

HYALURONIC ACID ACCUMULATION AND REDISTRIBUTION IN REJECTING RAT KIDNEY GRAFT

Relationship to the Transplantation Edema

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Hyaluronan (hyaluronate or hyaluronic acid [HA]¹ by older nomenclature [1]) is a linear polymer built up of the repeating disaccharide *N*-acetylglycosamine-glucuronic acid. This glycosaminoglycan is synthesized by mesenchymal cells and is an important stabilizing constituent of the loose connective tissue (2). The kidney matrix of several mammalian species is known to contain glycosaminoglycans and enzymatic degradation has revealed HA as the major component of the renal medulla (3, 4). The accumulation of HA in this region of the kidney may contribute to the mechanical stability of the tubular structures (4). The physico-chemical characteristics of the medullary and papillary matrix may modulate diffusion processes in these structures (5-7). Such effects may be dependent on the unique water-binding properties of HA (2, 8). One aim of the present study was to localize HA in the kidney of the rat by using a sensitive and specific radioassay. Another aim was to study the early response of the renal connective tissue during rejection. Previous studies on the formation and remodeling of the extracellular matrix in transplanted organs have focused mainly on the later stages of rejection and the development of fibrosis. However, the early remodeling process of the matrix may be of importance not only for a later granulation process but also for the function of the graft. We find an excessive accumulation of HA in the inner medullary portion and the papilla of the healthy kidney and a conspicuous progressive accumulation of HA in the interstitial tissue of the cortex and outer medulla during rejection. Furthermore, the progressive accumulation of HA in graft tissue is related to the development of transplantation edema, supporting the concept that abnormal accumulation of HA in interstitial tissue may expand the interstitial space by immobilizing water (9-12).

Materials and Methods

Lewis rats and DA rats (originally derived from Møllegaard, Skensved, Denmark; and from Bantin & Kingham, N. Humberside, UK, respectively) were bred in our animal department. The male rats used for the experiments weighed 150-200 g. They had free access to a standard pellet diet, R3 (ALAB, Sollentuna, Sweden) and tap water.

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¹ *Abbreviations used in this paper:* HA, hyaluronic acid (hyaluronan); HABP, HA-binding protein.

Kidney Harvesting Procedure. 1 h before harvesting the kidney, the donor animal was pretreated with phenoxybenzamine intravenously at a dose of 3 mg/kg body weight. Heparin was given at a dose of 300 IU i.v., 10 min before harvesting. The left renal vein and artery were dissected free. Ligatures were placed around the aorta proximally and distally to the renal vessels. Modified Sack's solution at 4°C was introduced via a catheter into the aorta and the renal vein was cut off. The kidney was perfused with at least 6 ml of perfusion solution for 10 min at a hydrostatic pressure of 70–80 cm of water. The modified Sack's solution consisted of 1.26 g NaHCO₃, 2.30 g KHCO₃, 4.76 g KH₂PO₄, 7.41 g K₂HPO₄, 37.50 g mannitol, and 1,000 ml sterile water. The kidneys were stored at 4 ± 1°C in Sack's solution until transplanted.

Transplantation Procedure. The recipient's left femoral vein was catheterized for continuous infusion of saline at a rate of 0.5 mg/h per 100 g body weight. The left kidney was mobilized and removed after clamping of the renal vein and artery. These vessels were then prepared using the "cuff" technique as described elsewhere (13). In brief, short polyethylene tubes ("cuffs"), ~3 mm in length, were inserted over the vessel of the recipient. The transected ends of the vessels were subsequently folded back over the tubes and fixed with ligatures around these cuffs and vessels. The arterial cuffs were made of nylon (ID 0.75 mm, OD 0.94 mm) and the vein "cuffs" were made of polyethylene tube PP 190 (ID 1.19 mm, OD 1.70 mm). The anastomosis was performed by slipping the graft vessels over the cuffs and tying them in position with one circular ligature. To avoid warming during the anastomosing procedure, the graft was placed in a Lucite cup filled with crushed ice.

Syngeneic graft transplantation was performed between Lewis rats and allogeneic graft transplantation with DA rats as donors and Lewis rats as recipients. 2, 4, and 6 d after transplantation, the anesthetized rats were exsanguinated. The kidney graft and the recipient's own kidney were immediately removed and used for either histopathological examination or for tissue analysis of total graft HA and water content.

Kidney Tissue Preparation, Extraction of Tissue Hyaluronan, and Calculation of Water Content. All preparations were made immediately after the animal's death. The kidneys and renal grafts were divided by a transversal cut. One part of the specimen was used for histopathology and fixed in buffered 4% formalin, pH 7.3, with 1% cetylpyridiniumchloride and stored at room temperature until embedded in paraffin and sectioned. The other part of the specimen was analyzed for HA and water content and was weighed immediately on filter paper at room temperature (wet weight [ww]) and later after lyophilization at -80°C for 4 d (dry weight [dw]). In one series of experiments, kidneys from healthy animals ($n = 9$) and allogeneic renal grafts ($n = 4$) harvested on day 6 after transplantation were microdissected as previously described (14) into cortex, outer stripe of the outer zone (outer medulla), and inner stripe of the outer zone (inner medulla) and inner zone (papilla). The microdissected renal specimens were analyzed for HA and water content as described above. The relative water content of kidney specimens were calculated according to the formula: $100 \times [(ww - dw)/(ww)]$. The HA was extracted from the pulverized dried renal tissue with 0.5 M NaCl. 20 mg of the kidney material were extracted with 2 ml of the buffer for 16 h with constant shaking at 4°C. The samples were then centrifuged for 15 min at 2,000 g. The supernatants were recovered and the HA concentrations analyzed in duplicate with a radiometric assay (Pharmacia Diagnostics, Uppsala, Sweden), according to the principles previously outlined (15). Briefly, 100 μ l sample or standard was mixed with 200 μ l ¹²⁵I-labeled protein (HABP, with specific affinity for HA and isolated from bovine nasal cartilage) and incubated for 60 min at 4–7°C. 100 μ l HA-Sepharose at a concentration of 1 mg/ml was then added and the tubes were incubated for a further 45 min at the same temperature. 2 ml of washing solution were added and the HA-Sepharose was recovered after centrifugation at 2,000 g for 10 min. Bound radioactivity in the pellet was measured in a gamma counter. Known amounts of HA were used in order to construct a standard curve. The radioactivity was plotted as a function of HA concentrations in the samples. The variability of the assay was <10%.

Histochemical Investigations. An avidin-enzyme, biotin-protein system was used for the detection of HA in tissue. The method is based upon the specific interaction between HA and the protein core of the cartilage proteoglycan (16). The technique consists of: (a) the specific binding of biotin-labeled protein to HA in the fixed tissue, (b) the binding of an avidin-enzyme complex to the biotin, and (c) visualization of bound enzyme with a substrate.

The HA-binding region of the cartilage proteoglycan (HABP) was prepared by affinity chromatography on HA-Sepharose as described earlier (17). The purified protein was then linked to biotin according to the principles outlined by Ripellino and colleagues (18). During the biotinylation, HA was added in order to protect the HA-binding site on the HABP. The HA was then removed by affinity chromatography on HA-Sepharose. HABP-biotin was stored at -20°C at a concentration of 150–160 μg protein/ml until used. HABP binds to HA with high specificity and is not crossreacting with other glycosaminoglycans, e.g., heparin (15, 16).

Before staining for HA, the kidney tissues were dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Serial sections, 5 μm thick, were cut at five levels and mounted on gelatin-coated slides, deparaffinized, and brought through a graded ethanol series to water. The staining for HA was performed according to a modification of the technique described by Ripellino et al. (18). All steps were performed at room temperature unless otherwise stated. The sections were first incubated with 3% H_2O_2 for 5 min to eliminate endogenous peroxidase activity and then with BSA (10 mg/ml, Fraction V, A4503; Sigma Chemical Co., St. Louis, MO) to block nonspecific binding sites. After washes (2×10 min) with PBS, pH 7.3, the sections were incubated overnight at 4°C with HABP-biotin (50–60 $\mu\text{g}/\text{ml}$). After 2×10 min washes in PBS, the sections were incubated with ABC Vectastain Reagent (Vector Laboratories, Burlingame, CA). After 3×10 min washes in PBS, the slides were incubated for 5 min in ethyl carbazole-DMSO (10 mg 3-amino-9-ethylcarbazole; Karl Roth, Karlsruhe, FRG) in 6 ml DMSO (Merck, Darmstadt, FRG) dissolved in a mixture of 40 ml acetate buffer, pH 5.0, and 5 μl H_2O_2 . The sections were mounted under glass coverslips in Kaiser's glycerin-gelatin. The sections were examined by light microscopy by one observer without knowledge of the experimental conditions of the individual animals. The specificity of the reaction was checked with Streptomyces hyaluronidase (Seikagaku Fine Biochemicals, Tokyo, Japan). Control sections were incubated for 4 h in a humidified chamber at 37°C with 500 U/ml of Streptomyces hyaluronidase in 100 mM sodium acetate buffer, pH 5.8, in the presence of protease inhibitors (1.8 mM EDTA; Merck), 1.8 $\mu\text{g}/\text{ml}$ soya bean trypsin inhibitor (Worthington Biochemical Corp., Freehold, NJ), 2.0 mM iodoacetic acid (Sigma), 0.18 mM ϵ -amino-*n*-caproic acid (Sigma), 9.0 mM bensamidine (Sigma), and 1.8 $\mu\text{g}/\text{ml}$ pepstatin A (Sigma).

The increase in the total renal graft water was calculated from the formula: $\text{ww of renal graft} \times 0.01 \times (\% \text{ renal graft water} - \% \text{ water in original kidney})$. The increase in the total HA content of the renal graft was estimated from the formula: $\text{dw of renal graft} \times [\text{HA in renal graft} (\mu\text{g}/\text{g dw}) - \text{HA in original kidney} (\mu\text{g}/\text{g dw})]$. By the use of the same formulas the increases in total water and HA in the microdissected portions of the kidney were calculated.

For histopathologic investigations, sections of kidney tissues were also stained with hematoxylin-eosin. Statistical analyses were performed by the use of Student's *t*-test on groups and paired values.

Results

In the normal kidney, no staining for HA was seen in the glomeruli or the interstitial tissue of the cortex and the outer medulla (Fig. 1, A, B). An intense staining for HA was present in the interstitial space of the inner medulla and the papilla (Fig. 1 C). The demarcation zone between HA positive and negative areas was very sharp and not more than 0.5 mm wide. The adventitia of renal arteries and veins also stained for HA (Fig. 1 D). In the inner medulla and the papilla, there was an even distribution of the HA staining to the entire interstitium and no apparent sub-localization to certain structures, such as vasa recta or Henley's loop (Fig. 2, A–D). The HA staining was present at the level of basal lamina of tubuli but no positive staining was seen in tubular cells or at their intercellular connections (Fig. 2 D). The original kidneys of the animals that received grafts revealed a similar staining pattern as did renal grafts just before transplant after being stored at 4°C for 1 h.

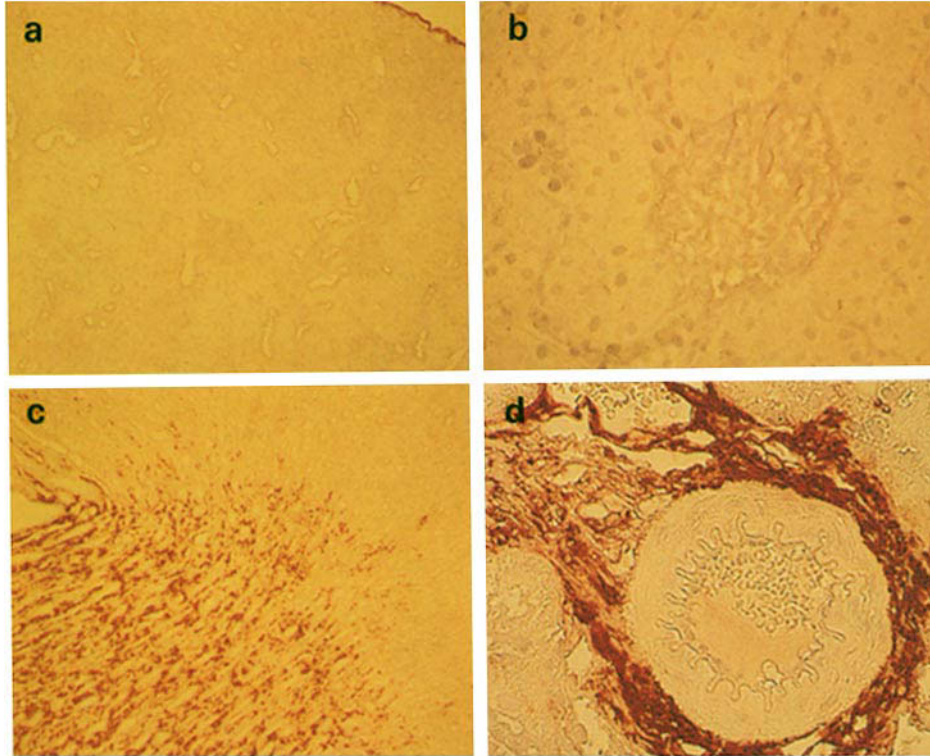


FIGURE 1. (*a-d*) Staining from HA in the normal rat kidney. (*a*) The cortex and the outer medulla show no staining for HA. A positive staining for HA (brown color) is seen in the renal capsule. Hyaluronan was detected by an indirect avidin-biotin-peroxidase technique (see Materials and Methods). The section was weakly counterstained with hematoxylin (original magnification, $\times 100$). (*b*) The thin connective tissue space separating tubuli and mesangium of the glomeruli are negative for HA staining ($\times 400$; weak counterstaining with hematoxylin). (*c*) An intense, evenly distributed staining for HA is seen in the renal papilla. The sharp delineation between the positively stained papilla and the negatively stained outer medulla is obvious ($\times 100$; weak counterstaining with hematoxylin). (*d*) Positive staining for HA is seen in the adventitia of a renal artery ($\times 400$; weak counterstaining with hematoxylin).

During the development of rejection in the allogeneic grafts, progressively larger areas of the cortex stained for HA (Fig. 3, 4). The accumulation of HA was localized to the interstitial tissue but no staining was seen in the glomeruli. It was apparent that HA accumulated in areas with round-cell infiltration and no staining for HA was present in areas without cell infiltration (Fig. 3, *A, B*). A patchy, faint staining for HA in the interstitial tissue of the outer medulla appeared on day 2 after transplantation. The staining intensity in this area increased during the progression of the rejection but no HA appeared in the tubuli cells (Fig. 4). 6 d after allogeneic transplantation the interstitial space had expanded conspicuously and was filled with invaded cells and an evenly distributed HA (Fig. 4, *C and D*). From day 2 to day 6 after transplantation the staining intensity for HA and its localization to the inner medulla and the papilla were similar to those in the control kidneys.

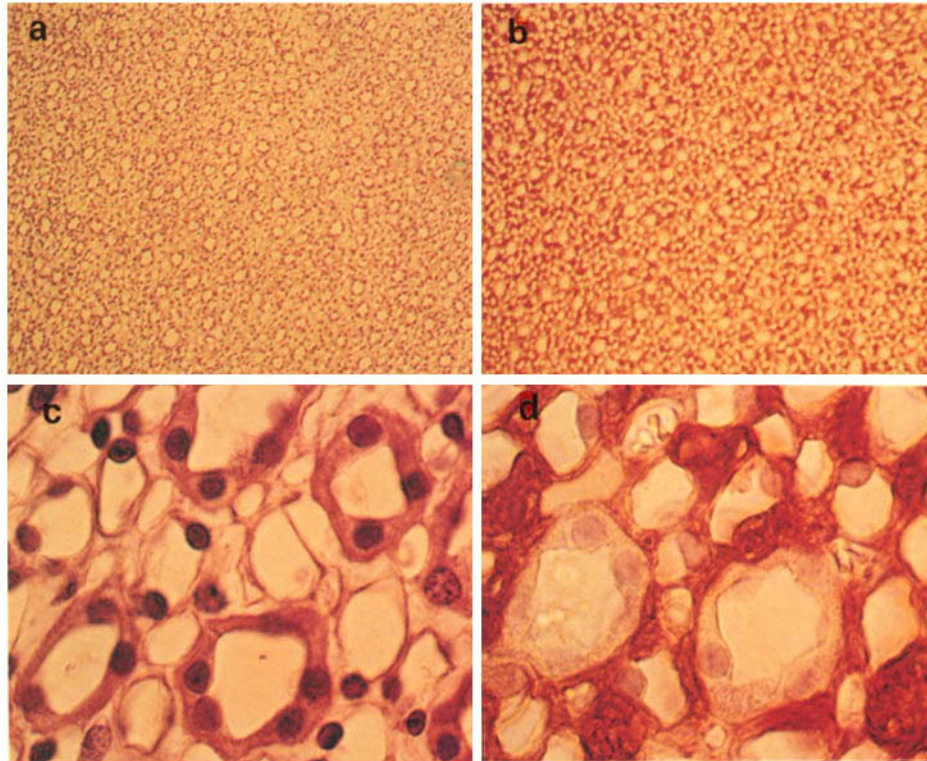


FIGURE 2. (a) The transected normal papilla (hematoxylin-eosin, $\times 100$) has an (b) even distribution of HA positive staining (brown color, $\times 100$). (c) On higher magnification (hematoxylin-eosin, $\times 1,000$ with oil immersion) it is shown that (d) the entire interstitium stains for HA ($\times 1,000$ with oil immersion; counterstaining with hematoxylin). The HA staining is sharply demarcated at the level of the basal lamina of tubuli but not staining for HA is seen in the intercellular connection between tubular cells.

The syngeneic grafts showed by day 2 a patchy inflammatory reaction and a similar stain pattern for HA as the allogeneic grafts did at this time in the cortex and the medulla. However, in contrast to the progressive changes seen in the allogeneic grafts, the abnormal findings in the syngeneic grafts were similar or became less apparent on days 4 and 6.

Determination of Hyaluronan in the Kidney Tissue. The renal HA content of healthy control rats ($n = 6$) was on average 75 ± 9 (SEM) $\mu\text{g/g}$ dry weight. In the syngeneic transplant group the HA content of the graft had increased by day 2 ($n = 4$) and was on average 2.5 times higher than in the controls ($193 \pm 23 \mu\text{g/g dw}$; $p < 0.001$). In the allogeneic transplant group ($n = 4$) the HA content of the grafts had increased to a similar extent by day 2, $184 \pm 16 \mu\text{g/g dw}$. By days 4 and 6 the increase of HA in the syngeneic grafts leveled off (Fig. 5). By contrast, the HA content of the allogeneic grafts increased progressively (Fig. 5) and was by day 6, on average, 4.5 times higher than in the healthy kidney. Significant differences between the HA content of the syngeneic and allogeneic grafts were recorded by day 4 ($p < 0.01$) and

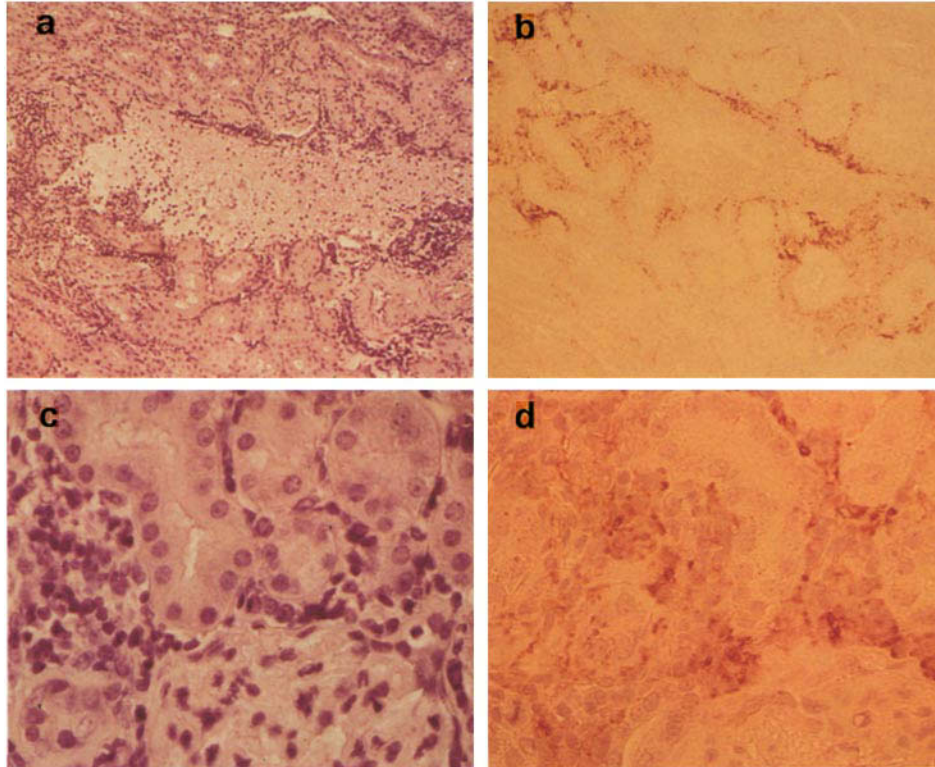


FIGURE 3. (a) 2 d after allogeneic transplantation focal areas in the cortex of the renal graft show a minor round-cell infiltration (hematoxylin-eosin, $\times 100$). (b) Patchy positive staining for HA is seen in the cell-infiltrated areas ($\times 100$; weak counterstaining with hematoxylin). (c) At higher magnification (hematoxylin-eosin, $\times 400$) the cell infiltration is seen in the periglomerular and peritubular interstitial tissue. (d) The HA staining is localized to these cell-infiltrated areas ($\times 400$; weak counterstaining with hematoxylin).

day 6 ($p < 0.001$); by day 6 the HA content of the allogeneic grafts was twice that of syngeneic grafts. The transplantation had no influence on the HA content of the animal's original, nontransplanted kidney (Fig. 5).

Allogeneic grafts ($n = 4$), removed on day 6 after transplantation, and control kidneys ($n = 9$) were microdissected. The cortex and outer stripe of the outer medullary zone in control kidneys contained minute amounts of HA, on average 4 and 6 $\mu\text{g/g}$ dw, respectively (Table I). Measurements in the corresponding portions in the grafts demonstrated on average a 40-fold increase in the HA concentrations; the mean concentration in the cortex was 216 $\mu\text{g/g}$ dw and in the outer stripe of the medullary zone 189 $\mu\text{g/g}$ dw. HA concentrations in the papilla were high and no differences were found between control kidneys and kidney grafts (Table I). In the control kidneys, the HA concentration increased gradually towards the papilla. The control papilla contained more than two times higher ($p < 0.01$) HA concentrations than the papilla of the grafts (Table I).

The Water Content of the Kidney Tissue. The relative water content of the kidneys in the control rats was on average $76.2 \pm 0.5\%$ (SEM). The transplantation proce-

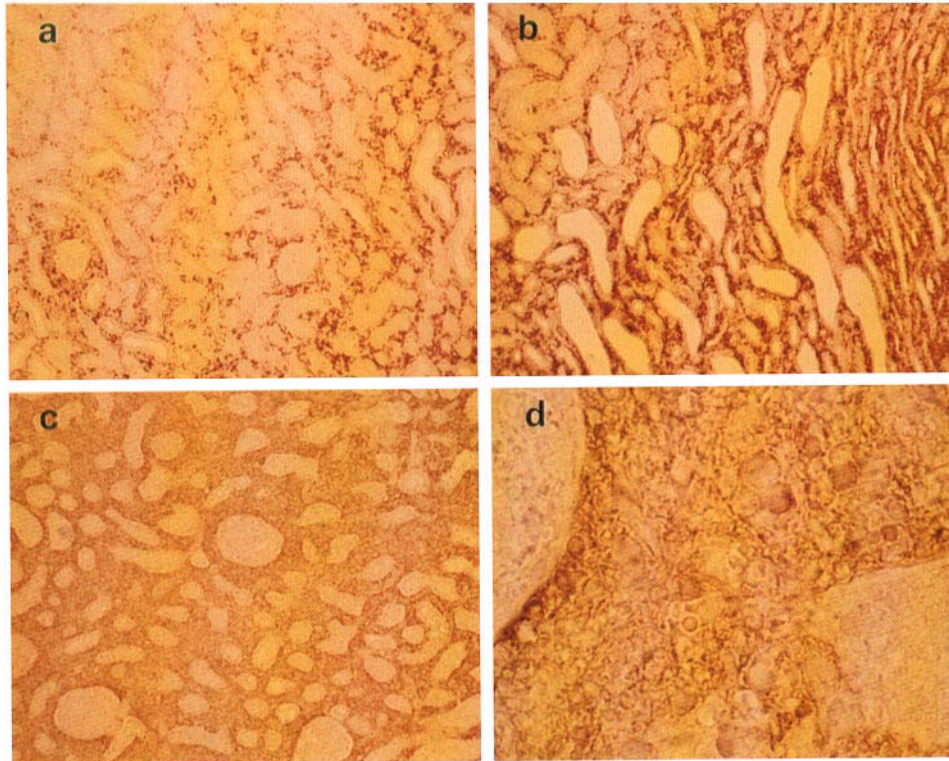


FIGURE 4. (a) 4 d after allogeneic transplantation a positive staining for HA is seen in the interstitial tissue of the entire cortex and the outer medulla but the intensity of the staining varies in different areas. An increased space between the parenchymal structures is present ($\times 100$). (b) The intensity of the HA staining was more apparent in the papilla ($\times 100$) and a delineation zone for the HA staining is still seen between the papilla and the medulla ($\times 100$). (c) 6 d after allogeneic transplantation the parenchymal structures were further separated by an expanding interstitial space evenly stained for HA ($\times 100$). (d) At higher magnification ($\times 1,000$) the swollen, cell-invaded interstitial tissue with accumulated HA is seen. No HA staining is seen in a glomerulus and its capsule (*left*) or in tubular cells (*right*). The sections *a-d* were weakly counterstained with hematoxylin.

dure did not influence the water content of the animal's original kidneys (Table II). The water content of both the syngeneic and allogeneic grafts had increased $\sim 2\%$ by day 2 after transplantation. Thereafter the relative water in the allogeneic grafts increased progressively ($p < 0.01$) to a maximum value of $84.3 \pm 1\%$ on day 6, while in the syngeneic grafts the increase leveled off (Table II). There was a significant correlation between the relative water and HA contents of the allogeneic grafts ($r = 0.51$, $p < 0.01$).

Measurements of the relative water in the dissected nontransplanted kidney portions demonstrated that the relative water content was highest in the papilla. Significant increases in the water content in the dissected allogeneic renal grafts were seen in the cortex and the outer medulla (Table II). The decrease in the water in the papilla of the graft was insignificant.

To estimate the water-binding capacity of accumulated HA during rejection, we

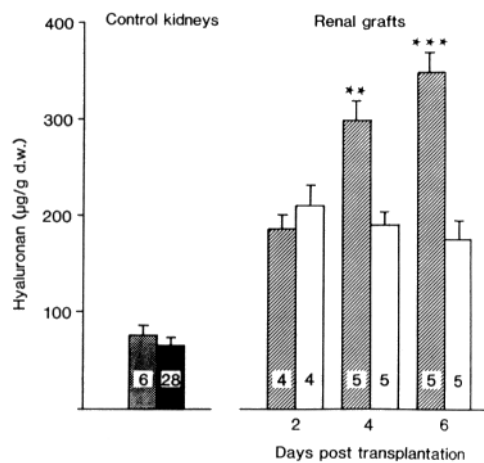


FIGURE 5. The concentrations of HA in allogeneic (*striped bars*) and syngeneic renal grafts (*open bars*) on day 2–6 after transplantation. The statistical differences between the HA contents of the two types of grafts are illustrated; (**) $p < 0.01$, (***) $p < 0.001$ (paired *t*-test). The HA concentrations in kidneys from healthy control rats (*shaded bar*) and from the original nontransplanted kidneys from rats undergoing allogeneic or syngeneic transplantation (*black bar*) are also shown.

calculated the increase in the total HA content and in the total renal water of the whole renal grafts and microdissected portions from cortex and medulla (see Materials and Methods). Based on the assumption that the increases in HA and water occur in the same compartment, the calculated values correspond to an HA concentration in the allogeneic graft (removed on days 4–6), of 0.6 ± 0.1 (SEM) mg/ml increased water volume. The corresponding concentrations in the allogeneic cortical and outer medulla tissues were 0.5 ± 0.1 and 0.6 ± 0.1 mg/ml, respectively.

Discussion

In the healthy rat kidney, HA was predominantly in the interstitium of the inner portion of the medulla and in particular in the papilla, while the interstitial tissue in the renal cortex and the outer medulla showed no staining or a very faint staining for HA. These findings were supported by our specific demonstration of extractable HA from various portions of the kidney. Thus, it was shown that the HA content of the inner medulla and the papilla was 50–100 times higher than that in the cortex and the outer medulla. Accumulation of HA was also seen in the perivascular tissue of large to small-sized vessels in the cortex and medulla. This is in accordance with our previous observations on HA in the adventitia of myocardial and alveolar vessel walls (10, 12) and reflects the established role of HA in the stabilization of the tissue matrix.

The observed accumulation of HA in the renal papillary interstitium is in accordance with previous findings. Ultrastructural studies using ruthenium red fixation and staining have shown that proteoglycans are widely distributed in the interstitium of the renal papilla (4). Analyses before and after enzyme digestion indicate that HA is the dominating glycosaminoglycan (4). Furthermore, it has been reported that intact papilla and isolated renomedullary interstitial cells, the predominant cells of the renal papillary interstitium, synthesize HA as the chief glycosaminoglycan (4). Therefore, the observed HA accumulation in the papilla and the inner medulla should mainly be due to enhanced synthesis and not to limited elimination. The HA in the renal papillary interstitium presumably provides mechanical support for

TABLE I
*Extracted Hyaluronan Amounts and Relative Water Contents
 (Means \pm SEM) of Microdissected Portions from
 Allogeneic Grafts Harvested on Day 6 after Transplantation*

| Portion | Hyaluronan | |
|---|------------------------------|-----------------------------------|
| | Allogeneic grafts (n = 4) | Nontransplanted kidney (n = 9) |
| | $\mu\text{g/g dw}$ | |
| Cortex | 216 \pm 14* | 4 \pm 3 |
| Outer medulla (outer stripe of the outer zone) | 189 \pm 12* | 6 \pm 3 |
| Inner medulla (inner stripe of the outer zone) | 193 \pm 28 | 209 \pm 23 |
| Papilla | 195 \pm 12† | 463 \pm 42 |
| | Water | |
| | % | |
| Cortex | 79.2 \pm 0.3‡ | 71.0 \pm 0.8 |
| Outer medulla (outer stripe of the outer zone) | 78.5 \pm 0.5* | 72.6 \pm 0.9 |
| Inner medulla (inner stripe of the outer zone) | 79.7 \pm 1.1 | 80.5 \pm 1.6 |
| Papilla (inner zone) | 80.0 \pm 1.4 | 82.2 \pm 0.6 |

* $p < 0.001$.

† $p < 0.01$.

‡ Statistical differences between HA or water contents of the different kidney sections from renal grafts and nontransplanted kidneys were tested for by use of Student's *t*-test. The HA amounts in specimens from nontransplanted control kidneys are also given. The extraction of HA was performed from freeze-dried kidney tissue with 0.5 M NaCl.

the tubules and blood vessels. It has also been proposed that the HA accumulation may have a physiological function in the renal concentrating process (5-7). Previous *in vitro* studies have also suggested that HA is the dominant nonsulphated glycosaminoglycan produced by glomerular epithelial and mesangial cells and that HA is a major glycosaminoglycans of the glomerular basement membrane (19, 20). It has been proposed that HA plays a key role in the permeability properties of the glomerular basement membrane (20, 21). We, however, were not able to demonstrate any positive staining for HA in the glomeruli. The minute HA amounts extractable from cortical tissue also indicate that the glomerular synthesis of HA is limited *in vivo*. Differences in results regarding the possible glomerular synthesis of HA may be explained by differences in phenotypical behavior of cells *in vitro* and *in vivo*.

The amounts of extractable HA in kidney grafts increased progressively during the development of rejection and by the sixth post-transplantation day the recovery from allogeneic graft had increased on average fivefold. The microscopic findings demonstrated that HA accumulated mainly in the interstitial tissue of the outer medulla and the cortex. This HA could be removed by specific enzymatic digestion. An accumulation of HA in the outer medulla was apparent from day 2 after transplanta-

TABLE II
Relative Water Contents (Means \pm SEM) of Allogeneic and Syngeneic Renal Grafts at Various Times after Kidney Transplantation in Rats

| Animals | Days after transplantation | n | Water | |
|----------------------------|----------------------------|---|----------------|-----------------|
| | | | Renal grafts | Original kidney |
| % | | | | |
| Allogeneic transplantation | 2 | 4 | 79.5 \pm 0.8 | 76.0 \pm 0.8 |
| | 4 | 5 | 83.3 \pm 0.4 | 75.3 \pm 0.5 |
| | 6 | 5 | 84.3 \pm 1.0 | 75.7 \pm 0.4 |
| Syngeneic transplantation | 2 | 4 | 79.3 \pm 0.6 | 74.9 \pm 0.4 |
| | 4 | 5 | 81.6 \pm 1.2 | 75.7 \pm 0.9 |
| | 6 | 5 | 81.4 \pm 0.5 | 75.2 \pm 0.6 |
| Controls | | 6 | | 76.2 \pm 0.5 |

The water contents in the recipient's original nontransplanted kidneys and in kidneys from healthy control rats are also given.

tion and the intensity and distribution of the HA staining in this part of the kidney remained constant during the progression of the rejection. In the cortical tissue, HA appeared in the edematous interstitial tissue infiltrated with invading cells, but not in the glomeruli. During the first days after transplantation, the accumulation of HA in the cortex was patchy but gradually involved larger areas concomitant with a more general cell invasion of the cortical tissue. It cannot be fully excluded that the accessibility of the probe to HA may be influenced by extracellular matrix remodeling in the transplanted kidney injured by invading inflammatory cells. However, the microscopic findings of an accumulation of HA in the cortex and outer medulla during rejection were confirmed by a biochemical assay of HA in microdissected renal specimens.

The elimination routes of HA in the kidneys are not known but presumably involve lymph drainage to the general circulation and uptake by the liver (22). The appearance of HA in the interstitial tissue of the kidney during rejection may partly be due to surgical damage to the lymph vessels and impaired elimination of HA, since we found that the extractable renal HA increased similarly by the second postoperative day after both syngeneic and allogeneic kidney transplantation. However, whereas the HA accumulation observed during rejection of the allogeneic grafts increased progressively, the HA content of the syngeneic grafts leveled off from day 2 after transplantation. The microscopic findings in syngeneic grafts indicate that the surgical trauma may contribute to a HA localization to the interstitial tissue of the cortex and the outer medulla. Enzymatic degradation of HA may also be of importance for the HA clearance. The enzyme hyaluronidase is known to occur in rat kidney (23). However, its activity is low in the cortex and outer medulla but increases markedly towards the papillary tip (24). Therefore, it seems unlikely that the observed accumulation in the cortex and outer medulla during rejection is due to a reduced hyaluronidase activity. Accumulated data instead indicate that the progressive HA accumulation in the renal cortex is due to an enhanced HA synthesis in the interstitial tissue.

The mechanisms for the enhanced cortical synthesis of HA during rejection are

not known; neither has the cellular source of cortical HA been identified in this study. It is conceivable that the enhanced HA production is a result of activation of the cortical interstitial cells by immune mediators released during the cell invasion of the transplant. The lack of a progressive HA accumulation in the renal medulla and the papilla might thus be explained by the weak cell invasion observed in these parts of the kidney during acute rejection. The early transient increase of HA in syngeneic grafts may also be dependent on inflammatory mechanisms since a patchy cell infiltration was present in these grafts. The inflammatory reaction seen in syngeneic grafts has been attributed to the traumas of the transplantation procedure (25, 26). Lymphokines and macrophage products can influence fibroblast activity (27, 28) and a number of inflammatory products with fibroblast-activating properties have been shown to stimulate fibroblast synthesis of HA in vitro (29–31). Furthermore, a connective tissue response to factors related to immunological and/or inflammatory events is not unique for the kidney or a rejection, but is also seen during rejection of heart graft (12) and in the lung tissue during the inflammatory active phase of alveolitis (9–11). However, it is noteworthy that the glomeruli did not accumulate HA during rejection indicating that not all mesenchymal cells show an enhanced HA synthesis.

The glycosaminoglycans in renal tissue have been implicated in physiological functions such as protein, electrolyte, and water filtration. The marked differences in the content of HA in the cortex and inner medulla and papilla of the healthy rat kidney may suggest that HA in the extratubular stroma provides mechanical support for the delicate tubular structures. The HA accumulation in the tubular interstitium might also influence the cell-to-cell or cell-to-substratum interactions and possibly the organization of the extracellular matrix (32). These effects may play an important role during the early stages of the formation and remodeling of embryonic tissue (32). Earlier studies in the developing chick embryo kidney have in fact demonstrated that hyaluronate accumulates in the immature metanephrosis containing undifferentiated mesenchyme and early stages of tubular epithelium formation. Differentiation of the epithelium into mature tubules occurs over a period when HA concentrations are decreasing (33). A matrix rich in HA may also promote cell infiltration and migration (34, 35). Such effects, central for embryonic development, may be of importance for the tissue reaction during an inflammatory/immunological attack. Thus, the early accumulation of HA in the cortical interstitial tissue during rejection may facilitate cell infiltration and thereby the rejection. Hyaluronan may also influence the rejection due to its regulatory effects on cells involved in inflammation (35–38).

Hyaluronan has unique water-binding properties (2, 8). Correlations observed in this study between HA tissue concentrations and hydration suggest that HA may cause expansion of intercellular spaces. In the healthy rat, the tissue hydration was highest in the HA-rich papilla. During rejection, the water content of the graft increased progressively and in parallel with the HA accumulation. A more intriguing finding was that the renal tissue hydration occurred only in the cortex and outer medulla and paralleled the pronounced HA accumulation in these regions. The average increase in HA and the water content in the cortical tissue of rejection grafts corresponds to an HA concentration of 0.6 mg/ml increased water volume. Previous physico-chemical studies on HA in vitro have shown that when the concentration

of high molecular HA exceeds 0.2 mg/ml, the molecules become entangled, forming a network that occupies the solvent space and excludes large molecules. This phenomenon is called steric exclusion and may influence water transport and osmotic activity in the intercellular matrix (2). Thus, our calculations on the HA and water contents in the graft tissue indicate that the HA accumulation is, theoretically, sufficient to induce steric exclusion and tissue edema. The increase in the water in the renal graft seems to be mainly induced by the rejection. The transplantation procedure may also contribute to the edema since we observed an increase in the graft water also after transplantation of syngeneic grafts. The extratubular accumulation of HA in the papilla may be of importance for the normal papillary function by serving as a retention lattice for the sodium gradient known to be present in this portion of the kidney.

The HA accumulation in the cortical interstitial tissue of rejecting kidney grafts may have clinical significance. Our present data support the concept that excessive HA accumulation in the rejecting kidney is a factor of importance for the transplantation edema. The more moderate accumulation of HA observed in syngeneic grafts was also linked to a less pronounced water accumulation, suggesting that HA also plays a role for the surgically induced tissue edema. An interstitial edema may increase the extracellular pressure and thereby compress the cortical microvasculature, possibly leading to ischemic necrosis. Whether regulation of HA synthesis by different immunosuppressive regimens or enhancing HA elimination by enzymatic treatment would offer clinical benefits remains to be explored.

Summary

By using biotin-labeled proteoglycan core protein and an avidin-enzyme system, hyaluronic acid (HA) was visualized in rat kidney. In the normal kidney, HA was localized in the extracellular space of the inner medulla and increased markedly towards the papillary tip. No staining for HA was seen in the interstitial tissue of the cortex or the outer medulla. During the development of rejection of allogeneic renal grafts, a progressive increase in accumulated HA was seen in the interstitial tissue of the cortex and outer medulla. The extractable amounts of HA increased, on average, 40 times in the cortex and outer medulla; no increase was measured in the inner medulla and papilla. The relative water content of the cortex and outer medulla also increased progressively and correlated with the HA accumulation. The extractable amounts of HA in syngeneic grafts increased by day 2 and then leveled off, indicating that surgical trauma may induce some transient HA accumulation after transplantation. Interstitial accumulation of HA, a glycosaminoglycan with unique water-binding qualities, would presumably influence water transport and osmotic activity and should thereby be implicated in the normal papillary function, but also in the development of the interstitial edema of the cortex and outer medulla during rejection of renal grafts.

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