agarose assay**

Petri dishes were coated overnight with control collagen or AGE-collagen (250 μg/ml). Agarose [indubiose; 2% (w/v)] was boiled in endotoxin-free sterile saline solution, followed by a 1:1 dilution with a solution containing MEM (minimal essential medium; Gibco), 10% (v/v) filtered plasma, 100 units/ml penicillin and 0.1 mg/ml streptomycin to achieve a final agarose concentration of 1%, which was then distributed in pre-coated Petri dishes. Using a plastic template and a bevelled punch, three 2-mm wells were created, each separated by a distance of 0.5 cm. The central well received 8 μl of prepared neutrophils at 40 × 106 cells/ml. A total of 8 μl of opsonized zymosan (Sigma) was placed in the upper-left well as a chemoattractant and 8 μl of MEM alone in the lower-right well (negative control). Dishes were incubated for 2.5 h at 37°C with 5% CO₂ and then fixed with formalin for 10 min. After removal of agarose, dishes were stained with binding buffer [137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 1.3 mM CaCl₂, 30 mM Hepes, 10 mM glucose and 0.1% BSA (pH 7.4)]. Cells that remained attached to the well were fixed with 1% glutaraldehyde before nuclear staining with 10% (w/v) Crystal Violet for 20 min at room temperature. After extensive washing, residual stain (reflecting the number of adherent cells) was dissolved in 0.5 M acetic acid and the absorbance was measured at 560 nm.
with May–Grunwald–Giemsa. Migration was measured under the microscope: the distance from the outer edge of the centre well to the leading edge of the migratory cells was measured. Both random (chemokinesis; distance towards MEM) and directed (chemotaxis; distance toward zymosan) migration were measured. Directed migration (response to chemoattractant) was defined as: (migration towards chemoattractant) — (migration towards PBS).

**Time-lapse video microscopy**

Neutrophils (5 × 10⁶ cells/ml) in DMEM containing calcium and magnesium were placed on polystyrene 24-well plates precoated with control collagen or AGE-collagen. Plates were incubated in a temperature-controlled chamber (37°C) with 5% CO₂ mounted on an inverted microscope (Axiovert; Zeiss). Spontaneous migration and migration in response to 10⁻⁸ M fMLP were recorded by acquisition of successive images of the wells (each 20 s) over 5 min. Images were used for quantification of migration speed using custom-made software. This software was developed at the INSERM Unit 514 to quantify the speed of migration of cells in two-dimensional cultures. In the first image, 20 cells were randomly selected and semi-automatic tracking of these cells was performed by indicating each cell in the subsequent images in the series. When all of the trajectories had been drawn, the rate of migration of the cells was then calculated using the computer software. Where indicated, migration was studied after pre-incubation with wortmannin or anti-RAGE F(ab)₂.

**Statistical analysis**

The amount of cell adhesion, and cell-surface and cell-migration rates were compared first using a Kruskal–Wallis test. If a difference was observed, a Mann–Whitney test was then performed for a group-by-group comparison. Statistical significance was defined as *P < 0.05, **P < 0.01 and ***P < 0.001.

**RESULTS**

**Adhesion of neutrophils to AGE-collagen is mediated by RAGE and PI3K**

In the initial series of experiments, we examined whether glycoxidation of collagen influences neutrophil adhesion. We found that adhesion of fMLP-activated neutrophils to AGE-collagen was significantly (*P < 0.01) increased when compared with control collagen (Figure 1). Pre-incubation of neutrophils with rabbit anti-RAGE antibodies before fMLP stimulation decreased cell adhesion, suggesting that RAGE was involved in the increased binding of neutrophils to AGE-collagen. We next analysed the role of ERK (extracellular-signal-regulated kinase), p38 MAPK (mitogen-activated protein kinase) and PI3K in fMLP-stimulated cell adhesion to AGE-collagen. The specific PI3K inhibitor wortmannin at 10 nM significantly inhibited neutrophil adhesion stimulated by fMLP. In contrast, no effect was observed with the p38 MAPK (SB20358) or ERK (PD98059) inhibitors (Figure 1).

To determine whether the effect of wortmannin on neutrophil adhesion to AGE-collagen was due to fMLP inhibition, we tested the ability of wortmannin to inhibit neutrophil adhesion to AGE-collagen in absence of stimulation. Adhesion of resting neutrophils to AGE-collagen was increased compared with adhesion to control collagen (Figure 2). Pre-incubation of resting neutrophils with rabbit anti-RAGE antibodies or with wortmannin significantly (*P < 0.01) reduced cell adhesion to AGE-collagen. Taken together, these results indicate that adhesion of resting neutrophils to AGE-collagen involves RAGE and PI3K. To confirm induction of the PI3K signalling pathway, we analysed in vitro PIP₃ biosynthesis in adherent resting neutrophils on AGE-collagen and control collagen. As shown in Figure 3, PIP₃ biosynthesis was increased on AGE-collagen in comparison with control collagen.

**Glycoxidation of collagen modulates migration of fMLP-stimulated neutrophils on the collagen surface**

In response to fMLP activation, neutrophils were morphologically spread on AGE-collagen, whereas they appeared polarized on control collagen (Figure 4A). Determination of cell-surface area confirmed that neutrophil shape was more extended on AGE-collagen than on control collagen, but without reaching statistical significance (Figure 4B). Because glycoxidation of collagen had

![Figure 1 Adhesion of stimulated neutrophils to AGE-collagen](image1)

![Figure 2 Adhesion of resting neutrophils to AGE-collagen](image2)
Figure 3 Activation of PI3K on AGE-collagen

Resting neutrophils, 10 × 10^6 cells/ml for each condition, were incubated in the presence of Ca^{2+} and Mg^{2+} with AGE-collagen or control collagen for 30 min. Cell lysates were then used for immunoprecipitation of phosphotyrosine proteins and in vitro formation of radiolabelled PIP3. P-Labelled lipids were then separated by chromatography on a silica plaque. PI3K activity is related to the amount of PIP3 formed. AGE-collagen greatly enhanced PIP3 formation of resting neutrophils compared with control collagen.

Figure 4 Morphological changes of fMLP-stimulated neutrophils on the collagen-coated surface

(A) Neutrophils were analysed on an inverted microscope 30 min after stimulation with 10^{-8} M fMLP and after fixation with formaldehyde and staining of polymerized actin with phallolidin. fMLP-activated neutrophils on control collagen have a migratory phenotype: cells are polarized with cytoplasmic expansions corresponding to pseudopod and uropod, and accumulation of polymerized actin in the pseudopod. On AGE-collagen, fMLP-activated neutrophils undergo a marked spreading; they are more flattened than on control collagen. Actin polymerization is diffuse. Magnification, ×20. (B) Cellular area was determined by manually tracing the cell perimeter in the digital image. Results are representative of 75% of the cells from more than three independent experiments. The cell surface of fMLP-activated neutrophils was increased on AGE-collagen compared with control collagen, but this was not significant (NS; P = 0.08). AU, arbitrary units.

Figure 5 Neutrophil migration on AGE-collagen

(A) Neutrophil chemotaxis on control-collagen- or AGE-collagen-coated surfaces in response to a chemotactic gradient (zymosan) was measured in an under-agarose assay. Directed migration in response to zymosan was measured after 2.5 h of incubation at 37°C with CO2. Migration in response to a chemotactic gradient was significantly decreased on AGE-collagen (10.6 ± 7.8 mm) compared with control collagen (34.4 ± 6.8 mm) (***P < 0.001). Results are means ± S.D. of three independent experiments, each performed in six replicates. (B) Neutrophil chemokinesis was recorded by time-lapse video microscopy. Neutrophil spontaneous migration rate was slightly increased on AGE-collagen (6.7 ± 1.36 μm/min) compared with native collagen (5.57 ± 0.8 μm/min), but this was not statistically significant. Stimulation with 10^{-8} M fMLP (fMLP) significantly increased neutrophil random migration rate (chemokinesis) on native collagen (11.6 ± 1.9 μm/min) (**P < 0.001), but not on AGE-collagen (8.2 ± 0.4 μm/min). Results are means ± S.D. of nine independent experiments. NS, no significance.

an effect on neutrophil morphologic polarity, we studied chemotaxis and chemokinesis. Directional migration in response to a chemoattractant gradient (chemotaxis) was performed using an under-agarose neutrophil migration assay. In this system, we found that the distance of neutrophil migration to a distant chemoattractant source was significantly (P ⩽ 0.001) reduced on AGE-collagen (10.6 ± 7.8 mm) when compared with control collagen (34.4 ± 6.8 mm) (Figure 5A). fMLP-induced random migration (chemokinesis) of neutrophils placed on the collagen-coated surface were recorded by time-lapse video microscopy. Unstimulated neutrophils had a slightly higher migration rate on AGE-collagen (6.7 ± 1.36 μm/min) than on control collagen (5.57 ± 0.8 μm/min; P value was not significant) (Figure 5B). In the presence of fMLP, cell migration rates were significantly (P < 0.001) increased on control collagen (from 5.57 ± 0.8 to 11.6 ± 1.9 μm/min), but not on AGE-collagen (from 6.7 ± 1.36 to 8.2 ± 0.4 μm/min; P > 0.05). These findings demonstrate that glycoxidation of the collagen surface modulates neutrophil migration. It slightly accelerates migration of resting neutrophils, but mainly blocks chemotaxis and chemokinesis of chemoattractant-stimulated neutrophils.

RAGE and PI3K modulate migration of fMLP-stimulated neutrophils on AGE-collagen

Next we examined whether inhibition of adhesion of neutrophils by anti-RAGE antibodies and PI3K inhibitors influenced migration on AGE-collagen. We found that pretreatment of neutrophils with anti-RAGE F(ab')2 led to a significant (P < 0.01)
RAGE modulates neutrophil migration on AGE-collagen

Figure 6 Role of RAGE on neutrophil migration on AGE-collagen

Effect of RAGE inhibition on neutrophil chemokinesis was studied by time-lapse video microscopy. (A) Neutrophils were pre-incubated with anti-RAGE F(ab)2 or control F(ab)2 (Fab2 Cont) (200 μg/ml) and allowed to migrate on AGE-collagen after stimulation with 10^{-8} M fMLP (FMLP-8). RAGE inhibition with anti-RAGE F(ab)2 led to a significant increase in fMLP-induced migration of neutrophils on AGE-collagen (**P < 0.01), whereas control F(ab)2 had no effect. (B) The same experiment was conducted on control collagen. RAGE inhibition with anti-RAGE F(ab)2 did not modify neutrophil migration on control collagen. Results are means ± S.D. of three independent experiments. NS, no significance.

DISCUSSION

Neutrophils are the first line of defence against infection. They exert bactericidal activity following successive steps, including activation, adhesion, migration and phagocytosis. AGE-proteins have been shown to modulate neutrophil production of ROS (reactive oxygen species), actin polymerization and phagocytosis [16,22]. In the present study, we were interested in the modulation of neutrophil adhesion and migration by glycoxidated collagen (AGE-collagen). We have shown that AGE-collagen modulates neutrophil function: adhesion was enhanced, whereas chemotactic migration was inhibited. RAGE and PI3K were shown to be involved in these processes.

Our present results suggest a very close relationship between adhesion and migration. Indeed, the strong adhesion of fMLP-stimulated neutrophils to AGE-collagen was associated with a lack of migration. Conversely, inhibition of neutrophil adhesion to the AGE-collagen-coated surface by anti-RAGE antibodies or by PI3K inhibitors was associated with an increase in migration rate. Cell motion requires adhesion to the substrate. Cell substratum attachment strength is a central variable governing cell migration speed. If the interaction of the substrate is too weak, the cells cannot gain traction, but, if it is too strong, they become stuck. Between these extremes, adhesive interactions of moderate affinity allow migration [26]. Our present results show that glycoxidation of collagen enhances the strength of adhesion of neutrophils. We hypothesized that this modulation of adhesion strength leads to modulation of the migration rate. As expected, inhibition of the strong adhesion of neutrophils by anti-RAGE antibodies and PI3K inhibitors restores migration on AGE-collagen. Our results are in agreement with previous studies showing that Src-family- and Syk-deficient neutrophils have a defect in integrin-mediated firm adhesion that is associated with an increase in fMLP-induced neutrophil migration [27]. In contrast, treatment of fMLP-activated neutrophils with the actin-polymerizing agent jasplakinolide strengthens neutrophil adhesion to their adhesive surface and considerably reduces their migration rate [28]. These
results suggest that neutrophil migration speed depends on the strength of transient cell substratum attachments. Modulation of adhesiveness may account for the change in neutrophil migration rate on AGE-collagen.

Glycoxidation of proteins, leading to the accumulation of AGEs, is accelerated during uraemia and diabetes. These proteins may behave like pro-inflammatory molecules following the interaction with their receptors. One of the best characterized receptors, RAGE, is a multiligand receptor and a member of the Ig superfamily. Collison et al. [16] have described the presence of RAGE on neutrophil membranes. In the present study, we show that RAGE is involved in the neutrophil adhesion and migration processes. Glycoxidation of collagen modulates neutrophil migration. Spontaneous migration was slightly increased, whereas chemotactic migration was significantly inhibited. The increase in spontaneous migration on AGE-collagen was not statistically significant, but taken together these results are in agreement with previous studies showing that AGE-proteins inhibit transendothelial migration of fMLP-activated neutrophils, whereas they increase transendothelial migration of unstimulated neutrophils [20]. Similar modulation of monocyte migration has been demonstrated with AGE-modified proteins. AGE-proteins increase migration of resting monocytes [19,29,30], but reduce chemotaxis of fMLP-activated monocytes by a RAGE-dependent pathway [19]. Our results show that RAGE-dependent modulation of strong adhesion may account for these variations in migration rate.

We have shown that PI3K is also involved in neutrophil adhesion and migration on AGE-collagen. RAGE signalling is usually mediated by activation of ERK, but activation of the p38 MAPK signalling pathways has also been reported [31–34]. Until recently, PI3K was not a classical signalling pathway involved in RAGE activation; however, Vincent et al. [35] have recently reported a direct activation of the PI3K pathway by RAGE in dorsal root ganglia neurons in response to stimulation with S100/calgranulin. That study shed light on the possible activation of PI3K by RAGE. Another hypothesis to explain PI3K activation in neutrophils during adhesion to AGE-collagen could be related to integrin engagement. Neutrophil adhesion to collagen is a multicomponent process including integrin ligation and post-binding events such as cell spreading. Cell spreading allows stronger adhesion. It is a fundamental event in which the contact area with a solid substrate increases because of actin polymerization. During this process, actin dynamics are coupled to the activation of PI3K, an enzyme that is stimulated by clustering of β1 and β2 integrins [36–38]. One may speculate that RAGE regulates integrin-mediated adhesion to AGE-collagen and that subsequent PI3K activation induced by integrin engagement reinforces neutrophil adhesion to AGE-collagen.

We tested the effect of PI3K inhibitors on chemokinesis. PI3K inhibition has been shown to partially reduce directed migration (chemotaxis) but not random migration (chemokinesis) of fMLP-activated neutrophils [39–41]. In agreement with these results, we confirmed in the present study that PI3K inhibition did not affect fMLP-induced chemokinesis on control collagen. However, we found that PI3K inhibition greatly enhanced fMLP-induced neutrophil chemokinesis on AGE-collagen. These results show that PI3K activation on AGE-collagen is a key step in the modulation of adhesion and migration. However, our results do not allow any conclusion on the mechanism leading to activation of the PI3K pathway. Studies are underway to establish whether the connection between RAGE and PI3K pathways is direct or secondary to integrin engagement.

Accelerated glycoxidation of collagen has been observed in patients with uraemia and diabetes [3,11]. Our present results show that AGE-collagen modulates neutrophil adhesion and migration in vitro. Animal studies are needed to demonstrate that this phenomenon is also true in vivo. As the directed motion of neutrophils to sites of infection is essential for protection against microbial infections, one may speculate that intensive glycoxidation of collagen is one mechanism that may contribute to the increased incidence of bacterial infections within the uraemic and diabetic population.

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