Restorative Neurology and Neuroscience 23 (2005) 355–365
IOS Press

RAGE: A journey from the complications of diabetes to disorders of the nervous system – striking a fine balance between injury and repair

Ling Ling Rong[a,e], Clifton Gooch[b], Mattias Szabolcs[c], Kevan C. Herold[d], Evanthia Lalla[a], Arthur P. Hays[c], Shi Fang Yan[a,e], Shirley Shi Du Yan[a,d] and Ann Marie Schmidt[a,e,*]
[a]*Department of Surgery, Columbia University Medical Center, NY 10032, USA*
[b]*Department of Neurology, Columbia University Medical Center, NY 10032, USA*
[c]*Department of Pathology, Columbia University Medical Center, NY 10032, USA*
[d]*Department of Medicine, Columbia University Medical Center, NY 10032, USA*
[e]*School of Dental & Oral Surgery, Columbia University Medical Center, NY 10032, USA*

Received 3 January 2005
Revised 4 August 2005
Accepted 15 August 2005

Abstract. The Receptor for Advanced Glycation End Products (RAGE) is a multiligand member of the immunoglobulin superfamily. RAGE interacts with AGEs, the products of nonenzymatic glycation/oxidation of proteins and lipids that accumulate in diverse settings, such as diabetes, inflammation, renal failure, pro-oxidant states and natural aging. In addition, RAGE is also a receptor for amyloid-β peptide and β-sheet fibril species. Recent studies underscore the premise that RAGE interacts with pro-inflammatory molecules, including S100/calgranulins and amphoterin, the latter also known as high mobility group box 1 (HMGB1). In chronic neurodegenerative disorders as well as in nerve tissue upon acute injury, evidence points to upregulation of both RAGE and these ligand families. In this review, we will discuss the implications of transient/self-limited upregulation of RAGE and its ligands, vs sustained/chronic upregulation of this axis in neurodegeneration vs repair in both the central and peripheral nervous systems. Experimental evidence supports the premise that RAGE bears both homeostatic and injurious properties in the nervous system, thereby highlighting "yin/yang" features of this receptor and its ligand families.

Keywords: Receptor, ligands, nerve crush, neurodegeneration, regeneration

1. Introduction

The Receptor for Advanced Glycation Endproducts (RAGE) is a multiligand member of the immunoglobulin superfamily [36,53]. RAGE was first identified as a signal transduction receptor for Advanced Glycation Endproducts (AGEs), the products of nonenzymatic glycation and oxidation of proteins and lipids, species that accumulate in a broad array of disease states, such as diabetes, renal failure, inflammation, neurodegenerative disorders such as Alzheimer's disease and amyotrophic lateral sclerosis (ALS), and natural aging [1,5,6,11,15,18,25–27,42,51,55,61,67]. AGEs may impart diverse consequences in the tissues via RAGE. Engage-

*Corresponding author: Dr. Ann Marie Schmidt, Columbia University Medical Center, 630 West 168 Street, P&S 17-501, NY 10032, USA. Tel.: +1 212 305 6406; Fax: +1 212 305 5337; E-mail: ams11@columbia.edu.

ment of RAGE by AGEs may activate multiple signal transduction pathways in diverse cell types; processes that may dramatically modulate cellular properties. Studies have indicated that RAGE also interacts with distinct molecules beyond AGEs, including amphoterin (also known as high mobility group box 1 (HMGB1), S100/calgranulins, amyloid beta peptide (Aβ) and β-sheet fibrils, and, most recently, Mac-1 [8,22,24,66, 69]. From this perspective, our data lead us to hypothesize that these families of ligands, at least in part via RAGE, may exacerbate injury in states of chronic and unremitting ligand/RAGE expression; and, as well, mediate repair when expressed in a transient/self-limited manner upon acute stress or injury. In this review, we will discuss the biology of RAGE and present evidence to suggest that RAGE may contribute to either tissue-destructive or reparative mechanisms in the injured nervous system.

2. RAGE: first identified as a Receptor for Advanced Glycation Endproducts (AGEs)

RAGE was first identified as a receptor for AGEs. AGEs are biochemical modifications of proteins and lipids that accumulate in multiple states, such as in hyperglycemia [36,53]. AGEs bound RAGE on key cell types integral to diabetic complications, including endothelial cells (EC), mononuclear phagocytes (MP) and vascular smooth muscle cells (SMC) [28,47,52, 54]. Although AGEs are a heterogeneous group of modifications, specific AGEs that bound RAGE were identified, most notably carboxymethyl lysine (CML) AGE adducts [28].

In diabetic lesions, both in human and experimental models, AGEs were found at higher levels compared to non-diabetic tissues susceptible to complications [29,60,65]. In parallel with increased accumulation of AGEs, expression of RAGE was upregulated in diabetic tissues [12,29,60,65]. To determine if upregulation of RAGE and AGEs in diabetic tissues was linked to the pathogenesis of complications and tissue injury, mouse or rat models of diabetic complications were developed and animals treated with soluble (sRAGE), the extracellular ligand binding domain of RAGE that acts as a decoy to bind ligands and prevent their access to, and activation of, the cell surface receptor [39]. Using sRAGE, we demonstrated that blockade of the ligand/RAGE axis suppressed accelerated atherosclerosis in diabetic apolipoprotein E null mice [7,29,39]; improved wound healing in diabetic db/db mice [19]; reversed diabetic vascular hyperpermeability [64]; suppressed albuminuria and mesangial expansion in db/db mice [65]; diminished alveolar bone loss in diabetic mice infected with the periodontal pathogen *Porphyromonas gingivalis* [31]; and significantly attenuated exaggerated neointimal expansion triggered by carotid artery balloon injury in diabetic rats [70].

These studies were the first to suggest that blockade of RAGE was associated with beneficial effects in tissues displaying chronic and sustained upregulation of both RAGE and its ligands.

A key facet of these studies was the demonstration that diabetic tissues displayed increased expression of inflammatory and tissue-destructive mediators compared to non-diabetic tissue. For example, diabetic skin wounds, periodontium and atherosclerotic plaques exhibited increased expression of deleterious cytokines and activation of matrix metalloproteinases (MMPs); in the presence of sRAGE, in parallel with decreased tissue injury, levels of such mediators were significantly reduced [7,19,31]. These data strongly suggested that RAGE was importantly involved in amplification of inflammatory mechanisms and led us to consider the question – were AGEs the sole ligands for RAGE?

3. S100/calgranulins and amphoterin: proinflammatory ligands of RAGE

In-depth investigation into non-AGE ligands of RAGE led to the identification of two molecule families, S100/calgranulins and amphoterin (HMGB1) as ligands for RAGE. The link between these molecules and inflammation transcended RAGE biology from diabetic complications to settings more broadly associated with inflammatory mechanisms.

3.1. S100/calgranulins

S100/calgranulins, a family of at least 20 polypeptide members, are characterized by EF hand, calcium-binding domains. S100/calgranulins may be expressed by a diverse array of cell types. In the specific context of inflammation and RAGE, S100/calgranulins may be produced and released by polymorphonuclear leukocytes, dendritic cells, MP and lymphocytes [16,50,71]. The first S100/calgranulin identified as a signal transduction ligand for RAGE on cells such as EC, SMC, MP and lymphocytes, was S100A12 (calgranulin C or Extracellular Newly-identified RAGE binding protein, EN-RAGE) [22]. In addition, S100s such as S100B

also bind RAGE; S100B is linked to nervous system stress [22]. Other S100s, such as S100P, are linked to key properties in transformed cells. In this context, RAGE-dependent ligation of S100P increases the proliferation and survival of cancer cells *in vitro* [4].

The direct evidence linking RAGE to the euglycemic inflammatory response was obtained from the following experiments. Blockade of RAGE suppressed delayed type hypersensitivity in euglycemic mice sensitized and challenged with methylated bovine serum albumin [22]. In other studies, treatment with sRAGE resulted in decreased colonic inflammation, and activation of NF-kB in euglycemic IL-10 deficient mice [22]. Blockade of RAGE suppressed joint inflammation and destruction in DBA/1 mice sensitized/challenged with bovine type II collagen [23]. In later studies, RAGE blockade, using pharmacologic approaches and transgenic mice expressing signal transduction deficient RAGE in CD4+ T cells suppressed experimental autoimmune encephalomyelitis (EAE) in mice exposed to encephalitogenic T cells or myelin basic protein [68]. In studies in EAE, the observation that administration of sRAGE to mice that spontaneously-developed EAE, that is, mice devoid of endogenous TCR-alpha and TCR-beta chains, suggested that RAGE was importantly involved in effector mechanisms that modulate T cell infiltration into the CNS [68].

Recent studies in a model of adoptive transfer of diabetogenic spleen cells into NOD/scid mice have suggested that RAGE may play important roles at more proximal steps in the immune response [9]. RAGE and S100 were expressed on islet cells with an inflammatory infiltrate in pancreata from diabetic NOD/scid mice that had received a transfer of diabetogenic splenocytes, but not from control NOD/scid mice. RAGE was expressed in endocrine cells, as well as in a population of T cells (CD4+ and CD8+) and B cells in the inflamed islets [9]. Treatment with sRAGE significantly reduced the rate of transfer of diabetes in this model, as demonstrated by a striking delay in the development of hyperglycemia. In parallel, the expression of destructive cytokines, IL-1beta and TNF-alpha, was significantly reduced in the sRAGE-treated islets compared with vehicle-treated animals, in parallel with upregulation of anti-inflammatory cytokines IL-10 and TGF-beta [9]. However, when the preactivated CD4+ T cell clone, BDC2.5 cells, was injected into mice, there was no impact of sRAGE on the development of hyperglycemia [9]. Such findings suggested that RAGE might be implicated in key aspects of T cell activation. Studies are underway at this time to elucidate the precise mechanisms underlying these findings.

Studies by Kevin Tracey's group elucidated that another RAGE ligand, amphoterin (or high mobility group box 1 (HMGB1)), displayed proinflammatory properties.

3.2. Amphoterin

Amphoterin was first identified within the cell as a DNA binding protein in the nucleus. In addition, it is well described that amphoterin may also exist extracellularly and on the surface of cells, especially cells actively migrating [41]. Studies from our laboratory have explored this molecule's interaction with RAGE in the context of neurite outgrowth and tumors [24,59]. As introduced above, Tracey and colleagues first thrust amphoterin directly into mechanisms linked directly to the inflammatory response. Like S100/calgranulins, amphoterin may be released from activated MPs, thereby leading to propagation of inflammatory responses [2, 63]. Key and direct roles for amphoterin in augmenting deleterious inflammatory responses were shown by administration of blocking antibodies to amphoterin generated by these investigators. Administration of these blocking antibodies to amphoterin enhanced survival of rodents subjected to conditions mimicking that of overwhelming septic shock [63]. It is important to note that studies suggest that amphoterin may also interact with other receptors, specifically toll-like receptor-4 and toll-like receptor 2 (TLR-4 and TLR-2) [38]. Although the precise interplay between RAGE and TLRs in mediating amphoterin signaling is yet to be fully uncovered, recent studies suggest that in amphoterin-enriched environments, such as arthritis, RAGE blockade plays important roles [23,40].

Taken together, these considerations strongly support the premise that RAGE activation in diabetes and immune-inflammatory states mediates chronic perturbation and injury. Thus, blockade of the receptor afforded benefit in such settings. Recent studies on RAGE and the nervous system demonstrate, however, that, not surprisingly, this paradigm may not be so straightforward.

4. RAGE & the CNS: Roles for amplification of amyloid-beta peptide (Aβ) toxicity

4.1. RAGE is a receptor for Aβ: Evidence from mouse models of Alzheimer's disease (AD)

Aβ contributes importantly to the pathogenesis of neuronal dysfunction in AD. Our first studies demon-

strated that RAGE bound Aβ in a dose-dependent and saturable manner, comparable to that of other ligands for the receptor [66]. Evidence suggestive of roles for RAGE in AD was elucidated by the enhanced expression of RAGE in AD brain vs that of age-matched controls [33,66]. RAGE expression was not limited to neurons in AD brain, but, as well, included increased expression in microglia. Importantly, studies also indicated that distinct RAGE ligands, AGEs, were also enhanced in AD brain [18,67]. Due to the chronicity of ligand/RAGE accumulation and expression in human AD and in mouse models, we tested the premise that such chronic upregulation of this axis mediated sustained perturbation and, thus, enhancement of neuronal stress.

To directly test this concept, we employed transgenic mice expressing both mutant amyloid precursor protein (mAPP) and functional RAGE in neurons. In the latter case, we employed the PDGF-B chain promoter to direct expression of full-length functional RAGE to neurons. As a first test of RAGE-dependent augmentation of stress in the brain, we examined activation of NF-kB in nuclear extracts prepared from wild-type, single transgenic (tg) mAPP, single transgenic PDGF RAGE, and double tg (mAPP/PDGF RAGE) mice. We found that activation of NF-kB in the brains of double tg mice was evident at 3–4 months of age, well before single tg mAPP mice displayed evidence of such activation [3]. Based on these findings, we subsequently tested the impact of simultaneous expression of RAGE and mAPP on key facets of neuronal function.

First, we assessed spatial learning/memory in the radial arm water maze. Although at 3–4 months of age, non-tg, tg mAPP, and tg PDGF RAGE mice displayed strong learning and memory capacity, double tg (mAPP/PDGF RAGE) mice displayed increased numbers of errors in this test, indicative of impaired spatial memory for platform location between trials [3]. Furthermore, synaptic transmission under basal conditions and during long-term potentiation (LTP) were also examined. Basal synaptic transmission (BST) impairment was accelerated in the double vs single tg mice; further, only the double tg mice displayed any evidence of impaired LTP (at 8–9 months of age). In contrast, single tg (mAPP or PDGF RAGE mice) failed to show evidence of altered LTP over this time period [3]. Such findings strongly support the premise that overexpression of RAGE in an Aβ-enriched environment amplifies perturbation of synaptic function in older mice, thus impairing plasticity.

Neuropathological studies further supported key roles for RAGE in augmenting Aβ-toxicity in the brains of these mice. We examined both acetylcholinesterase (AchE) activity-positive neurites (predominantly express cholinergic neurites) and synaptophysin (marker of presynaptic terminals) in the subiculum, CA1 and entorhinal cortex. At 3–4 months of age, evidence of accelerated neuropathological changes was demonstrated using both markers in double tg mice vs single tg mice or wild-type animals [3]. Importantly, our findings were extended to definitively ascribe roles for RAGE signaling in transducing the effects of Aβ, as transgenic mice expressing signal transduction deficient mutants of RAGE in neurons (using the PDGF-B chain promoter) bred into the mAPP background and studied at age 3–4 months displayed decreased activation of NF-kB in nuclear extracts, and strong spatial memory/learning [3]. Further, at later ages, neurological abnormalities (area occupied by AchE-positive neurites in the subiculum) were largely prevented in tg mAPP/PDGF DN RAGE mice compared with tg mAPP mice.

Taken together, these data strongly support roles for RAGE/RAGE signaling in mediating, at least in part, the adverse impact of Aβ in the brain triggered by the mAPP transgene [3]. Other studies have suggested key roles for RAGE in Aβ-transport across the blood-brain barrier (BBB).

4.2. RAGE, Aβ, and transport across the BBB

The impact of Aβ in the CNS is importantly modulated by interactions with the vasculature, as well as directly with neuronal and microglial cellular elements. Systemic Aβ infusion and studies in genetically-manipulated mice demonstrated that Aβ-RAGE interaction in cells of the vessel wall is essential for transport of Aβ across the BBB and influx into the CNS. Aβ-RAGE interaction in the vasculature results in upregulation of inflammatory cytokines and endothelin-1 (ET-1), mechanisms that induce vasoconstriction [13]. Consistent with key roles for RAGE in these processes, in a mouse transgenic model, inhibition of ligand-RAGE interaction suppressed accumulation of Aβ in brain parenchyma [13]. Thus, evidence strongly supports the premise that Aβ-RAGE is an important mechanism by which influx of Aβ into the CNS occurs. Importantly, the precise balance between Aβ influx- and efflux-mediating pathways, the latter specifically effected by low density lipoprotein receptor related protein-1 (LRP) in the CNS, is likely a critical gateway for design of optimal therapeutic interventions in AD [14]. Such efforts may be impacted upon by the ob-

servation that blocking RAGE on cultured EC, employing anti-RAGE IgG, results in upregulation of LRP expression, as illustrated by Western blotting [14]. These experiments underscore the possibility that the regulation and/or activities of RAGE and LRP may be linked.

4.3. RAGE, Aβ, AGEs antibodies in AD brain

Evidence from immunological studies is mounting to support Aβ-RAGE interaction *in vivo*. Mruthinti and colleagues showed that plasma samples from AD subjects displayed significantly higher titers of antibodies directed against Aβ, β-amyloid precursor protein (β-APP) and RAGE relative to controls without AD [34]. Other studies have suggested that in astrocytes in AD brain, epitopes for AGE, Aβ and RAGE co-localize [49]. Such findings do not specify cause and/or effect, however, they establish that these potentially key participants in the pathogenesis and progression of AD are in the right place and at the right time to contribute to cellular perturbation, from neurons to vascular cells and astrocytes.

Such findings also support the view that the biology of RAGE is not as simple as "one ligand – one disease process." Specifically, in the context of AD, it has been shown that when antibodies to glycated human brain neurofilament protein (HNF-AGE) were injected into mice, the animals unexpectedly developed IgGs against a peptide immunogenic fragment of RAGE and against human Aβ [35]. These observations suggest that the time-dependent formation of AGEs may play a role by modulating expression of Aβ-binding sites, such as RAGE and, thereby, possibly enhance the cellular toxicity of these species.

Taken together, these considerations indicate that both direct (cell surface binding) and immunogenicity properties of AGE, Aβ, and RAGE, alone or together, may contribute to cellular perturbation and toxicity in AD.

5. RAGE & other forms of chronic CNS stress & neurodegeneration

Pilot studies suggest roles for RAGE in mediating other forms of neurodegeneration in the CNS. Based on evidence that AGEs are enhanced in the spinal cord of human and experimental ALS [11,27,55], studies have been initiated to begin to test these premises. Employing mutant SOD1 (G93A) mice, we have found that overexpression of either neuronal RAGE (using the PDGF-B chain promoter) or microglial RAGE (using the scavenger receptor type A promoter) amplifies neuronal stress in the spinal cord of these animals [43, 46]. Further studies are ongoing to dissect the precise mechanisms underlying these findings, and to test the impact of genetic deletion of RAGE, or, alternatively, abrogation of RAGE signaling in neurons or microglia.

In another chronic neurodegenerative disease, Huntington's Disease (HD), selective neuron loss occurs in the striatum and cortex. Recent studies suggested that RAGE was expressed in at least two cell types in the caudate nucleus, medium spiny projection neurons and astrocytes [32]. Although the increase in RAGE immunostaining was associated with the increase in pathological grade of HD, no mechanistic link between RAGE and pathogenesis/progression of HD has yet been demonstrated.

Further, in one of the prion diseases, Creutzfeldt-Jakob disease (CJD), increased numbers of GFAP-positive astrocytes in the occipital cortex were reported to be immunopositive for prion protein, AGE epitopes and RAGE antigen. Astrocytes also were found to contain many AGE- and RAGE-immunopositive granules which displayed the characteristic pattern of prion protein expression [48]. Although such findings are not surprising given that RAGE has been found to bind β-sheet fibrils, the specific link between ligand/RAGE expression and CJD is yet to be elucidated and, thus, requires further study.

6. RAGE & the Peripheral Nervous System: Key roles in chronic stress

In addition to amyloid-type diseases of the CNS, amyloid diseases of the PNS also bear a RAGE link. For example, familial amyloid polyneuropathy (FAP) is a hereditary amyloidosis induced by mutated transthyretin which displays proclivity for PNS dysfunction. In FAP, studies have shown that transthyretin (TTR) is the main protein constituent of amyloid deposits in this disorder. Sousa and co-workers first identified that RAGE expression was increased in FAP tissues in the PNS [58]. In addition, increased activated NF-kB was also evident in these tissues, as demonstrated by increased nuclear translocation of p50. In parallel, *in vitro*, these investigators showed that RAGE bound TTR aggregates in cultured PC12 cells and stimulated increased activation of NF-kB. This process was dependent on RAGE, as indicated by blockade of these pathways in the presence of anti-RAGE IgG in RAGE-

expressing Chinese Hamster Ovary cells stimulated with TTR [57,58].

In addition to PNS amyloidoses, other studies have placed RAGE and its ligands in vasculitic neuropathies [21]. RAGE, CML-AGEs, NF-kB and IL-6 were localized in mononuclear cells, epineurial and endoneurial vessels and perineurium in sural biopsies of 12 human subjects with vasculitic neuropathies. In these studies, CML-AGE, RAGE, NF-kB and IL-6 were expressed by CD4+ and CD8+ T cells, as well as CD68+ MP invading the peripheral nerve [21].

Thus, evidence for augmentation of stress, in part via RAGE, in the CNS and PNS has been suggested in these chronic neurodegenerative disorders characterized, in part, by sustained upregulation of RAGE and its ligands. In this context, it thus became important to test the impact of RAGE in acute injury in the nervous system. From studies in the PNS, unexpected findings emerged from these experiments that highlighted for the first time innate roles for RAGE in restoration of homeostasis after acute injury.

7. RAGE & the PNS: Key roles in repair after acute injury

To delineate roles for RAGE in acute injury in the PNS, we employed a murine model of experimental axotomy. It is established that axotomy induced by nerve transection or crush activates an integrated series of cellular responses that mediate sharp but limited periods of inflammation that facilitate removal of myelin debris and damaged axonal elements distal to the site of injury (collectively termed "Wallerian degeneration)." In parallel, injury also triggers sprouting and elongation of regenerating axons as they grow from the proximal nerve stump into the distal stump in an attempt to reinnervate target tissue and, thus, potentially restore function [17, 30]. Effective Wallerian degeneration is essential for optimal axonal outgrowth and regeneration.

Since key roles for RAGE in inflammatory mechanisms were established in a distinct array of immune/inflammatory settings, even beyond hyperglycemia, it was thus logical to determine if RAGE and its ligands contributed to PNS repair triggered by acute axotomy. These studies were performed in non-diabetic mice. Both at baseline and at 18 hrs after unilateral crush of the sciatic nerve in C57BL/6 mice, segments within the first 3 mm distal to the site of crush displayed expression of RAGE in axons, as elucidated by colocalization with anti-neurofilament IgG [44]. At 18 hours after crush, RAGE was also expressed in MP in the distal segments, as shown by coexpression of immunoreactive RAGE and CD 68 [44]. Thus, RAGE was expressed in two important cell types linked to peripheral nerve regeneration, inflammatory MP and peripheral neurons. Importantly, these studies also elucidated that S100/calgranulins and amphoterin were also increased in the distal segments after crush in wild-type mice; importantly, by 21 days after crush, levels of these molecules began to return to those observed in homeostatic, uninjured states.

Based on these findings, we first employed pharmacological blockade of RAGE to test the impact of RAGE in peripheral nerve regeneration. Mice received either sRAGE or vehicle, murine serum albumin (MSA) or PBS. Compared to control treatment, sRAGE-treated animals displayed significantly decreased motor and sensory nerve conduction velocities on day 21 after crush, in parallel with diminished gait in a walking track analysis [44]. Definitive evidence of impaired regeneration was evident upon examination of myelinated fibers. In sRAGE-treated animals, decreased myelinated fiber density was noted compared to control-treated animals [44]. Additional strategies to test the role of RAGE and its ligands including the administration of blocking F(ab')$_2$ fragments prepared from anti-RAGE or anti-ligand (S100/calgranulin and amphoterin) IgG. Compared to non-immune F(ab')$_2$ fragments, treatment of mice with F(ab')$_2$ fragments derived from anti-RAGE, anti-S100/calgranulin or anti-amphoterin IgG resulted in decreased functional and morphological evidence of regeneration after sciatic nerve crush [44].

Although pharmacological blockade of RAGE impeded regeneration triggered by crush, these studies did not elucidate if the primary impact was on MP RAGE and/or axonal RAGE. To test these specific concepts, we employed non-diabetic mice expressing signal transduction deficient RAGE in either MP (using the scavenger receptor type A promoter) or peripheral neurons (using a fragment of the thy-1 promoter).

First, we studied the impact of RAGE signaling in MP. In tg mice expressing dominant negative (DN) RAGE in MP, on day 3 after crush, numbers of MP in the distal segments were significantly diminished compared to wild-type littermates [45]. Phosphorylation of p44/p42 MAP kinase was significantly reduced in the distal nerve segments at 18 hours in transgenic versus littermate mice, as well. To determine the effect of deficient RAGE signaling in peripheral neurons, we subjected tg mice to sciatic nerve crush. Levels of

phospho-p44/p42 MAP kinases were decreased 18 hrs after injury in distal segments in transgenic versus littermate animals [45]. Levels of phospho-STAT3 were significantly reduced on day 7 in the distal segments of sciatic nerve in transgenic thy-1 DN-RAGE mice compared to littermates [45]. These experiments elucidated that modulation of key signaling pathways linked to PNS regeneration were modulated by RAGE signaling.

To determine if such changes in RAGE signaling contributed to alterations in regeneration triggered by injury, nerve crush was then performed in single or double tg mice. Compared to wild type littermate mice, transgenic mice expressing DN RAGE in MP displayed an $\approx$ 40% and $\approx$ 45% decrease in motor and sensory nerve conduction velocities on day 21 after crush [45]. Compared to wild-type littermate mice, transgenic mice expressing DN RAGE in peripheral neurons displayed an $\approx$ 39% and $\approx$ 37% decrease in motor and sensory nerve conduction velocities on day 21 [45]. Simultaneous expression of DN RAGE in mononuclear phagocytes and neurons further exacerbated functional recovery after crush, as double transgenic mice displayed an $\approx$ 85% reduction in motor and sensory nerve conduction velocities on day 21 compared to wild-type controls, and an $\approx$ 70% decrease in motor and sensory nerve conduction velocities compared to single transgenic mice expressing DN RAGE in either MP or peripheral neurons [45].

Quantitative analyses of semithin sections demonstrated that mice expressing DN RAGE in MP (single or double transgenic) displayed increased myelin debris compared to wild-type mice, and mice expressing DN RAGE in neurons alone displayed increased myelin debris compared to wild-type mice [45]. Further, myelinated fiber density was significantly reduced by $\approx$ 42% in mice carrying the DN RAGE transgene in either MP or neurons compared to control littermates [45]. Mice expressing DN RAGE in both MP and neurons contained $\approx$ 67% less myelinated fibers in the sciatic nerve distal segments than wild-type mice, and $\approx$ 43% less myelinated fibers than single transgenic animals expressing DN RAGE in either MP or peripheral neurons [45].

These findings highlighted for the first time innate roles for RAGE in both inflammatory Wallerian degeneration and axonal outgrowth consequent to acute crush in the peripheral nerve. In the absence of diabetes, we posit that acute crush stimulates rapid and sharply-limited expression of RAGE and its ligands, S100/calgranulins and amphoterin that contribute, together, to mediating essential inflammatory mechanisms that remove myelin debris, and mechanisms that support axonal outgrowth.

In the context of peripheral nerve repair, it has been suggested that PNS injury triggers processes that recapitulate, in part, developmental mechanisms [37]. What is the evidence linking the ligand-RAGE axis to developmental programs?

8. The ligand-RAGE axis and development

Expression of RAGE is enhanced in developing CNS neurons and falls in the immediate post-natal period [24]. Studies have illustrated that at least two of RAGE's ligands, S100b and amphoterin, are expressed in developing brain where they may play roles as neurotropic, neuronal survival proteins, as well proteins involved in modulating neurite outgrowth and cell migration [10,20,62]. Importantly, studies in homozygous RAGE null mice do not support critical roles for this receptor in neuronal development, as RAGE null mice are viable and display normal reproductive capacity and growth [47]. However, it is possible that activation or upregulation or compensatory pathways have masked essential roles for RAGE in the process of development.

Although roles for RAGE in PNS development have been less well studied, it is notable that peripheral nerves appear to develop normally in RAGE null mice, and in transgenic mice expressing DN RAGE in neurons [45]. In the adult mouse, however, disruption of neuronal RAGE signaling in the latter mouse, suppressed optimal axonal regeneration following crush injury. In this context, evidence is emerging that signaling pathways linked to development vs the (adult) response to neuronal injury are distinct [56], based on divergent signal transduction responses via STAT3 and MAP kinases [56]. Thus, it is not surprising that RAGE-dependent developmental vs adult response-to-injury paradigms may, indeed, differ greatly.

9. RAGE, diabetes and the PNS: Impact of acute stress

Although our studies have addressed the role of RAGE in chronic diabetes and neuropathy, these findings raise the important question, what is the role of RAGE in acute injury in the diabetic peripheral nerve? Does RAGE mediate deleterious and/or beneficial mechanisms in such a setting? We posit that in

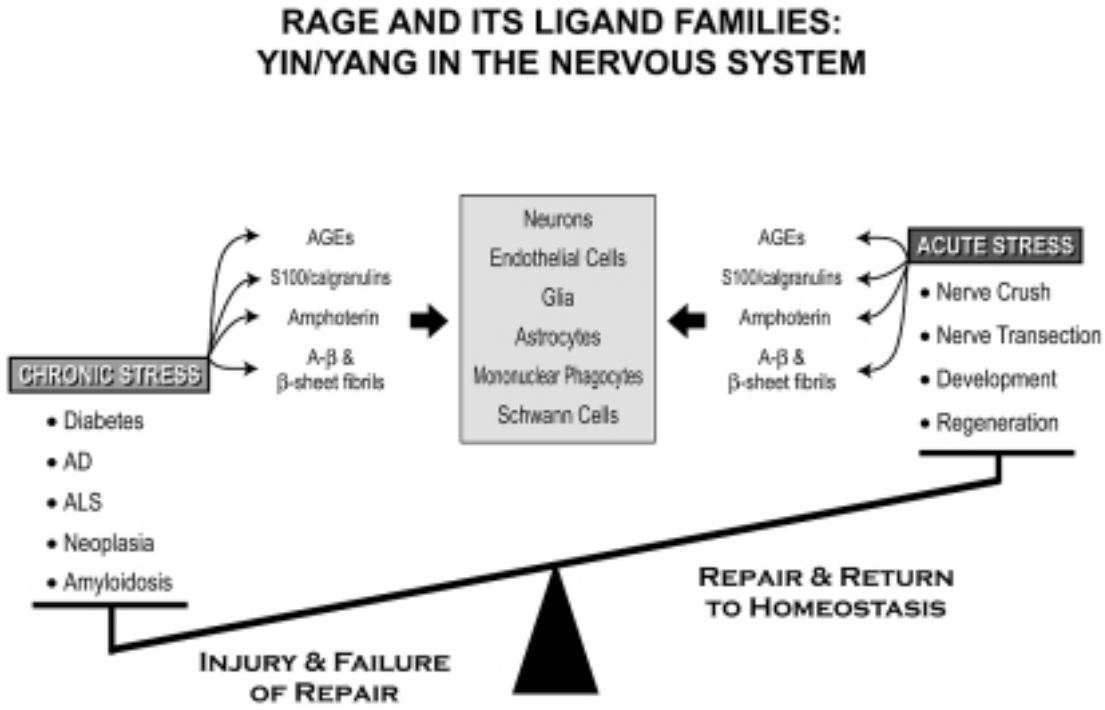

Fig. 1. RAGE: "Yin/Yang" in the Nervous System. Experimental evidence supports roles for RAGE-amplified injury and failure of restorative mechanisms in settings characterized by chronic upregulation of the ligand-RAGE axis, such as in long-standing diabetes, inflammation and neurodegenerative disorders, such as Alzheimer's disease. In contrast, in acute stress, such as that triggered by peripheral nerve crush in euglycemia, evidence supports the contention that transient and sharply-limited upregulation of the ligand-RAGE axis may contribute, in part, to repair and restoration of homeostasis. These findings elucidate the novel concept that RAGE, indeed, possesses key and innate functions. In future application of RAGE blockade in chronic disease, a fine balance must be struck between RAGE-dependent injurious- and RAGE-dependent regenerative pathways.

chronic diabetic neuropathy, long-standing accumulation of AGEs and perhaps other inflammatory ligands will result in chronic upregulation of RAGE. The impact of RAGE on repair mechanisms triggered by acute injury in chronic diabetes must, therefore, be dissected. Such studies are underway at this time.

10. Perspective & conclusion: A small dose of RAGE may be beneficial?

Figure 1 depicts the hypotheses underlying the apparently divergent roles for RAGE in the nervous system. Our studies suggest that in settings of chronic stress – whether it be diabetic tissues or the brain in AD – upregulation of the ligand-RAGE axis contributes critically to the pathogenesis of neuronal dysfunction and neurodegeneration. The accumulation of protein fragments, such as Aβ and perhaps AGEs, S100/calgranulins and amphoterin, within brain parenchyma and vasculature provides a chronically modified template in which homeostatic and reparative processes are sharply curtailed, at least in part, via RAGE. In stark contrast, in peripheral nerve, at least certain of these same cofactors, such as S100/calgranulins, amphoterin and RAGE, ignite essential mechanisms that augur effective inflammation and axonal outgrowth in repair pathways. Precisely how these same cofactors may have a pivotal role in innate, neuroprotective pathways that orchestrate reparative processes, versus highly deleterious neuro-injurious pathways that orchestrate chronic neurodegeneration, at least in part via RAGE, is the focus of intense study.

Acknowledgements

The authors gratefully acknowledge support from the U.S.P.H.S., the Juvenile Diabetes Research Foundation International, the Surgical Research Fund of Columbia University and the Burroughs Wellcome Fund. AMS is a recipient of a Burroughs Wellcome Fund Clinical Scientist Award in Translational Research.

References

[1] M. M. Anderson, J. R. Requena, J.R. Crowley, S.R. Thorpe and J. Heinecke, The myeloperoxidase system of human phagocytes generates Nepsilon-(carboxymethyl)lysine on proteins: a mechanism for producing advanced glycation endproducts at sites of inflammation, *J Clin Invest* **104** (1999), 103–113.

[2] U. Andersson, H. Wang, K. Palmblad, A.C. Aveberger, O. Bloom, H. Erlandsson-Harris, A. Janson, R. Kokkola, M. Zhang, M. Yang and K.J. Tracey, High mobility group 1 protein (HMG 1) stimulates proinflammatory cytokine synthesis in human monocytes, *J. Exp. Med.* **192** (2000), 565–570.

[3] O. Arancio, H.P. Zhang, X. Chen, C. Lin, F. Trinchese, D.Puzzo, S. Liu, A. Hegde, S.F. Yan, A. Stern, J.S. Luddy, L.F. Lue, D.g. Walker, A. Roher, M. Buttini, L. Mucke, W. Li, A.M. Schmidt, M. Kindy, P.A. Hyslop, D.M. Stern and S.S.D. Yan, RAGE potentiates Aβ-induced perturbation of neuronal function in transgenic mice, *EMBO J* **23** (2004), 4096–4105.

[4] T. Arumugam, D.M. Simeone, A.M. Schmidt and C.D. Logsdon, S100P stimulates cell proliferation and survival via RAGE, *J. Biol. Chem.* **279** (2004), 5059–5065.

[5] K.J. Bar, S. Franke, B. Wenda, S. Muller, R. Kientsch-Engel, G. Stein and H. Sauer, Pentosidine and N(epsilon)-(carboxymethyl)-lysine in Alzheimer disease and vascular dementia, *Neurobiol Aging* **24** (2003), 333–338.

[6] M. Brownlee, Glycation products and the pathogenesis of diabetic complications, *Diabetes Care* **15** (1992), 1835–1843.

[7] L.G. Bucciarelli, T.Wendt, W. Qu, Y. Lu, E. Lalla, L.L. Rong, M.T. Goova, B. Moser, T.K. Kislinger, D.C. Lee, Y. Kashyap, D.M. Stern and A.M. Schmidt, RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E null mice, *Circ* **106** (2002), 2827–2835.

[8] T. Chavakis, A. Bierhaus, N. Al-Fakhri, D. Schneider, S. Witte, T. Linn, M. Nagashima, J. Morser, B. Arnold, K.T. Preissner and P.P. Nawroth, The Pattern Recognition Receptor (RAGE) is a counterreceptor for leukocyte integrins: a novel pathway for inflammatory cell recruitment, *J. Exp. Med.* 198 (2003), 1507-1515.

[9] Y. Chen, S.S. Yan, J. Colgan, H.P. Zhang, J. Luban, A.M. Schmidt, D. Stern and K.C. Herold, Blockade of late stages of autoimmune diabetes by inhibition of the receptor for advanced glycation end products, *J. Immunol* **173** (2004), 1399–1405.

[10] D.K. Chou, J. Zhang, F.I. Smith, P. McCaffery and F.B. Jungalwala, Developmental expression of receptor for advanced glycation endproducts (RAGE), amphoterin and sulfoglucuronly (HNK-1) carbohydrate in mouse cerebellum and their role in neurite outgrowth and cell migration, *J Neurochem* **90** (2004), 1389–1401.

[11] S.M. Chou, C.Y. Han, H.S. Wang, H. Vlassara and R. Bucala, A receptor for advanced glycosylation endproducts (AGEs) is colocalized with neurofilament-bound AGEs and SOD1 in motoneurons of ALS: immunohistochemical study, *J Neurol Sci* **169** (1999), 87–92.

[12] F. Cipollone, A. Iezzi, M. Fazia, M. Zucchelli, B. Pini, C. Cuccurullo, D. De Cesare, G. De Blasis, R. Muraro, R. Bei, F. Chiarelli, A.M. Schmidt, F. Cuccurullo and A. Mezzetti, The Receptor RAGE as a progression factor amplifying arachidonate-dependent inflammatory and proteolytic response in human atherosclerotic plaques: role of glycemic control, *Circ* **108** (2003), 1070–1077.

[13] R. Deane, S. Du Yan, R.K. Submamaryan, B. LaRue, S. Jovanovic, F. Hogg, D. Welch, L. Manness, C. Lin, J. Yu, H. Zhu, J. Ghiso, B. Frangione, A. Stern, A.M. Schmidt, D.L. Armstrong, B. Arnold, B. Liliensiek, P. Nawroth, F. Hofman, M. Kindy, D. Stern and B. Zlokovic, RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain, *Nat Med* **9** (2003), 907–913.

[14] R. Deane, Z. Wu and B.V. Zlokovic, RAGE (yin) versus LRP (yang) balance regulates alzheimer amyloid beta-peptide clearance through transport across the blood-brain barrier, *Stroke* **35** (2004), 2628–2631.

[15] R. Dei, A. Takeda, H. Niwa, M. Li, Y. Nakagomi, M. Watanabe, T. Inagaki, Y. Washimi, Y. Yasuda, K. Horie, T. Miyata and G. Sobue, Lipid peroxidation and advanced glycation endproducts in the brain in normal aging and in Alzheimer's disease, *Acta Neuropathol* **104** (2002), 113–122.

[16] R. Donato, S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles, *Intl J Biochem Cell Biol* **33** (2001), 637–668.

[17] S.Y. Fu and T. Gordon, The cellular and molecular basis of peripheral nerve regeneration, *Mol Neurobiol* **14** (1997), 67–116.

[18] X. Girones, A. Guuimera, C.Z. Cruz-Sanchez, A. Ortega, N. Sasaki, Z. Makita, J.V. Lafuente, R. Kalaria and F.F. Cruz-Sanchez, N epsilon carboxymethyllysine in brain aging, diabetes mellitus, and Alzheimer's disease, *Free Radic Biol Med* **36** (2004), 1241–1247.

[19] M.T. Goova, J. Li, T. Kislinger, W. Qu, Y. Lu, L.G. Bucciarelli, S. Nowygrod, B.M. Wolf, X. Caliste, S.F. Yan, D.M. Stern and A.M. Schmidt, Blockade of Receptor for AGE (RAGE) restores effective wound healing in diabetic mice, *American J Pathol* **159** (2001), 513–525.

[20] S. Guazzi, A. Strangio, A.T. Franzi and M.E. Bianchi, HMGB1, an architectural chromatin protein and extracellular signaling factor, has a spatially and temporally restricted expression pattern in mouse brain, *Gene Expr Patterns* **3** (2003), 29–33.

[21] K.M. Haslbeck, A. Bierhaus, S. Erwin, A. Kirchner, P. Nawroth, U. Schlotzer, B. Neundorfer and D. Heuss, Receptor for advanced glycation endproduct (RAGE)-mediated nuclear factor kappa B activation in vasculitic neuropathy, *Muscle Nerve* **29** (2004), 853–860.

[22] M.A. Hofmann, S. Drury, C. Fu, W. Qu, A. Taguchi, Y. Lu, C. Avila, N. Kambham, A. Bierhaus, P. Nawroth, M.F. Neurath, T. Slattery, D. Beach, J. McClary, M. Nagashima, J. Morser, D. Stern and A.M. Schmidt, RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides, *Cell* **97** (1999), 889–901.

[23] M.A. Hofmann, S. Drury, B.I. Hudson, M.R. Gleason, W. Qu, Y. Lu, E. Lalla, S. Chitnis, J. Monteiro, M.H. Stickland, L.G. Bucciarelli, B. Moser, G. Moxley, S. Itescu, P.J. Grant, P.K. Gregersen, D.M. Stern and A.M. Schmidt, RAGE and arthritis: The G82S polymorphism amplifies the inflammatory response, *Genes Immun* **3** (2002), 123–135.

[24] O. Hori, J. Brett, T. Slattery, R. Cao, J. Zhang, J. Chen, M. Nagashima, D. Nitecki, J. Morser, D. Stern and A.M. Schmidt, The Receptor for Advanced Glycation Endproducts (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.* **270** (1995), 25752–25761.

[25] K. Ikeda, T. Higashi, H. Sano, Y. Jinnouchi, M. Yoshida, T. Araki, S. Ueda and S. Horiuchi, Carboxymethyllysine protein adduct is a major immunological epitope in proteins modified with AGEs of the Maillard reaction, *Biochem* **35** (1996), 8075–8083.

[26] T. Jono, T. Kimura, J. Takamatsu, R. Nagai, K. Miyazaki, T. Yuzuriha, T. Kitamura and S. Horiuchi, Accumulation of imidazolone, pentosidine, and N(epsilon)-(carboxymethyl)lysine in hippocampal CA4 pyramidal neurons in aged human brain, *Pathol Int* **52** (2002), 563–571.

[27] S. Kikuchi, K. Shinpo, A. Ogata, S. Tsuji, M. Takeuchi, Z. Makita and K.Tashiro, Detection of N epsilon-(carboxymethyl)lysine (CML) and non-CML advanced glycation end-products in the anterior horn of amyotrophic lateral sclerosis spinal cord, *Amyotroph Lateral Scler Other Motor Neuron Disord* **3** (2002), 63–68.

[28] T. Kislinger, C. Fu, B. Huber, W. Qu, A. Taguchi, S.D. Yan, M. Hofmann, S.F. Yan, M. Pischetsrider, D. Stern and A.M. Schmidt, Ne (carboxymethyl)lysine modifications of proteins are ligands for RAGE that activate cell signaling pathways and modulate gene expression, *J Biol Chem* **274** (1999), 31740–31749.

[29] T. Kislinger, N. Tanji, T. Wendt, W. Qu, Y. Lu, L.J. Ferran, Jr., A. Taguchi, K. Olson, L. Bucciarelli, M. Goova, M.A. Hofmann, G. Cataldegirmen, V. D'Agati, M. Pischetsrieder, D. Stern and A.M. Schmidt, RAGE mediates inflammation and enhanced expression of tissue factor in the vasculature of diabetic apolipoprotein E null mice, *Arterio Thromb Vasc Biol* **21** (2001), 905–910.

[30] P. Küry, G. Stoll and H.W. Müller, Molecular mechanisms of cellular interactions in peripheral nerve regeneration, *Curr Opin Neurol* **14** (2001), 635–639.

[31] E. Lalla, I.B. Lamster, M. Feit, L. Huang, A. Spessot, W. Qu, T. Kislinger, Y. Lu, D.M. Stern and A.M. Schmidt, Blockade of RAGE suppresses periodontitis-associated alveolar bone loss in diabetic mice, *J. Clin. Invest* **105** (2000), 1117–1124.

[32] M. Li and L.F.B. Nicholson, Expression of the receptor for advanced glycation end products in Huntington's disease caudate nucleus, *Brain Research* **1018** (2004), 10–17.

[33] L.F. Lue, D. Walker, L. Brachova, J. Rogers, Y. Shen, A.M. Schmidt, D.M. Stern and S.D. Yan, Involvement of RAGE-microglial interactions in Alzheimer's disease: *in vivo* and *in vitro* studies, *Exp. Neurol.* **171** (2001), 17810–17814.

[34] S. Mruthinti, J.J. Buccafusco, W.D. Hill, J.L. Waller, T.W. Jackson, E.Y. Zamrini and R.F. Schade, Autoimmunity in Alzheimer's disease: increased levels of circulating IgGs binding Abeta and RAGE peptides, *Neurobiol Aging* **25** (2004), 1023–1032.

[35] S. Mruthinti, W.D. Hill, S. Swamy-Mruthinti and J.J. Buccafusco, Relationship between the induction of RAGE cell-surface antigen and the expression of amyloid binding sites, *J Mol Neurosci* **20** (2003), 223–232.

[36] M. Neeper, A.M. Schmidt, J. Brett, S.D. Yan, F. Wang, Y.C. Pan, K. Elliston, D. Stern and A. Shaw, Cloning and expression of RAGE: a cell surface receptor for advanced glycosylation end products of proteins, *J Biol Chem* **267** (1992), 14998–15004.

[37] J. Nieke and M. Schachner, Expression of neural cell adhesion molecules L1 and N-CAM and their common carbohydrate epitope L2/HNK-1 during development and after transection of the mouse sciatic nerve, *Differentiation* **30** (1985), 141–151.

[38] J. S. Park, D. Svetkauskaite, Q. He, J.Y. Kim, D. Strassheim, A. Ishizaka and E. Abraham, Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein, *J. Biol. Chem.* **279** (2004), 7370–7377.

[39] L. Park, K.G. Raman, K.J. Lee, Y. Yan, L.J. Ferran, W.S. Chow, D. Stern and A.M. Schmidt, Suppression of accelerated diabetic atherosclerosis by soluble Receptor for AGE (sRAGE), *Nature Medicine* **4** (1998), 1025–1031.

[40] R. Pullerits, I.M. Jonsson, M. Verdrengh, M. Bokarewa, U. Andersson, N. Erlandsson-Harris and A. Tarkowski, High mobility group box chromosomal protein 1, a DNA binding cytokine, induces arthritis, *Arthritis Rheum* **48** (2003), 1693–1700.

[41] H. Rauvala and R. Pihlaskari, Isolation and some characteristics of an adhesive factor of brain that enhances neurite outgrowth in central neurons, *J. Biol. Chem.* **262** (1987), 16625–16635.

[42] S. Reddy, J. Bichler, K. Wells-Knecht, S.R. Thorpe, and J.W. Baynes, Carboxymethyllysine is a dominant AGE antigen in tissue proteins, *Biochem* **34** (1995), 10872–10878.

[43] L.L. Rong, A. Adebayo, Y. Lu, S. Przedborski, A.P. Hays, S.F. Yan and A.M. Schmidt, Microglial RAGE accelerates mortality and neuronal dysfunction in a murine model of familial amyotrophic lateral sclerosis, *Society for Neuroscience* (2004).

[44] L.L. Rong, W. Trojaborg, W. Qu, K. Kostov, S.D. Yan, C. Gooch, M. Szabolcs, A.P. Hays and A.M. Schmidt, Antagonism of RAGE suppresses peripheral nerve regeneration, *FASEB Journal* **18** (2004), 1812–1817.

[45] L.L. Rong, S.F. Yan, T. Wendt, D. Hans-Wagner, S. Pachydaki, L.G. Bucciarelli, A. Adebayo, W. Qu, Y. Lu, K. Kostov, E. Lalla, S.D. Yan, C. Gooch, M. Szabolcs, W. Trojaborg, A.P. Hays and A.M. Schmidt, RAGE modulates peripheral nerve regeneration via recruitment of both inflammatory and axonal outgrowth pathways, *FASEB Journal* **18** (2004), 1818–1825.

[46] L.L. Rong, S.F. Yan, S.D. Yan, D. Hans-Wagner, F. Song, D.M. Stern, S. Prezedborski, A.P. Hays and A.M. Schmidt, RAGE-dependent mechanisms accelerate neuronal dysfunction in a murine model of amyotrophic lateral sclerosis, *Society for Neuroscience* (2002).

[47] T. Sakaguchi, S.F. Yan, S.D. Yan, L.L. Rong, M. Sousa, D. Belov, M. Andrassy, S.P. Marso, S. Duda, B. Arnold, B. Liliensiek, P.P. Nawroth, D.M. Stern, A.M. Schmidt and Y. Naka, Arterial restenosis: central role of RAGE-dependent neointimal expansion, *J Clin Invest* **111** (2003), 959–972.

[48] N. Sasaki, M. Takeuchi, H. Chowei, S. Kikuchi, Y. Hayashi, N. Nakano, H. Ikeda, S. Yamagishi, T. Kitamoto, T. Saito and Z. Makita, Advanced glycation end products (AGE) and their receptor (RAGE) in the brain of patients with Creutzfeldt-Jakob disease with prion plaques, *Neurosci Lett* **326** (2002), 117–120.

[49] N. Sasaki, S. Toki, H. Chowei, T. Saito, N. Nakano, Y. Hayashi, M. Takeuchi and Z. Makita, Immunohistochemical distribution of the receptor for advanced glycation endproducts in neurons and astrocytes in Alzheimer's disease, *Brain Res* **888** (2001), 256–262.

[50] B.W. Schafer and C.W. Heinzmann, The S100 family of EF-hand calcium-binding proteins: functions and pathology, *Trends Biochem Sci* **21** (1996), 134–140.

[51] E. Schleicher, E. Wagner and A. Nerlich, Increased accumulation of glycoxidation product carboxymethyllysine in human tissues in diabetes and aging, *J Clin Invest* **99** (1997), 457–468.

[52] A.M. Schmidt, O. Hori, J. Chen, J.F. Li, J. Crandall, J. Zhang, R. Cao, S.D. Yan, J. Brett and D. Stern, Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1): a potential mechanism for the accelerated vasculopathy of diabetes, *J. Clin. Invest.* **96** (1995), 1395–1403.

[53] A.M. Schmidt, M. Vianna, M. Gerlach, J. Brett, J. Ryan, J. Kao, C. Esposito, H. Hegarty, W. Hurley, M. Clauss, F. Wang, Y.C. Pan, Y.C. Tsang and D. Stern, Isolation and characterization of binding proteins for advanced glycosylation endprod-

ucts from lung tissue which are present on the endothelial cell surface, *J Biol Chem* **267** (1992), 14987–14997.
[54] A.M. Schmidt, S.D. Yan, J. Brett, R. Mora and D. Stern, Regulation of mononuclear phagocyte migration by cell surface binding proteins for advanced glycosylation endproducts, *J. Clin. Invest.* **92** (1993), 2155–2168.
[55] N. Shibata, A. Hirano, S. Kato, R. Nagai, S. Horiuchi, T. Komori, T. Umahara, K. Asayama and M. Kobayashi, Advanced glycation endproducts are deposited in neuronal hyaline inclusions: a study on familial amyotrophic lateral sclerosis with superoxide dismutase-1 mutation, *Acta Neuropathol* (*Berl*) **97** (1999), 240–246.
[56] W.D. Snider, F.Q. Zhou, J. Zhong and A. Markus, Signaling the pathway to regeneration, *Neuron* **35** (2002), 13–16.
[57] M.M. Sousa, S. Du Yan, R. Fernandes, A. Guimaraes, D. Stern and M.J. Saraiva, Familial amyloid polyneuropathy: receptor for advanced glycation end products-dependent triggering of neuronal inflammatory and apoptotic pathways, *J Neurosci* **21** (2001), 576–586.
[58] M.M. Sousa, S.D. Yan, D. Stern and M.J. Saraiva, Interaction of the receptor for advanced glycation endproducts (RAGE) with transthyretin triggers nuclear transcription factor kB (NF-kB) activation, *Lab Invest* **80** (2000), 1101–1110.
[59] A. Taguchi, D.C. Blood, G. del Toro, A. Canet, D.C. Lee, W. Qu, N. Tanji, Y. Lu, E. Lalla, C. Fu, M.A. Hofmann, T. Kislinger, M. Ingram, A. Lu, H. Tanaka, O. Hori, S. Ogawa, D.M. Stern and A.M. Schmidt, Blockade of amphoterin/RAGE signaling suppresses tumor growth and metastases, *Nature* **405** (2000), 354–360.
[60] N. Tanji, G.S. Markowitz, C. Fu, T. Kislinger, A. Taguchi, M. Pischetsrieder, D. Stern, A.M. Schmidt and V.D. D'Agati, The expression of Advanced Glycation Endproducts and their cellular receptor RAGE in diabetic nephropathy and nondiabetic renal disease, *J. American Soc. Nephrol.* **11** (2000), 1656–1666.
[61] A. Tauer, T. Knerr, T. Niwa, T.P. Schaub, C. Lage, J. Passlick-Deetjen and M. Pischetsrieder, *In vitro* formation of N(epsilon)-(carboxymethyl)lysine and imidazolones under conditions similar to continuous ambulatory peritoneal dialysis, *Biochem Biophys Res Comm* **280** (2001), 1408–1414.
[62] L.J. Van Eldik and M.S. Wainwright, The Janus face of glial-derived S100B: beneficial and detrimental functions in the brain, *Restor Neurol Neurosci* **21** (2003), 97–108.
[63] H. Wang, O. Bloom, M. Zhang, J.M. Vishnuhakat, M. Ombrellino, J. Che, A. Frazier, H. Yang, S. Ivanova, L. Borovikova, K.R. Manogue, E. Faist, E. Abraham, J. Andersson, U. Andersson, P.E. Molina, N.N. Abumrad, A. Sama and K.J. Tracey, HMG-1 as a late mediator of endotoxin lethality in mice, *Science* **285** (1999), 248–251.
[64] J. L. Wautier, C. Zoukourian, O. Chappey, M-P. Wautier, P-J. Guillausseau, R. Cao, O. Hori, D. Stern and A.M. Schmidt, Receptor-mediated endothelial cell dysfunction in diabetic vasculopathy: soluble receptor for advanced glycation endproducts blocks hyperpermeability, *J. Clin. Invest.* **97** (1996), 238–243.
[65] T.M. Wendt, N. Tanji, J. Guo, T.R. Kislinger, W. Qu, Y. Lu, L.G. Bucciarelli, L.L. Rong, B. Moser, G.S. Markowitz, G. Stein, A. Bierhaus, B. Liliensiek, B. Arnold, P.P. Nawroth, D.M. Stern, V.D. D'Agati and A.M. Schmidt, RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy, *Am J Pathol* **162** (2003), 1123–1137.
[66] S.D. Yan, X. Chen, J. Fu, M. Chen, H. Zhu, A. Roher, T. Slattery, M. Nagashima, J. Morser, A. Migheli, P. Nawroth, G. Godman, D. Stern and A.M. Schmidt, RAGE and amyloid-b peptide neurotoxicity in Alzheimer's disease, *Nature* **382** (1996), 685–691.
[67] S.D. Yan, X. Chen, A.M. Schmidt, J. Brett, G. Godman, C.W. Scott, C. Caputo, T. Frappier, S.H. Yen and D. Stern, The presence of glycated tau in Alzheimer's disease: a mechanism for induction of oxidant stress, *PNAS* (*USA*) **91** (1994), 7787–7791.
[68] S.S.D. Yan, Z.Y. Wu, H.P. Zhang, G. Furtado, X. Chen, S.F. Yan, A.M. Schmidt, C. Brown, A. Stern, J. LaFaille, L. Chess, D.M. Stern and H. Jiang, Suppression of experimental autoimmune encephalomyelitis by selective blockade of encephalitogenic T-cell infiltration of the central nervous system, *Nature Medicine* **9** (2003), 287–293.
[69] S.D. Yan, H. Zhu, A. Zhu, A. Golabek, H. Du, A. Roher, J. Yu, C. Soto, A.M. Schmidt, D. Stern and M. Kindy, Receptor-dependent cell stress and amyloid accumulation in systemic amyloidosis, *Nature Medicine* **6** (2000), 643–651.
[70] Z. Zhou, K. Wang, M.S. Penn, S.P. Marso, M.A. Lauer, F. Forudi, X. Zhou, W. Qu, Y. Lu, D.M. Stern, A.M. Schmidt, A.M. Lincoff and E.J. Topol, Receptor for AGE (RAGE) mediates neointimal formation in response to arterial injury, *Circ* **107** (2003), 2238–2243.
[71] D.B. Zimmer, E.H. Cornwall, A. Landar and W. Song, The S100 protein family: history, function, and expression, *Brain Res Bull* **37** (1995), 417–429.