Chapter 8

Advanced Glycation Endproducts and Vascular Inflammation:

Implications for Accelerated Atherosclerosis in Diabetes

Giuseppina Basta*, Ann Marie Schmidt# and Raffaele De Caterina*°

*CNR Institute of Clinical Physiology, Pisa, Italy

#Columbia University, New York, NY

°Chair of Cardiology, "G. d'Annunzio" University, Chieti, Italy

Address for correspondence:

Raffaele De Caterina, MD, PhD
Chair of Cardiology
"G. d'Annunzio" University - Chieti
C/o Ospedale S. Camillo de Lellis
Via Forlanini, 50
I-66100 Chieti, Italy
Tel. +39-0871-41512
Fax: +39-0871-402817
E-mail: rdecater@unich.it

Abstract

The formation of advanced glycation endproducts (AGE) is an important biochemical abnormality that accompanies diabetes mellitus and, likely, inflammation. This chapter will summarize and discuss recent studies indicating that the effects of AGE on vessel wall homeostasis may account for the rapidly progressive atherosclerosis associated with diabetes mellitus. Driven by hyperglycemia and oxidant stress, AGE form to a greatly accelerated degree in diabetes. Within the vessel wall, collagen-linked AGE may "trap" plasma proteins, quench nitric oxide activity, and interact with specific receptors to modulate many cellular properties. On plasma low-density lipoproteins (LDL), AGE initiate oxidative reactions that promote the formation of oxidized LDL. Interaction of AGE with endothelial cells and other cells accumulating within the atherosclerotic plaque, such as mononuclear phagocytes and smooth muscle cells, provides a mechanism to augment vascular dysfunction. Specifically, the interaction of AGE with vessel wall components increases vascular permeability, expression of procoagulant activity, and the generation of reactive oxygen species, resulting in increased endothelial expression of endothelial leukocyte adhesion molecules. We propose that AGE potently modulate the initial steps in atherogenesis involving blood-vessel wall interactions, triggering an inflammatory-proliferative process and, furthermore, critically contribute to propagation of inflammation and vascular perturbation in established disease. Thus, a better understanding of the biochemical mechanisms by which AGE contribute to such processes in the vessel wall could be relevant to devise preventive and therapeutic strategies for diabetic atherosclerosis.

Introduction

Both type-1 and type-2 diabetes are powerful and independent risk factors for coronary artery disease, stroke, and peripheral arterial disease.[1,2]

Accelerated arterio- and atherosclerosis, as well as microvascular disease, are the major vascular complications of diabetes mellitus, constituting the main cause of morbidity and mortality in this common metabolic disorder. Atherosclerosis accounts for virtually 80% of all deaths among North American diabetic patients compared with one third of all deaths in the general population. Thus, prevention and treatment of chronic vascular disease is a central therapeutic problem in diabetes.

The primary causal factor leading to the pathophysiologic alterations in the diabetic vasculature is chronic exposure to high levels of blood glucose.[3,4] Data from the Diabetes Control and Complications Trial (DCCT), comprising two multicenter, randomized, prospective controlled clinical studies[3,5] definitely established a causal relationship between chronic hyperglycemia and diabetic microvascular disease long inferred from a variety of animal and clinical studies.[6] The update in 2003 from the DCCT and Epidemiology of Diabetes Interventions and Complications Research Group indicated that subjects treated early by intensive glycemic control displayed smaller carotid intima-media thickness than those treated with standard therapies.[7] Several other reports also support a relationship between chronic hyperglycemia and diabetic macrovascular disease in non-insulin-dependent diabetes mellitus patients.[8-10] Thus, prolonged exposure to hyperglycemia is the primary factor for the development of diabetes-specific vascular disease, although the relationship between deranged glucose metabolism and arterial disease is

complicated by many other factors that often coexist in diabetes, including hypertension, dyslipidemia and genetic determinants of tissue response to injury.[11]

Currently, three major mechanisms may explain the links between hyperglycemia and vascular complications, including: the increased intracellular oxidative stress induced by hyperglycemia itself, resulting in protein kinase C (particularly the beta isoform) activation and subsequent activation and nuclear translocation of the transcription factor nuclear factor (NF)-κB, leading to enhanced intracellular reactions;[12] increased activity of the sorbitol-aldose reductase pathway; [13] and formation of advanced glycation endproducts (AGE).[14,15] Although multiple studies support the direct adverse effects of glucose itself in modulating cellular properties,[16,17] the most important mechanism involved in the complex series of reaction associated with accelerated atherosclerosis in diabetes is the increase in non-enzymatic glycation of proteins and lipids, with the irreversible formation and deposit of reactive AGE. Indeed, recent studies by Brownlees's group suggest elevated levels of glucose that trigger these processes and indirectly cause increased intracellular generation of superoxide anion and other radicals.[12] This chapter specifically focuses on proposed mechanisms by which AGE accumulate in the extracellular space and within cells of the vessel wall, thus contributing to accelerated atherosclerosis in diabetes.

The early steps in atherosclerosis leading to plaque formation, extensively discussed in other chapter in this book (chapters 1-2, 5-6), involve alterations of blood-vessel wall interactions that initiate an inflammatory-proliferative process and comprise a number of interrelated "inflammatory" events.[18,19] First, a change of the normal function of the vascular endothelium

occur, including alterations in permeability and in adhesive properties towards circulating blood cells,. Other important steps in this process include entrapment of low-density lipoproteins (LDL) within the intima and their oxidative modification; migration of blood-borne monocytes into the intima and their conversion to activated macrophages by oxidized LDL; and the release of cytokines and proteases. In the intima, activated macrophages increase their lipid uptake, leading to the formation of "foam" cells characteristic of the early atherosclerotic lesion, the so-called "fatty streak." Secondly, a population of activated smooth muscle cells (SMC) migrates into the intima at sites of vascular lesion and proliferate, producing new extracellular matrix. Such events also concur to the formation of more advanced atherosclerotic lesions characterized by a “fibrous cap” covering an enlarged intima, which contains SMC, macrophages, matrix components, and deposits of intra- and extra-cellular lipids.

In this context, AGE may promote atherogenesis by oxidizing LDL and causing changes in the intimal collagen[20-24] A major contribution of AGE to atherogenesis also emerges, however, from important studies that isolated a receptor for AGE on cell surface, termed “RAGE", which functions as a signal transduction receptor.[25] Found on many cells throughout the body, RAGE is much more abundant in the endothelial cells (EC) of diabetic individuals than non-diabetic controls.[26] RAGE is a receptor not only for AGE but also for proinflammatory S100/calgranulins and amphoterins.[27-29] The overlapping accumulation and expression of RAGE and its ligands at sites of tissue lesions sustains RAGE-mediated cellular activation and the induction of multiple signaling pathways, including p21ras, MAP kinases, PI3 kinase, cdc42/rac, and transcription factor NF-κB[30-36] The importance of RAGE-ligand interaction is underscored by its suppression of early accelerated atherosclerosis and established atherosclerosis in a glycemia- and lipid-

independent manner in diabetic apoE null mice after treatment with the soluble extracellular domain of the receptor for AGE.[37-39]

Biochemical mechanisms leading to AGE production

Nonenzymatic glycation occurs through the covalent binding of aldehyde or ketone groups of reducing sugars to free aminogroups of proteins, such as the ε-aminogroups of the diamino-monocarboxylic aminoacid lysine or the lateral amino-groups of arginine, forming a labile Schiff's base (Figure 1). This normal phenomenon occurs constantly in most cellular and plasma proteins in multicellular organisms. During prolonged hyperglycemia, such as in diabetes, these early glycation products serve as a starting point for further rearrangements, which ultimately lead to AGE.[40,41] Such transformations can also occur on lipids, nucleotides, and even in an euglycemic state on proteins with sufficiently long half-life.

We formerly understood AGE to form only on long-lived extracellular macromolecules because the rate of their formation from glucose is so slow that more rapidly turned-over intracellular proteins would not exist long enough to permit their accumulation. However, AGE do, in fact, form normally on proteins *in vivo*.[42-44] Glucose, the most abundant carbohydrate in plasma, is the most common sugar attacking free aminogroups of proteins. In this way, the initial Schiff's base undergoes rearrangement over a period of days to a much more stable ketoamine, called an Amadori's product[40] (Fig. 1). For example, the reaction of glucose with protein aminogroups gives rise to 1-amino-1-deoxy-2-ketose as an Amadori's product. The reactive free carbonyl group of Amadori's products causes some of the biological consequences of glycation. In addition, Amadori's products can degrade into a variety of other highly reactive carbonyl

compounds such as 3-deoxy-glucosone, which can react again with free aminogroups to form intermediate glycation products. Recent studies suggest that the intermediates contributing to AGE formation include dicarbonyl intermediates such as 3-deoxy-glucosone, glyoxal and methyl-glyoxal[45] (Figure 1). Glucose auto-oxidation and products from glycolipids can also form glyoxal and methyl-glyoxal.[46,47] Over a period of weeks or months, these glycation products slowly undergo a complex series of further chemical rearrangements, including dehydration, condensation, fragmentation, oxidation, and cyclization to finally yield irreversible AGE. Amadori's products form crosslinks between each other, aggregating a heterogenous group of AGE, yellow-brown and fluorescent structures with a propensity to generate reactive oxygen species (ROS) and interact with specific cell surface structures.[41,48] AGE comprise a large number of chemical structures including 2-(2-furoyl)-4(5)-furanyl-1H-imidazole (FFI);[49] 1-alkyl-2-formyl-3,4-diglycosyl pyrroles (AFGP's);[50] N-ε-carboxy-methyl-lysine (CML);[51] pyrraline;[52] and pentosidine[53] (Figure 2). The best chemically-characterized AGE found in human are pentosidine and CML- modified protein/lipid adducts, whose levels correlate with the severity of complications in diabetic patients.[24,51,54-58] Patients with diabetes and renal failure have elevated serum levels of CML.[57,59] Biochemical and immunohistochemical studies suggest that CML modifications of proteins are predominant AGE that accumulate *in vivo*.[55-57]

A significant new fraction of total AGE that relevantly affect protein structure and function and also mediate biological responses have been characterized in tissues. These compounds include: the imidazolone adduct formed by reaction of 3-deoxy-glucosone with arginine residues in protein;[55-57,60,61] N-ε-carboxy-ethyl-lysine, an analogue of CML formed by the reaction of methyl-glyoxal with lysine;[62] glyoxal-lysine dimer (GOLD);[59] and methyl-glyoxal-lysine dimer (MOLD),[60] imidazolium crosslinks formed by reaction of glyoxal or methyl-glyoxal with lysine

residues in protein[63] (Figure 2). The presence of 3-deoxy-glucosone, methyl-glyoxal, and glyoxal *in vivo*, and the formation of the above AGE in model carbonyl-amine reaction systems suggest that these AGE also form *in vivo* and contribute to tissue damage.[60] In addition, the presence of white blood cell myeloperoxidase can enhance the formation of glycolaldehyde and 2-hydroxy-propanal from serine and threonine, respectively, even in the absence of sugars,[64] suggesting a role for AGE in inflammation.[65]

How AGE promote atherosclerosis: molecular mechanisms

AGE can be highly deleterious to the integrity and function of blood vessel walls in several ways (Figure 3). AGE crossbridges among vessel wall macromolecules cause purely mechanical dysfunction,[66] and AGE accumulation can cause circulating blood cells to adhere to the vessel wall, trapping molecules such as immunoglobulins and apolipoproteins, which already may have been modified by glycation[20,58,67] (Fig. 3). However, the impact and relevance of crossbridgings, trapping, and altered protein functions are by a third, non-mechanical source of damage: AGE can perturb cellular function. Such perturbation may occur through binding to a variety of receptors identified on various cell types including macrophages, EC, and SMC as well as renal and neuronal cells.[27,68-71]

Therefore, AGE formation may accelerate the atherosclerotic process through two general mechanisms classified as non-receptor dependant (Table 1) and receptor-mediated (Table 2).

Non receptor-mediated effects of AGE on atherogenesis

Effects of AGE on extracellular matrix

AGE formation alters the functional properties of several important matrix molecules. Collagen in the blood vessel wall has a relatively long biological half-life, and with time undergoes significant nonenzymatic glycation, which may have considerable bearing on atherosclerosis.[72-75] Glycation ultimately leads to increased intermolecular bonds; on type-I collagen, such crosslinking induces an expansion of the molecular packing.[76] Soluble plasma proteins, such as LDL and immunoglobulins(Ig)G, also become entrapped and covalently cross-linked by AGE on collagen.[20,77,78] In addition, the formation of AGE-mediated crosslinks leads to decreased solubility and susceptibility to enzymatic digestion, properties particularly relevant for collagen in the extracellular matrix.[79]

The luminal narrowing that characterizes diabetic vessels may arise, in part, from accumulation of subendothelial AGE-linked plasma proteins. AGE formation on type-IV collagen from the basement membrane inhibits lateral association of these molecules into a normal network-like structure.[80] Formation of AGE on laminin causes decreased polymer self-assembly, decreased binding to type-IV collagen, and decreased binding of heparan sulfate proteoglycans.[81] *In vitro*, AGE formation on intact glomerular basement membrane increases membrane permeability.[82] AGE-induced abnormalities in the function of extracellular matrix alter the structure and function of intact vessels. Thus, AGE decrease elasticity in large vessels of diabetic rats, even after the abolishment of vascular tone, and increase fluid filtration across the carotid artery.[83] Furthermore, glycation may modulate the function of molecules such as basic fibroblast growth

factor (b-FGF) . b-FGF mitogenic activity decreases markedly after post-translational modification of b-FGF induced by elevated intracellular glucose concentrations.[84] Such loss of functional properties due to AGEs is important for vascular homeostasis.

Effect of AGE on lipids

When amine-containing lipids such as phosphatidylethanolamine and phosphatidylserine incubate with glucose, a similar time- and concentration-dependent series of slow chemical reactions is observed as it occurs with proteins.[85] Quite interestingly, this formation of AGE associates with fatty acid oxidation, which occurs at a parallel rate. In contrast, lipids lacking free amines, such as phosphatidylcholine, cannot react with glucose or form oxidation products. Glycation occurs on the apoprotein B[86] and phospholipid[85] components of LDL, leading to functional alterations in LDL clearance and increased susceptibility to oxidative modifications. In fact, diabetic LDL samples revealed significantly elevated levels of both apo B- and lipid-linked AGE, which correlated with levels of oxidized LDL.[85,87] Intermediates such as glyoxales, glycolaldehydes, hydroxyaldehydes, or other carbonyl group-containing compounds may form during oxidation of carbohydrates and polyunsatured fatty acids.[88,89] These common intermediates (see above) can in turn react with free aminogroups of proteins such as LDL apoB to form AGE products, including imidazolone, CML, CEL, GOLD, MOLD, and others.[59,62-64,90] Glycation of LDL apoB occurs mainly on positively charged lysine residues within the putative LDL receptor binding domain, which are essential for the specific recognition of LDL by the LDL receptor.[87] Increased LDL glycation correlates with glucose levels, and AGE-ApoB binding results in a significant impairment of LDL receptor-mediated uptake, decreasing the *in vivo* clearance of LDL compared with native LDL.[91] Several studies have shown impaired

degradation of glycated LDL in cultured human fibroblast, which possess the LDL receptor, compared with normal LDL; such impairment is proportional to the extent of glycation.[91] In contrast to fibroblasts, human monocyte-derived macrophages recognize glycated LDL more frequently than native LDL.[92] The uptake of glycated LDL by these cells, however, is not mediated by the LDL pathways, but rather by a high-capacity, low-affinity receptor pathway.[92] Thus, glycated LDL are poorly recognized by the specific LDL receptor and preferentially recognized by a nonspecific ("scavenger") receptor present on human macrophages. Because LDL glycation enhances its uptake by human aortic intimal cells[93] and monocyte-derived macrophages[92] , with the resulting stimulation of foam cell formation, the recognition of glycated LDL by the scavenger receptor pathway may promote intracellular accumulation of cholesteryl esters and, consequently, atherosclerosis.

Effects of AGE on hemostasis: alteration of platelet function

In diabetes, an increase in *ex vivo* platelet response to aggregating agents has been described.[94] This can also be demonstrated by the increase of circulating levels of some *in vivo* markers of platelet activation, such as β-thromboglobulin [94] and platelet factor-4.[95] Therefore, non-enzymatic glycation of platelet membrane proteins, found in diabetic subjects, may cause the platelet hypersensitivity to aggregating stimuli.[96,97] Additionally, other studies have shown that increased glycation of platelet membranes reduces platelet membrane fluidity, but this apparently does not correlate with platelet sensitivity to aggregating agents.[97,98]

Effects of AGE on hemostasis: alterations of soluble proteins involved in coagulation and fibrinolysis

Several abnormalities in hemostatic mechanisms occur in diabetes, with an obvious tendency to thrombosis.[99] Such abnormalities involve all stages of hemostasis, including platelet function, fibrin formation, fibrinolysis, and endothelial hemostatic functions. The hypothesis that hyperglycemia may constitute a key factor of diabetic hypercoagulability and can induce changes promoting thrombosis is suggestive, and supported by several experimental pieces of evidence. Non-enzymatic glycation reduces the susceptibility of fibrin to degradation by specific enzymes such as plasmin.[77] A direct correlation between hyperglycemia and the increase of fibrinopeptide A (a marker of thrombin activation) occurs in patients with diabetes.[100,101] Finally, anti-thrombin III (AT-III), the likely most important physiological inhibitor of coagulation, shows diminished biological activity in patients with diabetes, probably directly due to glucose, which renders the molecule less active by occupying the lysine residue that allows the binding of AT-III to heparin, its natural cofactor.[102] Free radicals produced during glycation processes might also induce rapid inactivation of AT-III. Indeed, oxidative stress can reduce AT-III activity.[103] Moreover, hyperglycemia decreases the concentration and biological activity of protein C, another important physiological inhibitor of coagulation.[104]

Receptor-mediated effects of AGE in atherogenesis

Cellular uptake of AGE

Cell surface AGE-receptors mediate endocytosis and degradation of AGE-modified molecules, serving an important function in AGE catabolism and turnover. The search for removal mechanisms of AGE has led to the discovery of several cellular receptors binding these irreversibly modified macromolecules. A macrophage AGE receptor distinct from the mannose/fucose receptors involved in glycoprotein uptake and from previously described macrophage scavenger receptors recognizes *in vivo*-isolated and *in vitro*-synthesized AGE.[105,106] Two new proteins, 60-KDa and 90-KDa, of apparently unique aminoacid sequence, were later isolated from rat liver,[107] and subsequently an additional AGE-binding protein, lactoferrin-like 80-KDa protein, was identified.[108] Later, galectin-3, a 32-KDa macrophage protein, was identified as an AGE-binding protein.[109] The role of these binding proteins (therefore not necessarily "receptors") for AGE in AGE-mediated cellular activation remains undetermined.

Subsequent studies led to the identification, cloning, and analysis of RAGE, a multi-ligand member of the immunoglobulin superfamily and a receptor for AGE.[15,25,110] Current opinion increasing views RAGE, as an intracellular signal-transducing peptide rather than a simple receptor involved in AGE endocytosis and turnover.[111,112]

Structure of RAGE protein

RAGE is an approximately 45-KDa protein originally isolated from bovine lung endothelium on the basis of its ability to bind AGE ligands.[113] Subsequent molecular cloning revealed that RAGE as a newly identified member of the immunoglobulin superfamily of cell-surface molecules.[114] The entire mature receptor consists of 403 aminoacids in man, rat and mouse. The

extracellular region of RAGE consists of one V-type (variable) immunoglobulin domain, followed by two C-type (constant) immunoglobulin domains stabilized by internal disulfide bridges between cysteine residues. The V-type domain includes two putative N-linked glycation sites. In addition to the extracellular domain, RAGE displays a single putative transmembrane-spanning region and a short, highly charged cytosolic tail.

RAGE tissue expression, ligands and activation

RAGE is highly conserved across species and expressed in a wide variety of tissues.[114] Immunohistochemical methods including *in situ* hybridization and Northern analysis, have shown that RAGE antigen and mRNA localize at least in the endothelium, vascular SMC, mononuclear phagocytes, neural tissue, and glomerular visceral epithelial cells, or podocytes.[26,115] Enzyme-linked immunosorbent assay (ELISA) of various tissue extracts demonstrates that RAGE is most abundant in the heart, lung, and skeletal muscle. The presence of RAGE in multiple tissues suggests a potential relevance of ligand-RAGE interactions for the modulation of vascular properties, as well as neural, renal and cardiac functions, prominently affected in diabetes and aging. Indeed, RAGE expression increases at sites of diverse diseases including atherosclerosis, Alzheimer's disease, and amyotrophic lateral sclerosis.[15,116,117] In this context, other ligands for the receptor link to homeostatic and proinflammatory events. For example, RAGE binds to amphoterin, a developmentally expressed neurite-outgrowth promoting protein that intriguingly increases in tumors, where its interaction with RAGE facilitates tumor cell migration and invasion.[27,28] further, S100A12, a polypeptide of the S100/calgranulin family of pro-inflammatory cytokines also termed *extracellular newly identified RAGE binding protein* (EN-RAGE), interacts with RAGE in a dose-dependent and saturable manner, resulting in the

activation of cellular targets and competition with another member of the S100/calgranulin family, S100B, also capable of binding to RAGE.[118] Thus, RAGE is a receptor not only for AGE, but also for S100/calgranulins, molecules found in any inflammatory lesion, including the blood vessel wall of individuals with diabetes.[119-121] The overlapping presence of high levels of AGE, S100/calgranulins, and RAGE, together with dyslipidemia, might conspire to cause the rapid atherosclerosis observed in diabetes. Similarly, RAGE interacts with β-sheet fibrils composed of different subunits/monomers, including amyloid A, amyloid-β peptide, prion peptide, and amylin.[71,122] The binding by RAGE of seemingly diverse ligands deserves further research.

Another feature of RAGE is an unusual co-expression with its ligands in tissues. At sites where AGE and S100/calgranulins accumulate in the vascular lesions, RAGE expression increases in vessel wall cells, including endothelium, vascular SMC, and invading mononuclear phagocytes.[65,121,123] This overlapping distribution of the receptor and its ligands may lead to prolonged cellular activation, resulting in further increased expression of the receptor. Contrary to other receptors, such as the LDL receptor, which are downregulated by increased levels of their ligand, the RAGE-ligand interaction would thus lead to a positive feedback activation, which further increases receptor expression. Currently, the only means to substantially downregulate RAGE expression involve interrupting the cycle of ligand engagement of the receptor via soluble RAGE or blocking antibodies.

Signal transduction pathways activated by RAGE-ligand interaction

The most important pathological consequence of RAGE engagement with its ligands appear to be cellular activation, leading to the induction of oxidative stress and a broad spectrum of signaling mechanisms. Even if AGE were nothing more than accidental ligands for RAGE, interaction of RAGE with other ligands such as amphoterin or amyloid peptide likely induces similar consequences. In the vasculature, the principal pathological consequence of AGE interaction with RAGE is the induction of oxidative stress, leading to NF-κB activation and the induction of the endothelial expression of various cell adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin.[30,65,123]

The interaction of AGE with endothelial surface RAGE leads first to increased intracellular reactive oxygen species (ROS),[124,125] the generation of which seems linked, at least in part, to the activation of the NAD(P)H-oxidase system.[126] Our studies have shown that mitochondrial sources of ROS are also evoked secondary to AGE-RAGE interaction.[127] ROS in turn would activate the redox-sensitive transcription factor NF-κB, a pleiotropic regulator of many "response-to-injury" genes. Antibodies directed against either RAGE or AGE themselves can block this signal transduction cascade.[126]

NF-κB induced in response to oxidative stress leads to transcriptional activation of many genes highly relevant for inflammation, immunity, and atherosclerosis, including tumor necrosis factors (TNF-α and TNF-β), interleukins (IL)-1, -6, and -8, interferon-γ (IFN-γ), VCAM-1, ICAM-1, and E-selectin.[128] Importantly, the tethering of AGE to the cell-surface is not enough to generate ROS and cellular activation, since the RAGE carboxy-terminal cytosolic tail,

containing known signaling phosphorylation sites, kinase domains, and other activation sites, is critical for RAGE-dependent cellular activation. Indeed, a truncated form of RAGE lacking only the cytosolic tail and expressed in cells retains binding to various ligands identical to wild-type RAGE, but does not mediate the induction of cellular activation.[118]

Triggering of inflammatory effector mechanisms (generation of cytokines and chemokines, and expression of cell adhesion molecules) mediated by the AGE-RAGE interaction involves multiple intracellular signal transduction pathways, including p21ras, MAP kinases, PI3 kinase, cdc42/rac, Jak/STAT, NAD(P)H oxidase, and others.[31,33-36,126] Each of these pathways links closely with AGE binding to RAGE, because blockade of the receptor with either anti-RAGE IgG or excess soluble (s)RAGE prevents their activation.

The presence of RAGE in all cells relevant to atherosclerosis, including EC, monocyte-derived macrophages, lymphocytes, and SMC, suggests the relevance of RAGE engagement in these processes.

Interaction of AGE with mononuclear phagocytes and T cells

The mononuclear phagocyte (MP) AGE receptor system links closely to AGE turnover, and may represent a mechanism that responds to the increased levels of AGE that accompany aging and the degradation of senescent proteins.[129] The interaction of AGE with MP induces a phenotype of activated macrophages, manifested by the induction of platelet-derived growth factor, insulin-like growth factor-1, and proinflammatory cytokines such as IL-1β and TNF-α.[130-132] In MP, AGE-RAGE interaction prompts cell migration (chemotaxis) mediated by the interaction of

soluble RAGE ligands (AGE prepared *in vitro* or isolated from diabetic subjects, AGE-β2-microglobulin or CML-adducts) with RAGE. In contrast to the effect of soluble AGE, immobilized AGE such as those found in basement membranes retard MP migration, a process known as "apoptaxis". Anti-RAGE IgG or sRAGE block both chemotactic and apoptactic responses.[133,134]

More recently, EN-RAGE has been utilized as a stimulus to induce chemotaxis. The induced migration of MP has here been shown to be concentration- and RAGE-dependent. Similarly, the engagement of RAGE by EN-RAGE in cultured Bv2 cells (murine macrophages) induced production of IL-1β and TNF-α, in an NF-κB-dependent fashion.[118] On the other hand, when MP reach a site of immobilized AGE in the tissue, their migration slows, allowing them to bind to the AGE-modified surface and become activated. This could provide a mechanism for attracting and retaining MP at sites of AGE deposition in tissues, potentially contributing to the development of tissue lesions after AGE-induced MP migration.

An inducible system for RAGE expression also occurs in T cells.[135] The exposure to AGE of T cells prestimulated with phytohemagglutinin increases synthesis and release of IFN-γ, which represents the main activating factor for MP and enhances diverse effects of other cytokines, including TNF on EC.[135,136] Since activated T cells are present in atherosclerotic lesions,[137,138] combined AGE-activated T cells and MP may contribute to vascular damage in a hyperglycemic setting.

Interaction of AGE with vascular smooth muscle cells

In the presence of AGE, cultured SMCs increase proliferative activity and fibronectin production.[48,139] The precise mechanism of this response remains unclear. *In vivo*, cytokines or growth factors induced by AGE in the MP likely mediate indirectly the effects promoting SMC growth, at least in part. Transforming growth factor-β (TGF-β) might act as an intermediate factor in AGE-induced fibronectin production by SMC.[139] These studies suggest that stimulation of SMC by AGE may contribute to the proliferative lesions commonly observed in several tissues in diabetes.

Interactions of AGE with vascular endothelium: alterations of vascular permeability and hemostatic and adhesive properties

Due to its unique position and numerous properties, the vascular endothelium is particularly important in the regulation of permeability, the maintenance of blood fluidity, the regulation of vascular growth and tone, and metabolism of hormones and vasoactive mediators. Endothelium is exposed to AGE localized on circulating proteins or cells (e.g., diabetic red blood cells) as well as those present in the underlying subendothelial matrix. Receptors for AGE have been found on the EC surface, and they mediate both the uptake and transcytosis of AGE as well as internal signal transduction. AGE-RAGE interaction causes perturbation of two important homeostatic properties of the endothelium: barrier function and antihemostatic properties.

The alteration of barrier function has been demonstrated by showing increased permeability of EC incubated with AGE, with increased transit of macromolecules through the endothelial

monolayer. Alterations of the physical integrity of endothelium accompanies increased permeability, as shown by the destruction of organized actin structures and alterations of cellular morphology.[140,141]

AGE also determine alterations of endothelial antihemostatic functions *in vitro*, as shown by a reduction of thrombomodulin expression and the concomitant induction of tissue factor expression.[142,143] Tissue factor induction and the reduced thrombomodulin activity change the dynamic endothelial properties with regard to hemostasis from those of an anticoagulant to those of a procoagulant surface.

In addition, cytokine production (IL-1β and TNF-α), induced by interaction of AGE with macrophages, can indirectly alter the function of EC with specific receptors for these mediators.[142,143] Such cytokines downregulate endothelial expression of tissue plasminogen activator (t-PA) and induce the transcription of its main inhibitor (type-1 plasminogen activator inhibitor, PAI-1),[144,145] causing an imbalance between these two factors that results in a reduction of endothelial fibrinolytic properties.

Binding of AGE to endothelial RAGE also results in depletion of cellular antioxidant defense mechanisms (e.g., glutathione and vitamin C)[32] and the generation of ROS[124] (Figure 2). Increased cellular oxidative stress activates NF-κB and thus promotes the expression of NF-κB-regulated genes including procoagulant tissue factor and VCAM-1.[65,140,143] VCAM-1 expression may prime the diabetic vasculature towards enhanced interaction with circulating monocytes.[146,147] The incubation of EC with EN-RAGE or S100B causes VCAM-1 induction, in a RAGE-dependent manner, as confirmed by the inhibitory effect of anti-RAGE IgG or

sRAGE.[118] However, it has recently been found that the AGE preparations, which were essentially endotoxin free, were incapable of inducing VCAM-1 or TNF-α secretion regardless of RAGE binding affinity, AGE concentration or incubation time.[148] In contrast, the reported RAGE ligand S100b was confirmed to induce VCAM-1 expression on endothelial cells and TNF-alpha secretion.[148]

Alterations of endothelium-dependent vasodilatation

AGE linked to the vascular matrix can interfere chemically with the bioavailability of nitric oxide (NO), an important regulator of vascular tone inducing SMC relaxation.[149,150] An altered endothelium-dependent dilatation characterizes many physiopathologic settings, including diabetes,[151] aging,[152] atherosclerosis,[153] and some forms of hypertension.[154] AGE-modified proteins added to NO *in vitro* block NO activity in a concentration-dependent manner. Studies on animal models with experimentally induced diabetes demonstrate that an alteration of endothelium-dependent dilatation occurs quickly, within two months, from diabetes induction.[149] Presumably, the inactivation of NO occurs through a direct reaction of the NO radical with other free radicals formed during advanced glycation reactions. In parallel, AGE induce the expression of the potent vasoconstrictor endothelin-1 and shift endothelial function towards vasoconstriction.[155] Animal models *in vivo* have provided further evidence confirming the involvement of AGE in the modulation of vascular tone in diabetes. Indeed, healthy euglycemic rats and rabbits treated with AGE show an appreciable decrease of their vasodilatatory reserve.[142]

AGE, RAGE in experimental animals

The first evidence of the direct pathogenetic role of AGEs—independent of hyperglycemia and other possible contributory factors occurring in diabetes—has been obtained in animal models, namely healthy euglycemic rats treated with AGE. Such treatment spurs tissue deposition of AGE, accompanied by various changes in vascular function, including alterations of permeability, sub-endothelial sequestration of monocytes, and decreased sensitivity to vasodilatory agents.[142,156] Non-diabetic rabbits undergoing prolonged treatment with "physiological" amounts of AGE also manifest AGE deposition in aortic tissue and the expression of adhesion molecules such as VCAM-1 and ICAM-1.[157]

AGE administered by infusion to mice *in vivo* also induce new antigenic determinants such as malon-dialdehyde in the vessel wall, , markers of increased oxidative stress.[23,30,124]

Peculiarly, pathological lesions characterized by AGE accumulation associate closely with increased cellular expression of RAGE and the overlapping presence of AGE epitopes. Diabetic vessels with abundant AGE lie in close proximity to cells that express high levels of RAGE.[69] Thus, rather than decreasing RAGE expression, AGE may contribute to enhanced expression of the receptor, resulting in smoldering cellular activation in diabetic tissues. To dissect the contribution of RAGE-ligand interaction in the pathogenesis of diabetic vasculopathy, Wautier and colleagues first tested an acute animal model of diabetes-associated hyperpermeability by administering the decoy protein soluble (s)RAGE and then using reagents blocking either the receptor itself the access of ligands to RAGE.[141] After 9-11 weeks, rats rendered diabetic with streptozotocin showed increased vascular permeability in multiple organs, especially the

intestine, skin, and kidney. RAGE blockade with either sRAGE or mono-specific antibodies normalized tissue permeability. Because increased permeability in human subjects with diabetes associates with increased morbidity and mortality from cardiovascular complications, such diabetes-associated hyperpermeability, taken here as a "surrogate" marker for diabetic vasculopathy, is likely an "intermediate" endpoint of vascular disease.[157,158]

Murine models of atherosclerosis have significantly advanced our understanding of the development of accelerated diabetic macrovascular disease. Since mice inherently resist the development of atherosclerosis, in part due to their high plasma levels of high-density lipoproteins (HDL), researchers have used strains genetically susceptible to atherosclerosis. In apolipoprotein (apo) E-deficient mice, which develop spontaneous atherosclerosis on a normal chow diet, induction of diabetes with streptozotocin associated with an approximately fivefold increase in mean atherosclerotic lesion area at the aortic sinus after 6 weeks of diabetes compared with euglycemic apo E null mice of the same age.[38] In addition, diabetic animals displayed increased numbers of complex lesions (fibrous caps, extensive monocyte and smooth muscle infiltration, etc.) compared with euglycemic control mice. Diabetes-associated atherosclerotic lesions in this model featured increased AGE deposition and enhanced RAGE expression . Administration of sRAGE in diabetic apoE-null animals suppressed accelerated diabetic atherosclerosis. Here, lesions appeared arrested at the stage of fatty streak, and the number of complex atherosclerotic lesions reduced strikingly. In parallel, plasma levels of free AGE, the vascular expression of VCAM-1 and tissue factor, and the nuclear translocation of NF-κB all decreased in sRAGE-treated mice compared to vehicle-treated littermates. The effects of RAGE blockade, i.e., interrupting the cycle of sustained cellular activation, were independent of changes in other risk factors such as blood glucose, insulin, or lipoprotein levels. Interestingly,

euglycemic animals receiving sRAGE also demonstrated a trend towards diminished atherosclerosis compared to vehicle-treated animals.[38] Recent studies in diabetic apoE-null mice further support these observations. Among male apoE-null mice rendered diabetic with streptozotocin or treated with citrate buffer at the age of 6 weeks, certain mice were sacrificed or treated with once daily murine sRAGE or albumin at age 14 weeks and all mice were sacrificed at age 20 weeks .Compared with diabetic mice at the age of 14 weeks, albumin-treated animals showed increased atherosclerotic lesion area and features of plaque complexity. In diabetic mice treated with sRAGE from the age of 14 to 20 weeks, lesion area and complexity reduced significantly, not statistically different from diabetic mice at the age of 14 weeks. In these experiments, euglycemic mice treated with sRAGE also displayed a statistically-significant decrease in atherosclerosis. Taken together, these findings strongly suggest that AGE formation, which certainly forms even in the euglycemic environment exposed to oxidant stress, or plasma levels of other ligands, such as EN-RAGE, may participate in the initiation and progression of atherosclerosis, at least in part, in a RAGE-dependent manner.[39]

It is well-established that cellular proliferation, migration, and expression of extracellular matrix proteins and matrix metalloproteinases contribute to neointimal formation upon vascular injury.[159] In diabetic rats subjected to carotid artery injury induced by balloon angioplasty, administration of sRAGE resulted in a significantly lower intima/media ratio versus that seen in diabetic rats treated with vehicle.[160] Our studies have shown that wild-type C57BL/6 mice undergoing arterial endothelial denudation display a striking increase of RAGE in the injured vessel, particularly in activated SMC of the expanding neointima. In parallel, upregulation of AGE and S100/calgranulins is evident in the injured vessel wall.[161] Blockade of RAGE in homozygous RAGE null mice by soluble truncated receptor or antibodies resulted in

significantly decreased neointimal expansion after arterial injury and decreased SMC proliferation, migration ,and expression of extracellular matrix proteins. Mice bearing a transgene encoding a RAGE cytosolic tail deletion mutant, specifically in SMCs, driven by the SM22a promoter demonstrated a critical role for SMC RAGE signaling . Upon arterial injury, neointimal expansion was strikingly suppressed compared to wild-type littermates.[161] These data highlight key roles for RAGE in the modulation of SMC properties in the acutely and chronically injured vessel wall.

Recently, it has been shown that RAGE functions also as an endothelial adhesion receptor promoting leukocyte recruitment by a direct interaction of RAGE with the leukocyte beta2-integrin Mac-1.[162]In an animal model of thioglycollate-induced acute peritonitis, leukocyte recruitment was significantly impaired in RAGE-deficient mice as opposed to wild-type mice. In diabetic wild-type mice was observed enhanced leukocyte recruitment to the inflamed peritoneum as compared with nondiabetic wild-type mice; this phenomenon was abrogated in the presence of soluble RAGE and was absent in diabetic RAGE-deficient mice.[162] The RAGE-Mac-1 interaction defines a novel pathway of leukocyte recruitment relevant in inflammatory disorders associated with increased RAGE expression.

Studies in RAGE-deficient mice have confirmed an important role for RAGE as a mediator of diabetic complications and macrovascular and chronic disease, but it is not known yet which receptors compensate for the absence of RAGE in RAGE-deficient mice, thus ensuring normal development and a normal phenotype in these animals.

AGE, RAGE and vascular dysfunction in human: possible therapeutic interventions on AGE formation, AGE crosslinking and AGE-RAGE interaction

There is substantial evidence to support that AGE increase in aging, cardiovascular disease, and diabetes. The plasma levels of AGE correlate with the degree of coronary artery disease in nondiabetic and diabetic human subjects[163-166]. Elevated plasma levels of S100A12 protein, AGE and CML have been reported in type-2 diabetes patients.[167-170] Using a monoclonal anti-AGE antibody, immunohistochemical analyses of human atherosclerotic lesions have demonstrated diffuse extra-cellular as well as dense intracellular AGE deposition in macrophages and vascular SMC.[72,171,172] Tissue AGE concentration correlates with the severity of atherosclerotic lesions and the accumulation of plasma proteins, lipoproteins, and lipids in the vessel wall.[163,173,174]

Recently, evidence is accumulating to support the hypotesis that RAGE may be a contributing factor in cardiovascular disease, even in the absence of diabetes. Cipollone et al.[175] showed that RAGE was expressed in nondiabetic and diabetic human atherosclerosis and to enhanced degrees in diabetes. RAGE colocalized with cox-2, type 1/type 2 microsomal prostaglandin E2, and matrix metalloproteinases(MMPs) in the diabetic atherosclerotic plaques. [175]

Further, studies on polymorphisms on the RAGE gene have evidentiated that one particular promoter allele (-374A) is associated with minor degree of macrovascular disease in diabetic subjects.[176,177]

In nondiabetic subjects with the –374T/A or A/A genotypes displayed decreased severity of coronary atherosclerosis.[178] In one smaller study, no association between these variants and the incidence of macrovascular disease was found.[179]

Targeting of AGE-RAGE system mainly includes: AGE formation inhibitors, AGE breakers against AGE-derived protein crosslinking, RAGE competitors. The hydrazine compound aminoguanidine was the first AGE formation inhibitor discovered,[74] and it has been by far the most extensively studied compound of this kind thus far. Aminoguanidine has been evaluated in various animal models with different diabetic complications,[67,180,181] and is the prototype of a drug class that might eventually find use in the treatment of diabetic complications. Rather than interfering with Amadori's products on proteins, aminoguanidine and other AGE inhibitors likely function as nucleophilic traps for reactive carbonyl intermediates in the formation of AGE.[180] Although encouraging results in animal models of diabetic complications have demonstrated decreased AGE accumulation,[83] clinical studies must better define the place of aminoguanidine and other AGE inhibitors.[181] Moreover, clinical trials with aminoguanidine have shown a trend toward reduced renal dysfunction in human diabetic subjects with advanced nephropathy.[181] These findings may provide the first "proof of concept" in man that AGE importantly contribute to the pathogenesis of diabetic complications.

While aminoguanidine prevents AGE formation, it likely will not be effective in patients with a long history of disease and already extensive tissue AGE accumulation. The need to remove irreversibly bound AGE from connective tissues and matrix components has led to the development of AGE-cleaving agents.[182] Studies in animal models and preliminary clinical trials

have shown that pimagedine, an AGE-inhibitor, and the cross-link breaker ALT-711 can reduce the severity of pathological lesions associated with AGEs.[183,184]

RAGE expression increases in clinical settings characterized by enhanced cellular activation or oxidative stress, such as diabetes, and prolonged exposure of AGE to RAGE-expressing cells determines a chronic state of cellular activation.[185] Interference with the vicious cycle established by RAGE-ligand interaction might interrupt cellular activation and consequently lead to an improvement of various chronic disorders.[71,116,186,187] sRAGE treatment dose-dependently suppress the development of atherosclerosis in animal models, acting as a RAGE competitor in AGE binding thereby preventing the AGE-RAGE interactions. Reduced AGE level in sRAGE-treated diabetic mice suggest that sRAGE may enhance the removal of AGE from the plasma and tissues.[38]

Since endogenous sRAGE does exist in circulating blood, interesting studies on plasma levels of sRAGE further suggest a involvement of RAGE in the pathogenesis/severity of coronary artery disease. In age-matched Italian male subjects without diabetes, endogenously lower levels of plasma sRAGE were associated with enhanced risk of angiographically detected coronary artery disease.[188] When plasma sRAGE levels were divided into quartiles, the lowest levels of sRAGE were associated with the greatest overall risk for disease.[188] Circulating sRAGE levels were significantly lower in type 1 diabetic patients than in nondiabetic subjects and were inversely associated with the severity of some diabetic vascular complications.[189]

So far, AGE have been regarded as the main molecular targets in diabetic vascular complications; the inhibition of AGE-RAGE interactions and subsequent signal transduction would seem to be a promising way for overcoming diabetic vascular complications.

Conclusions

The experimental evidence gathered thus far demonstrates unequivocally that AGEs can alter vessel wall homeostasis in a pro-atherogenic fashion through multiple mechanisms, i.e., alterations of extracellular matrix permeability, release of inflammatory cytokines and growth factors, alterations of antithrombotic properties of the endothelium and of the vessel wall's ability to modulate vascular tone, and the increased expression of adhesion molecules and chemokines on vascular cells. Once initiated, a state of chronic vascular inflammation ensues, sustained by the migration and activation of inflammatory cells—mostly mononuclear phagocytes and T cells—that infiltrate the altered vessel wall. These processes thus trigger a cycle of ongoing cellular injury and vascular dysfunction, in part through the release of inflammatory peptides such as S100/calgranulins and amphoterin, also ligands of RAGE. The pivotal role of RAGE in these processes highlights this ligand-receptor axis as a logical and attractive candidate for therapeutic intervention to limit diabetic vascular damage and its long-term consequences.

References

1. Schwartz CJ, Valente AJ, Sprague EA, Kelley JL, Cayatte AJ, Rozek MM. Pathogenesis of the atherosclerotic lesion. Implications for diabetes mellitus. *Diabetes Care*. 1992;15:1156-1167.
2. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Interventetion Trial. *Diabetes care*. 1993;16:434-444.
3. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;329:977-986.
4. Laakso K. Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes*. 1999;48:937-942.
5. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. *New Engl J Med*. 2000;342:381-389.
6. Nathan D. Relationship between metabolic control and long term complications of diabetes. In: Kahn CR, Weir GC, eds. *Joslin's Diabetes Mellitus*. Philadelphia: Lea & Febiger; 1994:620-631.

7. Nathan DM, Lachin J, Cleary P, Orchard T, Brillon DJ, Backlund JY, O'Leary DH, Genuth S. Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N Engl J Med*. 2003;348:2294-303.

8. Kuusisto J, Mykkanen L, Pyorala K, Laakso M. NIDDM and its metabolic control predict heart disease in elderly subjects. *Diabetes*. 1994;43:960-967.

9. Kuusisto J, Mykkanen L, Pyorala K, Laakso M. Non-insulin-dependent diabetes and its metabolic control are important predictors of stroke in elderly subjects. *Stroke*. 1994;25:1157-1164.

10. Colwell JA. Multifactorial aspects of the treatment of the type II diabetic patient. *Metabolism*. 1997;46:1-4.

11. Brownlee M, Cerami A. The biochemistry of the complications of diabetes mellitus. *Annu Rev Biochem*. 1981;50:385-432.

12. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brouwnlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*. 2000;404:787-790.

13. Greene DA, Lattimer SA, Sima AA. Sorbitol, phosphoinositides, and sodium-potassium-ATPase in the pathogenesis of diabetic complications. *N Engl J Med*. 1987;316:599-606.

14. Wautier JL, Guillausseau PL. Diabetes, advanced glycation end products and vascular disease. *Vasc Med*. 1998;3:131-137.

15. Schmidt AM, Yan SD, Wautier JL, Stern DM. Activation of receptor for advanced glycation end products- a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circ Res*. 1999;84:489-497.

16. King GL, Shiba T, Oliver J, Inoguchi T, Bursell SE. Cellular and molecular abnormalities in the vascular endothelium of diabetes mellitus. *Annu Rev Med*. 1994;45:179-188.

17. Koya D, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes*. 1998;47:859-866.

18. Lusis A J. Atherosclerosis. *Nature*. 2000;407:233-241.

19. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105:1135-1143.

20. Brownlee M, Vlassara H, Cerami A. Nonenzymatic glycosylation products on collagen covalently trap low density lipoprotein. *Diabetes*. 1985;34:938-941.

21. Vishwanath V, Frank KE, Elmmets CA, Dauchot PJ, Monnier VM. Glycation of skin collagen in type I diabetes mellitus.Correlation with long-term complications. *Diabetes*. 1986;35:916-921.

22. Watanabe J, Wohltmann H, Klein R, Colwell J, Lopes-Virella MF. Enhancement of platelet aggregation by low density lipoproteins from IDDM patients. *Diabetes*. 1988;37:1652-1657.

23. Rosenfield M, Palinski W, Yla-Herttuala S, Butler S, Witztum J. Distribution of oxidation specific lipid-protein adducts and apolipoprotein B in atherosclerotic lesions of varying severity from WHHL rabbits. *Atheriosclerosis*. 1990;10:336-349.

24. Dyer DG, Dunn JA, Thorpe SR, Bailie KE, Lyons TJ, McCance DR, Baynes JW. Accumulation of Maillard reaction products in skin collagen in diabetes and aging. *J Clin Invest*. 1993;91:2463-2469.

25. Schmidt AM, Hasu M, Popov D, Zhang JH, Chen J, Yan SD, Brett J, Cao R, Kuwabara K, Costache G, Simionescu N, Simionescu M, Stern D. Receptor for advanced glycation end products (AGEs) has a central role in vessel wall interactions and gene activation in response to circulating AGE-proteins. *Proc Natl Acad Sci USA*. 1994;91:8807-8811.

26. Tanji N, Markowitz GS, Fu C, Kislinger T, Taguchi A, Pischetsrieder M, Stern D, Schmidt AM, D'Agati VD. Expression of advanced glycation end products and their cellular receptor RAGE in diabetic nephropathy and nondiabetic renal disease. *J Am Soc Nephrol*. 2000;11:1656-1666.

27. Hori O, Brett J, Nagashima M, Nitecki D, Morser J, Stern DM, Schmidt AM. RAGE is a cellular binding site for amphoterin: mediation of neurite outgrowth and co-expression of RAGE and amphoterin in the developing nervous system. *J Biol Chem*. 1995;270:25752-25761.

28. Taguchi A, Blood DC, del Toro G, Canet A, Lee DC, Qu W, Tanji N, Lu Y, Lalla E, Fu C, Hofmann MA, Kislinger T, Ingram M, Lu A, Tanaka H, Hori O, Ogawa S, Stern DM, Schmidt AM. Blockade of amphoterin/RAGE signalling suppresses tumor growth and metastases. *Nature*. 2000;405:354-356.

29. Tracey KJ. The inflammatory reflex. *Nature*. 2002;420:853-859.

30. Schmidt AM, Hori O, Chen JX, JF L, Crandall J, Zhang J, Cao R, Yan SD, Brett J, Stern D. Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1(VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. *J Clin Invest*. 1995;96:1395-1403.

31. Lander H, Tauras J, Ogiste J, Hori O, Moss R, Schmidt AM. Activation of the receptor for advanced glycation end products triggers a p21ras-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. *J Biol Chem*. 1997;272:17810-17814.

32. Bierhaus A, Chevion S, Chevion M, Hofmann M, Quehenberger P, Illmer T, Luther T, Berentshtein E, Tritschler H, Muller M, Wahl P, Ziegler R, Nawroth P. Advanced glycation end product-induced activation of NF-κB is suppressed by α-lipoic acid in cultured endothelial cells. *Diabetes*. 1997;46:1481-1490.

33. Simm A, Munch G, Seif F, Schenk O, Heidland A, Richter H, Vamvakas S, Schinzel R. Advanced glycation endproducts stimulate the MAP-kinase pathway in tubulus cell line LLC-PK_1. *FEBS Letters*. 1997;410:481-484.

34. Huttunen HJ, Fages C, Rauvala H. Receptor for Advanced Glycation Endproducts (RAGE)-mediated neurite outgrowth and activation of NF-kB require the cytoplasmic domain of the receptor but different downstream signaling pathways. *J Biol Chem*. 1999;274:19919-19924.

35. Treins C, Giorgetti-Peraldi S, Murdaca J, Van Obberghen E. Regulation of vascular endothelial growth factor expression by advanced glycation end products. *J Biol Chem*. 2001;276:43836-43841.

36. Guh JY, Huang JS, Chen HC, Hung WC, Lai YH, Chuang LY. Advanced glycation end product-induced proliferation in NRK-49F cells is dependent on the JAK2/STAT5 pathway and cyclin D1. *Am J Kidney Dis*. 2001;38:1096-1104.

37. Nakashima Y, Plump A, Raines E, Breslow J, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb*. 1994;141:133-140.

38. Park L, Raman KG, Lee KJ, Lu Y, Ferran L J Jr, Chow W-S, Stern D, Schmidt AM. Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. *Nat Med*. 1998;4:1025-1031.

39. Bucciarelli LG, Wendt T, Qu W, Lu Y, Lalla E, Rong LL, Goova MT, Moser B, Kislinger T, Lee DC, Kashyap Y, Stern DM, Schmidt AM. RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice. *Circulation*. 2002;106:2827-2835.

40. Harding JJ. Nonenzymatic covalent posttranslational modification of proteins in vivo. *Adv Protein Chem*. 1985;37:247-334.

41. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end-products in tissue and the biochemical basis of diabetic complications. *N Engl J Med*. 1988;318:1315-1321.

42. Higgins PJ, Bunn HF. Kinetic analysis of the nonenzymatic glycosylation of hemoglobin. *J Biol Chem*. 1981;256:5204-5208.

43. Larsen ML, Hørder M, Mogensen EF. Effect of long-term monitoring of glycosylated hemoglobin levels in insulin-dependent diabetes mellitus. *N Engl J Med*. 1990;323:1021-1025.

44. Makita Z, Vlassara H, Rayfield E, Cartwright K, Friedman E, Rodby R, Cerami A, Bucala R. Hemoglobin-AGE: a circulating marker of advanced glycosylation. *Science*. 1992;258:651-653.

45. Takeuchi M, Yanase Y, Matsuura N, Yamagishi Si S, Kameda Y, Bucala R, Makita Z. Immunological detection of a novel advanced glycation end-product. *Mol Med*. 2001;7:783-791.

46. Thornalley PJ, Langborg A, Minhas HS. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem J*. 1999;344:109-116.

47. Miyata T, Horie K, Ueda Y, Fujita Y, Izuhara Y, Hirano H, Uchida K, Saito A, van Ypersele de Strihou C, Kurokawa K. Advanced glycation and lipidoxidation of the peritoneal membrane: respective roles of serum and peritoneal fluid reactive carbonyl compounds. *Kidney Int*. 2000;58:425-435.

48. Vlassara H, Bucala R, Striker L. Pathogenic effect of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and aging. *Lab Invest*. 1994;2:138-151.

49. Pongor S, Ulrich PC, Benesath FA, Cerami A. Aging of proteins: isolation and identification of a fluorescent chromophore from the reaction of polypeptides with glucose. *Proc Natl Acad Sci USA*. 1984;81:2684-2688.

50. Farmar J, Ulrich P, Cerami A. Novel pyrroles from sulfite-inhibited Maillard reactions, insight into the mechanism of inhibition. *J Org Chem*. 1988;53:2346.

51. Ahmed MU, Thorpe SR, Baynes JW. Identification of N^{ε}-(carboxymethyl)-lysine as a degradation product of fructoselysine in glycated protein. *J Biol Chem*. 1986;261:4889-4894.

52. Hayase F, Nagaraj RH, Miyata S, Njoroge FG, Monnier VM. Aging of proteins: immunological detection of a glucose-derived pyrrole formed during maillard reaction in vivo. *J Biol Chem*. 1989;5:3758-3764.

53. Sell DR, Monnier VM. End-stage renal disease and diabetes catalyze the formation of a pentose-derived crosslink from aging human collagen. *J Clin Invest*. 1990;85:380-384.

54. Beisswenger P, Moore L, Brinck-Johnsen T, Curphey T. Increased collagen-linked pentosidine levels and AGEs in early diabetic nephropathy. *J Clin Invest*. 1993;92:212-217.

55. Reddy S, Bichler J, Wells-Knecht K, Thorpe S, Baynes J. Carboxymethyllysine is a dominant AGE antigen in tissue proteins. 1995;34:10872-10878.

56. Ikeda K, Higashi T, Sano H, Jinnouchi Y, Yoshida M, Araki T, Ueda S, Horiuchi S. Carboxymethyllysine protein adduct is a major immunological epitope in proteins modified with AGEs of the Maillard reaction. *Biochemistry*. 1996;35:8075-8083.

57. Schleicher E, Wagner E, Nerlich A. Increased accumulation of glycoxidation product carboxymethyllysine in human tissues in diabetes and aging. *J Clin Invest*. 1997;99:457-468.

58. Sakata N, Imanaga Y, Meng J, Tachikawa Y, Takebayashi S, Nagai R, Horiuchi S, Itabe H, Takano T. Immunohistochemical localization of different epitopes of advanced glycation end products in human atherosclerotic lesions. *Atherosclerosis*. 1998;141:61-75.

59. Degenhardt TP, Thorpe SR, Baynes JW. Chemical modification of proteins by methylglyoxal. *Cell Mol Biol (Noisy-le-grand)*. 1998;44:1139-1145.

60. Wells-Knecht KJ, Brinkmann E, Wells-Knecht MC, Litchfield JE, Ahmed MU, Reddy S, Zyzak DV, Thorpe SR, Baynes JW. New biomarkers of Maillard reaction damage to proteins. *Nephrol Dial Transplant*. 1996;11:41-47.

61. Niwa T, Katsuzaki T, Miyazaki S, Miyazaki T, Ishizaki Y, Hayase F, Tatemichi N, Takei Y. Immunohistochemical detection of imidazolone, a novel advanced glycation end product, in kidneys and aortas of diabetic patients. *J Clin Invest*. 1997;99:1272-1280.

62. Ahmed MU, Brinkmann Frye E, Degenhardt TP, Thorpe SR, Baynes JW. N-epsilon-(carboxyethyl)lysine, a product of the chemical modification of proteins by methylglyoxal, increases with age in human lens proteins. *Biochem J*. 1997;324:565-570.

63. Odani H, Shinzato T, Usami J, Matsumoto Y, Brinkmann Frye E, Baynes JW, Maeda K. Imidazolium crosslinks derived from reaction of lysine with glyoxal and methylglyoxal are increased in serum proteins of uremic patients: evidence for increased oxidative stress in uremia. *FEBS Lett*. 1998;427:381-385.

64. Anderson MM HS, Hsu FF, al. e. Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to convert hydroxy-amino acids into glycolaldehyde, 2-hydroxypropanal, and acrolein. A mechanism for the generation of highly reactive alpha-hydroxy and alpha-beta unsaturated aldehydes by phagocytes at sites of inflammation. *J Clin Invest*. 1997;99:424-432.

65. Basta G, Lazzerini G, Massaro M, Simoncini T, Tanganelli P, Fu C, Kislinger T, Stern DM, Schmidt AM, De Caterina R. Advanced glycation end products activate endothelium through signal-transduction receptor RAGE: a mechanism for amplification of inflammatory responses. *Circulation*. 2002;105:816-822.

66. Sell D, Monnier VM. Structure elucidation of senescence cross-link from human extracellular matrix: implication of pentoses in the aging process. *J Biol Chem*. 1989;264:21597-21602.

67. Brownlee M. Advanced glycosylation in diabetes and aging. *Annu Rev Med*. 1995;46:223-234.

68. Sugaya K, Fukagawa T, Matsumoto KI, Mita K, Takahashi EI, Ando A, Inoko H, Ikemura T. Three genes in the human MHC class III region near the junction with class

II: gene for RAGE, PBX2 homeobox gene and a notch homolog, human counterpart of mouse mammary tumor gene int-2. *Genomics*. 1994;23:408-419.

69. Ritthaler U, Roth H, Bierhaus A, Ziegler R, Schmidt AM, Waldherr R, Wahl P, Stern D, Nawroth P. Expression of RAGE in peripheral occlusive vascular disease. *Am J Pathol*. 1995;146:688-694.

70. Miyata T, Hori O, Zhang J, Yan SD, Ferran L, Iida Y, Schmidt AM. RAGE mediates the interaction of AGE-beta-2-microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway: implications for the pathogenesis of dialysis-related amyloidosis. *J Clin Invest*. 1996;98:1088-1094.

71. Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagashima M, Morser J, Migheli A, Nawroth P, Stern D, Schmidt AM. RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease. *Nature*. 1996;382:685-691.

72. Chappey O, Dosquet C, Wautier MP, Wautier JL. Advanced glycation end products, oxidant stress and vascular lesions. *Eur J Clin Invest*. 1997;27:97-108.

73. Kent MJC, Light ND, Bailey AJ. Evidence for glucose-mediated covalent cross-linking of collagen after glycosylation in vitro. *Biochem J*. 1985;225:745-752.

74. Brownlee M, Vlassara H, Kooney T, Ulrich P, Cerami A. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science*. 1986;232:1629-1632.

75. Bailey AJ, Paul RG, Knott L. Mechanisms of maturation and ageing of collagen. *Mech Ageing Dev*. 1998;106:1-56.

76. Tanaka S, Avigad G, Brodsky B, Eikenberry EF. Glycation induces expansion of the molecular packing of collagen. *J Mol Biol*. 1988;203:495-505.

77. Brownlee M, Pongor S, Cerami A. Covalent attachment of soluble proteins by nonenzymatically glycosylated collagen: role in the in situ formation of immune complexes. *J Exp Med*. 1983;158:1739-1744.

78. Meng J, Sakata N, Takebayashi S, Asano T, Futata T, Nagai R, Ikeda K, Horiuchi S, Myint T, Taniguchi N. Glycoxidation in aortic collagen from STZ-induced diabetic rats and its relevance to vascular damage. *Atherosclerosis*. 1998;136:355-365.

79. Schnider SL, Kohn RR. Effects of age and diabetes mellitus on the solubility and nonenzymatic glucosylation of human skin collagen. *J Clin Invest*. 1981;67:1630-1635.

80. Tsilbary EC, Charonis AS, Reger LA, Wohlhueter RM, Furcht LT. The effect of nonenzymatic glycosylation on the binding of the main noncollagenous NC1 domain to type IV collagen. *J Biol Chem*. 1990;263:4302-4308.

81. Charonis AS, Reger LA, Dege JE, Kouzi-Koliakos K, Furcht LT, Wohlhueter RM, Tsilbary EC. Laminin alterations after in vitro nonenzymatic glycosylation. *Diabetes*. 1988;39:807-814.

82. Cochrane SM, Robinson GB. In vitro glycation of a glomerular basement membrane alters its permeability: a possible mechanism in diabetic complications. *FEBS Lett*. 1995;375:41-44.

83. Huijberts MSP, Wolffenbuttel BRH, Struijker Boudier HAJ. Aminoguanidine treatment increases elasticity and decreases fluid filtration of large arteries from diabetic rats. *J Clin Invest*. 1993;92:1407-1411.

84. Giardino I, Edelstein D, M B. Nonenzymatic glycosylation in vitro and in bovine endothelial cells alters basic fibroblast growth factor activity. *J. Clin. Invest.* 1994;94:110-117.

85. Bucala R, Makita Z, Koschinsky T, Cerami A, Vlassara H. Lipid advanced glycosylation: Pathway for lipid oxidation *in vivo*. *Proc Natl Acad Sci USA*. 1993;90:6434-6438.

86. Bucala R, Mitchell R, Arnold K, Innerarity T, Vlassara H, Cerami A. Identification of the major site of apolipoprotein B modification by advanced glycosylation end products blocking uptake by the low density lipoprotein receptor. *J Biol Chem*. 1995;270:828-832.

87. Bucala R, Makita Z, Vega G, Grundy S, Koschinsky T, Cerami A, Vlassara H. Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency. *Proc Natl Acad Sci U S A*. 1994;91:9441-9445.

88. Loidl-Stahlhofen A, Hannemann K, Spiteller G. Generation of alpha-hydroxyaldehydic compounds in the course of lipid peroxidation. *Biochim Biophys Acta*. 1994;1213:140-148.

89. Glomb MA, Monnier V. Mechanism of protein modifications by glyoxal and glyceraldehyde, reactive intermediates of the Maillard reaction. *J Biol Chem*. 1995;270:10017-10026.

90. Fu MX, Requena JR, Jenkins AJ, Lyons TJ, Baynes JW, Thorpe SR. The advanced glycation end product, Nepsilon-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. *J Biol Chem*. 1996;271:9982-9986.

91. Steinbrecher UP, Witztum JL. Glucosylation of low-density lipoproteins to an extent comparable to that seen in diabetes slows their catabolism. *Diabetes*. 1984;33:130-134.

92. Klein RL, Laimins M, Lopes-Virella MF. Isolation, characterization, and metabolism of the glycated and nonglycated subfractions of low-density lipoproteins isolated from type I diabetic patients and nondiabetic subjects. *Diabetes*. 1995;44:1093-1098.

93. Sobenin IA, Tertov VV, Koschinsky T, Bunting CE, Slavina ES, Dedov II, Orekhov AN. Modified low density lipoprotein from diabetic patients causes cholesterol accumulation in human intimal aortic cells. *Atherosclerosis*. 1993;100:41-54.

94. Davis JW, Hartman CR, Davis RF, Kyner JL, Lewis HD, Phillips PE. Platelet aggregate ratio in diabetes mellitus. *Acta Haemat*. 1982;67:222-224.

95. Davì G, Rini GB, Averna M. Enhanced platelet release reaction in insulin-dependent and insulin-indipendent diabetic patients. *Haemostasis*. 1982;12:275-281.

96. Sampietro T, Lenzi S, Cecchetti P. Nonenzymatic glycation of human platelet membrane proteins in vitro and in vivo. *Clin Chem*. 1986;32:1328-1331.

97. Yatscoff RW, Mehta A, Gerrard JM. Glycation of platelet protein in diabetes mellitus: lack of correlation with platelet function. *Clin Biochem*. 1987;20:359-363.

98. Winocour PD, Watala C, Kinlough-Rathbone Rl. Reduced membrane fluidity and increased glycation of membrane proteins of platelets from diabetic subjects are not associated with increased platelet adherence to glycated collagen. *J Lab Clin Med*. 1992;120:921-928.

99. Colwell JA. Vascular thrombosis in Type II diabetes mellitus. *Diabetes*. 1993;42:8-11.

100. Jones RL. Fibrinopeptide A in diabetes mellitus: relation to levels of blood glucose, fibrinogen disappearance, and hemodynamic changes. *Diabetes*. 1985;34:836-841.

101. Ceriello A, Giugliano D, Quatraro A, Dello Russo P, Marchi E, Torella R. Hyperglycemia may determine fibrinopeptide A plasma level increase in humans. *Metabolism*. 1989;38:1162-1163.

102. Villanueva GB, Allen N. Demonstration of altered antithrombin III activity due to non enzymatic glycosylation at glucose concentration expected to be encountered in severely diabetic patients. *Diabetes*. 1988;37:1103-1107.

103. Gray E, Barrowcliffe TW. Inhibition of antithrombin III by lipid peroxides. *Thromb Res*. 1985;37:241-250.

104. Vukovich TC, Schernthaner G. Decreased protein C levels in patients with insulin-dependent type I diabetes mellitus. *Diabetes*. 1986;35:617-619.

105. Vlassara H, Brownlee M, Cerami A. High-affinity receptor-mediated uptake and degradation of glucose-modified proteins a potential mechanism for the removal of senescent macromolecules. *Proc Natl Acad Sci USA*. 1985;82:5588-5592.

106. Vlassara H, Brownlee M, Cerami A. Novel macrophage receptor for glucose-modified proteins is distinct from previously described scavenger receptors. *J Exp Med*. 1986;164:1301-1309.

107. Yang Z, Makita J, Mori Y, Brunelle S, Cerami A, Sehajpal P, Suthanthiran M, Vlassara H. Two novel rat liver membrane proteins that bind advanced glycosylation endproducts: relationship to macrophage receptor for glucose-modified proteins. *J Exp Med*. 1991;174:515-524.

108. Schmidt AM, Mora R, Cao R, Yan SD, Brett J, Ramakrishnan R, Tsang TC, Simionescu M, Stern D. The endothelial cell binding site for advanced glycation endproducts consists of a complex: an integral membrane protein and a lactoferrin-like polypeptide. *J Biol Chem*. 1994;269:9882-9888.

109. Vlassara H, Li Y, Imani F, Wojciechowicz D, Yang Z, Liu F, Cerami A. Galectin-2 as a high affinity binding protein for advamced glycation end products (AGE): a new member of the AGE-receptor complex. *Mol Med*. 1995;1:634-646.

110. Kislinger T, Fu C, Huber B, Qu W, Taguchi A, Yan SD, Hofmann M, Yan SF, Pischetsrieder M, Stern D, Schmidt AM. N^{ε}-(carboxymethyl)Lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. *J Biol Chem*. 1999;274:31740-31749.

111. Mackic JB, Stins M, McComb JG, Calero M, Ghiso J, Kim KS, Yan SD, Stern D, Schmidt AM, Frangione B, lokovic BV. Human blood-brain barrier receptors for Alzheimer's amyloid-beta 1- 40. Asymmetrical binding, endocytosis, and transcytosis at the apical side of brain microvascular endothelial cell monolayer. *J Clin Invest*. 1998;102:734-43.

112. Yan SD, Roher A, Schmidt AM, Stern DM. Cellular cofactors for amyloid beta-peptide-induced cell stress. Moving from cell culture to in vivo. *Am J Pathol*. 1999;155:1403-1411.

113. Schmidt AM, Vianna M, Gerlach M, Brett J, Ryan J, Kao J, Esposito C, Hegarty H, Hurley W, M C. Isolation and characterization of binding proteins for advanced glycosylation endproducts from lung tissue which are present on the endothelial cell surface. *J Biol Chem*. 1992;267:14987-14997.

114. Neeper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC, Elliston K, Stern D, Shaw A. Cloning and expression of a cell surface receptor for advanced glycosylation endproducts of proteins. *J Biol Chem*. 1992;267:14998-15004.

115. Brett J, Schmidt AM, Yan SD, Zou YS, Weidman E, Pinksy D, Nowygrod R, Neeper M, Przysiecki C, Shaw A, Migheli A, Stern D. Survey of the distribution of a newly characterized receptor for advanced glycation end products in tissues. *Am J Pathol*. 1993;143:1699-1712.

116. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J Clin Invest*. 2001;108:949-955.

117. Rong LL, Yan SF, Hans-Wagner D, Song F, Stern DM, Prezedborski S, Hays AP, Schmidt AM. RAGE-dependent mechanisms accelerate neuronal dysfunction in a murine model of amyotrophic lateral sclerosis. *Society for Neuroscience 32nd Annual Meeting*. 2002;719.2:53.

118. Hofmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell*. 1999;97:889-901.

119. Schmidt AM, Hofmann M, Taguchi A, Yan SD, Stern DM. RAGE: a multiligand receptor contributing to the cellular response in diabetic vasculopathy and inflammation. *Semin Thromb Hemost*. 2000;26:485-493.

120. Kislinger T, Tanji N, Wendt T, Qu W, Lu Y, Ferran LJ Jr, Taguchi A, Olson K, Bucciarelli L, Goova M, Hofmann MA, Cataldegirmen G, D'Agati V, Pischetsrieder M, Stern DM, Schmidt AM. Receptor for advanced glycation end products mediates inflammation and enhanced expression of tissue factor in vasculature of diabetic apolipoprotein E-null mice. *Arterioscler Thromb Vasc Biol*. 2001;21:905-910.

121. Wendt T, Bucciarelli L, Qu W, Lu Y, Yan SF, Stern DM, Schmidt AM. Receptor for Advanced Glycation Endproducts (RAGE) and Vascular Inflammation: Insights into the Pathogenesis of Macrovascular Complications in Diabetes. *Curr Atheroscler Rep*. 2002;4:228-237.

122. Yan SD, Zhu H, Zhu A, Golabek A, Du H, Roher A, Yu J, Soto C, Schmidt AM, Stern D, Kindy M. Receptor-dependent cell stress and amyloid accumulation in systemic amyloidosis. *Nat Med*. 2000;6:643-651.

123. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D. Cellular receptors for advanced glycation end products: Implications for Induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arterioscler Thromb*. 1994;14:1521-1528.

124. Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, D S. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem*. 1994;269:9889-9897.

125. Scivittaro V, Ganz MB, Weiss MF. AGEs induce oxidative stress and activate protein kinase C-β_{II} in neonatal mesangial cells. *Am J Physiol Renal Physiol*. 2000;278:F676-F683.

126. Wautier MP, Chappey O, Corda S, Stern DM, Schmidt AM, Wautier JL. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. *Am J Physiol Endocrinol Metab*. 2001;280:E685-E694.

127. Basta G, Lazzerini G, Del Turco S, Ratto GM, Schmidt AM, De Caterina R. At least 2 distinct pathways generating reactive oxygen species mediate vascular cell adhesion

molecule-1 induction by advanced glycation end products. *Arterioscler Thromb Vasc Biol*. 2005;25:1401-7.

128. Siebenlist U, Franzoso G, Brown K. Structure, regulation and function of NF-kappa B. *Annu Rev Cell Biol*. 1994;10:405-455.

129. Vlassara H. Receptor-mediated interactions of advanced glycosylation end products with cellular components within diabetic tissues. *Diabetes*. 1992;41:52-6.

130. Vlassara H, Brownlee M, Manogue KR, Dinarello CA, Pasagian A. Cachectin / TNF and IL-1 induced by glucose-modified proteins: role in normal tissue remodeling. *Science*. 1988;240:1546-1548.

131. Kirstein M, Brett J, Radoff S, Ogawa S, Stern D, Vlassara H. Advanced protein glycosylation induces transendothelial human monocyte chemotaxis and secretion of platelet-derived growth factor: role in vascular disease of diabetes and aging. *Proc Natl Acad Sci USA*. 1990;87:9010-9014.

132. Kirstein M, Aston C, Hintz R, Vlassara H. Receptor-specific induction of insulin-like growth factor I (IGF-1) in human monocytes by advanced glycosylation end product-modified proteins. *J Clin Invest*. 1992;90:439-446.

133. Schmidt AM, Yan SD, Brett J, Mora R, Nowygrod R, Stern D. Regulation of human mononuclear phagocyte migration by cell surface-binding proteins for advanced glycation end products. *J Clin Invest*. 1993;91:2155-2168.

134. Miyata T, Inagi R, Iida Y, Sato M, Yamada N, Oda O, Maeda K, Seo H. Involvement of beta 2-microglobulin modified with advanced glycation end products in the pathogenesis of hemodialysis-associated amyloidosis. Induction of human monocyte chemotaxis and

macrophage secretion of tumor necrosis factor-alpha and interleukin-1. *J Clin Invest*. 1994;93:521-8.

135. Imani F, Horii Y, Suthanthiran M, Skolnik EY, Makita Z, Sharma V, Sehajpal P, Vlassara H. Advanced glycosylation endproduct-specific receptors on human and rat T-lymphocytes mediate synthesis of interferon gamma: role in tissue remodeling. *J Exp Med*. 1993;178:2165-2172.

136. De Caterina R, Bourcier T, Laufs U, La Fata V, Lazzerini G, Neish AS, Libby P, JK L. Induction of endothelial-leukocyte interaction by interferon-γ requires coactivation of nuclear factor -κB. *Arterioscler Thromb Vasc Biol*. 2001;21:227-232.

137. Libby P, Hansson GK. Involvement of the immune system in human atherogenesis: current knowledge and unanswered questions. *Lab Invest*. 1991;64:5-15.

138. Stemme S, Rymo L, Hansson GK. Polyclonal origin of T lymphocytes in human atherosclerotic plaques. *Lab Invest*. 1991;65:654-660.

139. Sakata N, Meng J, Takebayashi S. Effects of advanced glycation end products on the proliferation and fibronectin production of smooth muscle cells. *J Atheroscler Thromb*. 2000;7:169-176.

140. Esposito C, Gerlach H, Brett J, Stern D, Vlassara H. Endothelial receptor-mediated binding of glucose-modified albumin is associated with increased monolayer permeability and modulation of cell surface coagulant properties. *J Exp Med*. 1989;170:1387-1407.

141. Wautier JL, Zoukourian C, Chappey O, Wautier MP, Guillausseau PJ, Cao R, Hori O, Stern D, Schmidt AM. Receptor-mediated endothelial cell dysfunction in diabetic

vasculopathy: soluble receptor for advanced glycation end products blocks hyperpermeability in diabetic rats. *J Clin Invest*. 1996;97:238-243.

142. Vlassara H, Fuh H, Makita Z, Krungkrai, Cerami A, Bucala R. Exogenous advanced glycosylation endproducts induce complex vascular dysfunction in normal animals: A model for diabetic and aging complications. *Proc Natl Acad Sci USA*. 1992;89:12043-12047.

143. Bierhaus A, Illmer T, Kasper M, Luther T, Quehenberger P, Tritschler H, Wahl P, Ziegler R, Muller M, Nawroth PP. Advanced glycation end product (AGE)-mediated induction of tissue factor in cultured endothelial cells is dependent on RAGE. *Circulation*. 1997;96:2262-2271.

144. Emeis JJ, Kooistra T. Interleukin 1 and lipopolysaccharide induce an inhibitor of tissue-type plasminogen activator in vivo and in cultured endothelial cells. *J Exp Med*. 1986;163:1260-1266.

145. Van Hindsberg VWM, Kooistra T, Van Den Berg EA, Princen HMG, Fiers W, Emeis JJ. Tumour necrosis factor increases the production of plasminogen activator in vivo and in cultured endothelial cells. *Blood*. 1988;72:1467-1473.

146. Cybulsky MI, Gimbrone MA Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science*. 1991;251:788-791.

147. Li H, Cybulsky MI, Gimbrone MA Jr, Libby P. Inducible expression of vascular cell adhesion molecule-1 by vascular smooth muscle cells in vitro and within rabbit atheroma. *Am J Pathol*. 1993;143:1551-1559.

148. Valencia JV, Mone M, Koehne C, Rediske J, Hughes TE. Binding of receptor for advanced glycation end products (RAGE) ligands is not sufficient to induce

inflammatory signals: lack of activity of endotoxin-free albumin-derived advanced glycation end products. *Diabetologia*. 2004;47:844-52.

149. Bucala R, Tracey J, Cerami A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J Clin Invest*. 1991;87:432-438.

150. De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MA Jr, Shin WS, JK L. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest*. 1995;96:60-68.

151. Oyama Y, Kawasaki H, Hattori Y, Kanno M. Attenuation of endothelium-dependent relaxation in aorta from diabetic rats. *Eur J Pharmacol*. 1986;131:75-78.

152. Brink C, Duncan PG, Douglas JS. Decreased vascular sensitivity to histamine during aging. *Agens Actions*. 1984;14:8-10.

153. Ludmer PL, Selwyn A, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction produced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med*. 1986;315:1046-1051.

154. Panza JA, Quyumi AA, Brush J E, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patient with essential hypertension. *N Engl J Med*. 1990;323:22-27.

155. Quehenberger P, Bierhaus A, Fasching P, Muellner C, Klevesath M, Hong M, Stier G, Sattler M, Schleicher E, Speiser W, Nawroth PP. Endothelin 1 transcription is controlled by nuclear factor-kappaB in AGE-stimulated cultured endothelial cells. *Diabetes*. 2000;49:1561-1570.

156. Vlassara H, Striker LJ, Teichberg S, Fuh H, Li Y, Steffes M. Advanced glycation endproducts induce glomerular sclerosis and albuminuria in normal rats. *Proc Natl Acad Sci USA*. 1994;91:11704-11708.

157. Jensen T, Borch-Johnsen K, Kofoed-Enevoldsen A, Deckert T. Coronary heart disease in young type 1 (insulin-dependent) diabetic patients with and without diabetic nephropathy: incidence and risk factors. *Diabetologia*. 1987;30:144-148.

158. Mattock MB, Morrish NJ, Viberti G KH, Fitzgerald AP, G J. Prospective study of microalbuminuria as predictor of mortality in NIDDM. *Diabetes*. 1992;41:736-41.

159. Park SH, Marso SP, Zhou Z, Foroudi F, Topol EJ, Lincoff AM. Neointimal hyperplasia after arterial injury is increased in a rat model of non-insulin-dependent diabetes mellitus. *Circulation*. 2001;104:815-9.

160. Zhou Z, Wang K, Penn MS, Marso SP, Lauer MA, Forudi F, Zhou X, Qu W, Lu Y, Stern DM, Schmidt AM, Lincoff AM, Topol EJ. Receptor for AGE (RAGE) mediates neointimal formation in response to arterial injury. *Circulation*. 2003;107:2238-43.

161. Sakaguchi T, Yan SF, Yan SD, Belov D, Rong LL, Sousa M, Andrassy M, Marso SP, Duda S, Arnold B, Liliensiek B, Nawroth PP, Stern DM, Schmidt AM, Naka Y. Central role of RAGE-dependent neointimal expansion in arterial restenosis. *J Clin Invest*. 2003;111:959-72.

162. Chavakis T, Bierhaus A, Al-Fakhri N, Schneider D, Witte S, Linn T, Nagashima M, Morser J, Arnold B, Preissner KT, Nawroth PP. The pattern recognition receptor (RAGE) is a counterreceptor for leukocyte integrins: a novel pathway for inflammatory cell recruitment. *J Exp Med*. 2003;198:1507-15.

163. Kilhovd BK, Berg TJ, Birkeland KI, Thorsby P, Hanssen KF. Serum levels of advanced glycation end products are increased in patients with type 2 diabetes and coronary heart disease. *Diabetes Care*. 1999;22:1543-8.

164. Aso Y, Inukai T, Tayama K, Takemura Y. Serum concentrations of advanced glycation endproducts are associated with the development of atherosclerosis as well as diabetic microangiopathy in patients with type 2 diabetes. *Acta Diabetol*. 2000;37:87-92.

165. Kanauchi M, Tsujimoto N, Hashimoto T. Advanced glycation end products in nondiabetic patients with coronary artery disease. *Diabetes Care*. 2001;24:1620-23.

166. Kitauchi T, Yoshida K, Yoneda T, Saka T, Yoshikawa M, Ozono S, Hirao Y. Association between pentosidine and arteriosclerosis in patients receiving hemodialysis. *Clin Exp Nephrol*. 2004;8:48-53.

167. Kosaki A, Hasegawa T, Kimura T, Iida K, Hitomi J, Matsubara H, Mori Y, Okigaki M, Toyoda N, Masaki H, Inoue-Shibata M, Nishikawa M, Iwasaka T. Increased plasma S100A12 (EN-RAGE) levels in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2004;89:5423-8.

168. Ahmed N, Babaei-Jadidi R, Howell SK, Thornalley PJ, Beisswenger PJ. Glycated and oxidized protein degradation products are indicators of fasting and postprandial hyperglycemia in diabetes. *Diabetes Care*. 2005;28:2465-71.

169. Shimada S, Tanaka Y, Ohmura C, Tamura Y, Shimizu T, Uchino H, Watada H, Hirose T, Nakaniwa T, Miwa S, Kawamori R. N-(carboxymethyl)valine residues in hemoglobin (CMV-Hb) reflect accumulation of oxidative stress in diabetic patients. *Diabetes Res Clin Pract*. 2005;69:272-8.

170. Kilhovd BK, Giardino I, Torjesen PA, Birkeland KI, Berg TJ, Thornalley PJ, Brownlee M, Hanssen KF. Increased serum levels of the specific AGE-compound methylglyoxal-derived hydroimidazolone in patients with type 2 diabetes. *Metabolism*. 2003;52:163-7.

171. Nakamura Y, Horii Y, Nishino T, Shiiki H, Sakaguchi Y, Kagoshima T, Dohi K, Makita Z, Vlassara H. Immunohistochemical localisation of advanced glycosylation end products in coronary atheroma and cardiac tissue in diabetes mellitus. *Am J Pathol*. 1993;143:1649-1656.

172. Vlassara H, Li YM, Imani F, Wojciechowicz D, Yang Z, Liu FT, Cerami A. Identification of galectin-3 as a high affinity binding protein for advanced glycation end products (AGEs): a new member of the AGE-receptor complex. *Mol Med*. 1996;1:634-646.

173. Monnier VM, Vishwanath V, Frank KE, Elmets CA, Dauchot P, Kohn RR. Relation between complications of type 1 diabetes mellitus and collagen-linked fluorescence. *N Engl J Med*. 1986;314:403-408.

174. Sims TJ, Rasmussen LM, Oxlund H, Bailey AJ. The role of glycation cross-links in diabetic vascular stiffening. *Diabetologia*. 1996;39:946-951.

175. Cipollone F, Fazia M, Iezzi A, Zucchelli M, Pini B, De Cesare D, Ucchino S, Spigonardo F, Bajocchi G, Bei R, Muraro R, Artese L, Piattelli A, Chiarelli F, Cuccurullo F, Mezzetti A. Suppression of the functionally coupled cyclooxygenase-2/prostaglandin E synthase as a basis of simvastatin-dependent plaque stabilization in humans. *Circulation*. 2003;107:1479-85.

176. Pettersson-Fernholm K, Forsblom C, Hudson BI, Perola M, Grant PJ, Groop PH. The functional -374 T/A RAGE gene polymorphism is associated with proteinuria and cardiovascular disease in type 1 diabetic patients. *Diabetes*. 2003;52:891-4.

177. Falcone C, Campo I, Emanuele E, Buzzi MP, Zorzetto M, Sbarsi I, Cuccia M. Relationship between the -374T/A RAGE gene polymorphism and angiographic coronary artery disease. *Int J Mol Med*. 2004;14:1061-4.

178. Falcone C, Campo I, Emanuele E, Buzzi MP, Geroldi D, Belvito C, Zorzetto M, Sbarsi I, Cuccia M. -374T/A polymorphism of the RAGE gene promoter in relation to severity of coronary atherosclerosis. *Clin Chim Acta*. 2005;354:111-6.

179. Kirbis J, Milutinovic A, Steblovnik K, Teran N, Terzic R, Zorc M. The -429 T/C and -374 T/A gene polymorphisms of the receptor of advanced glycation end products gene (RAGE) are not risk factors for coronary artery disease in Slovene population with type 2 diabetes. *Coll Antropol*. 2004;28:611-6.

180. Edelstein D, Brownlee M. Mechanistic studies of advanced glycosylation end product inhibition by aminoguanidine. *Diabetes*. 1992;41:26-28.

181. Freedman BI, Wuerth JP, Cartwright K, Bain RP, Dippe S, K H, Mooradian AD, Spinowitz BS. Design and baseline characteristics for the aminoguanidine Clinical Trial in Overt Type 2 Diabetic Nephropathy (ACTION II). *Control Clin Trials*. 1999;5:493-510.

182. Vasan S, Zhang X, Zhang X, Kapurniotu A, Bernhagen J, Teichberg S, Basgen J, Wagle D, Shih D, Terlecky I, Bucala R, Cerami A, Egan J, Ulrich P. An agent cleaving glucose-derived protein crosslinks in vitro and in vivo. *Nature*. 1996;382:275-278.

183. Cooper ME, Thallas V, Forbes J, Scalbert E, Sastra S, Darby I, Soulis T. The cross-link breaker, N-phenacylthiazolium bromide prevents vascular advanced glycation end-product accumulation. *Diabetologia*. 2000;43:660-664.

184. Vasan S, Foiles PG, Founds HW. Therapeutic potential of AGE inhibitors and breakers of AGE protein cross-links. *Expert Opin Investig Drugs*. 2001;10:1977-1987.

185. Bierhaus A, Schiekofer S, Schwaninger M, Andrassy M, Humpert PM, Chen J, Hong M, Luther T, Henle T, Kloting I, Morcos M, Hofmann M, Tritschler H, Weigle B, Kasper M, Smith M, Perry G, Schmidt AM, Stern DM, Haring HU, Schleicher E, Nawroth PP. Diabetes-associated sustained activation of the transcription factor nuclear factor-kappaB. *Diabetes*. 2001;50:2792-2808.

186. Miyata T, Hori O, Zhang J, Yan SD, Ferran L, Iida Y, Schmidt AM. The receptor for advanced glycation end products (RAGE) is a central mediator of the interaction of AGE-beta2microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway. Implications for the pathogenesis of dialysis-related amyloidosis. *J Clin Invest*. 1996;98:1088-1094.

187. Hofmann MA, Drury S, Hudson BI, Gleason MR, Qu W, Lu Y, Lalla E, Chitnis S, Monteiro J, Stickland MH, Bucciarelli LG, Moser B, Moxley G, Itescu S, Grant PJ, Gregersen PK, Stern DM, Schmidt AM. RAGE and arthritis: the G82S polymorphism amplifies the inflammatory response. *Genes Immun*. 2002;3:123-135.

188. Falcone C, Emanuele E, D'Angelo A, Buzzi MP, Belvito C, Cuccia M, Geroldi D. Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men. *Arterioscler Thromb Vasc Biol*. 2005;25:1032-7.

189. Katakami N, Matsuhisa M, Kaneto H, Matsuoka TA, Sakamoto K, Nakatani Y, Ohtoshi K, Hayaishi-Okano R, Kosugi K, Hori M, Yamasaki Y. Decreased endogenous secretory advanced glycation end product receptor in type 1 diabetic patients: its possible association with diabetic vascular complications. *Diabetes Care*. 2005;28:2716-21.

Table I: Non receptor-mediated effects of AGEs on atherogenesis

Extracellular Matrix
Collagen cross linking and high resistance to collagenases
Enhanced syntesis of extracellular matrix components with consequent narrowing of the vessel lumen
Decreased polymer self-assembly of laminin
Impairment of matrix-bound heparan sulfate proteoglycans
Glycated subendothelial matrix quenching of nitric oxide
Trapping of LDL and IgG in the sub-endothelium
Lipoprotein modifications
Reduced LDL recognition by cellular LDL receptor
Enhanced glycated LDL uptake by macrophage scavenger receptor
Increased LDL susceptibility to oxidative modifications
Alterations of Coagulation and Fibrinolysis
Reduced biological activity of AT-III
Reduced fibrin susceptibility to degradation
Increased platelet aggregation
Reduced platelet membrane fluidity

AGEs: advanced glycation endproducts; LDL: low density lipoproteins; IgG: Immunoglobulins G;
AT-III, antithrombin III

Table II: Receptor-mediated effects of AGEs on atherogenesis

Mononuclear Phagocytes
induction of PDGF, IGF-1, and proinflammatory cytokines such as IL-1β and TNF-α
Chemotaxis by soluble AGE-ligands
Apoptaxis by immobilized AGEs
Increased macrophage uptake of AGE-LDL
Smooth Muscle Cells
Increased proliferative activity
Increased production of fibronectin
Increased susceptibility to oxidative modifications
Endothelial Cells
Increased permeability
Decreased expression of t-PA and increased expression of PAI-I
Increased intracellular oxidative stress
Increased procoagulant activity
Induction of endothelin-1 and increased vasoconstriction
Increased expression of adhesion molecules

AGEs: advanced glycosylation end-products; PDGF: platelet-derived growth factor; IGF-1, insulin-like growth factor-1
TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β
t-PA, tissue-plasminogen activator; PAI-I: plasminogen activator inhibitor-I

Figure legends

Fig. 1: **Possible pathways in the formation of advanced glycation endproducts (AGEs).**

The initial interaction between a highly reactive aldehyde of glucose with any free aminogroup on proteins creates a Schiff's base, which spontaneously rearranges itself into an Amadori's product. Subsequent, slower changes (not shown) are progressively less reversible, and ultimately lead to the formation of AGEs. In addition, a variety of highly reactive carbonyl intermediates such as 3-deoxy-glucosone, glyoxal and methyl-glyoxal can be formed by glucose or Schiff's base or Amadori's product auto-oxidation, which can react again with free aminogroups to form AGE products such as imidazolone, N-ε-carboxy-methyl-lysine (CML), N-ε-carboxy-ethyl-lysine (CEL), glyoxal-lysine dimer (GOLD) and methyl-glyoxal-lysine dimer (MOLD).

Fig. 2: **Chemical structures of some advanced glycation endproducts (AGEs).**

FFI: 2-(2-furoyl)-4(5)-furanyl-1H-imidazole; **AFGP**: 1-alkyl-2-formyl-3,4-diglycosyl pyrrole; Pentosidine; pyrraline; **CML**: N-ε-carboxy-methyl-lysine; **CEL:** N-ε-carboxy-ethyl-lysine; Imidazolone; **GOLD**: glyoxal-lysine dimer; **MOLD**: methyl-glyoxal-lysine dimer.

Fig. 3: A schematic representation of the possible roles of advanced glycation endproducts (AGEs) in the development of accelerated atherosclerosis in diabetes.

(**1**) Circulating glucose enters into the intima, promotes the formation of AGEs on extracellular matrix collagen, creating new crosslinks, which stiffen the vessel wall and increase vascular permeability. (**2**) as a consequence of these phenomena, AGEs promote the intimal entrapment of low-density lipoproteins (LDL), which may already have been oxidized by circulating

glucose; (**3**): AGEs also promote the migration of blood-borne monocytes into the intima, their conversion to activated macrophages, and their release of cytokines and proteases. (**4**): within the intima, activated macrophages increase their lipid uptake, leading to the formation of foam cells. Secondly, a population of activated smooth muscle cells (SMCs) migrates into the intima at sites of vascular lesions, and here proliferates, producing new extracellular matrix. (**5**): the interaction of soluble AGEs with the endothelial cell RAGE (*receptor for AGEs*) increases the expression of tissue factor procoagulant activity, and (**6**): increases the endothelial expression of leukocyte adhesion molecules. **(7)**: the interaction of AGEs with monocytes promotes cell migration (chemotaxis) and **(8)** induces an activated macrophage phenotype, secreting proinflammatory cytokines, such as IL-1β and TNF-α. (**9**): AGE interaction with T cells also increases the synthesis and release of IFN-γ, which represents the main activating factor for monocytes.

Fig. 4: Signal transduction pathways activated by RAGE-ligand interaction

Activation of RAGE by AGEs, amphoterin and S100/calgrunulins induces the increased generation of oxygen radicals by a NAD(P)H oxidase. Free radicals then activate a Ras-MAP kinase pathway eventually leading to the activation and nuclear translocation of NF-κB. The RAGE promoter itself is controlled by NF-κB, and upregulation of the receptor provides an increasing number of binding sites for RAGE-ligands, perpetuating the cellular inflammatory response.

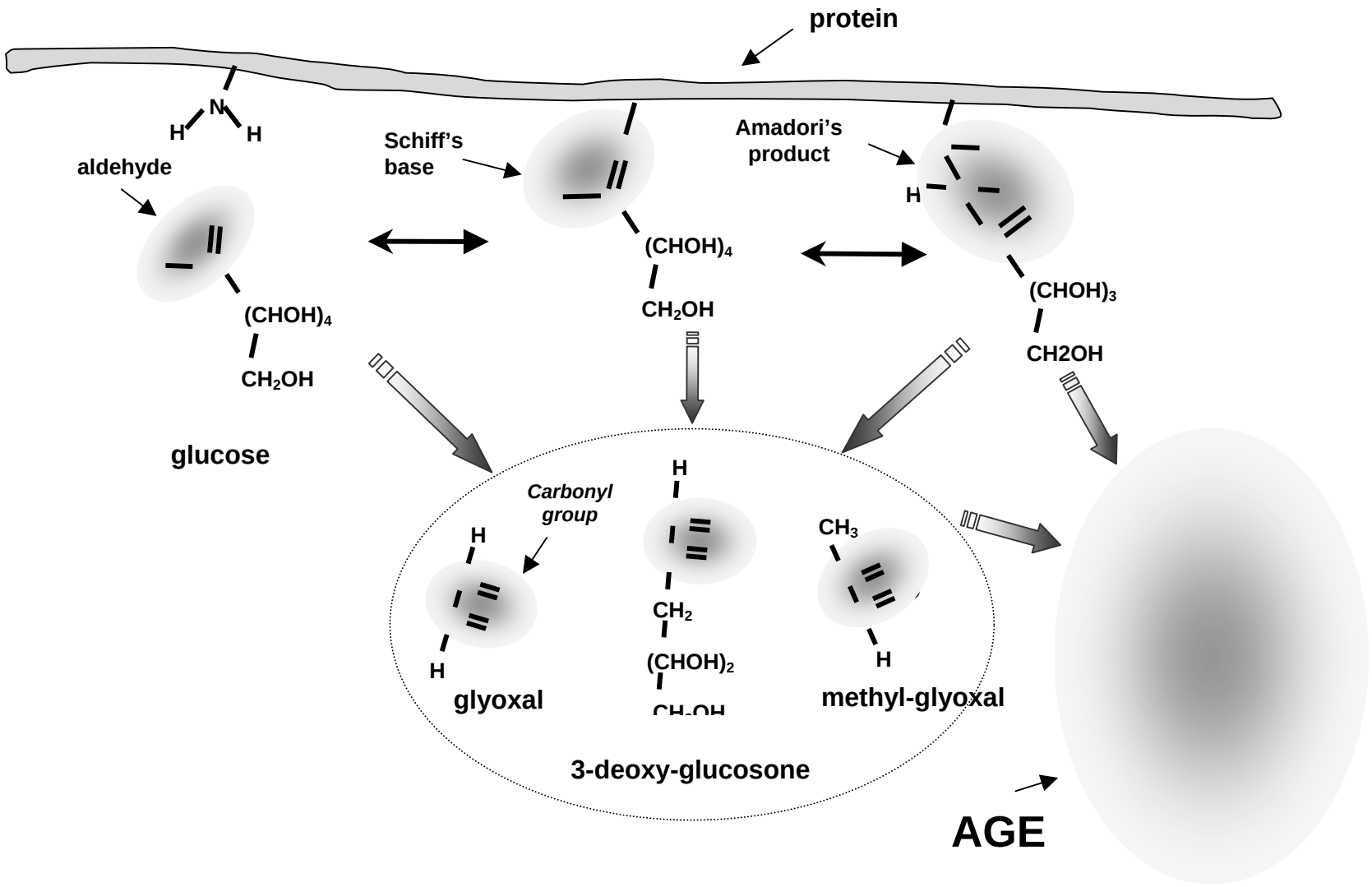
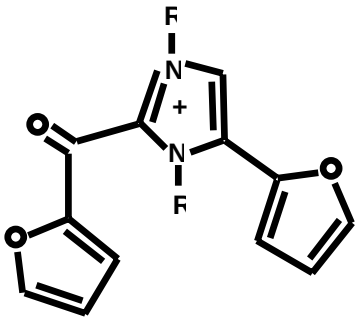
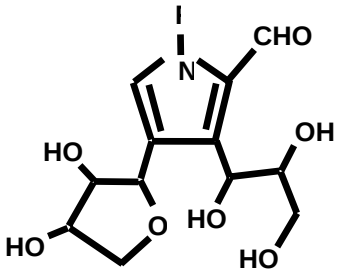

protein
N
H
H
aldehyde
(CHOH)4
CH2OH
glucose
Schiff's
base
(CHOH)4
CH2OH
Amadori's
product
H
(CHOH)3
CH2OH
Carbonyl
group
H
H
glyoxal
H
CH2
(CHOH)2
CH3
H
methyl-glyoxal
3-deoxy-glucosone
AGE

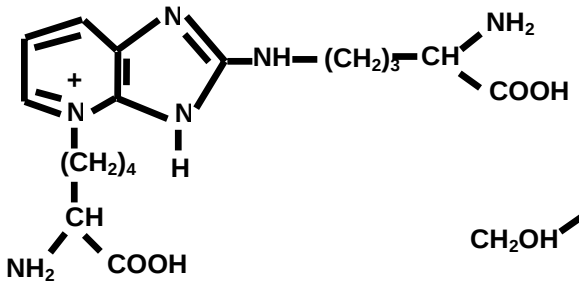
Fig. 1



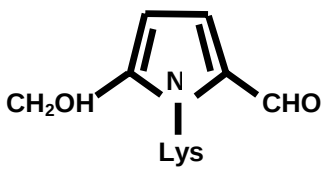
FFI



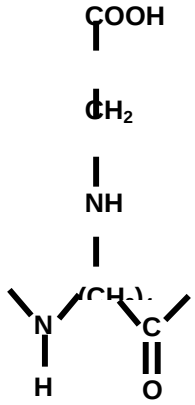
AFGP



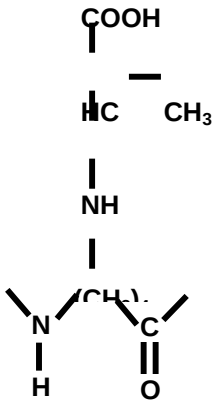
Pentosidine



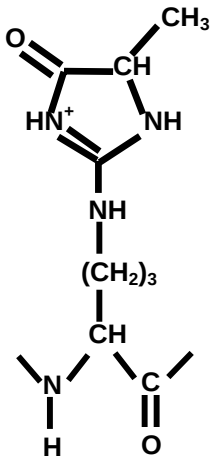
Pyrraline



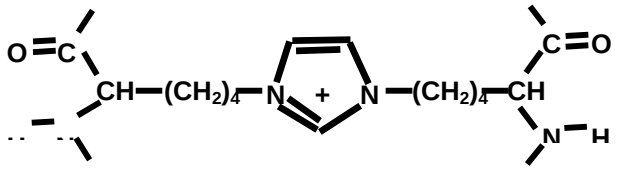
CML



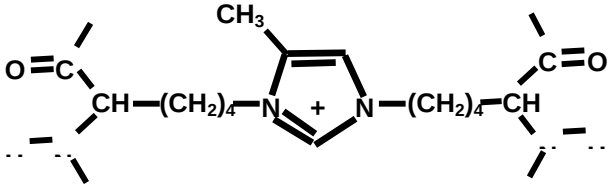
CEL



Imidazolon



GOLD



MOLD

Fig. 2

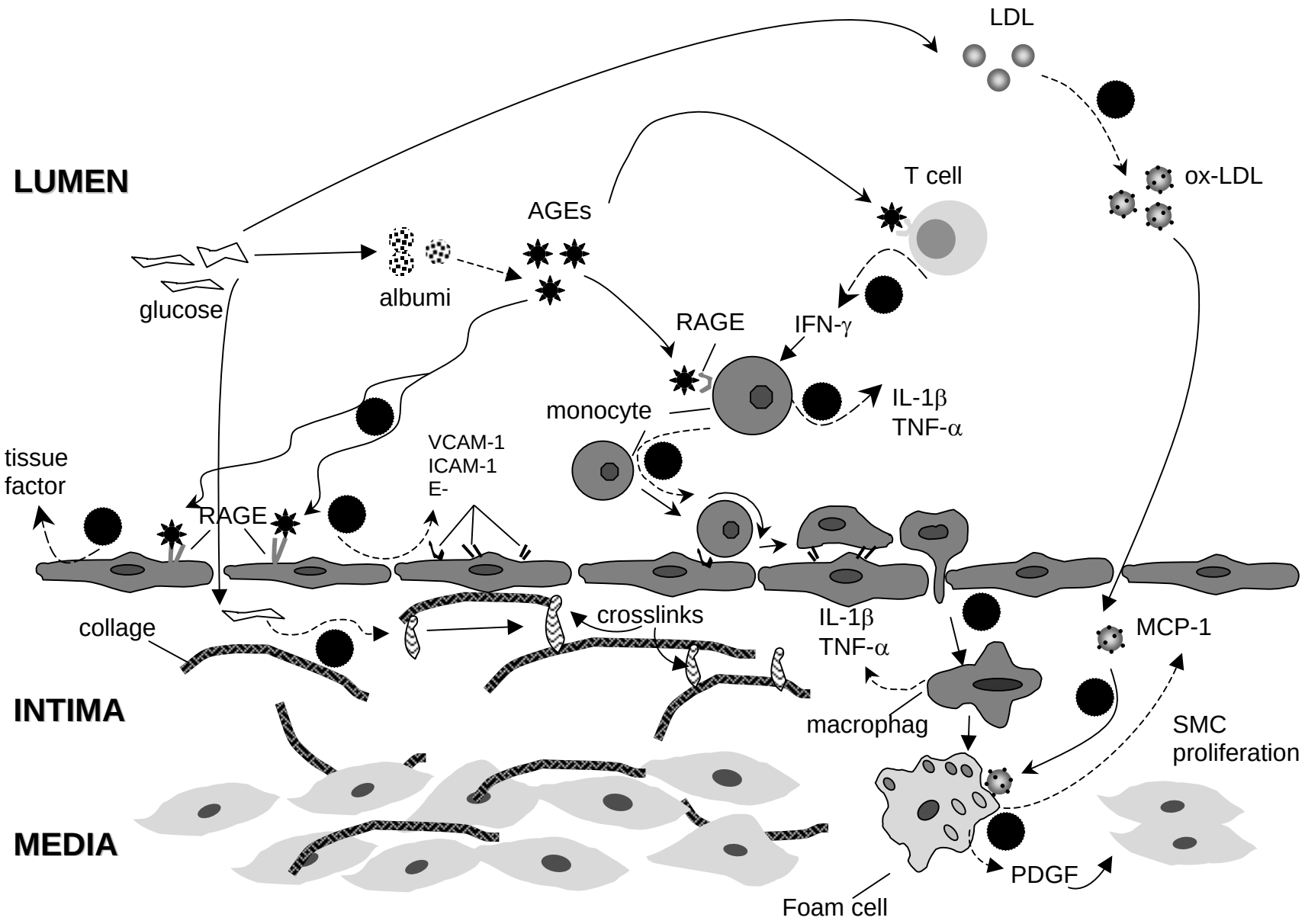

LDL
LUMEN
T cell
ox-LDL
AGEs
albumi
glucose
RAGE
IFN-γ
monocyte
IL-1β
TNF-α
tissue
factor
VCAM-1
ICAM-1
E-
RAGE
collage
crosslinks
IL-1β
TNF-α
MCP-1
INTIMA
macrophag
SMC
proliferation
MEDIA
PDGF
Foam cell

Fig. 3

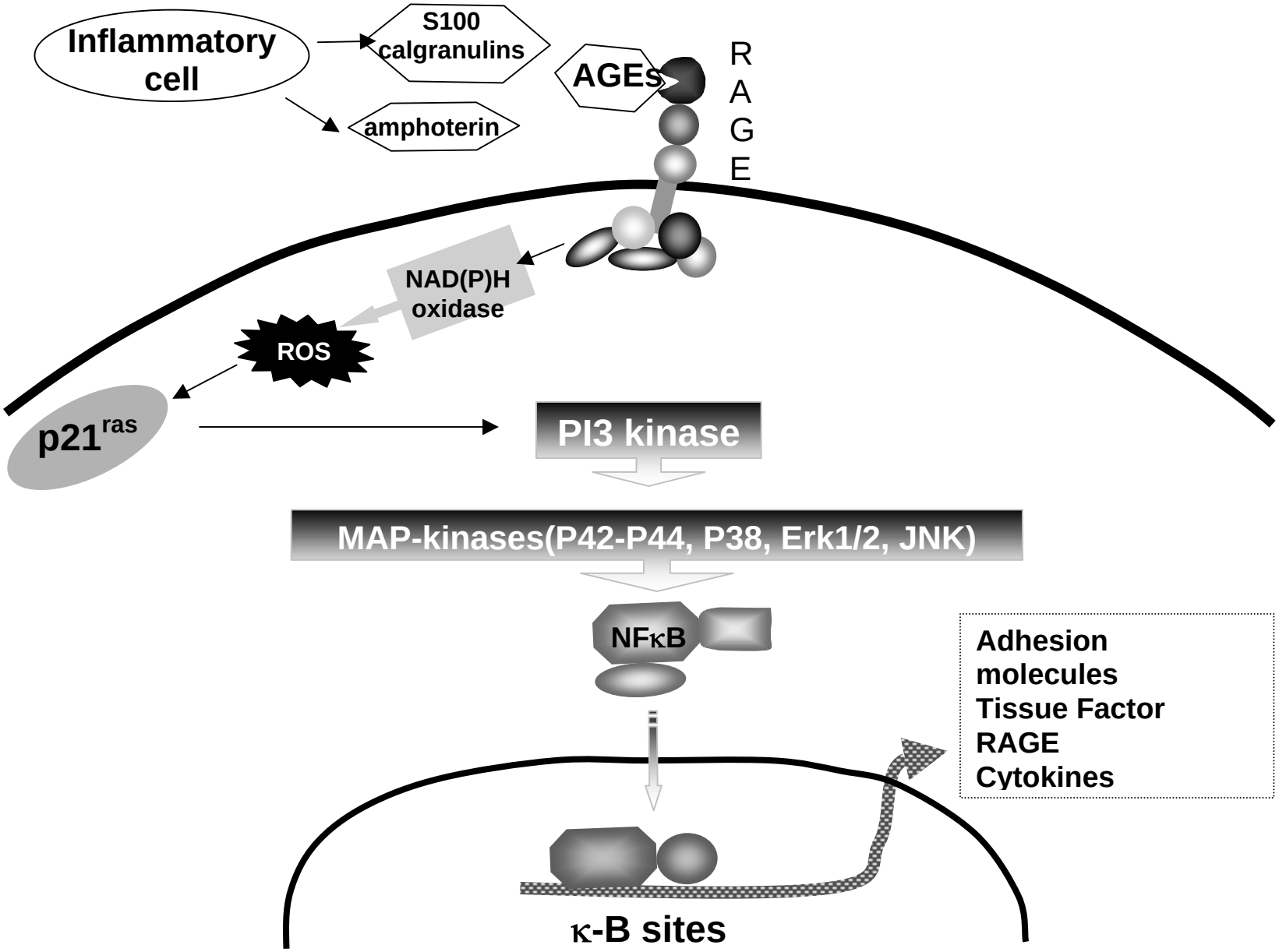

Inflammatory cell
S100 calgranulins
amphoterin
AGEs
RAGE
NAD(P)H oxidase
ROS
p21ras
PI3 kinase
MAP-kinases(P42-P44, P38, Erk1/2, JNK)
NFκB
Adhesion molecules
Tissue Factor
RAGE
Cytokines
κ-B sites

Fig. 4