

## MINIREVIEW

# ADVANCED GLYCATION END PRODUCTS AND THEIR RECEPTOR RAGE IN SYSTEMIC AUTOIMMUNE DISEASES - AN INFLAMMATION PROPAGATING FACTOR CONTRIBUTING TO ACCELERATED ATHEROSCLEROSIS

HANS L. A. NIENHUIS<sup>1</sup>, JOHANNA WESTRA<sup>1</sup>, ANDRIES J. SMIT<sup>2</sup>, PIETER C. LIMBURG<sup>1</sup>, CEES G.M. KALLENBERG<sup>1</sup>, MARC BIJL<sup>1</sup>

Univ. Medical Center Groningen, Dept. of Internal Medicine, <sup>1</sup>Div. of Rheumatology and Clinical Immunology, <sup>2</sup>Div. of Vascular diseases, Groningen, The Netherlands

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Corresponding author: Hans Nienhuis, Dept. of Internal Medicine, Div. of Rheumatology and Clinical Immunology, Univ. Medical Center Groningen, Hanzeplein 1, 9713 GZ or PO Box 30.001, 9700 RB, Groningen, The Netherlands; Phone: (31)50-3614006; Fax: (31)50-3619308; E-mail: [h.l.a.nienhuis@int.umcg.nl](mailto:h.l.a.nienhuis@int.umcg.nl)

**Running Title:** AGE-RAGE in systemic autoimmune diseases

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## **ABSTRACT**

Systemic autoimmune diseases are associated with inflammation and oxidative stress favouring the formation of AGE, able to modulate cellular functions by activation of RAGE. As RAGE expression is increased in an inflammatory milieu, present in patients with systemic autoimmune diseases, these patients are especially prone for the deleterious effects of AGE. Interaction of AGE with RAGE leads to intracellular signalling and subsequent expression of adhesion molecules, chemokines, proinflammatory cytokines and upregulation of RAGE itself. The AGE-RAGE interaction might act as a pro-inflammatory loop in these patients, contributing to chronic low grade inflammation rendering these individuals susceptible for development of accelerated atherosclerosis.

## **AGE**

AGE are a class of compounds resulting from non-enzymatic addition of saccharide derivatives to proteins, lipids or nucleic acids, partly under influence of oxidative stress. This leads to slow formation of intermediary Schiff bases and Amadori products and finally to irreversible AGE, that can be formed more rapidly via intermediate formation of reactive carbonyl compounds such as methylglyoxal or glyoxal in circumstances characterized by oxidative and carbonyl stress. Systemic autoimmune diseases, like SLE and RA are associated with increased oxidative stress due to local and systemic inflammation. Oxidative activity of myeloperoxidase produced by activated phagocytes for example can contribute to formation of AGE (rev. in [1]). This way of AGE formation is independent of hyperglycemia, and may explain increased AGE formation in inflammatory conditions such as SLE and RA [2]. AGE accumulate continuously on long lived proteins in the ECM, and are present in inflamed tissue, such as rheumatoid synovia and atherosclerotic blood vessels [1]. AGE are not simply innocent bystanders as they modulate the function of cells by activation of several receptors including RAGE. This may be an important mechanism contributing to the pathogenesis of vascular inflammation and atherosclerosis.

## **RECEPTORS FOR AGE**

Several receptors which bind AGE have been identified, these include RAGE, Mph SRA/B and receptors of the AGE-receptor complex: AGE-R1/2/3 (OST-48 / 80K-H / galectin-3) [1]. Mph SR and the AGE-receptor complex induce degradation of AGE, whereas RAGE triggers inflammation.

RAGE is a multiligand transmembrane receptor belonging to the Ig-SF. It consists of one V-type (variable) and two C-type (constant) extracellular Ig domains, a single pass transmembrane domain and a short negatively charged C-terminal intracellular signalling domain. Besides AGE a wide range of other endogenous ligands involved in inflammatory processes can bind to this receptor, including: S100/calgranulins, HMGB1,  $\beta$ -sheet fibrils, amyloid- $\beta$  peptide, transthyretin, serum amyloid A, and  $\beta$ 2-integrin. RAGE recognizes a three-dimensional structure rather than a specific amino acid sequence and therefore is considered being a PRR involved in recognition of DAMP. Under normal physiological conditions RAGE is expressed at low levels in several cell types including Mn/Mph, smooth muscle cells, neuronal cells, fibroblasts and ECs. Expression of the receptor can be upregulated by TNF- $\alpha$  and CRP as well as by AGE and other RAGE ligands [3, 4].

## **AGE-RAGE INTERACTION**

AGE have been described in association with a variety of pathological conditions and are implicated in vascular pathology by 3 general mechanisms. (I) cross-linking of AGE with proteins of the ECM decreases blood vessel elasticity. (II) intracellular AGE formation alters cellular functions. (III) AGE modulates cellular functions by activation of RAGE. Park et al

showed that AGE accelerates atherosclerosis by interaction with RAGE using the atherosclerosis model of diabetic apoE KO mice. Blockage of AGE-RAGE interaction stabilizes established atherosclerotic lesions and suppresses vascular inflammation [5]. Ligand binding to RAGE generates ROS, seemingly linked to activation of the NADPH-oxidase and mitochondrial electron transport system. ROS can in turn activate the redox-sensitive transcription factor NF- $\kappa$ B, leading to transcription of genes involved in inflammatory processes such as atherosclerosis and may also lead to formation of additional AGE. Besides NF- $\kappa$ B activation several other pathways link ligand-RAGE interaction to gene expression, including p21<sup>ras</sup>, ERK1/2 (p44/p42), p38 and SAPK/JNK MAPK and the JAK/STAT pathway (rev. in [6]). AGE induce expression of several inflammation related molecules such as VCAM-1, ICAM-1, E-selectin, VEGF, MCP-1, MMP and several IL as well as RAGE [3, 7]. MCP-1 and IL attract and activate monocytes which, via endothelial adhesion molecules migrate into the arterial wall. RAGE itself serves as adhesion molecule interacting with  $\beta$ 2-integrin [8]. AGE-RAGE interaction leads to increased vascular permeability and EC dysfunction, reflected by reduced production of NO and an imbalance in endothelium relaxing and contracting factors. EC activation and dysfunction are considered the first stages of atherosclerosis.

There are also studies not confirming the role of AGE-RAGE in driving inflammation. Valencia et al found that binding to RAGE of AGE was not sufficient to induce VCAM-1 and TNF- $\alpha$  secretion and HMEC grown *in vitro*. They postulated that inflammatory responses could be attributed to endotoxin and metal ion contamination of AGE preparations [9]. Ballinger et al reported no effect of AGE on vascular smooth muscle cells *in vitro* probably due to very low expression of RAGE in these cells [10]. Other studies found that AGE were not able to bind RAGE on HUVEC, Mph and lung epithelial cells [11], suggesting that not all AGE form the necessary structure(s) to interact with RAGE. Moreover, it must be stressed that the effects of AGE *in vitro* are modest compared to effects of physiological concentrations of cytokines. Other RAGE ligands, such as HMGB1 or S100b, seem stronger inducers of inflammation.

### **SOLUBLE RAGE (sRAGE)**

Several truncated forms of RAGE have been described of which C-truncated RAGE, also called sRAGE, has been studied most. sRAGE is generated by alternative splicing [12] or proteolytic cleavage of full-length RAGE (Raucci et al, in press) and lacks the transmembrane and intracellular domains. Circulating sRAGE binds ligands but does not lead to intracellular signalling. AGE are known to upregulate RAGE expression, and therefore may also be involved in regulation of sRAGE. A positive correlation between levels of sRAGE and AGE has been shown [13]. sRAGE is also associated with inflammation. In sepsis levels of sRAGE are elevated [14], in T2DM sRAGE was positively associated with

inflammatory markers [15] and sRAGE was increased in quiescent and even more in active SLE [2]. It has been suggested that the net effect of sRAGE can be either anti-inflammatory or pro-inflammatory depending on the milieu. In the absence of ligands, sRAGE possesses pro-inflammatory properties *in vitro* by interaction with Mac-1. In the presence of HMGB1 sRAGE suppressed inflammation by blocking the HMGB1-RAGE interaction [16]. Most studies support the concept that sRAGE has beneficial effects in a milieu rich in RAGE ligands such as AGE. sRAGE was found to block the effects of AGE on endothelial cells *in vitro* [12]. In diabetic apoE KO mice, administration of recombinant sRAGE was shown not only to suppress the development of atherosclerosis but also to stabilize established atherosclerosis [5] suggesting that exogenous sRAGE acts as decoy receptor. High serum levels of sRAGE were associated with longevity in humans [17]. Low levels of sRAGE are associated with increased mortality in renal transplant recipients [18] and were independently associated with coronary artery disease [19]. We assume that sRAGE is protective against AGE-elicited cellular activation. Compensatory anti-inflammatory mechanism may be responsible for increased sRAGE production during inflammation.

#### **PHARMACOLOGICAL INTERVENTIONS**

Many approaches may help counteracting the deleterious effect of RAGE activation in patients with systemic autoimmune diseases. (I) Reducing formation of AGE. Effective immunosuppression resulting in reduced inflammation and ROS limit AGE formation. ARB and ACEi lower AGE formation by scavenging ROS. Aminoguanidine, pyridoxamine, and OPB-9195, inhibit AGE formation by trapping the critical AGE precursors methylglyoxal and glyoxal [1]. (II) Prevention of AGE-RAGE interaction. Attempts to develop sRAGE as a treatment for humans have been made [20]. In addition, heparin binds to RAGE without inducing inflammation, suggesting that it may be applicable as a RAGE blocker (unpublished). (III) Application of AGE-breakers. These compounds are successful in cleavage of protein cross-links formed by AGE and through this improved artery compliance [1].

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## LIST OF ABBREVIATIONS

ACEi	angiotensin converting enzyme inhibitor
AGE	advanced glycation end products
AGE-R	advanced glycation end product receptor
ApoE	apolipoprotein E
ARB	angiotensin receptor blocker
CRP	C-reactive protein
DAMP	damage associated molecular pattern
EC	endothelial cell
ECM	extracellular matrix
ERK	extracellular signal-regulated kinase
HMEC	human microvascular endothelial cell
HMGB-1	high mobility group box 1 protein
HUVEC	human umbilical vein endothelial cells
ICAM-1	intracellular adhesion molecule-1
Ig	immunoglobulin
Ig-SF	immunoglobulin superfamily
IL	interleukine
JAK	Janus kinase
JNK	c-Jun N-terminal kinase
KN mice	knockout mice
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemotactic protein-1
MMP	matrix metalloproteinases
Mn	monocyte
Mph	macrophage
Mph SR	macrophage scavenger receptor
NADPH	nicotinamide adenine dinucleotide phosphate
NF- $\kappa$ B	nuclear factor- $\kappa$ B
NO	nitric oxide
PRR	pattern recognition receptor
RA	rheumatoid arthritis
RAGE	receptor for advanced glycation end products
ROS	reactive oxygen species
S100b	s100b calcium binding protein b
SAPK	stress-activated protein kinase
SLE	systemic lupus erythematosus
sRAGE	soluble receptor for advanced glycation end products
STAT	signal transducer and activator of transcription
T2DM	type 2 diabetes mellitus
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor