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CLINICAL IMPLICATIONS

The next generation of RAGE modulators: implications for soluble RAGE therapies in vascular inflammation

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Arterial inflammation is the consequence of an orchestrated response of cells within the arterial wall to multifactorial modes of injury; arterial inflammation is a fundamental mechanism contributing to atherosclerosis and restenosis. The remodeling of the arterial wall that results from the inflammation could either be obstructive (as in coronary and peripheral artery disease, or in-stent restenosis) or aneurysmal (as in thoracic and abdominal aortic aneurysms). It remains quite a challenge to decipher the distinct differences in local factors that lead to the varied possible outcomes of arterial inflammation. As a result, often we cannot fully explain the predilection to arterial inflammation of distinct vascular beds, or why, for example, obstruction occurs over dilatation.

Atop these considerations, there is overwhelming evidence that arterial inflammation is also a systemic process. Indeed, it is remarkable that although the initial description of atherosclerosis as an inflammatory disease was suggested nearly two decades ago [1], the first large clinical trial directly testing the inflammation hypothesis was launched only very recently. Specifically, the "Cardiovascular Inflammation Reduction Trial" will randomize 7,000 patients with prior myocardial infarction and either diabetes or metabolic syndrome to low-dose methotrexate or placebo. No doubt, the results of this pivotal trial will teach us important biology, and possibly irrevocably alter the means by which we treat patients with coronary artery disease [2].

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In the context of *common* pathways mediating arterial inflammation, the receptor for advanced glycation endproducts (RAGE) is certainly a strong candidate molecule. Emerging evidence links RAGE to the pathogenesis of vascular inflammation [3]. RAGE is expressed in vascular endothelial cells, smooth muscle cells, and immune cells and transduces the signals of ligands such as advanced glycation endproducts (AGEs), pro-inflammatory S100/calgranulins, and high mobility group box 1 (HMGB1); these ligands have been localized to atherosclerotic and restenotic tissues. Furthermore, RAGE is one of a family of receptors for lysophosphatidic acid (LPA) (Fig. 1). AGEs are a diverse group of compounds that are generated through nonenzymatic glycation or glycoxidations of proteins, lipids, and nucleic acids with the reactive carbonyl methylglyoxal considered as a major AGE precursor. AGE formation is directly accelerated by hyperglycemia and oxidative stress, but occurs also in normoglycemia during inflammation and oxidative stress. AGEs continue to accumulate in plasma and in tissues during natural aging. Other RAGE-ligands, including S100/calgranulins, HMGB1, and LPA are released from various cells during cell stress and injury and are indicators of cell damage. These ligands are, therefore, also known as "alarmins" or "damage associated-molecular pattern molecules" and are important activators of innate immune responses [4]. S100/calgranulins are endogenous proteins in the cytosol of myeloid cells, particular in granulocytes, and are released upon cell activation. In addition, S100/calgranulins are upregulated in smooth muscle and endothelial cells under pathological conditions, including acute myocardial infarction or aortic dissections [5]. Although heterogeneous in structure, these ligands commonly activate RAGE, which results in many downstream effects, including activation of adapter proteins, kinases, production of cytokines, adhesion molecules, migration, and proliferation of smooth muscle cells that collectively

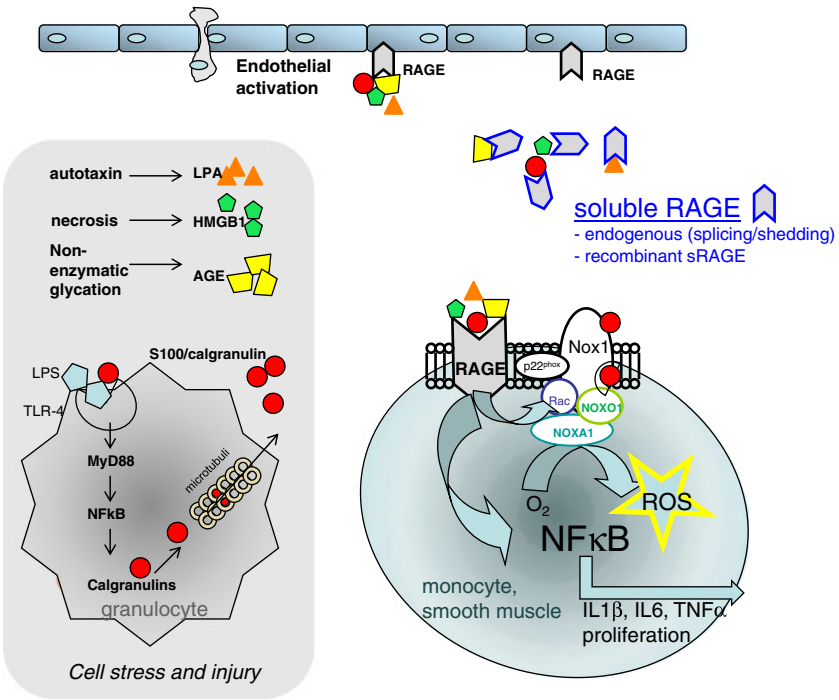

Fig. 1 Ligand-mediated activation of the receptor for advanced glycation endproducts (*RAGE*) mediates acute and sustained inflammation. Various ligands of RAGE, including advanced glycation endproducts (*AGEs*), high mobility group protein box 1 (*HMGB1*), S100/calgranulins, and lysophosphatidic acid (*LPA*) are released from various sources during cell stress and injury and bind to RAGE on endothelial cells, monocytes, and smooth muscle cells leading to activation of pathways resulting in translocation of transcription factor nuclear factor kappa B (*NFkB*) and increased oxidative stress, which in turn results in endothelial activation and proinflammatory cell phenotype

contribute to arterial inflammation. For example, transgenic expression of S100A12 in the vascular smooth muscle of ApoE deficient mice vastly accelerates atherosclerosis with enhanced formation of a necrotic core and other features commonly associated with vulnerable plaques (Fig. 2) [6]. Therefore, blocking the activation of RAGE should dampen arterial inflammation. Indeed, studies in ApoE deficient mice with genetic ablation of RAGE revealed a reduction of vascular inflammation and early atherosclerosis and provided important experimental evidence that RAGE is a suitable target for therapeutic interventions [7].

What about clinical application? In fact, a step forward for potential clinical translation was the development of soluble RAGE, a recombinant protein composed of the ligand-binding domain that inhibits the axis of ligand/RAGE/arterial

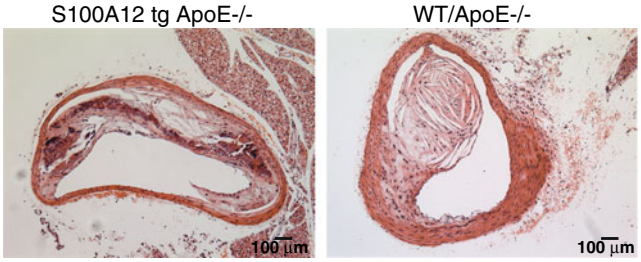

Fig. 2 Advanced remodeling with necrotic core, elastic fiber degradation, and calcification in atherosclerotic plaques of the innominate artery in ApoE-deficient mice with transgenic expression of hS100A12 targeted to the vascular smooth muscle and in wild-type ApoE-deficient littermate mice (modified from [6])

inflammation. Earlier studies showed that administration of soluble RAGE to rodents reduced atherosclerosis [8], particularly that accelerated by diabetes, and suppressed arterial wire injury-mediated neointimal proliferation [9] in other animal models. Indeed, these studies using soluble RAGE produced in insect cells provided the pivotal "proof of concept" linking ligation of RAGE ligands to protection against vascular perturbation.

In this issue of the *Journal of Molecular Medicine*, Tae and colleagues extend those findings and present a new variation of recombinant soluble RAGE using the *mammalian* CHO cell line (Chinese hamster ovary) for production of sRAGE [10], in contrast to insect cells (Sf9 cells) that were previously used to generate sRAGE for injection into animal models [8, 9]. The endoplasmic reticulum of mammalian cells adds *N*-linked glycans during protein translation, which, after extensive modifications and trimmings, are important for the proper folding, oligomerization, transport, and ultimately function of the proteins. Li and colleagues demonstrate that sRAGE-CHO displays important differences in N-glycans compared to sRAGE-Sf9, and this difference in posttranslational modifications might possibly explain that a single dose of 3 ng/g body weight injected at the time of balloon angioplasty reduced neointimal hyperplasia by 70 % in a rat carotid artery balloon injury model. N-glycan modification within the ligand-binding-domain of RAGE was previously shown to increase the ligand-binding capacity of RAGE, particularly its binding to S100A12 [11]. This study by Tae and colleagues reinforces the concept that quenching RAGE-ligands that are in excess at the site of arterial injury using a small but very effective dose

of sRAGE-CHO is an attractive avenue that should be further studied and possibly developed as a therapeutic protein applicable in multiple diseases linked to arterial inflammation.

Finally, Tae and colleagues refer to "high" versus "low" doses of insect cell versus CHO cell-produced material [10]. It is essential to note that such definitions may not be relevant to the ultimate application to clinical practice, as Li and colleagues have not yet performed detailed pharmacokinetic testing with their newest version of soluble RAGE. Indeed, in the case of insect cell- or CHO cell-produced material, it is possible that local delivery of either agent directly at the site of percutaneous coronary intervention might be the most logical means to block the vascular perturbing properties of RAGE ligands in atherosclerosis and restenosis.

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