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Commentary

Cellular Cofactors for Amyloid β-Peptide-Induced Cell Stress

Moving from Cell Culture to in Vivo

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Molecular mechanisms underlying Alzheimer's disease (AD) can be divided into inciting pathogenic factors and those more likely to be associated with amplification of cell stress once the disease process is already under way; the latter are termed progression factors. In view of the protracted clinical course of AD, evolving over years, both processes are relevant to the inexorable decline in neuronal function, both mechanistically and therapeutically. Mutations in β-amyloid precursor protein (βAPP) and presenilins 1 and 2, resulting in increased generation of amyloid β-peptide (Aβ) spanning residues 1–42, have been linked to the pathogenesis of familial AD.[1–4] An emerging role for compromised Aβ clearance and degradation, possibly involving the low density lipoprotein receptor-related protein (LRP), apolipoprotein E (apoE), and/or α_2-macroglobulin, has been suggested to contribute to sporadic AD.[5–8] Though other mechanisms certainly remain to be discovered, these data contribute to an increasing body of evidence connecting Aβ as a pathogenetic factor central to neuronal dysfunction underlying AD.

Although studies of βAPP biology have advanced to sophisticated analyses of presenilin association with intracellular signaling molecules[9] and subcellular compartmentalization of βAPP processing,[10–14] analysis of mechanisms of Aβ-induced cellular stress are at a less advanced stage. Despite our relative lack of knowledge, understanding how Aβ triggers changes in cellular properties is clearly an essential part of any formulation of the amyloid hypothesis.[15] Why, then, has the search for progression factors in the biology of Aβ been so elusive?

An analogy with the blood coagulation mechanism is pertinent. For many years, thrombin, the final enzyme in the procoagulant pathway, was known to cleave plasma protein C, forming an important antithrombotic regulator, activated protein C.[16] There was an apparent paradox as, *in vitro*, the amounts of thrombin required, the concentration of divalent cations, and the rate of activated protein C formation suggested that this reaction, as it occurs in a purified system, was physiologically irrelevant.[16] The solution to this quandary was provided by the identification of a novel endothelial cell cofactor, the integral membrane protein thrombomodulin. In the presence of thrombomodulin, thrombin-mediated activation of protein C occurred rapidly with physiological concentrations of reactants and cations.[16] The contribution of such cellular cofactors to many biological systems is accepted as a given. In this regard, what types of cellular cofactors have been identified based on their interaction with Aβ?

Progression factors relevant to AD exacerbate cell stress in an environment created by the pathogenetic factors. There are many candidate progression factors, such as cytokines,[17] complement activation,[18–20] reactive oxygen intermediates (ROIs),[21–24] and other products of activated microglia and/or astrocytes. Such mediators and other mechanisms, including elevated levels of cytosolic calcium,[24] are likely to be placed distally in pathways of cellular dysfunction. Thus, by the time neurons are bathed in proinflammatory cytokines and the environment is characterized by ubiquitous ROIs and elevated intracellular calcium, cellular dysfunction is likely to be quite advanced. However, another class of cell-associated progression factors relevant to AD are those selectively engaged by pathophysiologically relevant forms of Aβ at nanomolar concentrations. These are described below, grouped into several categories.

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Cell Surface Binding Sites for Aβ Monomer

Two classes of cell surface binding sites have been reported to interact selectively with Aβ monomer: the serpin enzyme complex receptor and $\alpha_5\beta_1$ integrin.[25–27] These Aβ interaction sites could participate in physiological functions of Aβ, though they might also have protective effects by lowering the concentration of Aβ monomer/dimer available for subsequent multimerization and β-sheet fibril formation. Further studies will be required to determine the contribution of these molecules to Aβ biology *in vivo* and whether they function as true receptors or cell surface tethering sites.

Heparan Sulfate Proteoglycans (HSPG)

The interaction of Aβ with proteoglycans, especially HSPG, has been appreciated for some time.[28–37] Several investigations have shown that Aβ residues 12–17 are involved in the binding to HSPG,[34,36,37] including proteoglycan present on the microglial cell surface and involving residues 13–16.[29] Binding of Aβ to cell surface proteoglycans could directly trigger signal transduction mechanisms, though the demonstrated association of Aβ with glypican, a glycosylphosphatidylinositol (GPI)-anchored heparan sulfate molecule,[38] emphasizes a likely role for such sites as coreceptors. This situation may turn out to be analogous to the biology of fibroblast growth factors, in which heparan sulfate proteoglycans serve as a reservoir of ligand strategically positioned for a handover to transmembrane-spanning signal transduction receptors.[39–42] The interaction of Aβ with proteoglycans may have a significant role in the sequence of events leading to Aβ-mediated cellular perturbation, as illustrated by inhibition of the inflammatory reaction in rat brain consequent to infusion of Aβ in the presence of the peptide HHQK (residues 13–16 from Aβ).[29]

Low-Density Lipoprotein Receptor-Related Protein

LRP has been suggested to participate in clearance mechanisms of Aβ, potentially mediated by complexes of amyloid with apoE and/or α_2-macroglobulin.[43,44] In this pathway, HSPG may also be involved as mediator between apoE and LRP.[45] The possible relevance of defects in this pathway to Aβ-induced neuronal dysfunction is emphasized by the association of mutations in each of the components of this pathway with AD.[5,6,46] However, considerably more experimental data are required to come to any conclusion on the role of these molecules in Aβ-cellular interactions.

Type A Macrophage Scavenger Receptor (MSR)

Experiments in cell culture strongly suggested an interaction of the type A MSR,[47,48] a receptor whose expression appears confined to cells of mononuclear phagocyte lineage,[49] with fibrillar Aβ. Especially in view of previous reports elegantly detailing the interaction of MSR with complex fibrillar structures and polyanionic ligands,[49,50] possible binding of MSR to Aβ with β-sheet fibrillar structure seemed to be a reasonable possibility. Consistent with a role for MSR in Alzheimer-type pathology was the increased expression of the receptor associated with senile plaques in Alzheimer's disease.[51] To complete this picture, proposed consequences of Aβ-MSR interaction included cellular uptake and degradation of Aβ fibrils by microglia, possibly providing insight into a long-sought clearance mechanism for fibrillar Aβ. Furthermore, it was suggested that Aβ binding to MSR triggered microglial activation.[47] However, these data were not substantiated by experiments with specific blocking antibodies in culture (only recently available),[52] and multiple other studies have not shown activation of intracellular signaling pathways to be a consequence of ligand binding to MSR.

In a more general context, MSR has been demonstrated to mediate uptake and disposal of potentially toxic ligands.[49] A particularly relevant example is the protective function of MSR in endotoxic shock. *In vivo*, MSR has a role in the hepatic clearance of lipid IV_A, the bioactive precursor of lipid A.[53] Mice deficient in MSR ($MSR^{-/-}$)[54] displayed increased vulnerability to infusion of bacillus Calmette Guerin (with decreased survival), accompanied by enhanced production of tumor necrosis factor-α and interleukin-6 (IL-6), compared with wild-type controls. In atherogenesis, in which MSR binding to oxidized LDL is thought to contribute importantly to foam cell formation,[49] it was predicted that atherosclerosis-prone $MSR^{-/-}$ mice would display attenuated formation of vascular lesions. This prediction initially gained support from experiments using apoE null mice crossed with $MSR^{-/-}$[54] to generate double knockout mice ($apoE^{-/-}$/$MSR^{-/-}$); the double knockouts displayed a decrease in lesion area compared with $apoE^{-/-}$ alone. However, subsequent studies in other atherosclerosis-prone murine models (for example, transgenic mice carrying the APOE3Leiden gene),[55] have shown more severe lesions in mice lacking MSR. This implies the existence of a more complex picture in which MSR may actually serve a protective role in atherosclerosis by promoting the clearance of modified lipoproteins.

In the context of these previous studies of MSR biology, and based on *in vitro* results suggesting that MSR did interact with Aβ fibrils, one could have predicted that crossbreeding mice overproducing a mutant form of βAPP (to create an Aβ-rich environment) with $MSR^{-/-}$ mice would have resulted in decreased clearance of Aβ, and, consequently, increased cytotoxicity attributable to Aβ; alternatively, absence of MSR might have prevented Aβ cytotoxicity if MSR had an important role in mediating toxic effects of the amyloid peptide on cells. The paper by Huang et al[56] in this issue of *The American Journal of Pathology*, from the laboratory of Dr. Lennart Mucke, addresses this issue using heterozygous transgenic mice overexpressing a minigene bearing two amyloidogenic mutations linked to familial AD (V717F and K670 Mol/L/N671).[57,58] Mice with this hAPP minigene, termed Tg

hAPP, were crossbred with $MSR^{-/-}$ mice.[54] In view of the predicted role of MSR in the processing of Aβ, this particular βAPP transgenic model was especially appropriate because the increase in brain Aβ content and accumulation of plaques in these mice has been carefully characterized.[59,60] Further studies in this model have shown i) a decrease in immunoreactive synaptophysin, a marker of presynaptic terminals, reflecting a reduction in the number of synapses, an important correlate of dementia in AD,[59,61–63] ii) a decrease in neuronal dendrites, based on MAP-2 staining in CA1 and CA3, and iii) a decline in basal synaptic transmission consistent with decreased functional synapses, though those remaining displayed appropriate responses (long-term potentiation was maintained up to 8 to 10 months of age).[59] This wealth of background information on the phenotype of Tg hAPP mice provides a rich setting for analyzing the early effects of progression factors proposed to modulate amyloid cytotoxicity and accumulation of Aβ.

A first look at the phenotype of Tg $hAPP^{+/-}/MSR^{-/-}$ mice,[56] with respect to Aβ deposition and synaptic integrity, indicates that there was no apparent difference in Aβ deposition in 6- and 12-month-old animals in terms of the number, extent, distribution, or age-dependence of amyloid plaques in Tg $hAPP^{+/-}/MSR^{-/-}$ as compared with Tg $hAPP^{+/-}/MSR^{+/+}$ mice. Furthermore, immunoreactive synaptophysin and the proportion of neuropil occupied by neuronal dendrites, based on staining for microtubule-associated protein 2 (MAP-2) was decreased over 12 months to the same extent in Tg $hAPP^{+/-}/MSR^{+/+}$ and Tg $hAPP^{+/-}/MSR^{-/-}$ mice, suggesting no effect of MSR. Of course, there is the possibility that other scavenger-like receptors might have substituted for the function of MSR in the Tg $hAPP^{+/-}/MSR^{-/-}$, and there are technical issues that perhaps render these results less than definitive. Some of these considerations include, for example, i) the number of mice was small (7–8 per group, though this was sufficient for statistical significance); ii) longer time points should also be tested (>12 months); iii) the genetic homogeneity of the Tg $hAPP^{+/-}/MSR^{-/-}$ in terms of being in an inbred mouse strain (the investigators used an apparently comparable random mixture of C57BL6, DBA/2, and ICR backgrounds) was not maximized; iv) early cell stress markers and electrophysiologic endpoints were not assessed; and v) other transgenic models resulting in enhanced Aβ generation should also be analyzed with respect to the impact of deleting MSR. The latter is an especially relevant issue as each of the models of AD-type pathology appears to have distinctive properties and potential limitations.[64–70] Perhaps most importantly, microglial activation in the Tg $hAPP^{+/-}/MSR^{-/-}$ mouse needs to be carefully compared with that observed in Tg $hAPP^{+/-}/MSR^{+/+}$ mice in a future study. Despite these reservations, one can conclude that MSR is not likely to be a central cofactor in Aβ clearance or Aβ-induced neurotoxicity in this transgenic model, subject to the caveats mentioned above. The key issue is that the current work from Dr. Mucke's laboratory[56] has put in place an impressive test system for ruling in or out the role of a proposed progression factor in a model with features of AD-like pathology.

With respect to other proposed progression factors in AD identified solely on the basis of *in vitro* studies, these results sound a cautionary note. As often applies, *in vitro* observations cannot be simply extrapolated to the *in vivo* setting. Rather, the *in vivo* study is a true experiment in a complex, multicellular, and interactive environment that can provide new insights, possibly along the lines suggested by *in vitro* results. We cannot limit our view to that suggested by data obtained from experiments in tissue culture. Indeed, based on the results obtained *in vivo* thus far, one could propose, with equal justification, that deletion of MSR might actually exacerbate or ameliorate the pathological picture at later time points. Thus, although data concerning induction of apoptosis after binding of Aβ to the p75 neurotrophin receptor *in vitro* is provocative,[71] it is essential to develop *in vivo* paradigms to test this concept. The same considerations apply to the other progression factors mentioned in this Commentary.

Two final progression factors under study in our laboratory will be considered because they appear to have passed an early test of *in vivo* relevance.

Receptor for Advanced Glycation Endproduct (RAGE)

RAGE is a multiligand member of the immunoglobulin superfamily. RAGE binds certain products of nonenzymatic glycoxidation,[72] Aβ (especially in β-sheet conformation), and other β-sheet fibrils, amphoterin, and S100/calgranulins.[73–78] The receptor is comprised of one V-type domain, which has a key role in ligand binding, followed by two C-type domains, a single transmembrane spanning region, and a short, highly charged cytosolic tail.[74,77] Although RAGE is most similar in structure to neural cell adhesion molecule (NCAM) and muc18 and has a V-C-C structure similar to that of the poliovirus receptor,[79] it does not share the same family of ligands as these other immunoglobulin-like molecules. RAGE is present at high levels early during development in a range of cells, but its expression falls off with maturity under homeostatic conditions in most tissues. However, with intervening pathology, RAGE is re-expressed at high levels. For example, in the developing rat central nervous system most cortical neurons stain strongly for RAGE.[75] With maturity, only occasional cortical neurons display RAGE antigen,[76,80] whereas in AD brain RAGE is expressed in neurons bearing neurofibrillary tangles and those proximal to deposits of Aβ.[76] In fact, RAGE is present at multiple sites of cellular perturbation in AD brain; it is also observed in activated microglia proximal to and invading plaques and is found in the vasculature, both in endothelium and smooth muscle of vessels with deposited Aβ.[76] Thus, the expression of RAGE in several cell types and its presence at sites of cellular pathology places it in a strategic location to participate in the cellular response.

A salient feature of RAGE biology is the chronicity of its expression. Other receptors, such as the LDL receptor, are down-regulated in the presence of ligand, thereby providing an endogenous negative feedback loop.[81] In

contrast, RAGE is up-regulated by its ligands and the increased amount of cell surface receptor enhances cellular activation in response to engagement by the ligands. In the case of advanced glycation endproducts (AGEs) and Aβ, a likely mechanism, at least in part, for ligand-mediated up-regulation of the receptor involves the two functional NF-κB sites in the RAGE promoter.[82] Thus, RAGE establishes a positive feedback loop resulting in a swirling spiral of cellular perturbation. The only way to intercept this cycle of cellular dysfunction, known at this time, is to block ligands from engaging the receptor. Once bound by ligand, the cytosolic tail of RAGE recruits intracellular signaling molecules and potently induces cellular activation; in contrast, RAGE does not effectively mediate cellular uptake and degradation of ligand, such as Aβ.[83]

One pathway through which RAGE-ligand interaction modulates cellular behavior involves activation of p21^{ras}, followed by mitogen-activated protein kinases (erk 1/2) and translocation to the nucleus of NF-κB, causing up-regulation of a range of genes.[84] In this context, Aβ binding to RAGE on neuroblastoma cells induces NF-κB activation and, consequently, increased expression of macrophage colony-stimulating factor (M-CSF).[85] Well known as a stimulator of cell proliferation, activation, and survival in cells of monocyte origin[86,87] that bear the receptor c-fms,[88,89] M-CSF can recruit microglia to sites of cellular perturbation. M-CSF-induced microglial/mononuclear phagocyte activation can generate neurotoxic products. In contrast, neurons do not express c-fms[89] and do not benefit from the positive effects of M-CSF; rather, they become immersed in a toxic environment favoring microglial expansion and activation. Consistent with this concept, increased levels of M-CSF have been observed in AD brain, and in cerebrospinal fluid from patients with AD,[85] although this does not imply that Aβ-RAGE interaction was the sole trigger for M-CSF expression *in vivo*.

In cell culture, various cell types display RAGE-dependent binding of Aβ (endothelium, rat cortical neurons, neuroblastoma cells, microglial cell lines, etc). Although the receptor appears to interact with Aβ in random conformation,[76,85] there is a greater affinity for the β-sheet structure, which presents a high density of surface ligand that potently activates RAGE. Changes in cellular properties due to ligand binding to RAGE on cells in culture can be blocked by i) monospecific antibodies to the receptor; ii) the presence of a truncated soluble form of the receptor spanning the extracellular domain (termed sRAGE, which functions as a decoy binding ligand, thereby preventing its interaction with normal cell surface receptor); and iii) on cells expressing a form of RAGE from which the cytosolic tail has been deleted (such cells bind ligand comparably to full-length RAGE, but do not induce cellular activation). The latter form of RAGE actually functions as a dominant negative, blocking RAGE-dependent cellular activation in cells also expressing wild-type receptor.[78] These data have established RAGE as a signal transduction receptor for its ligands, such as S100/calgranulins and Aβ, rather than solely as a cell surface tethering site.[76,78]

Preliminary results from *in vivo* studies have suggested that RAGE contributes to cell stress in several amyloidoses: 1) in a model of systemic amyloidosis (RAGE also interacts with amyloid A fibrils), RAGE blockade suppressed expression of cell stress markers heme oxygenase type 1 (HO-1), IL-6, and M-CSF, as well as accumulation of amyloid in the spleen, a major site of pathological changes (Yan S-D., Zhu, H., Zhu A., Golabek A, Wolozin B, Roher A, Yu J, Chaney M, Soto C, Schmidt A-M, Stern D, Kindy M, manuscript in preparation); and ii) in double-transgenic mice with targeted overexpression of RAGE in neurons and a mutant human βAPP transgene, expression of the stress markers, IL-6 and M-CSF, especially in neurons, occurred as early as 2.5 months, compared with their absence in single-transgenic or nontransgenic littermate control mice at this time point (Yan S-D, Stern D, Schmidt A-M, unpublished observation). This contrasts with the expression of the cell stress markers[90] only in older (21–25 months) transgenic mice expressing human mutant βAPP695 (the Swedish variant, K670N/M671L).[90] Although these results are encouraging, completion of the studies with RAGE transgenic mice, including analysis of electrophysiological, neuropathological, and behavioral endpoints, must be accomplished before any firm conclusions can be drawn.

Several points should be made when ascribing Aβ-induced changes in cellular properties to RAGE, or any receptor, based on studies in cell culture. First, engagement of RAGE is certainly not the only means through which Aβ mediates its cellular effects. At high concentrations of added Aβ (10–50 μmol/L), receptor-dependent effects, which become saturated in the nanomolar range, assume a secondary role to nonspecific, receptor-independent toxic properties of these peptides. This is especially true of cells that do not express RAGE. For example, this pertains to certain lines of PC12 cells do not express RAGE, but display toxic effects after incubation with Aβ^{25-35} at 10 μmol/L.[91] However, cultured rat cortical neurons and multiple other cells in culture do express RAGE, as detected at the mRNA or antigen levels,[75,76,80,85] and display RAGE-dependent effects of Aβ at lower concentrations.[76,85] Second, to ascribe an effect of Aβ to its interaction with RAGE, it is essential to block the receptor with specific antibodies or to use tail-deleted receptor to interrupt ligand-initiated cellular activation, as we have previously done in the context of its other ligands, AGEs (and particularly N$^{\epsilon}$-[carboxymethy]-lysine adducts of proteins) and members of the S100/calgranulin family of proinflammatory cytokines.[72,78] In studies using AGEs, it is important to note that AGE-modified proteins prepared *in vitro* have been sufficiently characterized to contain species that bind to RAGE (as these contain many glycoxidized species), thereby serving as RAGE agonists.

Taken together, these data lead us to propose that the properties of RAGE make it an ideal progression factor for AD. First, its expression is induced by factors in the AD environment. Second, it is present at sites of cellular perturbation, both in brain parenchyma and the vasculature. Third, the receptor interacts with at least two key elements in the AD milieu, Aβ (especially in β-sheet

fibrils) and S100/calgranulins,[92] both of which cause sustained receptor up-regulation and cellular activation. Most importantly, initial experiments in doubly transgenic mice overexpressing mutant βAPP and neuronal RAGE have shown increased expression of cell stress markers at very early times, suggesting that RAGE is likely to modulate cellular properties *in vivo*.

The use of genetically manipulated mice permits the performance of the critical experiments to test the involvement of RAGE in aspects of cellular stress relevant to AD pathology. Overexpression of full-length receptor on neurons and microglia early in the disease process would be predicted to exacerbate cellular stress. In contrast, similar experiments with a dominant-negative form of RAGE should confer a cytoprotective effect. Finally, generation of a RAGE knockout mouse by homologous recombination is now in its final stage of development. Although any firm conclusions regarding the participation of RAGE in AD-like pathology *in vivo* must await further results in transgenic models, the preliminary data are consistent with an active role for the receptor, and the means to perform definitive experiments are coming into place.

Aβ-Binding Alcohol Dehydrogenase (ABAD)

ABAD is a member of the family of short chain dehydrogenase/reductases localized in both endoplasmic reticulum and mitochondria whose salient features include potentiation of Aβ-induced cell stress and metabolism of a broad range of substrates (linear alcohols, 3-hydroxyacyl-Coenzyme A derivatives of fatty acids, steroids such as 17β-estradiol, as well as other molecules currently under study).[93–98] ABAD is unique among members of the short chain dehydrogenase/reductase family in that it binds Aβ in the nanomolar range, interacting with determinants present in the N-terminal portion of the amyloid peptide (residues 1–20).[93] In view of recent studies demonstrating βAPP processing in the endoplasmic reticulum, which leads to generation of Aβ(1–42),[10–13] as well as the presence of presenilins in this subcellular compartment, ABAD is strategically placed for engaging nascent amyloid peptide. The concept of an endoplasmic stress response in the pathogenesis of cellular perturbation in AD[99] therefore seems reasonable.

Based on *in vitro* studies, cells expressing ABAD display increased vulnerability to Aβ-induced cell stress, including generation of reactive aldehydes, such as 4-hydroxynonenal and malondialdehyde, suppression of the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, expression of cell stress markers such as HO-1, and, eventually, fragmentation of nuclear DNA.[93,94] Furthermore, the capacity of ABAD to metabolize 17β-estradiol suggests a possible role in modulating the neuroprotective effect of estrogens.[100–102] Expression of ABAD is increased in AD brain, especially in neurons near deposits of Aβ, consistent with the possible relevance of this enzyme in the propagation of cellular stress in AD brain.[93] There are important mechanistic questions to be addressed in cell culture concerning the means by which ABAD potentiates Aβ-induced cell stress: how does ABAD gain access to Aβ in intact cells? Is such an interaction required for ABAD amplification of Aβ-mediated cellular perturbation? Can ABAD-Aβ complex be demonstrated in cells? By what mechanism does this complex disturb cellular properties?

However, in the context of this discussion of the relevance of such findings in cell culture to events in AD, the most pressing issue is whether *in vivo* there is any evidence that ABAD exacerbates cellular dysfunction in an Aβ-rich environment. For this purpose, we have created transgenic mice overexpressing ABAD under control of the platelet-derived growth factor B chain promoter; these mice display high levels of ABAD in cortical neurons (Yan S-D and Stern DM, unpublished observation). Several double-transgenic mice have been bred by crossing these transgenic ABAD mice with animals overexpressing a mutant form of βAPP. Though preliminary, our first results demonstrate that by 4.5 months of age, double transgenics display high levels of HO-1 at the mRNA and protein level, compared with single transgenic and nontransgenic littermates. In addition, the double transgenics show increased 4-hydroxynonenal-lysine epitopes, consistent with generation of toxic reactive aldehydes.[103] These results suggest that ABAD may indeed prove to be a relevant cofactor in the Aβ-rich brains of the transgenic mice. However, any firm conclusion requires completion of these studies with full evaluation of the same endpoints used in the studies reported in this issue of the *Journal*.[56]

The Next Step(s)

The identification of cellular cofactors exacerbating cell stress in the AD milieu is likely to provide mechanistic insights and may produce benefits for future therapeutic approaches as well. As such progression factors are identified *in vitro*, it is essential that they be subjected to rigorous study *in vivo* using the most relevant models, at this time genetically manipulated mice bearing transgenes causing an Aβ-rich environment in brain parenchyma. In this context, it is not at all surprising that previous work *in vitro* has not elucidated the importance of such cellular cofactors in Aβ-induced cellular toxicity. This is true for several reasons: i) experiments with preparations of Aβ from different sources appear to have variable effects in cellular systems; ii) the pathophysiologically relevant macromolecular structure(s) into which Aβ assembles for inducing cellular dysfunction, whether it be dimer, Aβ-derived diffusible ligands, other oligomers, or β-sheet fibrils,[104–108] is not clear; iii) the endpoints in most cell-based studies focus on cell death within hours of exposure to Aβ, a circumstance not readily applicable to the much more protracted time course *in vivo*; and iv) micromolar concentrations (10–50 μmol/L) of Aβ(1–40/1–42) perturb properties of virtually any cell, though relevant levels of Aβ *in vivo* are more likely to be in the nanomolar range. In this regard, experiments showing equivalent cellular effects of D- or L-Aβ(1–40; 10 μmol/L) for induction of cell death suggest a

striking lack of specificity in Aβ-cellular interactions.[108] This view is emphasized by studies with the truncated form of Aβ spanning residues 25–35, which is not found *in vivo*,[109,110] but is a strong cytotoxic agent in the micromolar range *in vitro*.

Thus, the paper by Mucke and colleagues[56] is an important step in the direction of moving studies quickly from cell culture to the *in vivo* setting, because it provides a first test of the concept that the type A MSR has an important role in Aβ biology using a well-characterized transgenic model. Though the results are still at an early stage, the current data do not support a critical role for MSR. Experiments that produce a clear result, such as those described in this issue of the *Journal*,[56] propel the field forward. However, it is important to note that although their observations suggest that factors other than MSR are likely to be central to the pathobiology of AD, this should not be construed as negating the concept that cell-associated progression factors contribute to the cellular response, both cytoprotective and cytopathic, in amyloidoses. Rather, the transgenic models are ideal for identifying and prioritizing factors that modulate the effects of Aβ in complex cellular systems. Studies underway and completed involving cross-breeding of transgenic mice overexpressing mutant βAPP with those overexpressing factors such as transforming growth factor-β1, apoE, fibroblast growth factor, and superoxide dismutase are an important step in this regard.[60,111–113] Similarly, a potential role for other molecular scavengers in the Aβ-enriched environment *in vivo* should be rigorously tested.

In the inventory of progression factors that exacerbate cell stress in AD, it is critical to identify those acting at an early step in the cascade of events in Aβ-associated cellular dysfunction. A sole focus on mediators acting distally in successive cascades of cellular perturbation, such as ROIs, is not sufficient for an understanding of pathogenesis. For example, reactive oxygen species are integral to physiological intracellular signaling pathways,[114] and antioxidant therapies in a range of settings, such as atherosclerosis,[115–117] have been inconclusive or disappointing. A receptor such as RAGE, capable of interacting with two key factors for modulating cellular properties in AD brain, Aβ and S100/calgranulins, and, as a consequence, mediating sustained cellular dysfunction, seems to be worth intensive study. Similarly, ABAD's capacity to engage Aβ(1–42) at its very site of production in the endoplasmic reticulum is intriguing. Each of these issues must be subjected to the same careful analysis that the Mucke group has used to evaluate MSR.

The transgenic mouse models of AD-type pathology should not only be used to test mechanisms modulating Aβ production and deposition, but should also be exploited to identify and evaluate key cofactors modulating the cellular response in the complex *in vivo* environment. Proponents of the amyloid hypothesis should beware. The very transgenic mice first developed to prove that Aβ is where the action begins and ends in AD are more likely to produce many surprises as investigators analyze their phenotype, thereby advancing our understanding of the pathobiology of AD. The recent demonstration that immunization of transgenic mice overexpressing a variant of human βAPP with Aβ(1–42)[118] suppressed plaque formation in the brain provides a vivid reminder of the open-minded approach necessary to fully appreciate the significance of unexpected results. In this regard, it will be essential to determine whether the latter immunization method for diminishing Aβ accumulation in transgenic mice will have a cytoprotective effect in terms of functional and neuropathological endpoints.

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