Levels and Molecular Size Distribution of Serum Laminin in Adult Type I Diabetic Patients With and Without Microangiopathy

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The glycoprotein laminin, a cross-shaped complex of three genetically different polypeptide chains, is a structural component of the capillary basement membrane. Serum laminin concentrations of healthy controls (n = 60) and adult type I diabetic patients (n = 170) were not age-dependent. Laminin was correlated with hemoglobin A_1 (HbA_1) values in normoalbuminuric patients (r_s = .33, $P < .0005$, n = 116). Type I diabetic patients without nephropathy or retinopathy in good metabolic control had normal laminin levels. However, increasing stages of microangiopathy were associated with higher laminin levels. The molecular size distribution of serum laminin of control subjects (n = 4) and type I diabetic patients (n = 15) was analyzed by molecular-sieve chromatography. Laminin was eluted in two peaks with a molecular mass of 900 and 300 kd, most likely representing intact laminin and its P1 fragment, respectively. The areas of the two peaks were determined by two-gaussian function fitting. In patients without microangiopathy in poor metabolic control, an increase in the high-molecular weight (HMW) fraction could be detected as compared with healthy subjects and patients with acceptable metabolic control. Furthermore, the HMW laminin fraction and the ratio between the areas of the first and second peak increased with the stage of nephropathy ($P < .001$, Jonckheere-Terpstra test). These results provide evidence that (1) laminin concentration is increased in chronic hyperglycemia, (2) laminin may be a marker of microangiopathic lesions, and (3) elevated laminin levels may reflect an increased synthesis and/or a defective incorporation of laminin into the capillary basement membrane.
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BASEMENT MEMBRANES, the very thin, sheet-like structures of extracellular matrices, are composed of different collagenous and noncollagenous proteins such as collagens, heparan sulfate proteoglycans, laminins, and nidogen forming homotypic or heterotypic complexes.[1-9] Their supramolecular organization is crucial for providing mechanical strength, regulating cell attachment, growth, and differentiation, and building a filtration barrier.

Laminin, the most abundant noncollagenous component, and collagen IV, the major structural collagenous component of basement membranes, form homopolymeric networks. Diabetes mellitus leads to characteristic changes in the metabolism of these basement membrane proteins. However, the underlying cellular and molecular mechanisms of diabetic complications are poorly understood.[10] Increased amounts of collagen IV and decreased amounts of heparan sulfate proteoglycan and laminin were detected in diabetic basement membranes from humans and animals.[3,4,11-16] These findings are in agreement with the known morphological and functional changes of diabetic glomerular basement membranes, ie, basement membrane thickening and a decrease of its size- and charge-selective filtration properties. It is unclear whether these changes are based on an altered production and/or degradation of basement membrane components or are due to defective incorporation of these components into the basement membrane.

Laminin is a complex of three genetically different polypeptide chains (A, B1, and B2) that exist in genetic variants thus forming different laminin isoforms[9] with a molecular mass of approximately 850 to 900 kd.[17] The cross-shaped laminin molecule is built by three subunits forming a coiled-coil structure, and it has binding sites for collagen type IV, heparan sulfate proteoglycan, adhesive cells, and other laminin molecules.[17-19] Thereby, laminin plays a crucial role in the molecular organization of the capillary basement membrane.

Laminin levels can be measured in serum by radioimmunoassay.[20-22] After separation by column chromatography, high- and low-molecular weight (HMW and LMW) laminin fractions can be detected, which may most likely represent intact laminin or some oligomeric variants and degradation products.[21,23,24] In the present study, serum laminin levels of type I diabetic patients (insulin-dependent) without and with microangiopathy were measured to investigate whether the known structural changes of the basement membrane were reflected by serum laminin levels. Furthermore, laminin size heterogeneity was analyzed in sera from patients without and with microangiopathy to evaluate whether laminin synthesis or degradation is altered in diabetes mellitus.

SUBJECTS AND METHODS

Patients

Type I diabetic patients (82 women and 88 men; mean age, 38.5 years; range, 18 to 78; duration of diabetes, 17.0 years; range, 1 to 45; age at onset of diabetes, 22.0 years; range, 17 to 47) participated in the study after providing informed consent. All patients regularly attended the outpatient clinic of the Medical University Clinic of Heidelberg or the Diabetes Center of St. Josefs Hospital. They had no severe diseases such as cardiac insufficiency, cirrhosis of the liver, or infectious diseases or a history of nondiabetic kidney diseases.

Fundoscopy was performed every half-year. Retinopathy was classified as stage 0 (no pathological changes), stage I (background retinopathy), and stage II (proliferative retinopathy). Stages of nephropathy were defined according to Mogensen et al.[25] Stages I/II, III, and IV are characterized by normal albumin excretion (<20 mg/L), microalbuminuria (20 to 200 mg/L), and macroalbuminuria (>200 mg/L), respectively. An additional elevation of serum creatinine is present in stage V diabetic nephropathy. The classification was based on at least three measurements in the first morning urine sample, after excluding other reasons for increased albumin excretion.

Retinopathy stage I or II was present in 41% and 18% of type I diabetic patients. Nephropathy stage I/II, III, IV, or V was detectable,

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respectively, in 68.2% (women, 69.5%; men, 67.0%), 11.2% (women, 8.5%; men, 13.6%), 10.0% (women, 12.2%; men, 8.0%), and 10.6% (women, 9.8%; men, 11.4%) of 170 diabetic subjects under investigation. The 116 patients with nephropathy stage I/II had a mean age of 35 years (range, 18 to 66), a mean duration of diabetes of 14.0 years (range, 1 to 45), a mean hemoglobin A_1 (HbA_1) value of 8.5% (range, 5.2% to 12.0%), and a mean serum creatinine concentration of 0.9 mg/dL (range, 0.6 to 1.3).

The subgroup of patients without retinopathy (n = 67) had a mean age of 33.0 years (18 to 60), a duration of diabetes of 9.0 years (1 to 32), and HbA_1 8.4% (5.2% to 12.0%). The patients with retinopathy (n = 49) were older (46 years; 18 to 66) and suffered from diabetes for a longer time (19.0 years; 1 to 45), but had similar metabolic control (HbA_1 8.4%; 5.4% to 11.9%).

Control Group

Sixty nondiabetic apparently healthy individuals from the clinic staff (28 females and 32 males; mean age, 39 years; range, 16 to 65) served as controls. The age and sex distribution was comparable to that of the patient group.

Routine Measurements

HbA_1 was determined in all patients every 4 months by column chromatography (HbA_1 test combination; Boehringer, Mannheim, Germany) and had an upper reference limit of 8%. The serum creatinine level was measured by a modified Jaffé method with the Chem1 clinical chemistry analyzer (Bayer-Technicon, Tarrytown, NY). The albumin level in urine was measured by immunonephelometry (Nephelometer Analyzer; Behring Werke, Marburg, Germany).

Determination of Laminin

The serum concentration of laminin was quantified with a radioimmunoassay (LAM-RIA; Behring Werke). Antigens in the patient's serum compete with the ^{125}I-labeled P1 fragment from human placenta for the antibodies specific for epitopes of the pepsin-resistant laminin fragment P1. Coefficients of variation for within- and between-assay imprecision were 3.1% and 4.9%, respectively, and the inaccuracy ranged from −12% to +11%. Storage of serum samples at −20°C for a period of up to 1 year did not significantly affect laminin measurements (unpublished data, 1996). According to the manufacturer's instructions, the concentration of laminin is given in milliunits per milliliter because an international standard for laminin is not yet available. The median laminin content has been reported to be 1,300 mU/mL in healthy adults. In a group of obviously healthy subjects, a laminin antigen content of 1,000 mU produced a measuring signal corresponding to about 230 ng laminin.

Column Chromatography

Serum samples (2 mL) were chromatographed on a 1.6 × 100-cm column of Sephacryl S-400 High Resolution (Pharmacia, Uppsala, Sweden) in phosphate-buffered saline, pH 7.2, containing 0.05% Tween 20 with a flow rate of 20 mL/h. The duration of one separation was 10 hours, and a 2232 Microperex pump (Pharmacia) was used to guarantee a constant perfusion of the column. The column was calibrated with a gel filtration standard (Bio-Rad Laboratories, Munich, Germany). The effluent was collected in fractions of 4 mL. Each fraction was concentrated to volumes of about 250 µL (200 to 300 µL) with the Centricon-30 system (Millipore, Bedford, MA) by high-speed centrifugation at 5,000 × *g* applying a transmembrane pressure of 7.65 bar. Two 100-µL aliquots were used for laminin radioimmunoassay, and the results were corrected according to the efficiency of the concentrating procedure.

Evaluation of Column Chromatography Data

After chromatographic separation of the sera, we analyzed the function curves plotting laminin concentration versus effluent volume. We used the trapezoidal rule to numerically integrate the data plot from a baseline of zero. Thereby, the total area under the laminin outflow fraction concentration–effluent volume curve (AUC) was calculated for each serum. Moreover, the molecular size distribution of laminin was determined. Since no baseline separation of the laminin peaks was achieved, a software package was used to fit a curve with multiple gaussian peaks to the data plots (Origin 4.1, 1996; MicroCal Software, Northampton, MA). The implemented gaussian function is as follows: $y = [A/[w \cdot \text{sqrt}(\pi/2)]] \cdot \exp\{-2 \cdot [(x - xc)/w]\hat{}2]$, where xc, w, and A are the center, width, and area of the peak. With this tool, we fitted two gaussian distributions and calculated the AUC of the peaks and the ratio of the HMW to LMW peak of laminin. This curve-fitting analysis overcame the problems of the manual extrapolation method, and was objective. The suitability of this evaluation procedure was assessed by comparing the integral of the data plot with the fitted total AUCs.

Statistical Analysis

The mean ± SD and range are presented. Differences between groups were tested with the Kruskal-Wallis test, Wilcoxon's rank-sum test, or the Pearson χ^2 test (SAS 6.11, 1995; SAS Institute, Cary, NC). Correlations were assessed with a nonparametric procedure, Spearman's rank correlation coefficient (r_s), since this method does not require normal distribution of test scores. The level of significance (P) was set at .05.

The Jonckheere-Terpstra test, an exact nonparametric procedure for several independent samples from a priori–ordered populations (controls and stages of nephropathy), was used to test the hypothesis that, eg, laminin concentrations increase or decrease (two-sided P values) with the stage of nephropathy (SPSS Exact Test 6.1 for Windows, 1995; SPSS, Chicago, IL). In addition, results of the laminin fractionation experiments were evaluated with ANOVA within the framework of General Linear Models (GLM; SAS), since the sample size was small and the data were unbalanced. For this purpose, patients with nephropathy stage I/II or III and stage IV or V were combined and compared with the control subjects. Pairwise comparisons without α error adjustment are descriptive. The ratio of HMW and LMW laminin area as a main outcome measure was evaluated with Bonferroni-Holm adjustment for multiple comparisons (t test for independent variables) so that these statistical results can be interpreted as confirmatory.

RESULTS

Laminin Levels of Nondiabetic Controls

All control subjects had normal HbA_1 values. The mean laminin concentration was 1,324 ± 116 mU/mL.

Laminin Levels of Type I Diabetic Patients With Normoalbuminuria

Laminin levels of patients without renal late complications (n = 116) were not age-dependent, but showed a weak correlation with the duration of diabetes (r_s = .233, P = .012). No correlation existed between serum laminin and creatinine concentrations (r_s = .07, P = .49). In normoalbuminuric type I diabetic patients, the mean laminin concentration was 1,417 ± 240 mU/mL and the mean HbA_1 level was 8.4% ± 1.5%. Laminin and HbA_1 values were correlated (r_s = .33, P = .0004), and patients with HbA_1 greater than 9% (HbA_1 10.1% ± 0.9%,

n = 40) had higher laminin concentrations (1,503 ± 241 mU/mL) than patients with HbA_1 not greater than 9% (HbA_1, 7.6% ± 1.0%; laminin, 1,372 ± 229 mU/mL; n = 76, $\chi^2 = 7.94$, $P < .005$). Retinopathy was absent in 67 and present in 49 of these patients (Fig 1).

Laminin Levels of Type I Diabetic Patients Without Microangiopathy

Figure 2 shows laminin levels in healthy control subjects and diabetic patients with neither nephropathy nor retinopathy grouped according to metabolic control. Patients without microvascular complications (n = 67) had mean laminin levels of 1,394 ± 246 mU/mL, which were correlated with the HbA_1 values ($r_s = .25$, $P < .05$). Patients in acceptable metabolic control ($HbA_1 \leq 9\%$) had laminin concentrations (1,347 ± 242 mU/mL, n = 26) comparable to those of the nondiabetic controls. However, laminin levels in patients with poor metabolic control (1,468 ± 236 mU/mL, n = 41) were higher than in patients with acceptable metabolic control ($\chi^2 = 5.7$, $P < .02$; Fig 2).

Laminin Levels of Type I Diabetic Patients With Microangiopathy

Normoalbuminuric diabetic patients with retinopathy (n = 49) had mean laminin levels of 1,449 ± 232 mU/mL, which were correlated with the HbA_1 values ($r_s = .46$, $P = .001$).

Type I diabetic patients with background retinopathy and acceptable metabolic control ($HbA_1 \leq 9\%$, n = 35) showed normal laminin levels, whereas patients with poor metabolic control (n = 14) had significantly higher laminin concentrations (Fig 2). Patients with proliferative retinopathy exhibited significantly higher laminin levels than healthy controls. Laminin concentrations tended to be higher when HbA_1 values were at least 9%, but this difference did not gain statistical significance (Fig 2).

Diabetic patients with nephropathy stage III, IV, and V had significantly higher laminin levels than nondiabetic controls ($P < .0001$; Fig 3). Moreover, patients with stage III and stage IV diabetic nephropathy had higher laminin concentrations than stage I/II patients ($P < .001$ and $P < .002$, respectively). Laminin levels in stage IV were not different from those in stage III ($P = .92$), but were significantly lower than those in stage V ($P = .033$; Fig 3). In patients who developed incipient or overt nephropathy, serum laminin levels were no longer correlated with the quality of metabolic control ($.63 < P < .93$). In

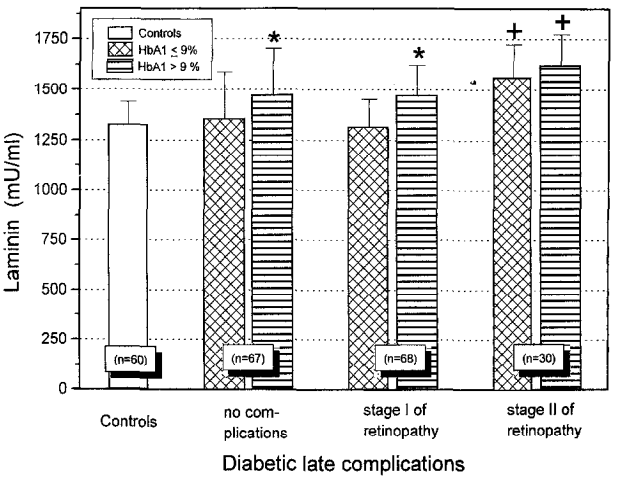

Fig 2. Laminin serum concentrations in healthy control subjects and type I diabetic patients without microvascular complications or with retinopathy stage I/II grouped by HbA_1 level as a measure of metabolic control. *$P < .05$ *v* control group. †$P < .05$ *v* respective group with acceptable metabolic control.

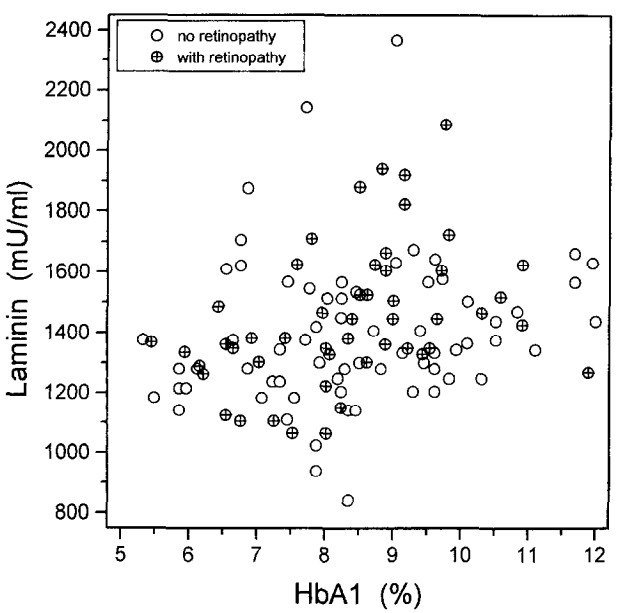

Fig 1. Laminin serum concentrations and HbA_1 values in type I diabetic patients with nephropathy stage I/II (n = 116). Laminin and HbA_1 were correlated in the whole group ($r_s = .32$, $P < .0005$) and in the subgroups without retinopathy ($r_s = .26$, $P < .05$, n = 67) and with retinopathy ($r_s = .46$, $P = .001$, n = 49).

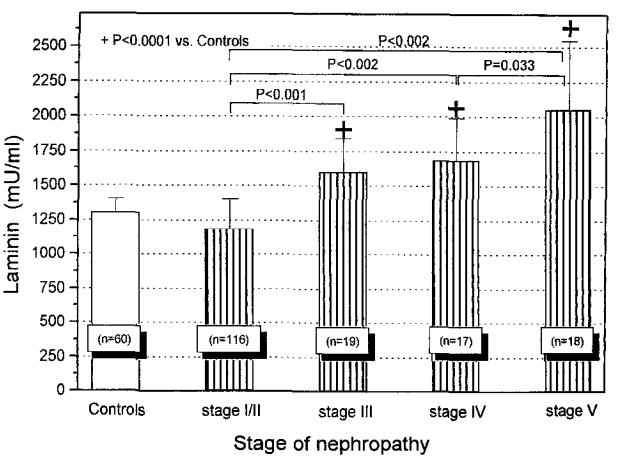

Fig 3. Laminin serum concentrations in type I diabetic patients grouped by stage of diabetic nephropathy. Laminin concentrations in patients with nephropathy stage III, IV, and V were higher than in control subjects ($P < .0001$). Laminin levels were higher in stage IV ($P < .002$) and stage V ($P < .002$) than in stage I/II, and moreover, were higher in stage V than in stage IV ($P = .033$). †$P < .0001$ *v* controls.

patients with nephropathy stage IV or V, laminin concentrations were not correlated with serum creatinine levels.

Molecular Size Distribution of Serum Laminin in Nondiabetic Controls and Diabetic Patients

Figures 4, 5, and 6 represent the elution characteristics of serum laminin from the size exclusion chromatography column. Two gaussian-like elution peaks of laminin with a molecular mass of 900 and 300 kd were separated. This double peak was best fitted with two gaussian functions giving a very good estimate of the proportion of the two laminin fractions. The quality of this fitting procedure may be demonstrated by the high correlation of the AUC calculated by integration of the data plot and the AUC determined by the two-gaussian function fitting (r_s = .998, n = 19). In addition, the mean difference between these two AUC determination methods was negligible (1.4% ± 0.57%, n = 19). Therefore, this objective and valid approach of curve-fitting for data evaluation was chosen.

Laminin concentrations were highly correlated with the AUCs (r_s = .87, $P < .0001$, n = 19). The mean ratio of the total AUC to the laminin concentration was 32.9 ± 1.1, which is near the expected ratio of 32 resulting from the sample volume applied (2 mL) and the 16-fold volume reduction of the elution fraction (4 mL to 250 µL) by means of the Centricon-30 filtration device.

Figure 4 shows the mean molecular size distribution of laminin in the sera of healthy persons (n = 4). The ratio of HMW to LMW laminin was calculated to be 0.64 ± 0.04 in healthy subjects. In type I diabetic patients, two separate laminin fractions with a similar molecular weight distribution could be found. However, the ratio of the HMW to LMW laminin fraction varied with the quality of metabolic control. The data for two normoalbuminuric diabetic patients without retinopathy are shown in Fig 5. The AUC of the LMW laminin peak remained nearly unchanged during a period of poor

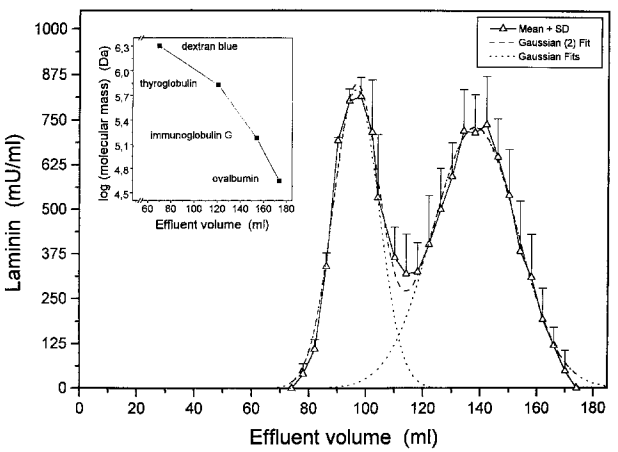

Fig 4. Molecular-sieve chromatography of sera from nondiabetic control subjects and radioimmunological determination of laminin in eluate fractions. The laminin elution profile is shown (Δ), and the positive standard deviation is indicated (n = 4). In addition, the 2-gaussian curve fitting (----) and the 2 underlying gaussian curves (.....) are presented. Inset, calibration of the column, with ovalbumin, immunoglobulin G, thyroglobulin, and dextran blue as molecular weight markers.

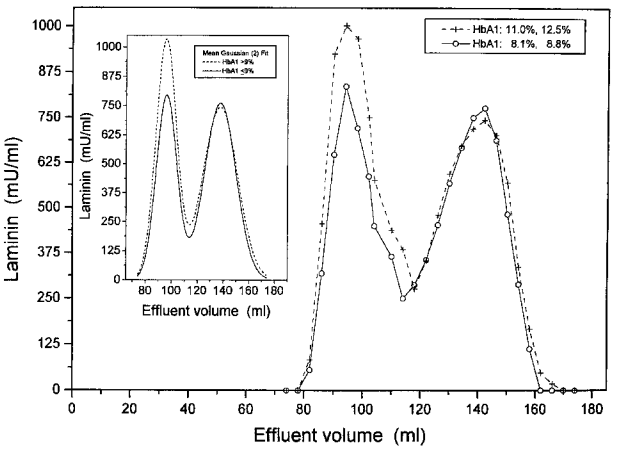

Fig 5. Molecular-sieve chromatography of sera from type I diabetic patients without microangiopathy either with acceptable (○) or insufficient (+) diabetes treatment. Mean values for radioimmunological measurements of column-fractionated laminin from 2 patients are shown. The mean ratio of peak areas during periods of acceptable ($HbA_1 \leq 9\%$) and poor ($HbA_1 > 9\%$) metabolic control was 0.67 and 0.82, respectively.

metabolic control (HbA_1, 11.0%; 12.5%), whereas the AUC of the HMW laminin peak was significantly higher than during a period of acceptable metabolic control (HbA_1, 8.1% ± 8.8%). Therefore, the mean ratio of the area of the HMW laminin peak to the LMW peak increased from 0.67 (0.65; 0.70) to 0.82 (0.73; 0.90) during deterioriation of metabolic control. Figure 6 demonstrates the serum laminin size heterogeneity at different stages of diabetic nephropathy. At progressing stages of nephropathy, the HMW laminin peak increased distinctly. The LMW peak showed no increase or only a minor increase, so the ratio of the area of the HMW laminin peak to the area of the LMW peak increased with the stage of nephropathy (Table 1). The ratio of the peak areas was significantly different between these five subject groups (ANOVA: $df = 4$, F = 6.92, $P < .005$, n = 17). The Jonckheere-Terpstra test (exact significance) con-

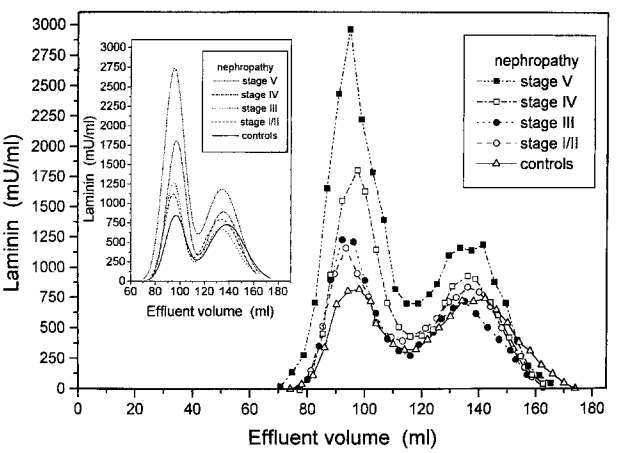

Fig 6. Molecular-sieve chromatography of serum laminin from nondiabetic controls (Δ, n = 4) and type I diabetic patients with nephropathy stage I/II (○, n = 4), stage III (●, n = 3), stage IV (□, n = 3), and stage V (■, n = 3). Mean values are shown. Inset, the corresponding 2-gaussian curve fits.

Table 1. Molecular Size Distribution of Serum Laminin in Control Subjects and Type I Diabetic Patients at Various Stages of Nephropathy (Mean ± SD)

Group	No. of Subjects	HbA_1 (%)	Laminin (mU/mL)	Total AUC (mL · mU/mL)	HMW AUC (mL · mU/mL)	LMW AUC (mL · mU/mL)	AUC Ratio
Controls	4	5.2 ± 0.3‡§	1,248 ± 56‡	44,022 ± 6,177*	17,059 ± 2,088‡	26,963 ± 4,220‖	0.636 ± 0.041‡
Stage I/II	4	8.0 ± 1.4	1,504 ± 401*	48,341 ± 10,726‡	20,462 ± 6,970‡	27,905 ± 6,098*	0.740 ± 0.224†
Stage III	3	8.5 ± 0.6	1,712 ± 407*	45,353 ± 4,868‡	22,370 ± 6,224‡	22,983 ± 1,379*	0.987 ± 0.339†
Stage IV	3	9.0 ± 1.3	1,913 ± 64	62,021 ± 5,520	33,631 ± 2,788	28,390 ± 4,815	1.210 ± 0.234
Stage V	3	8.7 ± 0.4	2,462 ± 516	97,539 ± 11,978	55,924 ± 8,503	41,615 ± 4,209	1.344 ± 0.143

NOTE. Patients with nephropathy stage I/II or III and stage IV or V were grouped for statistical comparison of mean levels with ANOVA. The AUC ratio of the control group ($P = .0002$) and patients with stage I/II or III nephropathy ($P = .0093$) differed significantly versus patients with stage IV or V, whereas control subjects and patients with stage I/II or III had similar ratios ($P = .18$).

*$P < .05$, †$P < .01$, ‡$P < .005$: *v* stage IV/V.

§$P < .001$ *v* stage I/II/III.

‖Nonsignificant.

firmed the hypotheses that the stage of diabetic nephropathy is associated with laminin concentration ($P < .001$), total laminin AUC ($P < .0005$), HMW laminin AUC ($P < .0001$), ratio of HMW to LMW laminin AUC ($P < .0005$), and HbA_1 ($P < .01$). In contrast, this test procedure failed to show any relation between the LMW laminin peak area and the stage of renal disease ($P = .15$).

DISCUSSION

The aim of the study was to investigate the laminin concentration and metabolism in type I diabetic patients and its possible role as an early indicator of the diabetes-induced disturbance of the capillary basement membrane. Therefore, we measured serum concentrations of laminin in adult type I diabetic patients without and with late complications. In addition, the molecular size distribution of laminin in the serum of these patients was characterized by size-exclusion chromatography.

In accordance with the data of Niemelä et al[23] but in contrast to other reports on Japanese patients,[26-28] the present results demonstrate that neither in adult nondiabetic controls nor in adult type I diabetic patients were laminin levels age-dependent. Elevated laminin levels have been reported in microalbuminuric[29] and in 18 normoalbuminuric[30] type II diabetic patients. In the sera from our type I diabetic patients, laminin levels were already increased in patients without microalbuminuria. In these 116 patients, laminin levels were positively correlated with HbA_1 values. In patients with poor metabolic control, laminin levels were significantly higher than in patients with an acceptable HbA_1 value. These data are in agreement with the reports of Danne et al[31,32] showing an elevation of laminin in greater than 23% of diabetic children with poor metabolic control.

However, the influence of metabolic control decreased with progression of diabetic vascular complications. Differences in laminin levels between type I diabetic patients with good metabolic control and those with poor metabolic control were no longer significant when microalbuminuria or macroalbuminuria were present.

In type I diabetic patients, we demonstrate a relation of the laminin level with the stage of nephropathy. As compared with the controls, diabetic patients had significantly higher laminin levels already at the stage of microalbuminuria. In contrast, Högemann et al[33] could not find significantly different laminin levels in patients with diabetic microangiopathic lesions (n = 14) compared with those without clinical signs of microangiopathy (n = 12).

Laminin and serum creatinine concentrations were not correlated in our type I diabetic patients at stage IV and V nephropathy. These data are in agreement with those of Horikoshi and Koide,[26] but are in contrast to those of Pietschmann et al.[34] A positive correlation would attest to an influence of the glomerular filtration function on the laminin level. However, glomerular filtration of a molecule with a molecular weight of 900,000 through the glomerular basement membrane should be associated with a complete loss of size selectivity and severe proteinuria. The association between high serum levels of laminin and proliferative retinopathy, as well as incipient or overt nephropathy, may indicate that laminin is a marker of the development of microangiopathy.

Size-exclusion chromatography followed by radioimmunological laminin measurement revealed that serum laminin is not homogenous. We observed two gaussian-like elution peaks of laminin characterizing the molecular size of these two laminin proportions. The width of the second elution peak was larger than that of the first peak. This peak-broadening might be explained by a longer retention time. However, it cannot be excluded that a true polydispersity in the samples is responsible for this observation. One elution fraction had a molecular mass of 900 kd, most likely representing intact laminin molecules, and the second fraction had a molecular mass of 300 kd. Other study groups[21,24] found similar results in healthy subjects or tumor patients. Brocks et al[21] found HMW laminin antigens and a smaller laminin fragment that coeluted with the laminin P1 fragment that has a molecular mass of 300 kd. However, both laminin molecules eluted before a globular protein of 670 kd when using a Biogel A5m column (Biogel Technology, Indianapolis, IN) for molecular-sieve chromatography. In the present study with high-resolution chromatography media, two separate laminin fractions with molecular weights of 900 and 300 kd could be demonstrated with an expected elution profile. The size of the smaller laminin fragment we found in diabetic patients and healthy subjects corresponds to that of laminin P1 and E8. The pepsin-digested laminin P1 fragment contains the rod-like portions of the short arms of the molecule. The laminin E8 fragment consisting of the carboxy-terminal half of the long arm of laminin is obtained after elastase digestion of the molecule.[8,35]

However, in the present investigation, the laminin determina-

tion was performed with an assay that uses antibodies specific for the P1 fragment. Therefore, we presume that the 300-kd antigen is the P1 fragment. Diabetic patients with good metabolic control and without vascular late complications had normal serum laminin concentrations and a similar ratio of HMW to LMW laminin fractions as in healthy subjects. However, metabolic control and severity of microangiopathy were associated with a disproportionate increase of intact laminin. The above-mentioned increase in total serum laminin levels in patients with poor metabolic control and/or microangiopathy is predominantly caused by an increase in the HMW laminin fraction. Jonckheere-Terpstra test statistics for the association between stage of nephropathy and laminin, HMW laminin, and the ratio of HMW to LMW laminin were highly significant, whereas the test did not show an association between the LMW laminin peak and the stage of nephropathy.

These results argue against an increased degradation of laminin, and might be explained with either an upregulated production of laminin or a reduced incorporation of laminin into the basement membrane. Under hyperglycemic conditions, tissue levels of mRNA coding for the sequence of the laminin molecule were elevated as compared with normal metabolic conditions.[36-38] A reduced incorporation of laminin into the basement membrane might be proved by comparing the amount of laminin in the basement membrane of healthy persons versus diabetic patients. In fact, Shimomura and Spiro[4] found 30% to 60% less laminin in the basement membrane of diabetic patients versus nondiabetic controls. Karttunen et al[12] were able to show that laminin was reduced in the kidney cortex of diabetic patients. These findings support the assumption of reduced incorporation of laminin into the basement membrane independent from the dispute concerning increased or unchanged laminin production.

Yurchenco et al,[39] Tarsio et al,[19] and Charonis and Tsilbary[40] demonstrated with in vitro experiments that nonenzymatic glycosylation of laminin leads to a significant reduction of its capacity to interact with other molecules. It remains to be clarified whether this mechanism is of any importance in vivo. The period between laminin synthesis and its incorporation into the basement membrane, during which nonenzymatic glycosylation could happen, is considerably shorter than the period used in the in vitro experiments. On the other hand, it can be assumed that in vivo, newly synthesized laminin molecules interact with binding partners in the basement membrane that are already glycated during chronic hyperglycemia and therefore might lead to a reduced incorporation of laminin into the basement membrane. Prospective longitudinal studies are needed to clarify whether laminin or laminin fragments might be useful as indicators of early diabetic microangiopathy or even as predictors of progression of diabetic nephropathy.

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