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ACE Over Expression in Myelomonocytic Cells: Effect on a Mouse Model of Alzheimer's Disease

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Abstract

While it is well known that angiotensin converting enzyme (ACE) plays an important role in blood pressure control, ACE also has effects on renal function, hematopoiesis, reproduction, and aspects of the immune response. ACE 10/10 mice over express ACE in myelomonocytic cells.

Macrophages from these mice have an increased polarization towards a pro-inflammatory phenotype that results in a very effective immune response to challenge by tumors or bacterial infection. In a mouse model of Alzheimer's disease (AD), the ACE 10/10 phenotype provides significant protection against AD pathology, including reduced inflammation, reduced burden of the neurotoxic amyloid- β protein and preserved cognitive function. Taken together, these studies show that increased myelomonocytic ACE expression in mice alters the immune response to better defend against many different types of pathologic insult, including the cognitive decline observed in an animal model of AD.

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Conflict of Interest

Yosef Koronyo, Ellen Bernstein, Jorge F. Giani, Tea Janjulia, Keith L. Black, and Sebastien Fuchs declare that they have no conflict of interest.

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Keywords

ACE 10/10; Alzheimer's disease; macrophages; monocytes; neuroinflammation; blood pressure

Introduction

Dr. Leonard T. Skeggs, Jr., the man who discovered angiotensin converting enzyme (ACE), expressed the belief that “...most important scientific discoveries are in large part accidental” [1]. Undoubtedly this opinion was influenced by how in 1956 ACE and its role in producing angiotensin II were uncovered [2]. Though science today is more sophisticated, the importance of accidental findings cannot be underestimated. In fact, one aspect of scientific acuity is the ability to recognize and embrace ideas outside accepted wisdom.

Skeggs and scientists of his era discovered that ACE is a zinc dependent peptidase produced by endothelium, renal proximal tubules and many other tissues [3]. It is a relatively non-specific enzyme and can cleave angiotensin I, bradykinin and several other small peptide hormones. Further, the physiologic regulation of ACE expression appears to play little role in blood pressure regulation. Thus, ACE may seem functionally in the shadow of renin, an enzyme with one substrate produced in a highly regulated fashion only by juxtaglomerular cells. Even the development of the ACE inhibitor captopril in 1981, a seminal event, has never really focused attention away from renin as the master physiologic regulator of angiotensin II production, at least as far as blood pressure is concerned. And yet, the ability of ACE to cleave many peptide substrates has endowed this enzyme with diverse physiologic roles, some of which were only recently discovered (some accidentally). This review will discuss several roles of ACE as recognized by mouse models in which normal ACE expression is either eliminated or augmented from natural expression levels.

ACE knockout mice

Techniques to target genes using embryonic stem cells led to the creation of mice lacking all ACE expression (ACE KO mice) [4,5]. Such mice have systolic blood pressures that average less than 75 mmHg, a reduction of 35 mmHg from levels observed in wild-type (WT) mice. ACE KO mice cannot effectively concentrate urine due to both low renal perfusion pressures and a functional defect in the transport of urine along the ureter. This defect leads to expansion of the renal pelvic space and compression of the renal medulla [6]. However, the inability to concentrate urine is due to more than just this anatomic abnormality, since another strain of mouse that produces a limited amount of soluble ACE has enough enzyme to avoid hydronephrosis and pelvic expansion, but still has low blood pressure, low renal perfusion and a defect in urine concentration [7, 8].

Male mice (and all male vertebrates) normally produce two isoforms of ACE [3]. Somatic ACE is produced by endothelium and other somatic tissues and is responsible for most of the functional actions of ACE. The second isoform, produced by developing male germ cells, is often called testis ACE. The lack of this isoform in male ACE KO mice markedly reduced the reproductive capacity of these animals by mechanisms that are still not well understood.

In addition to renal and reproductive defects, ACE KO mice have defects in hematopoiesis. ACE KO mice are mildly anemic due to the lack of angiotensin II production [9]. They also have defects in myelopoiesis due to both the lack of angiotensin II production and, in the absence of ACE, a defect in substance P elimination [10].

Analysis of ACE KO mice also revealed a very unexpected role for this enzyme in shaping the peptide repertoire displayed by cells as part of the MHC class I complex [11]. Apart from red blood cells, all cells express MHC class I molecules on their surface, which serves to identify tissues as 'self' to the immune system. It is the incompatibility of MHC class I molecules that is the primary cause of transplant rejection. Within the endoplasmic reticulum, MHC class I proteins are loaded with peptides that come from the degradation of cellular proteins. Under normal circumstances, the display of these peptides on the surface of cells by MHC class I molecules does not activate the immune response. However, in the presence of viral infection (or following transplantation) MHC class I display of foreign peptides triggers CD8⁺ T cell activation and ultimately destruction of the 'non-self' tissues.

Within the endoplasmic reticulum, a critical part of preparing peptides for MHC class I binding, is the trimming of these peptides to an appropriate size. Early studies identified a role of ACE in this process using systems in which ACE was purposefully overexpressed [12]. However, the normal physiologic role of ACE in shaping the MHC class I peptide repertoire was investigated using cross immunization studies [11]. For these experiments, macrophages or splenocytes from a donor mouse were transferred to a recipient. The recipient was then monitored for MHC class I peptide incompatibility, as indicated by CD8⁺ T cell activation. When WT recipients were transplanted with WT cells, or when ACE KO recipients were immunized with cells from an ACE KO donor, T cell activation was not detected. However, the immunization of WT mice with ACE KO cells or the immunization of ACE KO mice with ACE WT cells resulted in T cell activation, including IFN γ production and CD8⁺ T cell proliferation. This and many other experimental approaches strongly indicated that under natural physiologic conditions, ACE plays an important role in the C-terminal trimming of MHC class I peptides. While the role of ACE in shaping the peptide repertoire is easily detected in mice, equivalent studies have not been performed in humans. It must be noted that there is no evidence of autoimmunity in humans taking ACE inhibitors, undoubtedly due to the many redundancies in the immune response.

Mice over expressing ACE in myelomonocytic cells

In addition to effects on MHC peptide processing, ACE has been recognized as having several effects on the immune response [3]. One of the most dramatic examples of ACE and its ability to affect immune function is a mouse model, called ACE 10/10, in which the natural ACE promoter was functionally replaced by the promoter for the c-fms gene [13]. As a result of this manipulation, ACE 10/10 mice markedly over express ACE in monocytes, macrophages and other myelomonocytic derived cells such as Kupfer cells and brain microglia. On the other hand, the c-fms promoter is not recognized by such natural sources of ACE as vascular endothelium or renal proximal tubular epithelium. Yet despite the lack of ACE expression by endothelium, ACE 10/10 mice have a normal level of plasma ACE activity, normal blood pressure and normal renal structure and basal renal function.

ACE 10/10 mice were originally conceived as a means of studying the role of the reninangiotensin system in atherosclerosis. However, in one of the serendipitous events that seems characteristic of ACE research, challenge of these mice with the mouse B10 melanoma cell line revealed that the ACE 10/10 model has an immune response quite different from normal mice. Typically, the intradermal injection of tumor cells leads in two weeks to the growth of a local melanotic nodule. However, in ACE 10/10 mice, the tumors were very much smaller than in WT [13] (Figure 1). The B10 model is an aggressive tumor that normally evades immune surveillance. In contrast, the small tumors that did arise in ACE 10/10 mice were accompanied by large numbers of monocytic cells attached to tumor blood vessel endothelium. Also, there were significant numbers of macrophages within the tumor that appeared to be phagocytizing tumor cells.

The ACE 10/10 model is characterized by over expression of ACE by myelomonocytic cells but near total lack of ACE production by vascular endothelium. Conceivably, either of these changes could contribute to the very slow growth of the B16 tumor. To test this, mice were treated with an ACE inhibitor or an angiotensin II AT1 receptor antagonist during exposure to tumor. These experiments showed that it was the presence of ACE catalytic activity in the ACE 10/10 model that was important [13]. Further, the ACE inhibitor reversed the effect and resulted in larger tumors, but the AT1 receptor antagonist (blocking the effects of angiotensin II on the AT1 receptor) had no effect on tumor size. The conclusion that the immune modulation was independent of angiotensin I and angiotensin II was reinforced by study of ACE 10/10 mice that were also angiotensinogen knockouts; such animals retain an enhance immune response despite a total lack of angiotensin II formation.

Careful characterization of the immune response in ACE 10/10 mice suggested that ACE over expression results in a phenotypic change in monocytes and macrophages, producing cells that are highly effective in their inflammatory response. Such pro-inflammatory macrophages are often referred to as 'classically activated' or 'M1' type cells [14, 15]. Upon stimulation, ACE 10/10 macrophages excrete large amounts of the pro-inflammatory molecules TNF α , IL-12 and iNOS. They also excrete reduced amounts of the anti-inflammatory molecule IL-10 [3]. Typically, tumor bearing WT mice have a mix of M1 proinflammatory monocytic cells and myeloid derived suppressor cells (MDSC), a cell population that inhibits the anti-tumor immune response [16]. In response to tumor, ACE 10/10 mice demonstrate a change in the pro- and anti-inflammatory balance of monocytic cells in favor of a more pro-inflammatory cell population [17].

In addition to the B16 melanoma model, ACE 10/10 mice were also challenged with an intradermal injection of leukemic cells, infection with *Listeria monocytogenes* and intradermal infection with methicillin resistant *Staphylococcus aureus* (MRSA) (Figure 2) [13,18]. In all instances, the ACE 10/10 mice responded with a much more effective immune response than WT mice. A simple holistic hypothesis concerning this model is that the over expression of ACE in myelomonocytic cells renders these cells generally more efficient at eliminating the pathogens and cell debris that are the natural role of monocytic cells. This has profound implications since several serious human illnesses are due to the unchallenged accumulation of unwanted and pathologic detritus. For example, atherosclerosis is hypothesized to arise in part due to the lack of effective clearance of lipids

by monocytic derived cells [19]. Studies are now under way to investigate whether ACE 10/10 cells outperform equivalent WT cells in mouse models of atherosclerosis.

ACE, immunity, and Alzheimer's disease

Another illness that is thought due to misfolding and aggregation of proteins and other debris is Alzheimer's disease (AD). Currently more than 5.2 million Americans are affected with this disease, and it is estimated that this number will exceed 13 million in 2050 unless effective treatments are developed [20, 21]. While the precise origins of AD are not fully understood, there is a consensus that the peptide breakdown products of amyloid precursor protein are pathogenic in this disease [22]. These are referred to as amyloid- β protein ($A\beta$) peptides which are commonly 38-43 amino acids in length and which form the extracellular amyloid plaques characteristic of this disease [23, 24]. Substantial evidence indicates that the peptide $A\beta_{1-42}$ is particularly neurotoxic in both soluble oligomeric and insoluble forms [22, 25]. Age-dependent accumulation of amyloidogenic $A\beta$ in the brain appears as a net result of an imbalance between its production and clearance [26]. Recent clinical reports indicate that while the familial AD patients have increased $A\beta_{1-42}$ production [27], in the most common sporadic cases there is inadequate $A\beta$ clearance [28]. Cerebral $A\beta$ clearance is mediated by multiple mechanisms, including phagocytosis and enzymatic degradation by inflammatory cells [29, 30]. If AD results in part from the accumulation of $A\beta$ peptides due to inadequate removal, then ACE 10/10 monocytic cells could be envisioned as having several features that may ameliorate disease. First, as discussed, ACE 10/10 monocytes are highly effective at eliminating debris. Second, the over expression of ACE on the surface of monocytic cells, the feature which endows them with an enhanced immune response, is also a peptidase capable of the cleavage of $A\beta_{1-42}$ into $A\beta_{1-40}$ and other less amyloidogenic peptides [31-34]. To investigate this hypothesis, ACE 10/10 mice were crossed with a mouse strain genetically prone to Alzheimer's-like pathology (cortical and hippocampal amyloid plaques, astrogliosis, and progressive cognitive defects with increasing age) [35]. Systematic evaluation of the pathologic features of AD, including cognitive assessment, showed a huge difference between AD mice with the ACE 10/10 phenotype and AD mice with a WT ACE phenotype (Figure 3). For example, analysis of mice 7 to 8 months old indicated that cortical $A\beta$ plaque area was reduced approximately 80% in AD ACE 10/10 mice as compared to AD ACE WT mice.

In both human AD patients and animal models, neurologic damage attracts inflammatory cells to clear debris and restore homeostasis. However, in AD, inflammatory cells (activated microglia and reactive astrocytes) are chronically activated and instead of supporting tissue healing, they become cytotoxic to neurons; they secrete high levels of pro-inflammatory cytokines, contributing to disease progression [36, 37]. Several studies suggest that improved disease outcome in animal models of AD was associated with lower brain levels of proinflammatory cytokines or higher expression of anti-inflammatory cytokines [38, 39]. The AD ACE 10/10 mice had significantly less cerebral inflammation than AD ACE WT mice. Thus, it appears that in AD ACE 10/10 mice, less neurologic injury due to lower levels of soluble and insoluble $A\beta_{1-42}$ may result in less secondary ancillary inflammatory damage. Interestingly, in the ACE 10/10 mice, monocyte-derived macrophages were selectively more abundant surrounding amyloid plaques and there was histologic evidence

of plaque phagocytosis [35]. These data suggest that, while overall inflammation is reduced, the inflammation in AD ACE 10/10 mice may be quite effective in clearing A β and forestalling progressive neurologic injury.

One of the most interesting findings in the AD ACE 10/10 study was the evaluation of perivascular amyloid deposition in AD mice having either an ACE WT/WT, ACE 10/WT or ACE 10/10 genotype (Figure 4). Among these mice, the heterozygous AD ACE 10/WT group had an intermediate phenotype, with less pathology than the AD ACE WT/WT mice but more pathology (plaques area for example) than the AD ACE 10/10 mice [35].

However, in terms of perivascular A β deposition (assessed by quantitative immunohistochemistry), the AD ACE 10/WT mice had significantly less than either the AD ACE WT/WT or the AD ACE 10/10 mice. In humans, cerebral amyloid angiopathy is a neurological condition characterized by amyloid deposition in blood vessel walls of the central nervous system [40]. Cerebral amyloid angiopathy increases the risk of hemorrhagic stroke and dementia, and it is extremely common among AD patients [41-43]. In the murine transgenic models of AD, perivascular A β depositions are abundantly found in cerebral arterial walls [44]. The AD ACE 10/10 mice are homogenous for the mutation resulting in high myelomonocytic expression of ACE, but these animals make essentially no endothelial ACE [13]. This compares to heterozygous AD ACE 10/WT mice in which one ACE allele targets high ACE expression to myelomonocytic cells while the other allele (WT) targets ACE to its natural locations, including endothelium. Thus, the data comparing these two groups implies that the natural expression of endothelial ACE may play an important role in protecting cerebral blood vessels from vascular amyloid deposition and progressive vascular pathology. If this is true, then one may speculate if ACE inhibitors are appropriate for hypertensive patients with early dementia or a family history of dementia, particularly when AT1 receptor antagonists have an excellent outcome in treating hypertension and heart disease. The question of ACE, ACE inhibitors and AD is very complex because human epidemiological studies have suggested that hypertension itself may affect the onset of AD [45]. As reviewed by Kehoe and Passmore, there are now several human studies strongly suggesting that AT1 receptor antagonists may reduce the incidence of AD [46]. Perhaps not surprising, data with ACE inhibitors is less convincing, consistent with the idea that while ACE inhibition lowers blood pressure (reducing the incidence of AD), ACE inhibitors may inhibit endothelial ACE and may reduce the natural defenses against perivascular amyloid deposition.

In the evaluation of ACE 10/10 mice and AD, the most striking finding was the cognitive assessment of the mice [35]. This was performed on two separate cohorts of animals, aged 11 and 12 months old, using a Barnes maze which tested the ability of the mice to learn and remember the location of an escape box using available spatial cues. Each of the two cohorts was evaluated by a separate group of evaluators blinded to the genotype of the animals. The test was performed over a period of 9 days. Mice were initially trained in the maze during which the time required to find the escape box progressively diminished. The mice were then rested for two days and reassessed for their ability to find the escape box, a measure of hippocampal-based spatial memory. Finally, the mice were required to re-learn the maze with the escape box in a new position. In all these tasks, AD ACE 10/10 mice showed

cognitive learning abilities that were essentially equivalent to those of similarly aged *non-AD* mice. The AD ACE 10/WT mice, while assessed only at 11 months, also showed cognitive abilities equivalent to non-AD control mice. This compares to AD ACE WT/WT mice which were markedly less efficient in learning, remembering, and re-learning the solution to the maze. Thus, while the ACE WT/WT mice demonstrated the expected cognitive decline observed in this model of AD, AD ACE 10/10 and AD ACE 10/WT mice - animals with an equivalent genetic predisposition for AD - essentially performed equivalently to control non-AD mice.

Despite considerable progress in understanding the etiology of AD, an effective treatment remains elusive. AD studies using ACE 10/10 mice provide a rationale for immunotherapy, particularly since ACE over expression in monocytic cells results in the complementary advantages of increased immune function and local delivery of ACE, an A β degrading enzyme. Hypertension is a common disease of the elderly and a risk factor for AD. Often, ACE inhibitors or angiotensin receptor blockers (ARBs) are used to control it. While these drugs are similar in their effects on blood pressure and heart disease, ARBs do not inhibit ACE activity, including ACE mediated cleavage of A β .

Summary

The renin-angiotensin system plays a major role in the control of blood pressure due to the production of angiotensin II by ACE. *In vivo* studies of mice have indicated that it is the C-terminal catalytic domain of ACE that is predominantly responsible for this production of angiotensin II *in vivo* [3]. Why then has ACE maintained a structure composed of two distinct catalytic domains since the original genetic duplication of this gene hundreds of millions of years ago? While such questions are impossible to answer with certainty, recent data on the diverse functions of ACE indicate that this enzyme plays an important physiologic role in many different physiologic processes, almost certainly through a somewhat different substrate specificity for each of the two ACE domains. The role of ACE in the immune response is still under investigation but the heightened immune response of the ACE 10/10 mice suggests that not only is there more to learn about ACE, but its manipulation may provide novel future approaches to treat a variety of diseases apart from hypertension and cardiovascular injury. This may even include ACE manipulation in the treatment of AD.

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Figure 1.

Reduced tumor size in ACE 10/10 mice. Both ACE WT and ACE 10/10 mice received an equivalent intradermal injection of B16-F10 myeloma cells and were sacrificed 14 days later. In the top panel (left) a typical tumor in ACE WT mice. In contrast, ACE 10/10 mice (right) have much smaller tumors. The bottom panel shows individual data points as well as the group means and the standard error of the mean. Adapted from reference 13.

Figure 2.

Increased resistance to MRSA in ACE 10/10 mice. ACE WT and ACE 10 mice received equivalent subcutaneous injections of methicillin resistant *S. aureus* (MRSA, clone USA 300). After 4 days, mice were sacrificed and bacterial counts in the skin lesions were determined. This is presented in the figure as the log of colony forming units (cfu) per mg tissue for individual data points, group means, and standard error of the mean. ACE 10/10 mice were substantially more resistant to MRSA growth than WT mice. Treatment of the mice with the ACE inhibitor lisinopril eliminated the difference between the groups. Adapted from reference 18.

Figure 3. Reduced Alzheimer's disease-like pathology in ACE 10/10 mice. (Top) All mice were on a background genetically prone to develop Alzheimer's-like disease; the mice were either ACE WT (left panels) or ACE 10/10 (right panels) for ACE expression. All mice were sacrificed at 255 days. Brain sections were immunohistochemical stained for human amyloid-β protein (Aβ) using the monoclonal antibody 6F/3D. (Bottom) Quantitative analysis of 6E10-labeled Aβ substantiates that there are far fewer amyloid plaques in the

brains of ACE 10/10 mice than ACE WT mice. Reprinted with permission from reference 35.

Figure 4.

Reduced perivascular Aβ deposits in AD ACE 10/WT mice. Quantitative immunofluorescence was used to measure perivascular Aβ deposition in sections from the hippocampus of 8 month old mice. This showed that Thioflavine-S (Thio-S)-labeled Aβ deposition was significantly less in AD ACE 10/WT mice as compared with either AD ACE WT/WT or AD ACE 10/10 mice. The figure shows data for individual mice, group means and SEM, as well as the percent reduction in the means. ***P<0.001. Reprinted with permission from reference 35.