

# Advanced Glycation End Products in Kidney Transplant Patients: A Putative Role in the Development of Chronic Renal Transplant Dysfunction

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• Chronic renal transplant dysfunction is one of the leading causes of graft failure in kidney transplantation. A complex interplay of both alloantigen-related and alloantigen-unrelated risk factors is believed to underlie its development. We propose that advanced glycation end products (AGEs) are involved in the development of chronic renal transplant dysfunction. AGE formation is associated with different alloantigen-unrelated risk factors for chronic renal transplant dysfunction, such as recipient age, diabetes, proteinuria, hypertension, and hyperlipidemia. In vitro studies have shown that AGEs induce the expression of various mediators associated with chronic renal transplant dysfunction. Furthermore, AGE-induced renal damage has been found in multiple experimental studies. This renal damage shows similarity to the damage found in chronic renal transplant dysfunction. Together, several lines of evidence support a role of AGEs in the development of chronic renal transplant dysfunction and suggest that preventive therapy with AGE inhibitors may be helpful in preserving renal function in transplant recipients. *Am J Kidney Dis* 43:966-975.

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**INDEX WORDS:** Kidney transplantation; advanced glycation end products (AGEs); chronic renal transplant dysfunction; chronic allograft nephropathy; oxidative stress; carbonyl stress.

**T**HE DEVELOPMENT of new immunosuppressive drugs has improved short-term graft survival in kidney transplant recipients substantially.<sup>1,2</sup> Although overall long-term graft survival is improving slowly, it does not parallel improvements in short-term survival.<sup>2</sup> Approximately 60% of patients receiving cadaveric donor kidneys will develop graft failure within 10 years after transplantation.<sup>1</sup>

Chronic renal transplant dysfunction, also known as chronic allograft nephropathy, is one of the leading causes of late graft failure. Chronic renal transplant dysfunction is characterized clinically by a slow, but steady, decline in function of the transplanted kidney, associated with the development of hypertension and proteinuria.<sup>3</sup> Histopathologic characteristics of chronic renal transplant dysfunction include arteriosclerosis of the

intrarenal vasculature, glomerulosclerosis, and interstitial fibrosis with tubular atrophy.<sup>4</sup> A complex interplay of both alloantigen-dependent and alloantigen-independent risk factors is believed to underlie the development of chronic renal transplant dysfunction.<sup>3</sup> Alloantigen-dependent factors include episodes of acute rejection, inadequate immunosuppression, and increased HLA mismatching.<sup>3</sup> Alloantigen-independent factors include recipient and donor age,<sup>5</sup> impaired renal function,<sup>6</sup> hypertension,<sup>7</sup> the presence of diabetes,<sup>8</sup> proteinuria,<sup>9</sup> hyperlipidemia,<sup>10</sup> obesity,<sup>11</sup> transplant ischemia,<sup>12</sup> and use of calcineurin inhibitors.<sup>13</sup> The extent of their contributions is largely unknown.

Interestingly, to a certain extent, alloantigen-independent risk factors for the development of chronic renal transplant dysfunction overlap risk factors for the accumulation of advanced glycation end products (AGEs). This overlap is well established for age,<sup>14</sup> renal function impairment,<sup>15,16</sup> and diabetes.<sup>17</sup> Although less conclusive, evidence exists that associates hypertension,<sup>18,19</sup> proteinuria,<sup>20</sup> and hyperlipidemia<sup>21</sup> with enhanced AGE accumulation. This led us to believe that AGEs might be involved in the pathogenesis state of chronic renal transplant dysfunction. In this report, we summarize the evidence for a role of AGEs in the development of chronic renal transplant dysfunction. First, we

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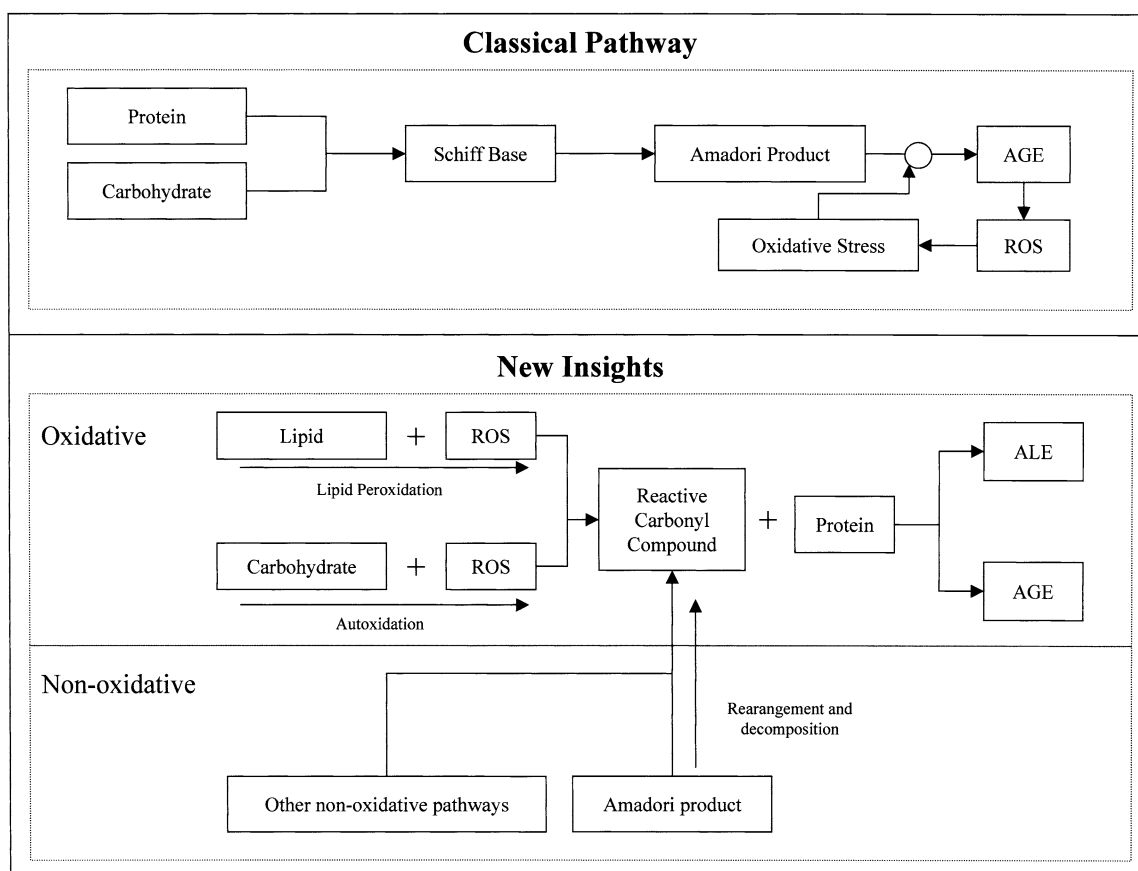
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**Fig 1. Classical pathway of AGE formation and new insights. Abbreviations: ROS, reactive oxygen species; ALE, advanced lipoxidation end product.**

discuss recent insights in AGE kinetics. Second, we discuss data on plasma and tissue AGE levels in patients with a kidney transplant. Third, we propose mechanisms through which AGEs may be involved in the development of chronic renal transplant dysfunction. Finally, we discuss studies on AGE-induced renal tissue damage.

#### AGE KINETICS

Historically, AGEs have been considered end products from nonenzymatic reactions between sugars and proteins, called the Maillard reaction.<sup>22</sup> The final steps in the Maillard reaction are driven by oxidative stress, defined as the steady-state level of reactive oxygen species.<sup>23</sup> Because AGEs are able to accelerate oxidation strongly, they favor their own production.<sup>23,24</sup> Figure 1 shows classical and newly discovered pathways of AGE formation. Currently, it is known that

some AGEs are derived from lipid peroxidation; therefore, advanced lipoxidation end products would be a better name for this subgroup of AGEs. However, we use the term AGEs when referring to both AGEs and advanced lipoxidation end products. Furthermore, it was discovered that in addition to oxidative stress, carbonyl stress, ie, the steady-state level of reactive carbonyl compounds, is thought to be centrally involved in AGE formation.<sup>25,26</sup> Reactive carbonyl compounds are derived from the reaction of lipids or carbohydrates with reactive oxygen species. These compounds subsequently react with proteins to form AGEs and advanced lipoxidation end products. Examples of reactive carbonyl compounds include methylglyoxal and glyoxal.<sup>25,26</sup>

The formation and accumulation of AGEs in tissue, the amount of AGEs circulating in the

bloodstream, and the excretion of AGEs by the kidney seem to be in dynamic equilibrium. AGEs form cross-links with long-living tissue proteins, which enable them to accumulate in the body.<sup>27</sup> AGE accumulation in tissue is associated with aging,<sup>14</sup> renal function impairment,<sup>28</sup> and the presence of diabetes.<sup>17</sup> External sources of AGEs include AGE precursors in cigarette smoke and alimentary intake of AGEs.<sup>29,30</sup> Detoxification of AGEs depends on both the degradation of AGEs to AGE peptides by macrophages<sup>31</sup> and renal clearance of AGEs. There is evidence for filtration of AGE compounds through glomeruli and active reabsorption in proximal tubuli. After modification or degradation in proximal tubuli, AGEs eventually are cleared in urine.<sup>32,33</sup>

Although several methods to determine AGE accumulation have been described, no commercial assay or tool is available yet. Classically, AGEs are determined by using their characteristic fluorescence properties.<sup>34</sup> Currently, gas chromatography mass spectrometry is considered the most accurate technique to determine AGE levels.<sup>35</sup> High-performance liquid chromatography also is accurate, but is relatively time consuming.<sup>36</sup> Several difficulties exist with standardization if an enzyme-linked immunosorbent assay is used.<sup>37</sup> Furthermore, fluorescent techniques have been adapted to enable their use in clinical studies.<sup>38</sup> In addition to biochemical assays and fluorescent techniques, several immunohistochemical techniques have been described to determine AGE levels.<sup>39</sup> One should consider differences in accuracy of the techniques used when interpreting data on AGE levels.

#### AGE LEVELS IN KIDNEY TRANSPLANT RECIPIENTS

Before exploring AGE accumulation in kidney transplant recipients, it is important to realize that most transplant recipients have experienced a long period of impaired renal function before transplantation. AGEs accumulate during the period of gradual renal function loss and during dialysis treatment.<sup>28</sup> Thus, kidney transplant recipients most often have high AGE levels before transplantation. AGE levels in transplant donors are unknown. Presumably, a wide variability in donor kidney AGE levels exists because of the heterogeneity of donors. However, it is reasonable to assume that donors will have lower

tissue AGE levels than transplant recipients. Thus, a kidney with presumably low AGE levels is transplanted into an AGE-rich environment. Kidney transplantation aims to restore renal function and thereby is thought to lower AGE levels. Questions are to what extent AGE accumulation will resolve after kidney transplantation and how the transplanted kidney behaves in an AGE-rich environment. Several research groups have investigated the influence of kidney transplantation on AGE levels in tissue and blood. Unfortunately, only data on extrarenal AGE levels have been published. No data are available on AGE levels in kidneys of transplant recipients, either with or without chronic renal transplant dysfunction. Thus, we do not know how the transplanted kidney handles the AGE-rich environment it is placed in. Although it is interesting to hypothesize that the transplanted kidney is more prone to AGE formation because of local proinflammatory stimuli, the current lack of data on renal AGE levels limits us to expand on this thought. The different studies on AGE levels in pretransplantation and posttransplantation patients are listed in Table 1.

Blood AGE levels are increased strongly in patients on dialysis therapy compared with controls. Although transplantation reduces blood AGE levels, these generally remain greater than normal. Interestingly, studies evaluating blood AGE levels within the first 6 months after transplantation showed that blood AGE levels decreased by 70% to 80%. This suggests that a decrease in blood AGE levels occurs early after improvement of renal function.<sup>40,41</sup> Some investigators reported disproportionally high blood AGE levels after transplantation when related to renal function.<sup>42,43</sup> Thus, other factors not already present in patients with chronic renal insufficiency and unrelated to renal function may influence AGE formation after transplantation as well. One explanation could be that enhanced AGE accumulation in relation to renal function reflects an enhanced nutritional status.<sup>44</sup> Another explanation could be the use of calcineurin inhibitors, especially cyclosporine, in transplant recipients. Use of cyclosporine has been associated with enhanced oxidative stress and thus might be of influence on AGE levels found in kidney transplant recipients.<sup>45</sup>

Results of studies on the influence of kidney

**Table 1. Effect of Kidney Transplantation on AGE Levels in Tissue and Blood**

Reference	Study Design		Pretransplantation*			Posttransplantation*			Post-v Pretransplantation		Remarks
	Endpoint	Method	Type (duration)	No. of Patients	Fold v Controls	No. of Patients	Time	Fold v Controls	Reduction (%)	P	
Data on blood AGEs											
Makita et al, <sup>40</sup> 1991	Se-AGE	RRA	HD (12 ± 17 mo)	6	5.31	16	2-9 y	1.56	71	<0.001	Diabetic
	Se-AGE	RRA	D	2	4.62	2	14 d	1.36	71	—	Prospective
Hricik et al, <sup>15</sup> 1993	PI-Pent	HPLC	D	41	27.5	39	24 mo	3.3	88	<0.05†	Prospective
Hricik et al, <sup>49</sup> 1996	PI-Pent	HPLC	HD + PD (16.3-38.1 mo)	88	21.2	15	6-80 mo	2.2	89	0.002	Prospective
Miyata et al, <sup>41</sup> 1997	PI-Pent-Alb	HPLC	HD (12.4 ± 7.9 y)	29	10.7	7	6 mo	1.5	86	<0.05†	Prospective
	PI-Pent-Alb	HPLC	HD (12.4 ± 7.9 y)	29	10.7	12	6.2 y	1.0	91	<0.05†	
Sebekova et al, <sup>42</sup> 2001	PI-Fluor	Sp	HD + PD (8-68 mo)	10	4.8	9	34 mo	2.4	50	<0.01	Pediatric
Misselwitz et al, <sup>16</sup> 2002	PI-CML	ELISA	HD + PD (8-68 mo)	10	3.3	9	34 mo	3.0	15	NS	Pediatric
	Se-Pent	HPLC	PD + HD	9	16.4	12	0.5-6 y	2.4	85	<0.01	Pediatric
	Se-CML	ELISA	PD + HD	9	2.2	12	0.5-6 y	1.0	53	<0.01	Pediatric
Data on tissue AGEs											
Lee et al, <sup>46</sup> 1995	Skin-Ti-CLF	Sp	CRF	18	2.45	16	11 wk	1.26	49	0.003	Nondiabetic
	Perit-Ti-CLF	Sp	CRF	13	1.89	15	11 wk	1.20	37	NS	Nondiabetic
Hricik et al, <sup>49</sup> 1996	Skin-Ti-Pent	HPLC	HD + PD (16.3-38.1 mo)	88	59.7 ± 21.7 pmol/mg‡	15	6-80 mo	57.9 ± 17.3 pmol/mg‡	3	NS	Prospective
Yoshida et al, <sup>47</sup> 1998	Card-Ti-CML	IH	HD + PD (5.8 ± 1.6 y)	10	3.6	8	5.8 y	2.2	39	<0.05	
Shaw et al, <sup>48</sup> 1998	Card-Ti-AGE	IH	HD + PD (5.8 ± 1.6 y)	10	0.96	8	5.8 y	0.55	43	NS	
	Skin-Ti-CLF	Sp	CRF	26	11.7 ± 4.51 AU/mg‡	18	3-43 mo	5.0 ± 3.13 AU/mg‡	57	<0.00001	Nondiabetic
	Skin-Ti-Pent	HPLC	CRF	13	245.4 ± 77 pmol/mg‡	9	3-43 mo	65.9 ± 40 pmol/mg‡	73	<0.001	Nondiabetic

Abbreviations: Perit, peritoneal; Card, cardiac; Se, serum; PI, plasma; Ti, tissue; Pent, pentosidine; CML, carboxymethyllysine; Alb, albumine; Fluor, fluorescent AGEs; CLF, collagen linked fluorescent; RRA, radio receptor assay; HPLC, high-performance liquid chromatography; Sp, spectrometry, ELISA, enzyme-linked immunosorbent assay; IH, immunohistochemistry; HD, hemodialysis; PD, peritoneal dialysis; CRF, chronic renal failure; D, dialysis (non-specified); NS, not significant.

\*AGE levels in patients were divided by AGE levels in healthy controls to calculate fold difference from control.

†Although differences were significant, significance levels were not given.

‡No controls available, concentrations are given in picomoles per milligram or arbitrary units per milligram.

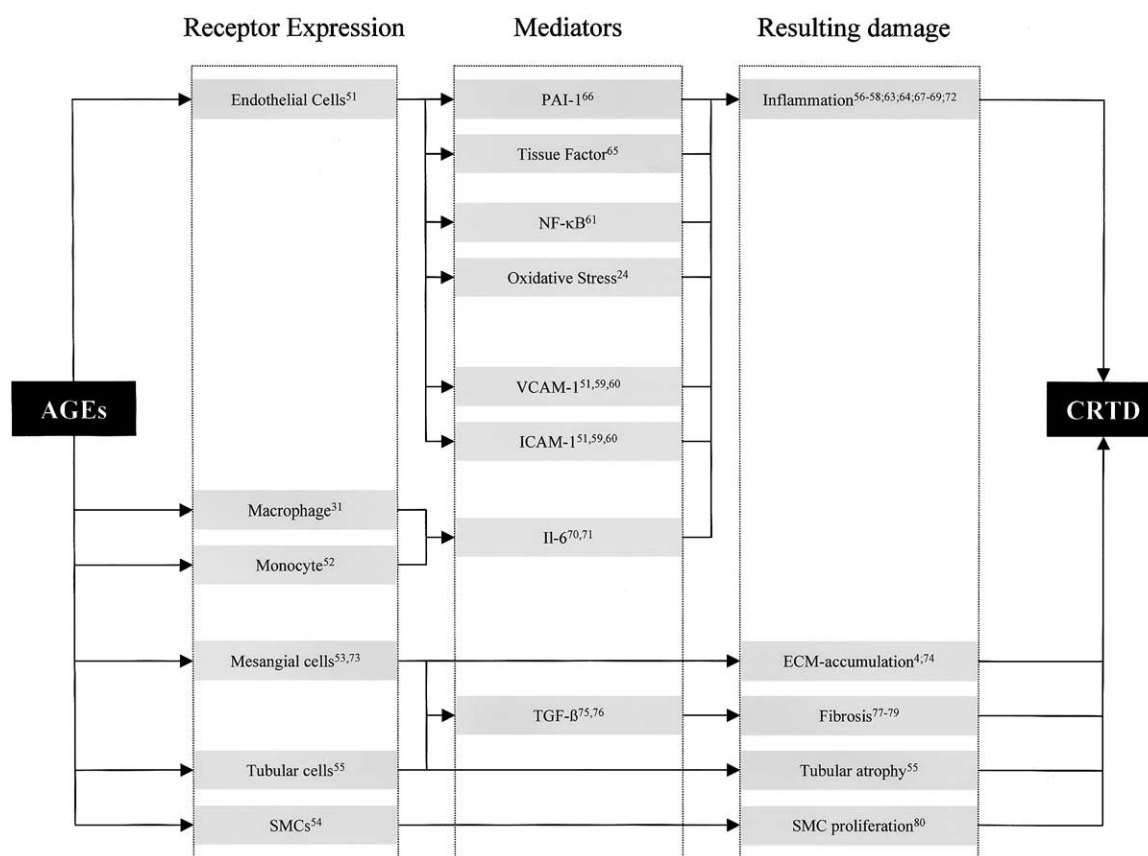
transplantation on AGE accumulation in tissue are inconclusive. Although the idea that kidney transplantation decreases tissue AGE accumulation is supported by some studies,<sup>46-48</sup> Hricik et al<sup>49</sup> showed that kidney transplantation does not correct tissue AGE accumulation. They found an increase in tissue AGE levels in the majority of patients studied. Although there is reason to believe that a decrease in blood AGE levels eventually is reflected in a decrease in tissue AGE accumulation, studies on tissue AGE levels are limited in number, size, and duration after transplantation (mostly <4 to 5 years).

Few data currently are available on the kinetics of tissue AGE accumulation in the long run after transplantation. Recently, data were published on extrarenal AGE levels in patients who developed chronic renal transplant dysfunction.<sup>50</sup> Patients with biopsy-proven chronic renal transplant dysfunction had greater AGE levels compared with transplant recipients with normal

renal function and patients with chronic renal failure of their native kidneys. These findings argue that the increased AGE levels in patients with chronic renal transplant dysfunction cannot be attributed solely to the effect of decreased renal function in patients with chronic renal transplant dysfunction.

#### AGE-INDUCED CELLULAR RESPONSES

We wonder whether AGEs are innocent bystanders or contribute actively to the pathophysiological processes underlying the development of chronic renal transplant dysfunction. In Fig 2, we propose a cascade of events that may be involved. It refers to cell types that express AGE receptors, mediators released in response to activation of these receptors, and tissue damage that resulted from those mediators in different in vitro experiments. AGE receptor expression has been found in a wide range of cells, such as endothelial cells,<sup>51</sup> monocytes,<sup>52</sup> macrophages,<sup>31</sup>



**Fig 2.** Effect of AGEs on different cell types involved in the development of chronic renal transplant dysfunction. Abbreviations: CRTD, chronic renal transplant dysfunction; PAI-1, plasminogen activator inhibitor 1; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; IL-6, interleukin-6; NF-κB, nuclear factor-κB; ECM, extracellular matrix; TGF-β, transforming growth factor-β; SMC, smooth muscle cell.

mesangial cells,<sup>53</sup> smooth muscle cells,<sup>54</sup> and tubular cells.<sup>55</sup> The various cells release different mediators when stimulated by AGEs, inducing an inflammatory response that may lead to tissue damage.

#### Endothelial Cells

Endothelial cells are thought to be centrally involved in the process of inflammation. Different inflammatory mediators are released after activation of receptors on endothelial cells. When stimulated by AGEs, endothelial cells release the inflammatory mediators vascular cell adhesion molecule-1 and intercellular adhesion molecule-1.<sup>51,56</sup> Release of these inflammatory mediators is influenced by oxidative stress and nuclear factor-κB expression,<sup>57-60</sup> which are both enhanced by AGEs in vitro.<sup>4,61</sup> Oxidative stress is

enhanced in patients with end-stage renal failure<sup>62</sup> and kidney transplant recipients.<sup>63</sup> In addition, it was shown that transplant recipients with chronic rejection experience significantly more oxidative stress than patients without chronic rejection.<sup>63</sup> Inflammation also has been associated with chronic rejection. An immunohistochemical study of transplant biopsy specimens showed enhanced expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in chronic rejection.<sup>64</sup> In endothelial cells, AGEs induce the production of tissue factor and plasminogen activator inhibitor-1, as well.<sup>65,66</sup> Tissue factor is the major cellular trigger of blood coagulation. Activated plasminogen activator inhibitor-1 inhibits the activation of plasminogen to plasmin, resulting in thrombosis.<sup>67</sup> In addition to their involvement in blood

coagulation, plasminogen activator inhibitor-1 and tissue factor are thought to have important proinflammatory capabilities.<sup>68,69</sup>

#### *Monocytes and Macrophages*

Monocytes and macrophages are actively involved in the inflammatory process after their attraction and activation by endothelial cells. Interleukin-6, produced by both cell types when stimulated by AGEs in vitro, stimulates the liver to produce acute-phase proteins.<sup>56,70,71</sup> In a rat model of chronic kidney allograft rejection, enhanced interleukin-6 expression was associated with graft failure.<sup>72</sup> Furthermore, human monocytes stimulated by AGEs produce insulin-like growth factor, which is known to stimulate mesangial cells.<sup>73</sup>

#### *Mesangial Cells, Smooth Muscle Cells, and Tubular Cells*

In response to AGEs, mouse mesangial cells showed increased expression of collagen type IV messenger RNA, leading to accumulation of extracellular matrix.<sup>74</sup> Accumulation of extracellular matrix is one of the histological findings in chronic renal transplant dysfunction.<sup>4</sup> Furthermore, both mesangial cells and tubular cells stimulated by AGEs produce transforming growth factor- $\beta$ .<sup>75,76</sup> Transforming growth factor- $\beta$  mediates the formation of fibrosis.<sup>77</sup> Transforming growth factor- $\beta$  expression in a renal allograft correlates with the development of interstitial fibrosis.<sup>78</sup> Moreover, increased transforming growth factor- $\beta$  expression has been found in renal biopsy specimens of patients with chronic renal transplant dysfunction.<sup>79</sup> Finally, smooth muscle cell proliferation and tubular atrophy, both found in chronic renal transplant dysfunction, have been associated with AGE accumulation.<sup>55,80</sup>

#### AGE-INDUCED RENAL TISSUE DAMAGE

Although several AGEs, such as pentosidine, *N*<sup>ε</sup>-carboxymethyllysine, and *N*<sup>ε</sup>-carboxyethyllysine, have been characterized, differences in the pathogenic role between specific AGEs are not yet clear. The pathogenic role of AGEs on renal tissue has been tested in various experimental studies.

In a study by Vlassara et al.,<sup>81</sup> 50 healthy male Sprague-Dawley rats were administered AGE-

modified rat albumin, native rat albumin, or AGE-modified rat albumin in combination with aminoguanidine. Repeated injections with AGEs resulted in increased AGE levels in blood and kidney. AGE-injected animals showed an increase in glomerular volume, glomerular basement widening, and mesangial extracellular matrix, indicating global and segmental glomerulosclerosis. These structural changes were less pronounced in rats administered aminoguanidine.<sup>81</sup> Furthermore, AGE injections resulted in increased total urinary protein excretion, which was almost completely prevented with aminoguanidine treatment.

In another experiment by Vlassara's group, the effect of aminoguanidine on age-related renal pathological characteristics was examined. Non-diabetic female Sprague-Dawley rats and Fischer-344 rats were treated with aminoguanidine for 18 months. Aminoguanidine significantly decreased renal AGE accumulation compared with non-treated controls. Moreover, aminoguanidine partly inhibited age-related albuminuria and proteinuria. In Sprague-Dawley rats, the age-related decrease in glomerular number, accompanied by progressive glomerular sclerosis, was significantly ameliorated by aminoguanidine treatment. In Fischer-344 rats, observed age-related changes were less pronounced. Consequently, no significant structural effects of aminoguanidine were found in this strain.<sup>82</sup>

More recently, Vlassara's group tested whether a diet low in glycoxidation products could prevent diabetic nephropathy in mice.<sup>83</sup> Nonobese diabetic mice were randomly assigned to an AGE-rich or low-AGE diet. Both serum and kidney AGE levels were significantly lower in the low-AGE diet group. Rats fed an AGE-rich diet developed progressive diabetic nephropathy and had short survival, whereas rats fed a low-AGE diet developed only minimal glomerular pathological characteristics and had a significantly extended survival. It remains questionable whether observed effects could be attributed to the toxicity of AGEs alone. One should consider possible effects of other toxic compounds formed under similar conditions as AGEs.<sup>84</sup> Furthermore, in relation to alimentary AGEs, antioxidant effects of some of the Maillard reaction products formed also should be anticipated.<sup>85</sup>

Results from Vlassara's group<sup>81-83</sup> are in line

with those reported by others. Soulis-Liparota et al<sup>86</sup> examined the effect of aminoguanidine on the development of albuminuria, mesangial expansion, and tissue fluorescence in streptozocin-induced diabetic rats during a 32-week period. Compared with untreated controls, aminoguanidine prevented diabetes-induced increased fluorescence in isolated glomeruli and renal tubules, but not in the whole kidney. Furthermore, aminoguanidine treatment attenuated the increase in albuminuria and mesangial expansion.

The use of other AGE inhibitors in experimental studies, such as *N*-(2-acetamidoethyl)-hydrazine-carboximidamide-hydrochloride (ALT-946),<sup>87</sup> ( $\pm$ )-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195),<sup>88</sup> and pyridoxamine,<sup>89</sup> has confirmed the results of studies mentioned. In conclusion, AGE-induced renal tissue damage is well established in both diabetic and nondiabetic animal models. Although observed changes are often nonspecific, they are similar to lesions observed in chronic renal transplant dysfunction. Although heavily modified proteins were used in the first study described from Vlassara's group,<sup>81</sup> recent studies examined the effect of more clinically relevant age-related or diabetes-related increases in AGE accumulation on renal tissue. To date, no results of experimental studies with AGE inhibitors have been published in chronic renal transplant dysfunction rat models. Moreover, no clinical trials have been performed in kidney transplant recipients using AGE-lowering treatment modalities.

### CONCLUSION

We discussed evidence for a pathogenic role of AGEs in the development of chronic renal transplant dysfunction. First, AGE levels are elevated in the presence of some risk factors involved in the development of chronic renal transplant dysfunction. Although few data currently are available on the kinetics of tissue AGE accumulation in the long run after transplantation, increased AGE levels were found in blood of patients who developed chronic renal transplant dysfunction. In vitro data showed that AGEs may stimulate various cells to release mediators that contribute to the renal damage found in chronic renal transplant dysfunction. Based on these findings, we proposed a pathophysiological mechanism of AGE-induced renal tissue dam-

age. Finally, we discussed results of experimental studies on AGE-induced renal tissue damage. To date, no studies, experimental or clinical, have been performed to examine the effect of AGE-lowering treatment modalities on the development of chronic renal transplant dysfunction.

Opponents of AGE-related hypotheses argue that AGEs are detectable only in trace concentrations in tissue proteins and therefore could not be important pathogenic constituents. Proponents argue that new AGEs are still being discovered and little is known about AGE effector mechanisms. However, various studies have associated AGE accumulation with vascular disease processes. Together, these studies illustrated the pathogenic potential of AGEs in vitro and in vivo. We expect clinical studies to confirm the role of AGEs in the development of chronic renal transplant dysfunction. In the future, therapy with AGE-formation inhibitors or AGE cross-link breakers may be warranted to preserve renal function in transplant recipients.

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