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Receptor for advanced glycation end products (RAGE) in a dash to the rescue: inflammatory signals gone awry in the primal response to stress

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Abstract: The multiligand receptor for advanced glycation end products (RAGE) of the Ig superfamily transduces the biological impact of discrete families of ligands, including advanced glycation end products, certain members of the S100/calgranulin family, high mobility group box-1, membrane-activated complex-1, and amyloid-β peptide and β-sheet fibrils. Although structurally dissimilar, at least at the monomeric level, recent evidence suggests that oligomeric forms of these RAGE ligands may be especially apt to activate the receptor and up-regulate a program of inflammatory and tissue injury-provoking genes. The challenge in probing the biology of RAGE and its impact in acute responses to stress and the potential development of chronic disease are to draw the line between mechanisms that evoke repair versus those that sustain inflammation and tissue damage. In this review, we suggest the concept that the ligands of RAGE comprise a primal program in the acute response to stress. When up-regulated in environments laden with oxidative stress, inflammation, innate aging, or high glucose, as examples, the function of these ligand families may be transformed from ones linked to rapid repair to those that drive chronic disease. Identification of the threshold beyond which ligands of RAGE mediate repair versus injury is a central component in delineating optimal strategies to target RAGE in the clinic. *J. Leukoc. Biol.* 82: 000–000; 2007.

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THE LIGAND FAMILIES OF RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS (RAGE)

Advanced glycation end products (AGEs)

RAGE was first described as a receptor for AGEs, the products of nonenzymatic glycation and oxidation, which form as post-translational modifications of proteins and lipids, primarily on lysine and arginine groups within the backbone protein. AGEs may transform the configuration and function of the backbone protein; depending on the site and context, AGEs may modify long-lived protein species of the vessel wall and tissues, leading to extensive cross-linking and virtual insolubility [1–3]. AGEs, a heterogeneous group of structures, including such specific species as carboxy methyl lysine (CML), pentosidine, and pyralline AGEs, may form in settings such as aging, hyperglycemia, oxidative stress, renal failure, and inflammation. AGE modifications may impart gain-of-function properties in their substrates. For example, AGE modification of lipoproteins enhances their atherogenic potential [4, 5]. In chronic disease, AGEs may beget further AGE formation; AGE interaction with RAGE increases oxidative stress [6–8]. In turn, oxidative stress increases AGE formation; mice deficient in NADPH oxidase fail to generate the same amount of CML AGE, as generated by wild-type mice in an inflammatory milieu [9, 10]. Among the heterogeneous AGEs, CML AGEs have been shown to be specific AGE ligands of RAGE [11] (**Fig. 1**). In cultured endothelial cells (EC), smooth muscle cells (SMC), and monocytes/macrophages, CML-AGE-RAGE interaction activated NF-κB and up-regulated genes linked to inflammation. Upon infusion into wild-type mice, CML AGE resulted in increased expression of VCAM-1 in lung tissue, a process dependent on RAGE, as pretreatment of the animals with anti-RAGE IgG suppressed CML AGE-mediated up-regulation of VCAM-1 [11].

AGEs are formed in diverse organisms, from bacteria, yeast, and Drosophila to higher-order mammals as a consequence of the inevitable production of a key pre-AGE methylglyoxal through the metabolism of D-glucose [12–14]. AGEs are linked integrally to damage, but are there salutary roles for AGEs?

Potential hints to innate functions for AGEs in modifying immune responses may be inferred from findings in in vitro analyses. AGEs may be found on the surface of aging lymphocytes [15, 16]. Further, findings in dendritic cells (DC) suggest that DC glycation may promote their development but impair

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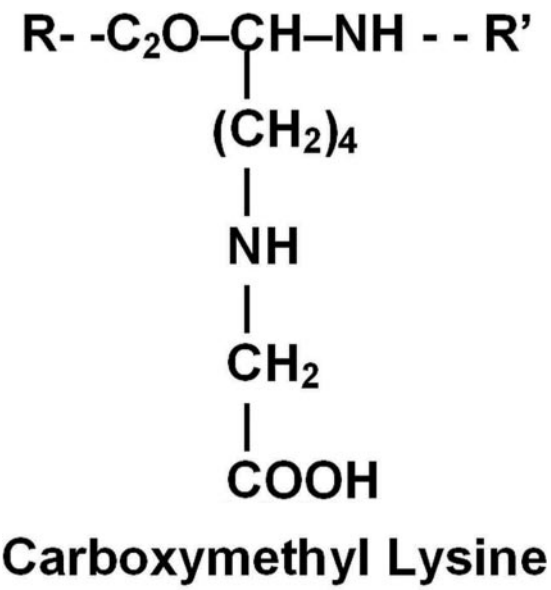

Fig. 1. CML structure. Previous studies indicated that CML AGEs are prevalent products, which accumulate in the tissues in such settings as inflammation, hyperglycemia, and renal failure. CML adducts are specific ligands of RAGE. The structure of this prevalent species is depicted in the figure.

their ability to stimulate primary T cell responses [17]. Although potentially injurious in impeding the organism's response to antigen stress, it is conceivable that in certain settings, T cell or DC glycation may suppress untoward immune responses.

S100/calgranulins

The family of S100/calgranulins is composed of multiple members. Although not all members of the family likely bind RAGE, increasing evidence suggests that S100/calgranulins beyond S100A12 and S100b bind this receptor. S100/calgranulins may be expressed by a wide variety of cell types, including cells linked to rapid and sustained inflammatory responses, such as neutrophils, monocytes/macrophages, lymphocytes, and DC [18]. The expression of S100/calgranulins by EC, neurons, and transformed cells suggests that a diverse array of responses may be elicited by release of these species. S100/calgranulins are primarily intracellular molecules; however, upon their release into the extracellular space by autocrine and/or paracrine interactions, they may gain new functions via their ability to bind cell surface receptors such as RAGE [19].

In vitro analyses in cultured neurons suggested that one measure potentially distinguishing the adaptive versus injurious impact of S100b was the "dose" to which the cells were exposed. Whereas low (nM) concentrations of S100 mediated survival, exposure of cultured neuronal and glioma cells resulted in recruitment of proinjury pathways [20].

Thus, these concepts suggest that far from being inert, intracellular molecules, linked solely to calcium binding and its consequences inside the cell, S100/calgranulins possess distinct functions and importance in the biology of the cell through homeostasis to crisis.

High mobility group box-1 (HMGB1)

Akin to S100/calgranulins, HMGB1 usually is expressed in the intracellular space, specifically in the nucleus, where these molecules play roles as nonhistone, DNA-binding molecules. Like S100/calgranulins, "activation" stimuli may trigger release of HMGB1 onto the surface of highly activated cells or directly into the extracellular space [21, 22]. It is in that context, we propose, that HMGB1 may be freed to interact with RAGE.

Studies reported in the mid-1990s uncovered for the first time that HMGB1, or amphoterin, was a signal transduction ligand of RAGE. In the first studies, RAGE and HMGB1 were found to colocalize in the developing cerebral cortex of embryonic rats; cell culture analyses suggested that the HMGB1-RAGE interaction contributed to outgrowth of neurites in neurons from prenatal rat brain [23]. It is intriguing that the HMGB1-RAGE interaction exerted its influence in migrating cells; specifically, HMGB1 and RAGE are expressed by transformed cells, and their interaction is linked to activation of cell migration and possibly, mechanisms linked to tumor metastasis, such as activation of matrix metalloproteinases (MMPs). Studies in vivo in murine tumors indicated that administration of a soluble receptor decoy of RAGE, sRAGE, sharply limited local tumor growth and particularly, metastases in vulnerable mice [24]. In parallel, activity of MMPs and MAPK activation was reduced greatly by RAGE blockade [24].

The studies of Tracey and colleagues [25] elucidated for the first time the provocative possibility that HGMB1 was linked to amplification of inflammatory mechanisms as a late mediator of the impact of endotoxin. Treatment of RAW 264.7 murine macrophages with LPS evoked up-regulation and release of HMGB1 8 h after incubation. In vivo, blocking antibodies to HMGB1 protected rodents against the impact of overwhelming sepsis [25]. Recent studies suggest the possibility that HMGB1 may not only interact with RAGE but as well, certain Toll receptors, such as TLR2 and TLR4 [26].

DC express HMGB1 and RAGE; release of HMGB1 by these cells provokes clonal expansion, survival, and functional polarization of differentiating T cells, at least in part through activation of MAPKs and NF-κB [27]. Further experimentation reveals that at least in vitro, HMGB1 stimulates up-regulation of CCR7 and CXCR4 chemokine receptors in DC and their migratory ability [22]. Although these studies were limited to the in vitro milieu, they nevertheless suggest the possibility that the RAGE axis plays critical roles in the adaptive immune response. These implications from cell culture analyses require rigorous validation in vivo.

Membrane-activated complex-1 (Mac-1)

RAGE is an endothelial adhesion receptor, which mediates direct interaction with the β2 integrin Mac-1. RAGE-Mac-1 interaction is enhanced by incubation with the proinflammatory RAGE ligand, S100B [28]. Recent studies indicated that HMGB1-mediated recruitment of neutrophils was dependent on Mac-1 but not on LFA-1. In bone marrow chimera experiments, Mac-1-dependent neutrophil recruitment induced by HMGB1 required the presence of RAGE on neutrophils but not on EC [29]. Thus, a HMGB1-dependent pathway for inflam-

matory cell recruitment and activation requires the interplay between RAGE and Mac-1. These findings establish mechanisms by which RAGE and Mac-1, via HMGB1, may be linked to acute inflammatory responses initiated by neutrophil recruitment and activation.

Amyloid-β peptide (Aβ) and β-sheet fibrils

In addition to AGEs, S100/calgranulins, HMGB1, and Mac-1, RAGE is also a signal transduction receptor for Aβ and β-sheet fibrils [30, 31]. An emerging concept is that although seemingly dissimilar, common features envelop many of the ligand families of RAGE, perhaps leading to recognition by identical or at least closely neighboring sites within the extracellular domain of RAGE, specifically, but possibly not exclusively, in the V-type Ig domain of RAGE [19]. Many of ligands of RAGE are found in monomeric and oligomeric forms. Although soluble, monomeric ligands interacted with RAGE in ARPE-19 cells, their ability to stimulate signal transduction and modulation of gene expression via RAGE was enhanced significantly in the oligomeric state [32].

Furthermore, the ligands of RAGE may potentiate each other's formation and aggregation; AGE precursor species methylglyoxal and glyoxal may increase the aggregation and cytotoxicity of intracellular Aβ carboxy-terminal fragments [33]. In addition, it has been suggested that glycation stimulates amyloid formation [34, 35]. These concepts suggest that the ligands of RAGE may indeed supermodify each other and that the ligands may be more similar than distinct. Studies are gaining first insights into the structure of the receptor and providing physical evidence for the basis of ligand-RAGE interactions [36].

SIGNAL TRANSDUCTION—CENTRAL TO THE BIOLOGICAL IMPACT OF LIGAND-RAGE INTERACTION

The ligands of RAGE share common properties upon their binding to the receptor. First, among the known ligands, such as AGEs, S100A12 and S100b, and HMGB1, Aβ and β-sheet fibrils, there is cross-competition in radioligand-binding assays [11, 19]. Second, RAGE ligands bind to the V-type Ig domain, as elucidated by radioligand-binding assays to recombinant V domain [11]. Although we were unable to show that ligands bound C-type domains directly, recent studies suggested hexameric forms of S100A12 bound the C-type Ig domain of RAGE [36]. Third, the binding affinities, as established in radioligand-binding assays for ligand binding to RAGE, are quite similar in the nM range (~50 nM). Fourth, extensive evidence indicates that each of the RAGE ligands exerts its impact as a consequence of RAGE-mediated signal transduction. The cytoplasmic domain of RAGE is essential for RAGE-mediated changes in gene expression and cellular properties [11, 19]. These studies suggested that the interaction of ligands with RAGE activates signaling pathways and thus, the platform to alter patterns of gene expression in the cell.

RAGE is expressed in multiple, distinct cell types; thus, it is not surprising that diverse signal transduction pathways may be impacted by RAGE. An important but not sole means by which RAGE exerts effects on gene expression is via activation of NF-κB [7, 37], which impacts proinflammatory/prodeath and prosurvival pathways depending on the cell type and context. Multiple experiments have suggested that the ligand-RAGE interaction in cells such as EC, SMC, monocytes/macrophages, and neurons activates NF-κB. Generation of reactive oxygen species (ROS) is a key intermediate step, at least in certain cases, as pretreatment of EC, for example, with antioxidants, suppresses AGE-RAGE-mediated activation of NF-κB [38]. RAGE-mediated activation of NADPH oxidase may account, at least in part, for these observations [6, 8].

Multiple members of the MAPK family have been shown to be activated by RAGE; the ligand-RAGE interaction activates p44/p42 (ERK) MAPK, p38 MAPK, and JNK MAPK [24, 39, 40]. In addition, other studies have illustrated that AGE-mediated activation of ras and src kinase via RAGE in SMC is a key step in activation of NF-κB [37, 41]. The specific signaling pathways triggered by RAGE are influenced by the context of stimulatory signals; in the setting of arterial injury, for example, RAGE-mediated activation of JAK/STAT pathways critically impacts on SMC proliferation and migration [42]. In monocytes/macrophages, NF-κB is a central target of ligand-RAGE. Recent studies suggested that in cultured microglial cells, RAGE-mediated up-regulation of cyclooxygenase-2 required recruitment of cdc42/rac and JNK MAPK signal transduction [43].

Consistent with the concept that in distinct cell types and forms of stress, diverse signaling may be impacted by RAGE, it has been shown that in cultured mesangial cells, AGE-RAGE-mediated generation of ROS triggered TGF-β/Smad signaling [44]. In other cell types, cultured, primary, sensory neurons exposed to S100 displayed increased caspase-3 activity and nuclear DNA degradation, at least in part via activation of PI-3K signaling [45].

Under intense investigation at this time is the precise means by which the short, highly charged, cytoplasmic domain of RAGE signals. Earlier reports suggested that this domain interacted with ERK MAPK [46]; however, it is unlikely that such findings explain or underlie the diverse signal transduction repertoire of RAGE. Further, essential to establish will be if and how distinct ligands of RAGE may stimulate specific (or not) signaling pathways. In addition, the complexity of this system is enhanced by the concept that the ligands of RAGE may interact with distinct binding molecules themselves. For example, HMGB1 may interact with TLR2 and -4; thus, it is possible that RAGE-distinct signaling may be characteristic of those ligands [26]. In other settings, it was suggested that S100b activation of myotubes was independent of RAGE, although the specific, distinct receptors were not identified [47]. Furthermore, it has been reported that AGEs may bind CD36 and other scavenger receptors (SRs) [48]. Taken together, these observations suggest that specific tools, such as RAGE antagonism and RAGE null mice, would be essential in dissecting the specific role for RAGE in biological responses.

Based on the striking ability of RAGE ligands to activate signal transduction and thereby, alter gene expression patterns, it was reasonable to test these concepts in vivo. Integral to the biology of RAGE and its ligands is their up-regulation and

increased accumulation in multiple biological and disease settings. In the sections to follow, we present evidence linking ligand-RAGE signaling to fundamental mechanisms in the inflammatory response.

TESTING THE ROLE OF RAGE IN INFLAMMATION—FIRST STUDIES TESTING PHARMACOLOGICAL ANTAGONISM OF THE LIGAND-RAGE AXIS

Our earliest work suggested that the biological repertoire of the AGE-RAGE interaction contributed to the pathogenesis of diabetic complications. The discovery of S100/calgranulins and HMGB1 as putative ligands of RAGE compelled us to consider that RAGE played roles in inflammatory responses, even in the absence of hyperglycemia. Using sRAGE, an extracellular ligand-binding decoy of RAGE, prepared and purified in a baculovirus expression system, and $F(ab')_2$ fragments of anti-RAGE IgG or anti-S100A12 IgG, experimentation revealed that blockade of ligand-RAGE suppressed the challenge phase of footpad edema in which infiltration of inflammatory cells and granuloma formation developed in mice sensitized and challenged with methylated BSA. In parallel, nuclear extracts retrieved from RAGE-antagonized mouse foodpads revealed strikingly diminished activation of the proinflammatory transcription factor NF-κB [19]. In mice highly vulnerable to colitis mediated by genetic deficiency of IL-10, chronic administration of sRAGE reduced gut inflammation and activation of NF-κB [19]. In parallel, gene expression was altered in sRAGE-treated mice, as animals treated with sRAGE displayed decreased levels of TNF-α in plasma [19].

These proinflammatory effects of RAGE signaling were probed further in a murine model of bovine Type II collagen-induced arthritis in DBA/1 mice; significant reduction in joint swelling and erythema was noted when mice were treated with sRAGE. In the context of inflammatory arthritis, a possible genetic link to RAGE was uncovered by the observation that a genetic variant of RAGE, the G82S polymorphism, was in linkage disequilibrium with HLA-DR4. In in vitro analyses, transfected cells expressing G82S displayed increased binding affinity to RAGE ligand S100A12 and enhanced generation of cytokines and MMPs upon transfection with G82S versus the wild-type allele in the presence of S100A12 [49]. Studies in human subjects with rheumatoid arthritis (RA) supported this genetic link. However, based on association studies in cell culture, future experiments are required to probe if the presence of this variant is linked to the extent or temporal appearance of joint and bone destruction in RA.

RAGE AND IMMUNE RESPONSES: STUDIES PROBING CELLULAR CONTRIBUTIONS OF LIGAND-RAGE AXIS

The next step in probing the mechanisms linked to ligand-RAGE signaling in immune/inflammatory consequences was to specifically dissect the cellular mechanisms underlying these observations. Administration of sRAGE and studies in homozygous RAGE null mice revealed that the impact of sepsis induced by cecal ligation and puncture was attenuated significantly by antagonism or deletion of RAGE [50]. When homozygous RAGE null animals were reconstituted with endothelial or hematopoietic cell expression of the receptor, the protective impact of the global deletion mutant was reversed [50]. One interpretation of these findings was that RAGE was linked to amplification of inflammation in sepsis but not to the adaptive immune response. Studies using distinct, cell-specific mutants of RAGE, however, suggest that in specific settings, RAGE may play roles in adaptive immune mechanisms.

RAGE and the pathogenesis of Type 1 diabetes: studies in NOD mice

Roles for RAGE in inflammatory signaling were further studies in NOD/scid mice subjected to adoptive transfer of diabetogenic spleen cells. Compared with baseline, pancreata from diabetic NOD/scid mice, which had received a transfer of splenocytes, revealed marked up-regulation of RAGE and S100 on islet cells containing an inflammatory infiltrate. In parallel, it was shown that RAGE was also expressed in a population of T cells (CD4+ and CD8+) and B cells [51].

To test the potential role of RAGE in mediating autoimmune diabetes, NOD/scid mice receiving a transfer of splenocytes from a diabetic NOD donor were treated with sRAGE or vehicle, murine serum albumin. Animals treated with sRAGE displayed significant reduction in the rate of transfer of diabetes. By Day 36 after transfer, 92% of control animals but only 10% of mice treated with sRAGE became diabetic. In parallel, gene expression patterns were altered when RAGE signaling was impacted; levels of cytokines IL-1β and TNF-α were reduced significantly in the sRAGE-treated islets compared with murine serum albumin-treated animals. The expression of IL-10 was increased in sRAGE-treated mice islets, along with increased TGF-β, compared with vehicle-treated animals [51]. In contrast to injection of diabetogenic splenocytes, when preactivated, diabetogenic BDC2.5 cells were injected into mice, sRAGE displayed no effect on prevention of diabetes [51]. These investigations suggested that the ligand-RAGE axis contributed, in part, to T lymphocyte priming or cognate DC-T lymphocyte interactions. Studies are underway to address these concepts; first studies in cultured T cells suggest that blockade of RAGE attenuates T lymphocyte proliferation in response to allostimulation [52].

Roles for RAGE in T lymphocyte responses in the adaptive immune response

Prompted by these findings in vitro, we probed roles for RAGE in T lymphocytes in an established model of heterotopic, allogeneic heart transplantation, wherein fully mismatched grafts (donor, CD1 strain, $H2^q$) were transplanted into C57BL/6 recipients ($H2^b$). Mice were treated with sRAGE, 100 or 200 μg/day, beginning 1 day prior to transplantation and continued once daily until sacrifice. Control animals received equal volumes of PBS. The mean graft survival time in vehicle (PBS)-treated mice was 7.3 ± 0.7 days. Mice treated with sRAGE, 100 μg/day, displayed significantly increased graft survival,

11.7 ± 1.7 days. In animals treated with the higher dose of sRAGE, 200 μg/day, graft survival time was even greater, at 19.5 ± 2.8 days [52].

Immunofluorescence microscopy revealed a significant reduction in cells expressing RAGE in sRAGE-treated graft recipients. In parallel, S100 and HMGB1-expressing cell staining was reduced significantly in hosts that received sRAGE. Inflammation was, in turn, reduced significantly in the sRAGE-treated grafts. Compared with PBS-treated animals, mice treated with sRAGE displayed significantly less edema and inflammatory cell infiltration, including reduced numbers of T lymphocytes [52].

The finding that significantly less T cells were present in the sRAGE-treated allografts led us to test the hypothesis that RAGE modulated alloimmune responses directly. We used purified T cells and MHC Class II^+ APC retrieved from MHC-mismatched mice and used sRAGE and blocking antibodies to the receptor. Incubation with sRAGE resulted in a statistically significant, dose-dependent decrease in lymphocyte proliferation versus IgG control-treated cultures. To substantiate the specific role of RAGE, cells in the mouse allogeneic mixed lymphocyte culture were incubated with blocking antibodies to RAGE. Compared with nonimmune IgG, incubation with monoclonal anti-RAGE IgG resulted in a statistically significant, dose-dependent decrease in lymphocyte proliferation [52].

These findings suggested that sRAGE suppressed donor-reactive, T cell-priming responses and led us to test the effect of RAGE antagonism on human lymphocyte proliferation triggered by alloresponses. Incubation with sRAGE resulted in a statistically significant decrease in lymphocyte proliferation versus control-treated cultures. Similar effects were noted with anti-RAGE IgG. We propose that ligands released from or presented on the surface of the irradiated cells provided the stimulus for proliferation and activation of lymphocytes [52]. Studies are underway at this time to elucidate the precise signaling mechanisms impacted by RAGE in T cell-proliferative and cytokine responses.

Further studies suggested the importance of RAGE in modulating T cell infiltration into immune/inflammatory sites. In a murine model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), RAGE, and its inflammatory ligand S100 were overexpressed. Blockade of RAGE, using sRAGE, suppressed EAE disease induced by myelin basic protein (MBP) peptide or encephalitogenic T cells or when EAE occurred spontaneously in the TCR-transgenic mice devoid of endogenous TCR-α and TCR-β chains. In these studies, the striking impact of RAGE antagonism was evident by markedly decreased infiltration of the spinal cord by immune and inflammatory cells. In parallel, nuclear extracts retrieved from spleen tissue revealed marked reduction in activation of NF-κB, thereby suggesting that RAGE-mediated signaling accounted, at least in part, for genes in gene expression and cellular properties [53].

Thus, to address the role of CD4 T cell RAGE signaling directly in these processes, transgenic mice were generated with targeted overexpression of dominant-negative (DN) RAGE in CD4+ T cells. DN RAGE consists of the extracellular and membrane-spanning domain of RAGE; solely, the RAGE cytosolic domain is deleted. Thus, although RAGE ligands may bind the truncated receptor, they are unable to affect signal transduction via RAGE. Compared with wild-type littermate mice, transgenic CD4 DN RAGE mice were resistant to MBP-induced EAE [53]. These data reinforced the significant impact of CD4+ T cell signaling in RAGE-dependent, adaptive immune responses and underscored roles specifically for RAGE in mediating migration of effector cells into immune foci.

To further probe the specific mechanisms linking RAGE to T cell responses, we recently used OT-II T cells reactive with OVA. Preliminary studies using RAGE-expressing versus RAGE null OT-II cells suggest that RAGE is required for effective T cell priming. Experiments are underway to delineate the specific signal transduction mechanisms underlying these findings [54].

Roles for RAGE in DC/macrophage responses in the adaptive immune response

Extensive studies, particularly in vitro, highlighted roles for RAGE signaling in monocyte/macrophage migration and activation, the latter as defined by up-regulation of proinflammatory factors such as cytokines and MMPs. These concepts were probed in vivo in a murine model of massive liver injury. The ability of the liver to regenerate is finite; in experimental systems, 70% resection of the liver triggers a fully effective regeneration program in which liver mass is restored in parallel with function. However, in contrast, when 20% more liver tissue is removed (85% resection), the threshold for recruitment of effective regeneration programs is exceeded in rodents, such that overwhelming inflammation and apoptosis of the remnant ensue. Given the strong link to inflammatory mechanisms in this setting, we probed the role of the RAGE axis.

The first suggestion that RAGE might be implicated in massive liver injury was the observation that consequent to resections, RAGE mRNA transcripts were up-regulated selectively in the 85% but not 70% resection setting. Immunohistochemistry using anti-RAGE IgG revealed the intriguing finding that the principal site of RAGE expression in the remnant after massive resection was in cells expressing CD11c and CD68, thus suggesting that RAGE was expressed largely in mononuclear phagocyte (MP)-derived DC (MPDDC) [55]. These observations led us to posit that MPDDC, in part via RAGE, modulated inflammatory responses in the liver remnant, which contributed to massive apoptosis and failure of regeneration.

To address these concepts, we first administered sRAGE to the animals undergoing massive resection. Compared with vehicle-treated mice, a significant increase in survival of wild-type C57BL/6 mice was noted in sRAGE-treated mice; the effects of sRAGE were dose-dependent. To target RAGE and its inflammatory ligands directly, we prepared $F(ab')_2$ fragments of anti-RAGE IgG, anti-S100 IgG, and anti-HMGB1 IgG. Compared with administration of nonimmune $F(ab')_2$ fragments, mice treated with anti-RAGE, anti-S100, or anti-HMGB1 $F(ab')_2$ fragments displayed significantly increased survival. Consistent with the concept that sRAGE trapped RAGE ligands, we found that when plasma of sRAGE-treated mice was subjected to immunoprecipitation with anti-RAGE IgG, followed by immunoblotting of bound material with anti-

bodies to S100/calgranulin, S100/calgranulin epitopes were revealed. Thus, studies using sRAGE and these antiligand antibody fragments strongly suggested that the ligand-RAGE axis contributed to impaired survival and failure of regeneration in these remnants [55].

In liver resection, it is well-established that inflammation must be tempered appropriately, such that proregenerative and not prodeath pathways would be recruited selectively. Our findings revealed that administration of sRAGE modulated cytokine expression in the remnant and drove early up-regulation of NF-κB activity, in parallel, with reduction in TUNEL-expressing cells and decreased activation of caspase-3. As sRAGE and anti-RAGE $F(ab')_2$ fragments would be expected to target the remnant globally, we prepared transgenic mice in which RAGE signaling would be mutated in cells of MP lineage, including cells expressing CD11c and CD68, as driven by the macrophage SR Type A (SR-A) promoter, referred to as SR DN RAGE mice. Compared with wild-type mice, we found that transgenic SR DN RAGE mice displayed significantly improved survival, regeneration of the hepatic remnant, modulation of proregenerative cytokines, and early up-regulation of NF-κB activity. It is important to note that by using this promoter, it was not possible to dissect the specific impact of infiltrating monocytes/macrophages, Kupffer cells, or MPDDC. However, it was evident that blunting RAGE impact in cells of MP lineage restored effective regeneration, even in the face of massive resection of the liver [55].

Thus, in these studies, blockade of RAGE in massive liver resection restored beneficial, inflammatory responses and activation of NF-κB, suggesting that RAGE played key roles in modulation of these central pathways in inflammatory mechanisms. These considerations prompted us to probe the hypothesis that at least in certain settings, RAGE might contribute to beneficial, inflammatory mechanisms.

RAGE and inflammatory mechanisms linked to nerve regeneration

The specific hypothesis that RAGE-dependent mechanisms might mediate adaptive repair as a consequence of inflammatory signaling was addressed in a murine model of unilateral crush of the sciatic nerve. Upon crush of the nerve, rapid recruitment of proinflammatory mechanisms ensues, which contributes to adaptive remodeling in the crushed nerve segments, along with up-regulation of regenerative pathways. Our studies revealed that RAGE and its ligands, particularly S100/calgranulins and HMGB1, were up-regulated rapidly within hours of sciatic nerve crush. RAGE expression was up-regulated in macrophages and in axonal elements within the crushed nerve segment [56]. As these experiments placed RAGE and its ligands at the site of peripheral nerve crush, it was logical to explore the outcome of RAGE blockade in this context. Did RAGE contribute to repair versus failure of regeneration?

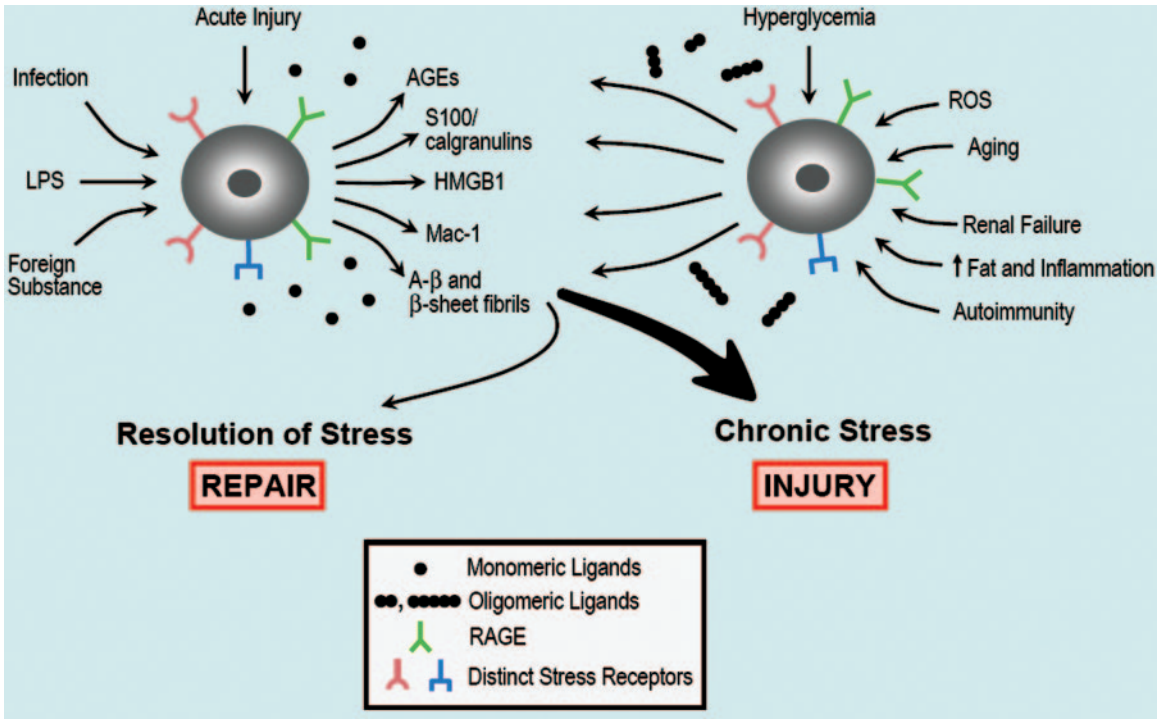

Fig. 2. RAGE and key roles in acute stress versus pathways linked to chronic disease—hypotheses and unifying concepts. We hypothesize that the ligands of RAGE possess adaptive roles in primal responses to short and self-limiting stresses, which accompany host existence within their environment. In uncomplicated environments, such stresses may promote release of RAGE ligands, largely in monomeric forms, in a manner linked to their rapid engagement of primarily innate immune receptors and to a degree, RAGE. Rapid detoxification and removal of these ligands after the burst of release and response to stress ensure repair and return to homeostasis. In contrast, in complex settings, chronic inflammation, hyperglycemia, and innate aging prime these ligands to undergo supermodification, in which oligomeric forms may predominate. We predict that under such conditions, these ligand configurations recognize and activate RAGE preferentially and chronically. In turn, the consequences of such interactions favor long-term tissue stress. Identification of strategies to retain primal RAGE responses to stress, yet derail amplification pathways, which damage tissues irrevocably, holds great promise in harnessing lessons learned from the biology of RAGE to clinical trials.

To address these questions, we administered sRAGE or blocking $F(ab')_2$ fragments derived from anti-RAGE IgG to wild-type mice subjected to acute crush to the sciatic nerve. Motor and sensory conduction velocities, walking tract analyses, and regeneration, as assessed by myelinated fiber densities, were impaired in RAGE-blocked mice. Histological and molecular analysis revealed that macrophage infiltration and thus, Wallerian degeneration were reduced in RAGE-blocked mice [56]. To determine if macrophage and/or neuronal RAGE signaling participated critically in the response to nerve crush, transgenic mice expressing DN RAGE in macrophages or peripheral neurons, driven by the SR-A or thy-1 promoter, respectively, were used. Compared with littermate controls, regeneration was delayed in the transgenic mouse but especially, in double-transgenic mice expressing DN RAGE in macrophages and peripheral neurons [57].

These studies were the first to demonstrate that recruitment of RAGE facilitated beneficial inflammatory mechanisms. Certainly, however, the biology of tissue repair in the context of RAGE is complex, as administration of sRAGE to diabetic mice subjected to full-thickness excisional wounds accelerated wound healing, largely by suppression of exaggerated inflammatory mechanisms and blunting of excessive MMP activity. In contrast, when sRAGE was administered to nondiabetic mice, no impact, beneficial or deleterious, was observed on wound closure, perhaps as the wounds close quite rapidly [58]. Further, in bacterially challenged mice, periodontal wound healing was improved in diabetic mice treated with sRAGE; in parallel, cytokine generation and MMP activity in gingival tissue were reduced significantly [59].

PERSPECTIVES AND HYPOTHESES

In the full context of the biological response to stress, exquisite control of inflammatory mechanisms is critical to executing the best balance between rapid resolution of injury versus amplification of proinjury pathways. Thus, it is not surprising that multiple receptors and pathways and their cross-talk must be involved in the primal response to acute stress (**Fig. 2**). In the biology of RAGE, we propose that the ligand families, which may mediate rapid repair in such responses, are likely one and the same, sustaining and mediating chronic tissue perturbation and injury. We predict that the distinction will lie in the specific environments and forms of presentation of RAGE ligands. Common to many of the ligand families of RAGE is the ability to form oligomers, rendering them more apt to bind and activate RAGE. Environmental modulation of the ligand families, such as in chronic diseases beset by hyperglycemia, aging, obesity, chronic inflammation, or autoimmunity, as examples, may supermodify these ligands, thus tipping the balance between "forms" likely to mediate rapid repair versus those that engage the receptor chronically (Fig. 2). Indeed, we predict that in simpler, monomeric forms, such as in uncomplicated and rapidly resolving acute stresses, these ligands may activate RAGE and other innate receptors but that the environment facilitates rapid removal of these ligands as repair ensues. However, in chronically stressed environments, we propose that these ligands may be more apt to achieve higher order forms that cross the threshold to recognize RAGE strongly and perhaps selectively. It is possible that aggregation mechanisms may overwhelm natural clearance systems and over-run the chronically injured environment and lead to sustained recruitment and activity of RAGE.

In conclusion, striking the optimal balance in therapeutic targeting of RAGE will require crossing a fine line of resolution and repair between acute and chronic stimulation and thus, resolution and repair or irreversible tissue injury. If our predictions are correct, then a chief challenge of ongoing work is to identify the threshold for the ability of ligands to recruit RAGE in distinct stresses. Once so identified, the optimal pathways to targeting the receptor selectively in maladaptive stress are likely to be uncovered.

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