Diabetologia (1997) 40: 619–628

Diabetologia

Originals

Advanced glycation end products and their receptors co-localise in rat organs susceptible to diabetic microvascular injury

T. Soulis[1], V. Thallas[1], S. Youssef[1], R. E. Gilbert[1], B. G. McWilliam[2], R. P. Murray-McIntosh[2], M. E. Cooper[1]

[1] Department of Medicine, University of Melbourne, Austin and Repatriation Medical Centre (Repatriation Campus), West Heidelberg, Australia
[2] Department of Medicine/Biochemistry, Otago University, Wellington, New Zealand

Summary Advanced glycation end products (AGEs) are believed to play an important role in the development of diabetic complications. AGEs are increased in experimental diabetes and treatment with the inhibitor of advanced glycation end products, aminoguanidine, has been shown to attenuate the level of these products in tissues undergoing complications. Recently, an AGE-binding protein has been isolated from bovine lung endothelial cells and termed the receptor for advanced glycated end products (RAGE). The present study sought to determine the distribution of AGE and RAGE in tissues susceptible to the long-term complications of diabetes including the kidney, eye, nerve, arteries as well as in a tissue resistant to such complications, the lung. Using polyclonal antisera both AGE and RAGE were found to co-localize in the renal glomerulus. AGE staining was clearly increased with age and was further increased by diabetes. Aminoguanidine treatment reduced AGE accumulation in the kidney. Co-localisation of AGE and RAGE was demonstrated in the inner plexiform layer and the inner limiting membrane of the retina and in nerve bundles from mesenteric arteries. In the aorta, both AGE and RAGE were found in the intima, media and adventitia. Medial staining was increased in diabetes and was reduced by aminoguanidine treatment. A similar pattern was observed for RAGE in the aorta. In the lung, RAGE was found widely distributed throughout the lung whereas the distribution of AGE staining was more limited, primarily localising to macrophages. The co-localisation of AGEs and RAGE in sites of diabetic microvascular injury suggests that this ligand-receptor interaction may represent an important mechanism in the genesis of diabetic complications. [Diabetologia (1997) 40: 619–628]

Keywords Glycation, aminoguanidine, kidney, retina, aorta.

Non-enzymatic glycation involves the condensation of free aldehyde groups of sugars with the amino groups of proteins [1]. This reaction occurs very slowly resulting in the formation of cross-linked, modified long-lived proteins referred to as advanced glycation end-products (AGEs) [2]. As a consequence of increased substrate (glucose) availability, AGEs accumulate in diabetes mellitus where they are believed to be involved in the pathogenesis of long-term complications as well as some of the manifestations of aging in normal subjects [3–6]. An inhibitor of advanced glycation, aminoguanidine, has been shown to retard or prevent the development of nephropathy [7], retinopathy [8] and neuropathy [9] in the streptozotocin-induced diabetic rat model.

Recently, a protein which can bind to AGEs has been cloned from bovine lung [10, 11]. This protein with significant homology to the IgG superfamily

Received: 16 October 1996 and in final revised form: 17 February 1997

Corresponding author: Dr. M. Cooper, Department of Medicine, University of Melbourne, Austin and Repatriation Medical Centre (Repatriation Campus) Banksia Street, West Heidelberg, 3081 Victoria, Australia

Abbreviations: AGEs, Advanced glycation end products; RAGE, receptor for advanced glycation end products; KLH, keyhole limpet hemocyanin; CML, carboxymethylysine; TGF, transforming growth factor; BSA, bovine serum albumin; PDGF, platelet derived growth factor; IL, interleukin.

has been termed the receptor for advanced glycated products or RAGE [12]. The present study sought to explore possible sites of interaction of AGE products and this receptor with a particular focus on tissues and organs which are susceptible to long-term diabetic complications.

Materials and methods

Keyhole limpet hemocyanin (KLH) (Boeringher Mannheim, Mannheim, Germany), 20 mg/ml, was incubated for 12 weeks with 1 mmol/l glucose 6-phosphate to form AGE-KLH. Incubation was performed at 37 °C under dark room conditions using glass vials containing 0.4 mmol/l sodium phosphate buffer pH 7.4, 100 U/ml penicillin (Sigma St. Louis, Mo, USA), 40 μg/ml gentamycin sulphate (Sigma) and 0.5 mmol/l EDTA (Sigma). AGE-KLH was dialysed extensively against phosphate buffered saline pH 7.4 (PBS) and stored at –70 °C prior to use.

Polyclonal antiserum was raised against AGE-KLH as follows. New Zealand white rabbits were inoculated with 1 mg AGE-KLH in complete Freund's adjuvant weekly for 6 weeks with a 0.5 mg booster in incomplete adjuvant 2 weeks later [13]. Antisera titre was determined by non-competitive ELISA and by the Ouchterlony double diffusion assay [14]. With dot blots and non-competitive ELISAs, the antiserum was shown to bind to AGE-bovine serum albumin (BSA) glycated with glucose 6-phosphate. AGE-RNase (using glucose), AGE-poly-L-lysine and AGE-N-acetyl-L-lysine methyl ester (using glucose 6-phosphate), AGE-human serum albumin (using glucose or glucose 6-phosphate) but not to the unmodified proteins, peptides or amino acids. Furthermore, the antiserum does not detect carboxymethylysine (CML) or pentosidine. These experiments also involved assessment of increasing concentrations of BSA-CML (up to 500 μg/ml) by radioimmunoassay provided by Dr. O. Bjerrum (Novo Nordisk, Bagsvaerd, Denmark). Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded sections (4 μm) of rat kidney, eye, lung and aorta. Sections were rehydrated and treated with 1% H_2O_2/methanol followed by incubation in Protein Blocking Agent (Lipshaw-Immunon, Pittsburgh, Penn., USA) for 20 min at room temperature. Sections were then washed in PBS and incubated with biotinylated goat anti-rabbit immunoglobulin (Dako, Carpinteria, Calif., USA) followed by peroxidase conjugated strepavidin (Dako). Localisation of peroxidase conjugates was revealed using diaminobenzidine tetrahydrochloride (DAB) as the chromogen. As a control for anti-AGE immunoreactivity, AGEs formed by the addition of glucose and BSA proteins and incubated for more than 60 days at 37 °C were preincubated with AGE antiserum and used in place of the AGE antiserum alone in the normal staining procedure (Fig. 1 F).

To explore whether AGE staining reflected AGE levels in vivo, AGE staining in glomeruli was quantitated in various rat groups as outlined below. Male Sprague Dawley rats weighing between 200 and 250 g were randomised to control or diabetic groups. Rats were rendered diabetic via an intravenous injection of the beta-cell toxin, streptozotocin (55 mg/kg) and then further randomised into untreated or aminoguanidine treated (1 g/l in drinking water) groups. To be included in the study, rats had to have blood glucose levels greater than 20 mmol/l one week after induction of diabetes. Control rats were also randomised into untreated or aminoguanidine-treated (2 g/l in drinking water) groups. The doses of aminoguanidine used were based on previous studies carried out in our laboratory [7] and reflect the increased water consumption in diabetic rats. Rats were killed 3 and 32 weeks after induction of diabetes. Before killing, rats were weighed and blood taken for the measurement of plasma glucose by the glucose oxidase method [15] and for glycated haemoglobin by a HPLC technique (Biorad, Richmond, Calif., USA).

Table 1. Body weight and glycaemic control at 32 weeks

Group	*n*	Body weight (g)	Glucose (mmol/l)	HbA_{1c} (%)
Control	6	543 ± 20	4.9 ± 0.1	2.8 ± 0.3
Control + AG	6	537 ± 15	5.2 ± 0.3	2.4 ± 0.4
Diabetic	8	379 ± 24[a]	32.9 ± 1.2[a]	5.2 ± 0.5[a]
Diabetic + AG	8	329 ± 39[a]	35.6 ± 1.4[a]	5.6 ± 0.2[a]

Data are shown as mean ± SEM at week 32. [a] $p < 0.01$ vs control

Staining for AGE was quantitated in the glomeruli of rat kidneys, the major site of the renal lesion in diabetes, using a videoimaging system, (Video Pro 32; Leading Edge, Bedford Park, South Australia, Australia) connected to a Zeiss AXIOPHOT microscope (Stuttgart, Germany) [16]. The amount of staining for AGEs was defined as the area of brown pigment detected by the videoimaging system [17]. The degree of AGE staining within glomeruli was expressed as a proportion, which was calculated as the quotient of the area of the AGE staining and the total glomerular area. This was defined by using the inner lining of the Bowman's capsule as the outer margin of the glomerulus. From each rat, 50 randomly selected glomeruli were quantitated. Measurements were performed in a blinded manner by one observer (S. Y.). The coefficient of variation for this method was 4.5%.

To explore the presence of RAGE protein in the various tissues, an antiserum to human recombinant RAGE (gift of Dr. K Jansen, Merck Sharp and Dohme, West Point, Penn., USA) was used at a working concentration of 1/50. The description of this antiserum is as previously reported [18]. Since RAGE is non-covalently bound to a molecule with sequence identity to lactoferrin [12], studies were also performed with an anti-lactoferrin antiserum (Sigma) at a working concentration of 1/50. Immunohistochemical studies were performed on the adjacent sections to those stained with AGE antibody. The methods used did not differ from those described above for evaluation of AGE staining. As a control for anti-RAGE immunoreactivity, positively stained tissues were subjected to blocking experiments in which anti-RAGE antiserum was preincubated with recombinant RAGE (gift of Dr. K Jansen, Merck Sharp and Dohme) at a concentration of 60–65 μg/ml for 3 h at 37 °C (Fig. 2 C). Similarly designed experiments were performed to assess the specificity of the staining to the anti-lactoferrin antisera by preincubation with lactoferrin (Fig. 2 D).

Results

Metabolic parameters at the various timepoints are shown in Table 1. Diabetic rats had reduced body weight and had elevated plasma glucose and glycated haemoglobin. Aminoguanidine treatment did not affect body weight or glycaemic control.

Rat kidney. Figure 1 shows AGE staining renal glomeruli and in the tubointerstitium. The predominant

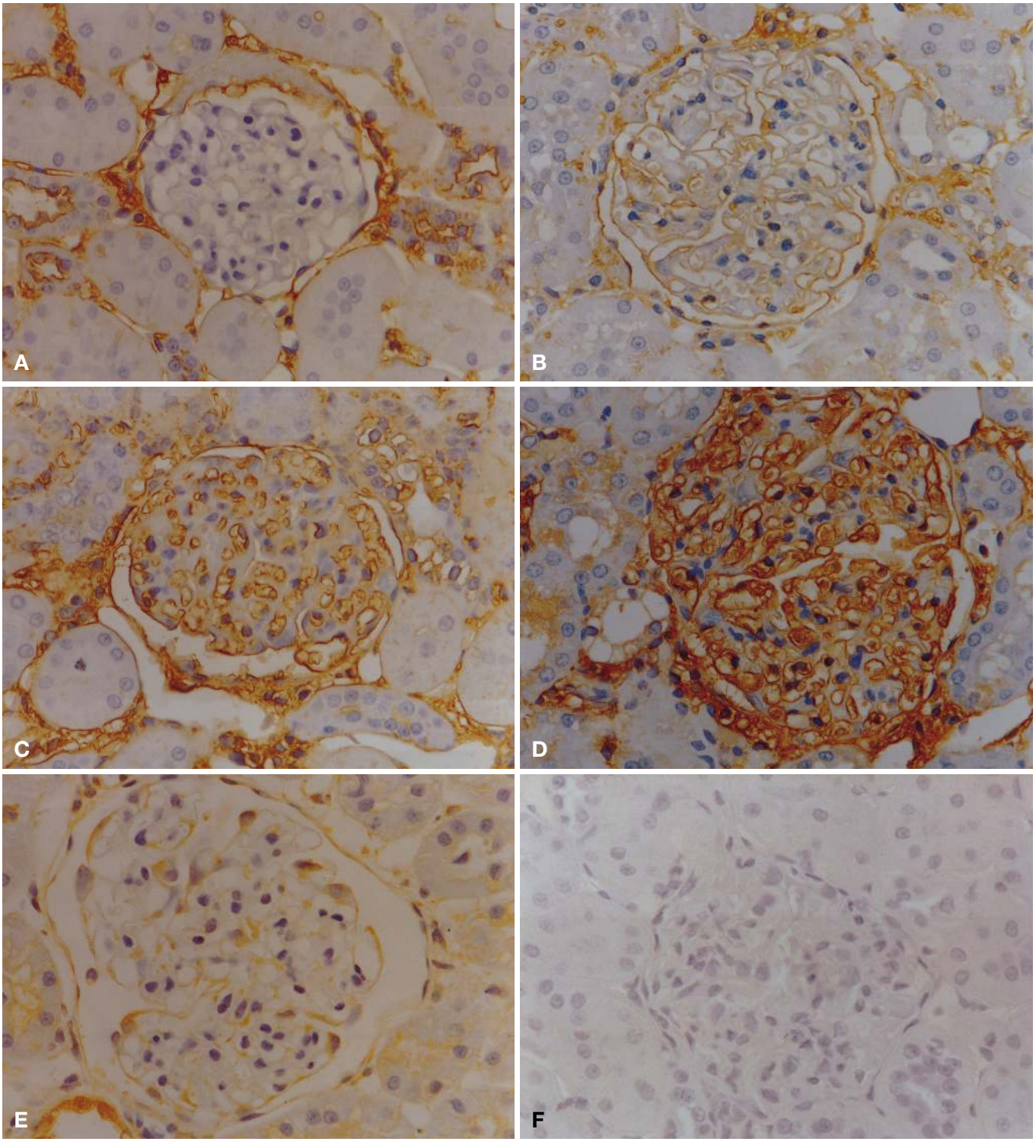


Fig. 1. A–F Staining for AGEs in glomeruli from control rats after 3 (**A**), and 32 weeks (**B**), diabetic rats after 3 (**C**) and 32 weeks (**D**) and diabetic + aminoguanidine rats after 32 weeks (**E**). The negative control which includes pre-incubation with excess AGEs is also shown (**F**). Magnification × 100

site of AGE staining within glomeruli was to endothelial cells in control rats (Fig. 1 A). Staining within the glomeruli was increased over the 32-week study period in control rats (Fig. 1 B). There was an increase in AGE staining within glomeruli from diabetic rats (Fig. 1 C) which was more clearly evident after 32 weeks of diabetes (Fig. 1 D). This increase in AGE staining was not observed in diabetic rats receiving aminoguanidine (Fig. 1 E). Quantification of AGE

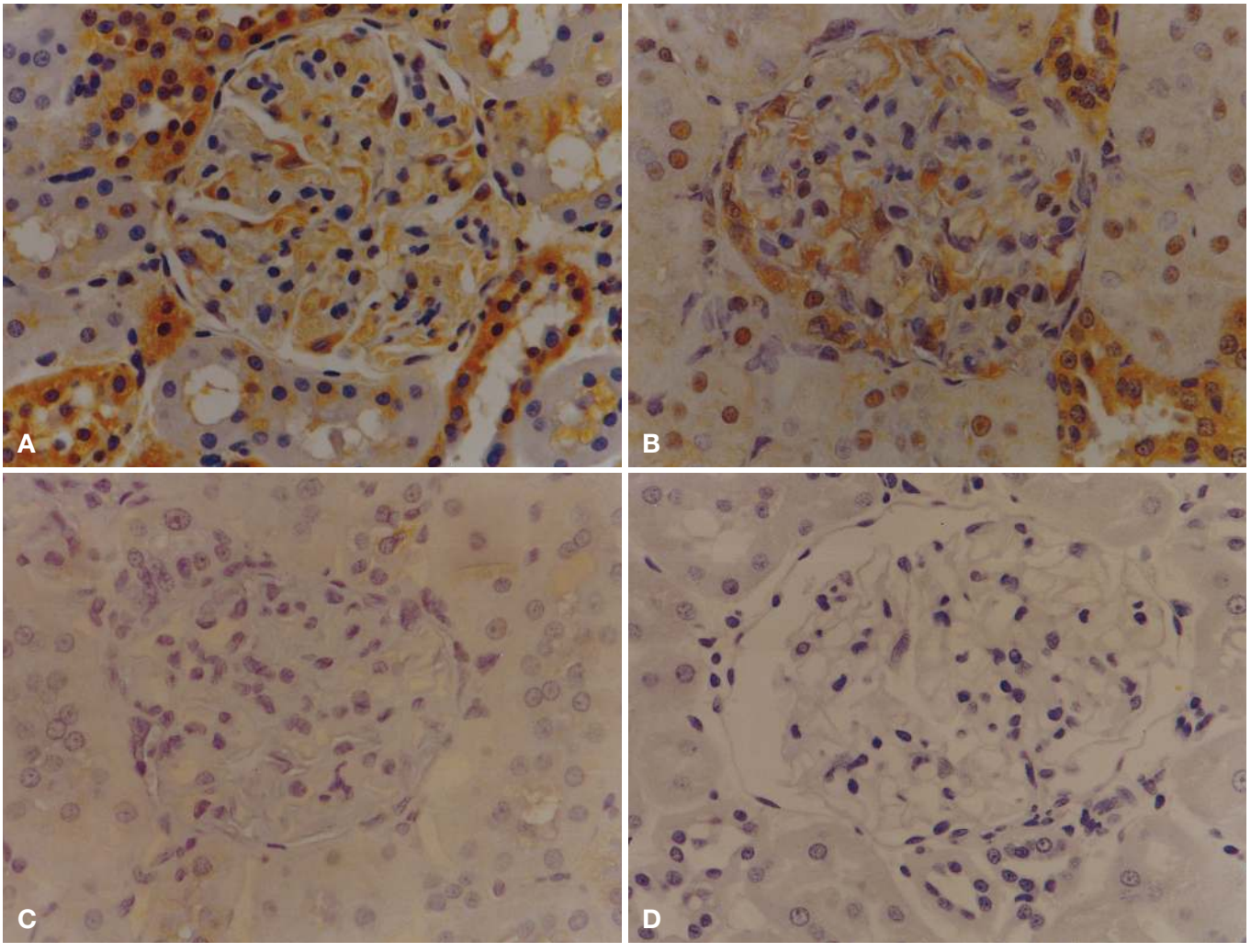


Fig. 2. A–D Staining for RAGE (**A**) and lactoferrin (**B**) in control rat glomeruli. Negative controls for RAGE (**C**) and lactoferrin (**D**) are also shown. Magnification × 100

staining data in glomeruli confirmed this pattern (AGEs staining; control, 21 ± 4%, control+aminoguanidine, 25 ± 6%, diabetic, 44 ± 4%, diabetic+aminoguanidine 27 ± 2%, $n = 6$/group, $p < 0.01$ diabetic vs all other groups). Preincubation of the antibody with AGEs resulted in no positive staining (Fig. 1F).

Staining for RAGE was observed in glomeruli as well as in distal tubules and collecting ducts (Fig. 2A). A similar pattern was observed with respect to staining for lactoferrin in the kidney (Fig. 2B). Tissue sections stained with RAGE or lactoferrin were uniformly negative if preincubated with recombinant RAGE or lactoferrin, respectively (Fig. 2C and D).

Lung. AGE staining was predominantly localised to the interstitium with prominent staining of alveolar macrophages (Fig. 3A). There was widespread staining for RAGE antigen in rat lung parenchymal tissue (Fig. 3B). RAGE was detected in respiratory epithelium, the lining of alveoli and bronchial cells.

Retina and nerves. Figure 4 depicts staining of both AGE (Fig. 4A–C) and RAGE (Fig. 4D) in the retina. AGE and RAGE were co-localized to the inner-limiting membrane and inner plexiform layers of the retina. Although not readily quantifiable, there appeared to be increased AGE staining in the inner plexiform layers of diabetic rats (Fig. 4B) which was prevented by aminoguanidine treatment (Fig. 4C). Although RAGE co-localised with AGE in the retina, no clear-cut differences in RAGE staining in the presence of diabetes could be detected. There was also co-localisation of AGE and RAGE in nerve bundles located in the adventitial layer of large arteries including the mesenteric arteries (Fig. 5A and B).

Aorta. Staining of AGE and RAGE was detected in the intimal and adventitial layers of the aorta in control rats (Fig. 6A and B). Although there was minimal staining of AGE in the smooth muscle layer of

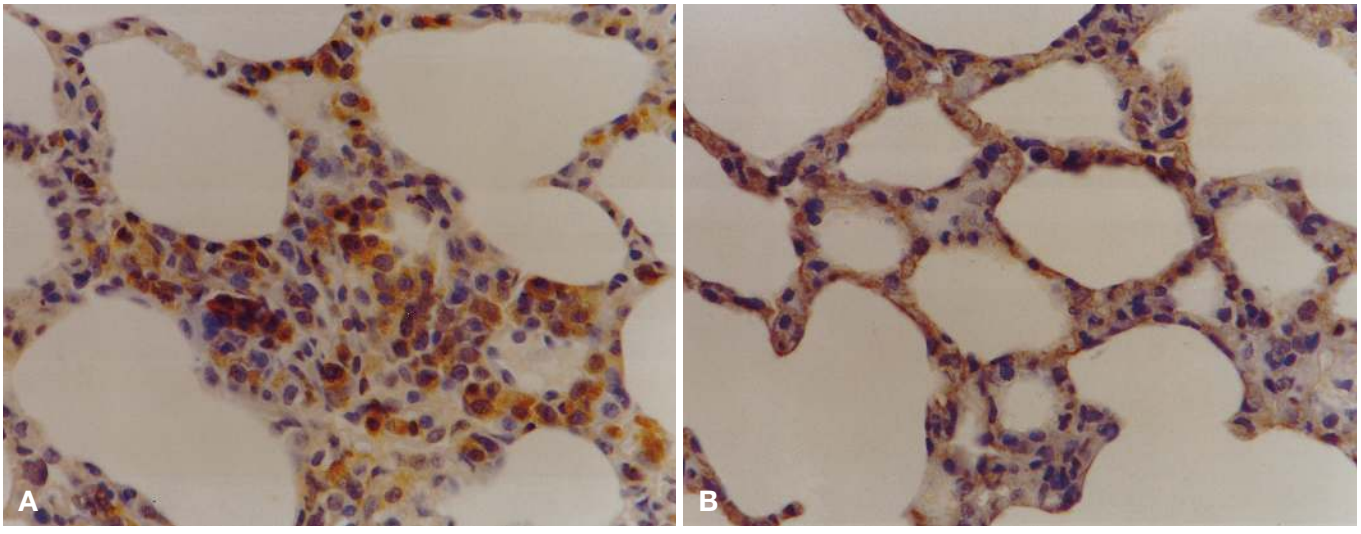
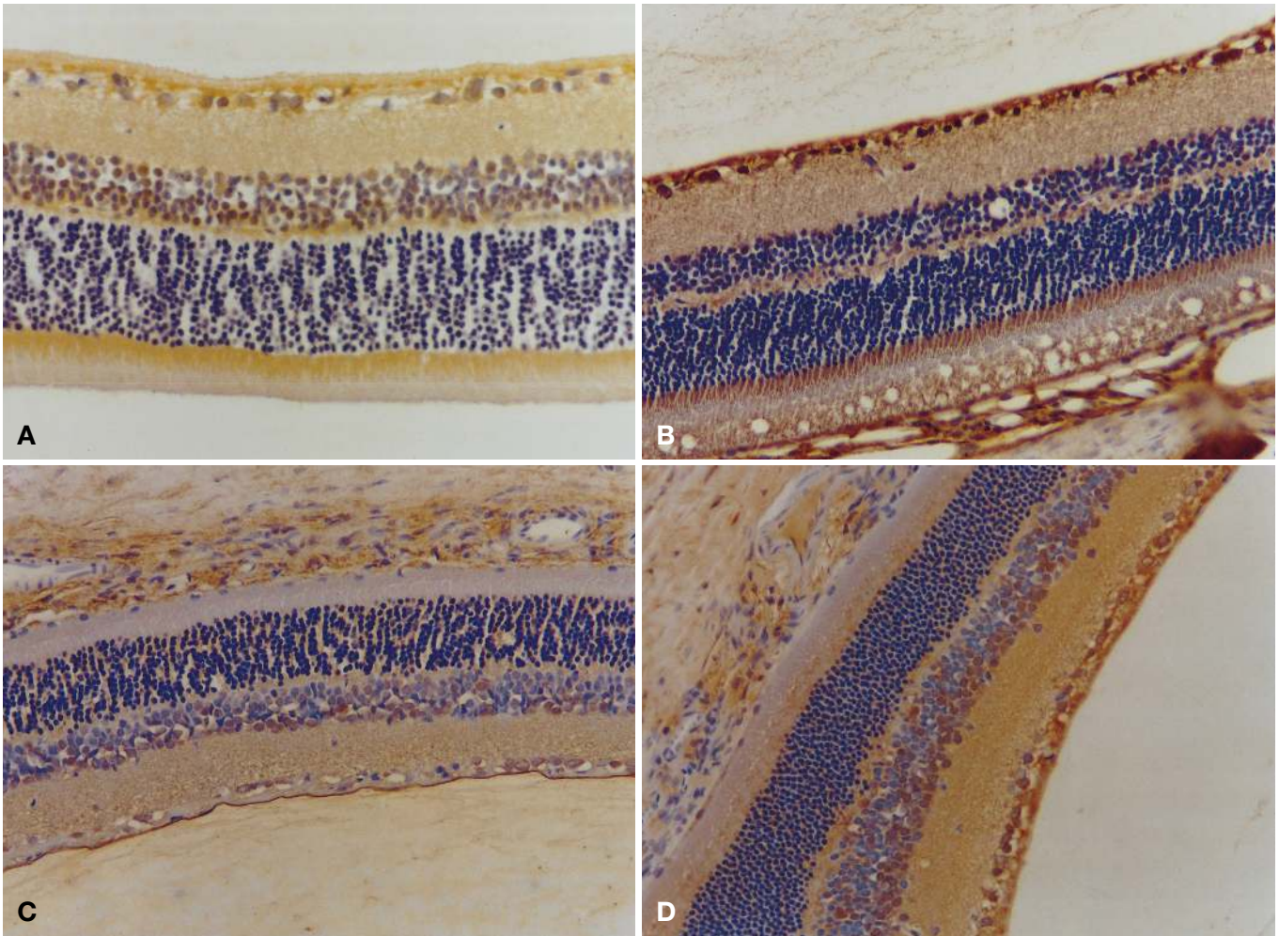


Fig. 3. **A, B** Staining for AGEs (**A**) and RAGE (**B**) in control lung. Magnification × 50

Fig. 4. **A–D** Staining for AGEs (**A**) in retina from control (**A**), diabetic (**B**) and diabetic + aminoguanidine (**C**) rats. Staining for RAGE (**D**) in control rat retina. Magnification × 100



control rats, AGE staining was prominent in this layer in diabetic rats (Fig. 6C). Aminoguanidine treatment prevented the increased AGE staining in the medial layer (Fig. 6E). There was colocalisation of staining to both AGE and RAGE antibodies in the aorta. Furthermore, the effects of diabetes and aminoguanidine on RAGE staining reiterated the pattern observed with the AGE antibody (Figs. 6B, D and F).

Discussion

This study has confirmed that advanced glycated proteins and the receptor for AGEs (RAGE) are present in sites which are commonly affected by diabetic complications. AGE and RAGE are both detected in the kidney, eye, nerve and blood vessels. A previous report has described the localisation of RAGE in human and bovine tissues, but did not evaluate whether the receptor co-localised with AGE proteins [18]. The co-localisation of AGE and RAGE in the retina, aorta, nerve and certain sites within the kidney is consistent with a ligand-receptor interaction. If this interaction represents a clearance mechanism for AGEs, a pathway mediating diabetic vascular injury or both remains to be delineated.

Long-lived proteins such as collagen are modified by prolonged exposure to glucose resulting in AGE formation. In diabetes, a state of chronic hyperglycaemia, the accumulation of AGEs is accelerated [19]. Proteins that have undergone advanced glycation have a characteristic fluorescence which may be used for quantitation of AGEs [7]. However, it has now been shown that AGEs represent a family of protein adducts, some of which, such as CML do not fluoresce [20].

The antisera to AGEs in the present study does not detect CML. However, pilot studies by our group have been performed using another antibody which does detect CML. Both antibodies revealed similar localisation of AGPs within the kidney (data not shown). CML has been shown to be closely linked to diabetic complications including nephropathy [20]. These findings are consistent with the results of a previous study which clearly shows accumulation of CML-reactive proteins in the glomerulus in experimental diabetes [21].

Previous studies by several groups have measured AGE levels by immunoassay as well as by specific fluorescence in tissues undergoing diabetic complications [22, 23]. The advent of these antibodies to AGEs has allowed immunohistochemical techniques to localise AGE proteins in various sites [24–26]. The present study has extended these reports by assessing the effects of age and diabetes on AGE accumulation in various tissues. It could be clearly shown in the kidney that AGEs accumulate in the glomerulus over time and that this phenomenon is accelerated in diabetes. Furthermore, aminoguanidine treatment attenuates glomerular AGE accumulation. These immunohistochemical findings are consistent with our recent report of AGE accumulation as measured by a specific radioimmunoassay in glomerular homogenates from diabetic rats in the absence and presence of aminoguanidine treatment [21]. In addition, in that study, the presence of AGEs in tubular homogenates was detected. This is consistent with the findings in the present study that the antisera to AGEs also stained tubulointerstial structures.

It has been postulated that the accumulation of AGEs contributes to the development of diabetic nephropathy [27]. Several studies in our laboratory have shown that the increased AGE levels in the diabetic kidney are associated with the development of mesangial expansion and albuminuria [7, 21, 28]. Furthermore, aminoguanidine not only prevented AGE formation in the kidney but also retarded the development of albuminuria and mesangial expansion. In the present study, AGE accumulation in long-term diabetic rats was clearly evident in the mesangial region of the glomerulus (Fig. 1D). In humans with diabetic nephropathy, AGEs have been detected immunohistochemically within glomerulosclerotic lesions [29].

Recently, an AGE binding protein known as RAGE was cloned from bovine lung [11]. This 35 kDa polypeptide was found to bind with high affinity to an 80 kDa protein, identical in amino acid sequence to lactoferrin. Both these proteins can bind to AGEs. It has been suggested that RAGE functions as a cell surface receptor for AGEs and mediates their cellular effects [30]. For example, AGE-induced increases in expression of vascular cell adhesion molecule-1 can be inhibited by either antibodies to RAGE or soluble RAGE [31]. Using antibodies to both RAGE and lactoferrin and staining adjacent kidney sections, it could be clearly shown that the lactoferrin-like and RAGE proteins are located at the same sites within the kidney including distal tubules and collecting ducts and to a lesser extent glomeruli. The co-localisation of the antibody staining for both proteins is consistent with the previous report that RAGE is non-covalently bound to the lactoferrin-like molecule [32]. RAGE expression has recently been reported in the human kidney [33]. It is intriguing that staining of RAGE appeared to be increased in inflammatory forms of renal disease rather than in diabetic nephropathy. This is compatible with the results of the present study in which it was not possible to clearly detect a difference in RAGE staining between control and diabetic kidney. However, a recent preliminary report has suggested an increase albeit modest in gene expression for RAGE in the diabetic kidney using reverse transcriptase-polymerase chain reaction techniques [34]. Whether this increase

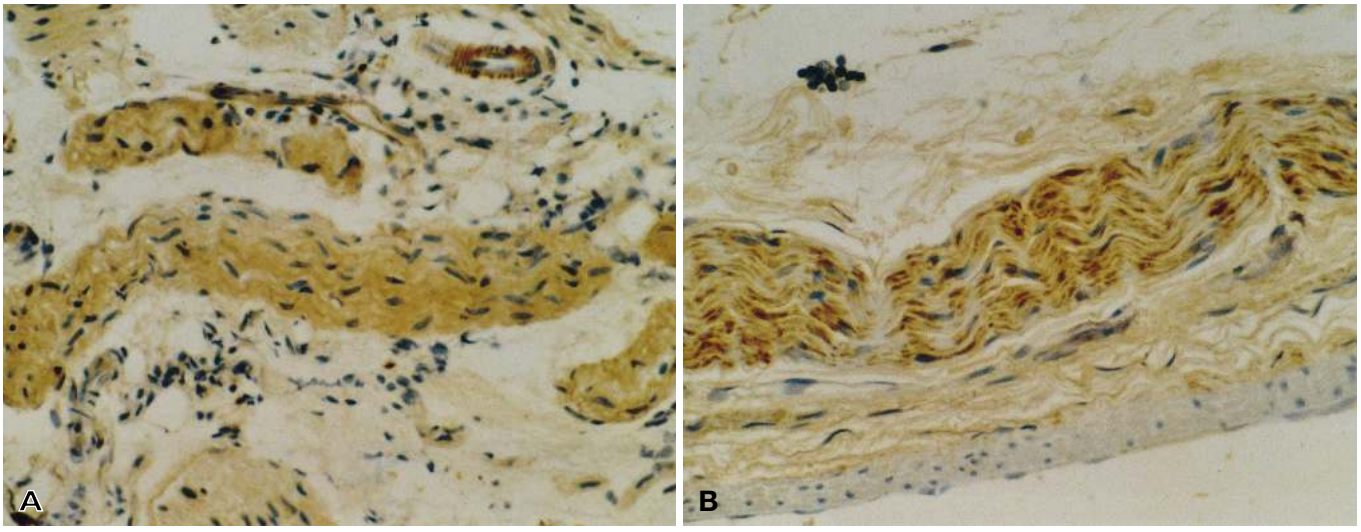


Fig. 5. **A, B** Staining for AGEs (**A**) and RAGE (**B**) in nerves from the adventitial layer of the superior mesenteric artery. Magnification × 100

in mRNA can be translated to an increase in RAGE protein is as yet unknown.

Abel et al. [33] have suggested that the endogenous ligand for this receptor may not be AGE proteins. Consistent with these findings is the proposal by Schmidt et al. [12] that the primary function of RAGE may be as part of the immune response and that the reaction to AGE protein is 'accidental'. This is further suggested by the recent report that one of the endogenous ligands for RAGE may be amphoterin and that the interaction of amphoterin with RAGE modulates neurite formation [35].

AGEs may play a role in the genesis of experimental diabetic retinopathy. Hammes et al. have reported in both normotensive [8] and hypertensive [36] diabetic rats that aminoguanidine prevented the development of retinopathy, characterised by acellular capillaries and pericyte loss. The co-localisation of AGEs and RAGE within the retina may represent a ligand-receptor interaction involved in the genesis of diabetic retinopathy. There appeared to be increased AGE staining in the retina from diabetic rats which was not observed in the animals treated with aminoguanidine. It has been difficult to quantify retinal AGE levels although Hammes et al. [8] have suggested that there is an increase in AGE levels in retinal homogenates from long-term diabetic rats, as assessed by the relatively non-specific fluorescence method. There are as yet no reports of inhibition of this interaction either with soluble receptor or antibodies leading to amelioration or prevention of experimental diabetic retinopathy. AGE and RAGE also co-localised in nerves consistent with a role for advanced glycation in diabetic neuropathy. Aminoguanidine has been reported to ameliorate both functional and structural markers of nerve dysfunction in diabetic rats [9, 37, 38].

An important finding in the present study was the co-localisation of AGEs and RAGE in the aorta. Many studies have focused on the central role of advanced glycation in diabetic vascular disease [23, 25]. Previous studies have reported increased AGE levels in the aorta from diabetic rats and reduced AGE levels if the rats received aminoguanidine [7, 39]. In the present study, we have confirmed the presence of AGEs in the aorta and that diabetes and aminoguanidine therapy influence aortic AGE levels. Using immunohistochemical techniques, AGEs have been shown to be present in atheromatous plaques, particularly in the setting of diabetes [25].

Not only did AGE and RAGE co-localise in the aorta but the pattern of staining for RAGE was similar to that observed with AGE in the setting of diabetes and aminoguanidine treatment. The similarity in the pattern and intensity of AGE and RAGE staining in these vessels suggests that AGE levels may modulate RAGE expression in the aorta. Ritthaler et al. [40] have recently shown that in patients with a range of peripheral occlusive vascular diseases with or without diabetes there was enhanced endothelial RAGE expression in the blood vessels. It is feasible that in diabetes there is a further increase in RAGE expression in blood vessels and that this phenomenon could be involved in the genesis of diabetes associated vascular injury. Since AGEs and RAGE are present in blood vessels in the absence of diabetes [40, 41] it is possible that this ligand-receptor interaction may have a role in the progression of atherosclerosis. Indeed, a preliminary report has indicated that aminoguanidine treatment will reduce lipid accumulation in the aorta from cholesterol-fed non-diabetic rabbits [42].

The exact function of RAGE remains to be elucidated. It has been suggested that RAGE promotes

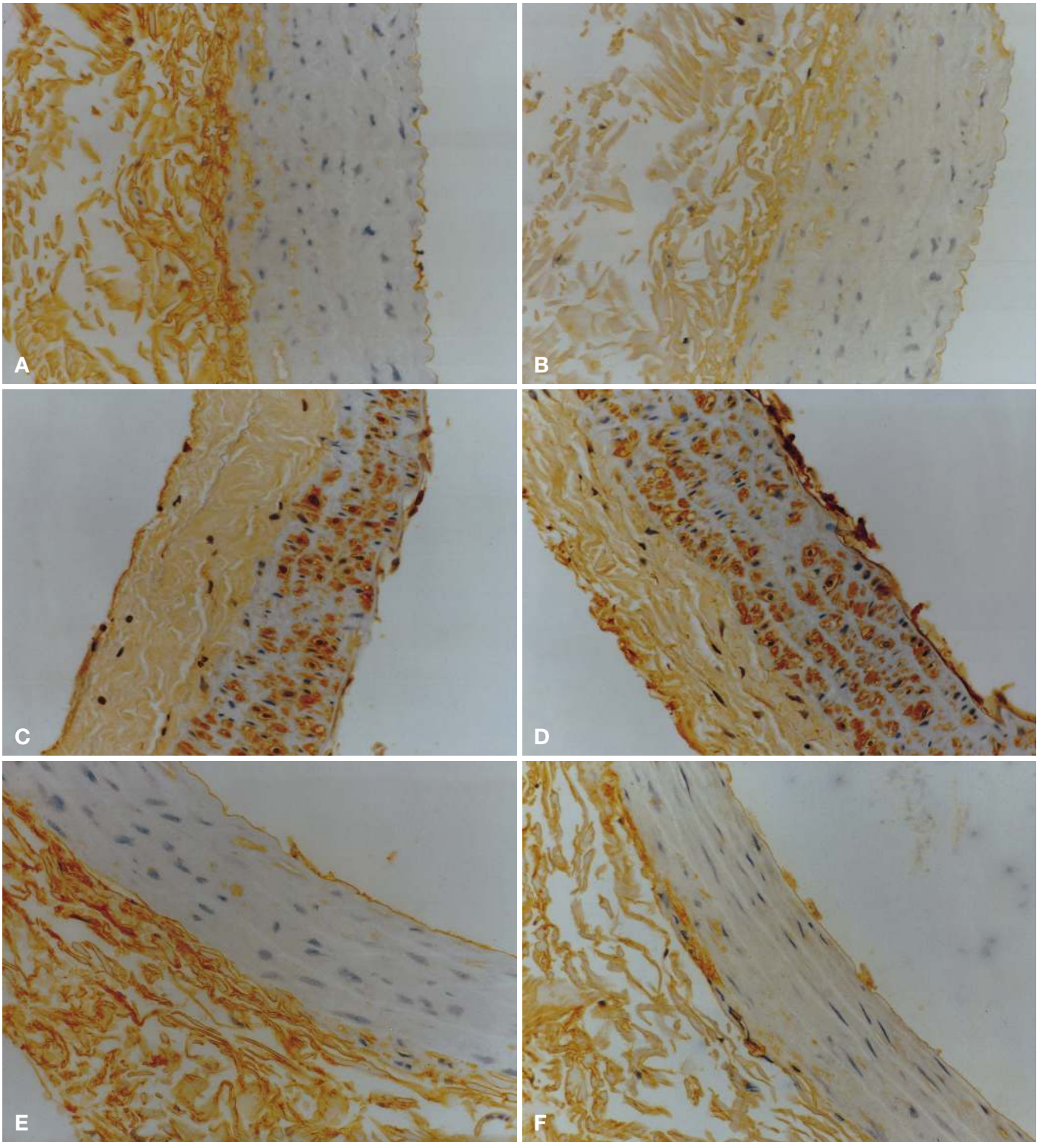


Fig. 6. A–F Staining for AGEs in the aorta from control (**A**), diabetic (**C**) and diabetic + aminoguanidine (**E**) rats. Staining for RAGE in the aorta from control (**B**), diabetic (**D**) and diabetic + aminoguanidine (**F**) rats. Magnification × 100

AGE clearance from plasma since the administration of a soluble form of RAGE, which acts functionally as an antagonist, retards the clearance of radiolabelled AGE-albumin from plasma [43]. AGE proteins may be cleared by other, possibly non-receptor mediated mechanisms as suggested in a recent study assessing AGE handling in the kidney [44]. That study reported that the predominant site of clearance was the proximal tubule, a site with minimal RAGE.

Nevertheless, this does not exclude a role for RAGE in the clearance of AGE proteins. One possibility is that this ligand binding site acts as a clearance mechanism for AGE proteins by breaking down the products into smaller peptides which could then be cleared by the circulation via non-receptor mediated mechanisms. Low molecular weight AGE peptides which are formed after receptor internalisation have been shown to remain reactive with protein side chains and accumulate in diabetes, particularly in the setting of renal failure [45].

The mechanisms by which AGE binding to its receptor may lead to the pathological features of diabetic complications have begun to be explored. For instance, administration of AGE proteins to normal mice results in upregulation of the prosclerotic cytokine transforming growth factor (TGF)β in glomeruli, possibly mediating the increase in extracellular matrix gene expression and glomerular hypertrophy observed in this model [46]. In vitro studies by Doi et al. [47] have suggested that platelet derived growth factor (PDGF) may also mediate the increase in extracellular matrix production by mesangial cells on exposure to AGE proteins. Indeed, recent studies indicate that interventions which inhibit PDGF and TGF-β prevent AGE stimulated collagen III production by cultured mesangial cells [48]. However, the effect of PDGF may be mediated by stimulation of TGF-β production. Other growth factors and cytokines which are upregulated in response to AGEs include interleukin (IL)-1 [49], IL-6, tumour necrosis factor (TNF)-alpha [49] and insulin-like growth factor-1 (IGF)-1 [50]. The precise mechanism by which AGE binding alters gene transcription is unknown although preliminary evidence suggests that AGE binding to cells may lead to activation of the transcription factor, NF-$\varkappa$b [31].

Although this study has focussed on RAGE, previous studies have isolated other receptors for AGEs including 60 kDa and 90 kDa proteins from rat liver macrophages [51]. More recently one of these receptors has been shown to have homology with the protein, galectin-3 [52]. The role of these AGE binding proteins has not been fully delineated. An analysis of the distribution of these proteins which were not evaluated in the present study would assist in the further understanding of the handling and fate of AGE proteins in various pathophysiological states.

Although the present study has clearly defined the distribution of advanced glycated proteins and one of their putative receptors, RAGE, the importance of this ligand-receptor interaction in the genesis of diabetic complications remains unproven. Recent studies which blocked the action of RAGE using recombinant RAGE prevented the early increase in permeability that is observed in diabetic rats [53]. Further assessment of the functional and morphological sequelae to interference of this receptor-ligand interaction with modalities such as recombinant RAGE are warranted.

Acknowledgements. The authors wish to thank Leanne Thomas for expert technical assistance. The authors also wish to thank the late Professor T. J. Neale for his advice during preparation of the AGE antibody and Dr. O. Bjerrum for provision of CML and assistance in further characterisation of our antibodies. This work was supported by grants from Juvenile Diabetes Foundation International, Hoechst Diabetes Research Development Board, the Australian Kidney Foundation, the National and Central Health and Medical Research Councils of Australia, the Health Research Council of New Zealand and the Lotteries Board of Control, New Zealand.

References

1. Brownlee M (1994) Lilly Lecture 1993. Glycation and diabetic complications. Diabetes 43: 836–841
2. Fu MX, Knecht KJ, Thorpe SR, Baynes JW (1992) Role of oxygen in cross-linking and chemical modification of collagen by glucose. Diabetes 2: 42–48
3. Monnier V, Vishwanath V, Frank K, Elmets G, Dauchot P, Kohn R (1986) Relation between complications of type I diabetes mellitus and collagen-linked fluorescence. N Eng J Med 314: 403–408
4. Monnier VM, Sell DR, Nagaraj RH, et al. (1992) Maillard reaction-mediated molecular damage to extracellular matrix and other proteins in diabetes, aging, and uremia. Diabetes 2: 36–41
5. Sensi M, Pricci F, Pugliese G, et al. (1995) Role of advanced glycation end-products (AGE) in late diabetic complications. Diabetes Res Clin Pract 28: 9–17
6. Nicholls K, Mandel T (1989) Advanced glycosylation end products in experimental murine diabetic nephropathy: effect of islet grafting and of aminoguanidine. Lab Invest 60: 486–489
7. Soulis Liparota T, Cooper M, Papazoglou D, Clarke B, Jerums G (1991) Retardation by aminoguanidine of development of albuminuria, mesangial expansion, and tissue fluorescence in streptozocin-induced diabetic rat. Diabetes 40: 1328–1334
8. Hammes H, Martin S, Federlin K, Geisen K, Brownlee M (1991) Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. Proc Natl Acad Sci USA 88: 11555–11558
9. Cameron NE, Cotter MA, Dines K, Love A (1992) Effects of aminoguanidine on peripheral nerve function and polyol pathway metabolites in streptozotocin-diabetic rats. Diabetologia 35: 946–950
10. Neeper M, Schmidt A, Brett J et al. (1992) Cloning and expression of a cell surface receptor for advanced glycation end products of proteins. J Biol Chem 267: 14998–15004
11. Schmidt A, Vianna M, Gerlach M et al. (1992) Isolation and characterisation of two binding proteins for advanced glycation end products from bovine lung which are present on the endothelial cell surface. J Biol Chem 267: 14987–14997
12. Schmidt AM, Mora R, Cao R et al. (1994) The endothelial cell binding site for advanced glycation end products consists of a complex: an integral membrane protein and a lactoferrin-like polypeptide. J Biol Chem 269: 9882–9888
13. McWilliam B, Ruger B, Neale T, Murray-McIntosh R (1994) Detection of glucose-6-phosphate derived advanced glycosylation end products in diabetic nephrophathy. Kidney Int 46: 939 (Abstract)
14. Brownlee M, Vlassara H, Cerami A (1985) Nonenzymatic glycosylation products on collagen covalently trap low-density lipoprotein. Diabetes 34: 938–941
15. Schmidt F (1961) Enzymatic determination of glucose and fructose simultaneously. Klin Woch 39: 1244–1247
16. Cooper M, Rumble J, Komers R, Du H, Jandeleit K, Chou S (1994) Diabetes-associated mesenteric vascular hypertrophy is attenuated by angiotensin-converting enzyme inhibition. Diabetes 43: 1221–1228

17. Shankland S, Scholey J, Ly H, Thai K (1994) Expression of transforming growth factor-beta 1 during diabetic renal hypertrophy. Kidney Int 46: 430–442
18. Brett J, Schmidt AM, Yan SD et al. (1993) Survey of the distribution of a newly characterized receptor for advanced glycation end products in tissues. Am J Pathol 143: 1699–1712
19. Sell DR, Monnier VM (1990) End-Stage renal disease and diabetes catalyze the formation of a pentose-derived crosslink from aging human collagen. J Clin Invest 85: 380–384
20. McCance DR, Dyer DG, Dunn JA et al. (1993) Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. J Clin Invest 91: 2470–2478
21. Soulis T, Cooper M, Vranes D, Bucala R, Jerums G (1996) The effects of aminoguanidine in preventing experimental diabetic nephropathy are related to duration of treatment. Kidney Int 50: 627–634
22. Nakayama H, Mitsuhashi T, Kuwajima S et al. (1993) Immunochemical detection of advanced glycation end products in lens crystallins from streptozocin-induced diabetic rat. Diabetes 42: 345–350
23. Makita Z, Vlassara H, Cerami A, Bucala R (1992) Immunochemical detection of advanced glycosylation end products in vivo. J Biol Chem 267: 5133–5138
24. Kume S, Takeya M, Mori T et al. (1995) Immunohistochemical and ultrastructural detection of advanced glycation end products in atherosclerotic lesions of human aorta with a novel specific monoclonal antibody. Am J Pathol 147: 654–667
25. Shikata K, Makino H, Sugimoto H et al. (1995) Localization of advanced glycation endproducts in the kidney of experimental diabetic rats. J Diabetes Complications 9: 269–271
26. Nakamura Y, Horii Y, Nishino T et al. (1993) Immunohistochemical localisation of advanced glycation end products in coronary atheroma and cardiac tissue in diabetes mellitus. Am J Path 143: 1649–1656
27. Bucala R, Vlassara H (1995) Advanced glycosylation end products in diabetic renal and vascular disease. Am J Kidney Dis 26: 875–888
28. Soulis-Liparota T, Cooper ME, Dunlop M, Jerums G (1995) The relative roles of advanced glycation, oxidation and aldose reductase inhibition in the development of experimental diabetic nephropathy. Diabetologia 38: 387–394
29. Noshino T, Horii Y, Shiiki H (1995) Immunohistochemical detection of advanced glycation end products within the vascular lesions and glomeruli in diabetic nephropathy. Human Path 26: 308–313
30. Wautier JL, Wautier MP, Schmidt AM et al. (1994) Advanced glycation end products (AGEs) on the surface of diabetic erythrocytes bind to the vessel wall via a specific receptor inducing oxidant stress in the vasculature: a link between surface-associated AGEs and diabetic complications. Proc Natl Acad Sci USA 91: 7742–7746
31. Schmidt AM, Hori O, Chen JX et al. (1995) Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. J Clin Invest 96: 1395–1403
32. Schmidt AM, Hasu M, Popov D et al. (1994) Receptor for advanced glycation end products (AGEs) has a central role in vessel wall interactions and gene activation in response to circulating AGE proteins. Proc Natl Acad Sci USA 91: 8807–8811
33. Abel M, Ritthaler U, Zhang Y et al. (1995) Expression of receptors for advanced glycosylated end-products in renal disease. Nephrol Dial Transplant 10: 1662–1667
34. Lin S, Huang Y (1996) Receptor for advanced glycation endproducts is upregulated within diabetic kidney tissue: potential role in diabetic nephropathy. JASN 7: 1874
35. Hori O, Brett J, Slattery T et al. (1995) The receptor for advanced glycation end products (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: 25752–25761
36. Hammes HP, Brownlee M, Edelstein D, Saleck M, Martin S, Federlin K (1994) Aminoguanidine inhibits the development of accelerated diabetic retinopathy in the spontaneous hypertensive rat. Diabetologia 37: 32–35
37. Kihara J, Schmelzer J, Pudusio J, Curran G, Nickander K, Low P (1991) Aminoguanidine effects on nerve blood flow, vascular permeability, electrophysiology, and oxygen free radicals. Proc Natl Acad Sci USA 88: 6107–6111
38. Yagihashi S, Kamijo M, Baba M, Yagihashi N, Nagai K (1992) Effect of aminoguanidine on functional and structural abnormalities in peripheral nerve of STZ-induced diabetic rats. Diabet Metab 41: 47–52
39. Brownlee M, Vlassara H, Kooney A, Ulrich P, Cerami A (1986) Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. Science 232: 1629–1632
40. Ritthaler U, Deng Y, Zhang Y et al. (1995) Expression of receptors for advanced glycation end products in peripheral occlusive vascular disease. Am J Pathol 146: 688–694
41. Palinsky W, Koschinsky T, Butler S (1995) Immunological evidence for the presence of advanced glycosylation end products in atherosclerotic lesions of euglycemic rabbits. Arterioscler Thromb Vasc Biol 15: 571–582
42. Jerums G, Soulis T, Panagiotopoulos S, Cooper M (1995) In vivo effects of aminoguanidine in experimental diabetes and atherosclerosis. Diabetes Medical International Series 1100: 299–305
43. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D (1994) Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. Arterioscler Thromb 14: 1521–1528
44. Gugliucci A, Bendayan M (1996) Renal fate of circulating advanced glycated end products (AGE): evidence for reabsorption and catabolism of AGE-peptides by renal proximal tubular cells. Diabetologia 39: 149–160
45. Makita Z, Bucala R, Rayfield EJ et al. (1994) Reactive glycosylation endproducts in diabetic uraemia and treatment of renal failure. Lancet 343: 1519–1522
46. Yang CW, Vlassara H, Peten EP, He CJ, Striker GE, Striker LJ (1994) Advanced glycation end products up-regulate gene expression found in diabetic glomerular disease. Proc Natl Acad Sci USA 91: 9436–9440
47. Doi T, Vlassara H, Kirstein M, Yamada Y, Striker G, Striker L (1992) Receptor specific increases in extracellular matrix productions in mouse mesangial cells by AGEP is mediated via platelet growth factor. Proc Natl Acad Sci USA 89: 2843–2877
48. Throckmorton D, Brogden A, Min B, Rasmussen H, Kashgarian M (1995) PDGF and TGF-beta mediate collagen production by mesangial cells exposed to advanced glycation end product. Kidney Int 48: 111–117
49. Vlassara H, Brownlee M, Manogue K, Dinarello C, Pasagian A (1988) Cachetin/TNF and IL-1 induced by glucose modified proteins: role in normal tissue remodelling. Science 240: 1546–1548
50. Kirstein M, Aston C, Hintz R, Vlassara H (1992) Receptor specific induction of IGF-1 in human monocytes by AGEP-modified proteins. J Clin Invest 90: 439–446
51. Skolnik EY, Yang Z, Makita Z, Radoff S, Kirstein M, Vlassara H (1991) Human and rat mesangial cell receptors for glucose-modified proteins: potential role in kidney tissue remodelling and diabetic nephropathy. J Exp Med 174: 931–939
52. Vlassara H, Li YM, Imani F et al. (1995) Identification of galectin-3 as a high-affinity binding protein for advanced glycation end products (AGE): a new member of the AGE-receptor complex. Mol Med 1: 634–646
53. Wautier JL, Zoukourian C, Chappey O et al. (1996) Receptor-mediated endothelial cell dysfunction in diabetic vasculopathy. Soluble receptor for advanced glycation end products blocks hyperpermeability in diabetic rats. J Clin Invest 97: 238–243