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Research Article

Nephrin in human lymphoid tissues

E. Åström[a, b], J. Rinta-Valkama[a, b], M. Gylling[a], H. Ahola[a, b], A. Miettinen[a, c], T. Timonen[b] and H. Holthöfer[a, b, *]

[a] Department of Bacteriology and Immunology, University of Helsinki (Finland)
[b] Biomedicum, University of Helsinki, PB 63 Haartmaninkatu 8, 00014 University of Helsinki (Finland),
Fax: +358 9 19125501, e-mail: harry.holthofer@helsinki.fi
[c] Department of Immunology, HUSLAB, Helsinki University Hospital, Helsinki (Finland)

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Abstract. When nephrin, the protein product of *NPHS1*, was cloned, it was proposed to be specific for the kidney glomerular podocytes. Recently, however, new reports have emerged verifying additional nephrin expression sites, particularly the insulin-producing β cells of the pancreas, as well as the central nervous system. In this study, we demonstrate nephrin expression in lymphoid tissues, specifically the tonsil, adenoid and lymph node. Nephrin mRNA expression levels were 4-fold higher in tonsils and adenoids than in thymus or B lymphocytes, and 20-fold higher than in T lymphocytes or monocytes, as shown by quantitative RT-PCR analysis. Anti-nephrin antibodies recognised a specific 165-kDa band in lysates of tonsil and adenoid. In immunofluorescence and immunohistochemichal stainings of adenoid and lymph node sections, nephrin-positive cells were detected in the germinal centres of the lymphoid follicles in a staining pattern typical for interdigitating cells. These results indicate a definite and additional presence of nephrin in lymphoid tissue.

Keywords. Nephrin, germinal centre, tonsil, adhesion molecule, lymphoid tissue, molecular biology.

NPHS1, coding for the nephrin protein, was cloned in 1998 and shown to be the causative gene mutated in congenital nephrotic syndrome of the Finnish type (CNF) [1]. Nephrin is a transmembrane protein, with similarities to cell adhesion molecules, structurally belonging to the immunoglobulin (Ig) superfamily [2]. The predicted amino acid sequence of nephrin is 1241 residues. The gene contains 29 exons, coding for a signal peptide, eight Ig-like domains, a fibronectin III-like domain, as well as a transmembrane and a cytosolic region [1–4]. In addition to the full-length nephrin mRNA, *NPHS1* encodes a spliced α form, lacking the membrane-spanning exon 24, which constitutes a potentially secreted form of nephrin [5].

Nephrin has been localised to the kidney glomerulus, preferentially to the interpodocyte filtration slit area [6–8]. Recently, nephrin was additionally found in the pancreas [9–11], particularly in the insulin producing β cells [10], in specific cells of the brain [9] as well as Sertoli cells in the testis [12]. There are tissue-specific enhancers in the mouse nephrin gene and a new alternative exon 1B of the nephrin gene has been found in the mouse brain [13]. The function of nephrin in these sites has not been characterised in detail.

Nephrin has ten putative N-glycosylation sites on the extracellular domain [1]. The reported molecular mass of nephrin in the kidney is 185 kDa [6, 7, 14], and 165 kDa in the pancreas [10]. Mutations leading to deletion of the cytosolic part give rise to effacement of podocyte foot processes and massive, treatment-resistant proteinuria [1]. Since the intracellular part of nephrin has nine tyrosine residues, nephrin has been proposed to participate in outside-in signal transduction [1, 8]. We and others have shown that nephrin is a lipid-raft-associated molecule with a distinct signalling function in the glomerulus [15, 16]. Nephrin is proposed to extend from adjacent podocytes to

* Corresponding author.

interact in a homotypic manner and thus form the dynamic slit diaphragm of the glomerular filtration barrier [6, 17]. Slit diaphragms are special cell adhesion structures, and many cell junction proteins, together with IQGAP1, MAGI-1, CASK, spectrins, α-actinin [18], P-cadherin [19] and the tight junction protein zonula occludens-1 [20, 21], have been localised to the slit diaphragm area. Other molecules, including the CD2-associated protein first described in T lymphocytes [22], Neph1 [23], densin [24] as well as podocin [25], have also been reported as components of the slit diaphragm.

We have earlier reported expression of nephrin mRNA in rat spleen [8] and preliminary evidence of nephrin mRNA in mouse thymus has been shown [12]. Here we extend these observations with a systematic study of nephrin in human lymphoid cells and tissues by comparing nephrin mRNA as well as protein levels, and localising nephrin expression in the lymphoid tissues.

Materials and methods

Isolation of cells from human peripheral blood. Human peripheral blood mononuclear cells were isolated from buffy coat preparations, obtained from the Central Laboratory of the Finnish Red Cross Blood Transfusion Service, by Ficoll/Hypaque (d = 1.077 g/cm^3; Pharmacia, Uppsala, Sweden) gradient separation according to the protocol provided with the Ficoll/Hypaque reagent. The monocyte population was enriched by 2 h adherence to culture plates. The adherent cells were incubated in RPMI 1640 medium (BioWhittaker, Verviers, Belgium) supplemented with 10% foetal calf serum (Life Technologies, GibcoBRL, Karlsruhe, Germany), 100 U/ml penicillin/streptomycin (Department of Virology, University of Helsinki, Finland), 2 mM glutamine (Department of Virology, University of Helsinki) in a humidified atmosphere with 5% CO_2 at 37 °C. Incubation of adherent cells in Macrophage-SFM medium (Life Technologies, Gaithesburg, MD) for 7 days in the presence of 600 U/ml granulocyte-macrophage-colony-stimulating factor (GM-CSF), gave rise to macrophages [26], whereas the presence of 500 U/ml GM-CSF (Roche Diagnostics GmbH, Mannheim, Germany) and 250 U/ml IL-4 (Roche Diagnostics) for 7 days gave rise to dendritic cells [27]. By a further incubation with added lipopolysaccharide (LPS) (50 ng/ml) or tumour necrosis factor-α (TNF-α) (10 ng/ml) for 48 h, dendritic cells were matured [27] and macrophages were activated [28].

Different lymphocyte populations were isolated from peripheral blood mononuclear cells using a magnetic cell separation system, (Miltenyi Biotec, Bergisch Gladbach, Germany) according to instructions supplied by the manufacturer. For the isolation of T lymphocytes, monocytes and B lymphocytes, fluorescin (FITC)-conjugated antibodies against CD3 (Pharmingen, San Diego, Calif.), CD14 (Dako, Glostrup, Denmark) and CD19 (Becton Dickinson, San Jose, Calif.) were used together with magnetic beads coated with anti-FITC or anti-mouse Ig antibodies. The purity of the isolated cell populations was over 90% as verified by FACS analysis.

Tissue samples. Human thymic tissue was obtained at open-heart surgery at the Hospital for Children and Adolescents, Helsinki University Central Hospital. Human kidney tissue was from cadaver donors. Human tonsil and adenoid tissues were kindly provided by Dr. P. Mattila (Department of Otorhinolaryngology, Helsinki University Central Hospital, Finland). The ethical committee of the University Central Hospital approved the study. The lymph nodes for immunohistochemical stainings were selected from the routine diagnostic biopsy material of the Central Laboratory of Pathology.

Extraction of total RNA. Total RNA was isolated from the cultured dendritic cells, macrophages and the magnetically isolated cells or from frozen pieces of human tissue samples such as thymus, kidney, adenoid and tonsil with the Trizol reagent (Life Technologies, GibcoBRL, Paisley, UK) according to the manufacturer's instructions. Total RNA was treated with DNaseI (Promega, Madison, Wis.) and transcribed to cDNA using Moloney murine leukaemia virus (M-MLV) reverse transcriptase (Promega). For each sample, a control sample was prepared without the reverse transcriptase to confirm the specificity of the signal and exclude DNA contamination.

RT-PCR. The human nephrin (GenBank AF035835) sense (5′-cccatcactacccccggtct) and antisense (5′-gagacaacacgactggcac) primers flanking the transmembrane portion of *NPHS1* were used for the PCR reactions. The β-actin gene (GenBank accession AB004047) served as a control gene.

All PCR reactions were amplified using AmpliTaq Gold DNA polymerase (Perkin Elmer, Norwalk, Conn.) and the following amplification programme: an initial denaturation at 94 °C for 10 min followed by 35 cycles with 94 °C for 30 s, 56 °C for 45 s, 72 °C for 30 s with a final elongation at 72 °C for 5 min. The amplification cycles were reduced to 25 for β-actin because of its abundance. The PCR products were analysed on 0.8% agarose gels.

Sequencing. Specific primer pairs along the human nephrin sequence were used in PCR. The PCR products were run on agarose gels, purified using the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany) and sequenced with Amersham Thermo Sequanase II dye terminator cycle sequencing premix kit (Amersham Pharmacia Biotech, Uppsala, Sweden) and with an ABI Prism 373A sequencer (Perkin Elmer, Applied Biosystems, Forster

City, Calif.) according to the instructions of the manufacturer.

Real-time quantitative PCR. For the amplification of human nephrin with the Taqman quantitative RT-PCR, a FAM-labelled probe (5′-tccacaatgcactggtaagcgcca-3′) was used, together with the sense (5′-caactgggagagactgggagaa-3′) and antisense (5′-aatctgacaacaagacggagca-3′) primers. The ubiquitin-conjugating enzyme (*UbcH5B*) gene (GenBank U39317), used as the endogenous control, was amplified using the sense (5′-tgaagagaatccacaaggaattga-3′) and antisense (5′-caacaggacctgctgaacactg-3′) primer pair as well as the VIC-labelled probe (5′-tgatctggcacgggaccctcca-3′) [29]. The UbcH5B values were used to normalize the amounts of nephrin. The ABI Prism 7700 sequence detector, which was used for the signal detection, was programmed to an initial step of 2 min at 50 °C and 10 min at 95 °C, followed by 40 thermal cycles of 15 s at 95 °C and 1 min at 60 °C.

Production of nephrin antibody. The generation of the affinity-purified Aff338 antibody against human nephrin has been described earlier [24]. Briefly, the antibody was originally raised against the major splicing variant designated as α-nephrin using as antigen the recombinant fusion protein α-435, which lacks exon 24, but carries parts of the extra- and intracellular regions (amino acids 1031–1055 and 1096–1215).

Protein extraction and immunoblot. Crushed frozen parts of thymus, kidney, adenoid and tonsil tissues were solubilised, using a glass homogeniser, in RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate, 50 mM Tris-Cl, pH 8.0) containing complete protease inhibitor (Boehringer Mannheim, Mannheim, Germany) and incubated on ice for 30 min. Cell extracts were centrifuged at 14,000 rpm for 30 min to remove the insoluble material and to collect the supernatant, which was stored at –20 °C until used. Proteins from the magnetically separated cell populations, the monocyte-derived dendritic cells and the monocyte-derived macrophages, isolated from peripheral blood were, extracted from the phenol phase, obtained when isolating RNA with Trizol (Life Technologies) reagent, according to instructions of the manufacturer. Protein concentration in the different protein extracts was determined using the BCA Protein Assay reagent according to instructions supplied by the manufacturer (Pierce, Rockford, Ill.).

Protein extracts, all diluted to the same concentration, were mixed with 4× Laemmli sample buffer and run under reducing conditions on 8–10% polyacrylamide gels using a Protean Minigel electrophoresis system (Bio-Rad Laboratories, Richmond, Calif.). Separated proteins were transferred to nitrocellulose membranes (Amersham, Life Science, Amersham, UK) for 1 h at 100 mA using a Mini Trans-Blot cell (Bio-Rad). After blocking the membranes with 2% skimmed milk (Valio, Valio, Finland) in PBS at room temperature for 1 h, the membranes were immersed overnight at +4 °C with the primary antibodies diluted in 2% bovine serum albumin (BSA) (ICN Biomedicals, Aurora, Ohio) in PBS. The following antibodies were used; affinity-purified anti-human nephrin antibody (10 µg/ml) and the nephrin antibody preabsorbed with the antigen used for immunisations (10 µg). Membranes were washed with PBS-Triton X-100 (0.2%) before the addition of the horseradish-peroxidase-conjugated swine anti-rabbit Ig (1:4000 dilution in 2% skimmed milk in PBS) (Valio). After incubation at room temperature for 45 min and thorough washing with PBS-Triton X-100 (0.2%), the bound antibodies were detected using the SuperSignal West Pico Chemiluminescent substrate kit, according to the manufacturer's instructions.

Immunofluorescence. To study the distribution of nephrin protein in lymphoid tissues, frozen sections of human adenoids were cut at 4 µm and fixed in acetone for 10 min at –20 °C before washings in PBS. The tissue sections were incubated for 1 h with the following antibodies and dilutions (diluted in 10% normal human serum): affinity-purified anti-human nephrin (0.8 mg/ml, dilution 1:20), nephrin antibody absorbed with the antigen used for immunisations (10 µg), FITC-conjugated anti-human CD3 (1:10) (Becton Dickinson), FITC-conjugated anti-human CD19 (1:10) (DAKO) and monoclonal anti-human CD21 antibody (DAKO, clone 1F8). After washes in PBS, the sections were incubated for 45 min with a FITC-conjugated goat anti-mouse Ig (1:50) (Jackson Immuno Research, Baltimore, Pa.) and/or a rhodamine (TRITC)-conjugated goat anti-rabbit Ig (1:150) (Jackson Immuno Research). Before embedding in Immu-mount embedding medium (Shandon, Pittsburgh, Pa.), the sections were washed in PBS. The tissue sections were studied with an Olympus OX50 fluorescence microscope (Olympus Optical, Hamburg, Germany) equipped with epi-illumination and filter combinations for FITC and TRITC. For documentation of the results, an Orca IIIm CCD-camera (Hamamatsu Photonics, Hamamatsu City, Japan) and Openlab version 2.3.3 software (Improvision, Coventry, UK) were used.

Immunohistochemistry. Lymph node sections were deparaffinized in xylene and rehydrated through graded concentrations of ethanol to distilled water. The slides were incubated in a microwave oven in 10 mM Tris-HCl, 1 mM EDTA buffer, pH 9.0 for 20 min. They were allowed to cool and rinsed in phosphate-buffered saline (PBS). The immunohistochemical staining was performed using Lab Vision Autostainer (Lab Vision Corporation, Fremont, Calif.) and ChemMate DAKO Envision

Detection Kit (DakoCytomation, Glostrup, Denmark). The primary antibody against nephrin (diluted 1:500) was incubated for 30 min. Rabbit IgG and antibody dilution buffer were used instead of primary antibody as negative controls. The staining was visualised using ChemMate DAB chromogen (DakoCytomation) and finally the sections were counterstained in Mayer's haematoxylin (Merck, Darmstadt, Germany).

Results

Nephrin mRNA levels in lymphoid cells and tissues. Nephrin mRNA expression was detected in the human adenoid, tonsil and thymus as well as T lymphocytes (CD3+), B lymphocytes (CD19+) and monocytes (CD14+) isolated from human peripheral blood and from monocyte-derived dendritic cells and monocyte-derived macrophages after TNF-α stimulation (Fig. 1). Using the primer pair comprising the sense primer upstream and the antisense primer, downstream of the transmembrane exon, a dominant PCR product of approximately 310 bp could be seen in all samples. A smaller product of around 190 bp, which was also detected in kidney cortex, was found at much lower amounts especially in the T lymphocytes, B lymphocytes, monocytes and monocyte-derived dendritic cells (Fig. 1). These two sizes correspond to the full-length nephrin mRNA and the α-nephrin mRNA lacking the transmembrane region, as verified by sequencing [5]. The sequence from the PCR products was identical to the nephrin sequence in the kidney. The monocyte-derived macrophages, produced in the presence of GM-CSF after 7 days of *in vitro* culture, did not show any nephrin mRNA expression (Fig. 1).

Real-time quantitative RT-PCR was used to compare nephrin-specific mRNA levels. Normalised values of nephrin mRNA expression levels were obtained by comparing them to the amount of ubiquitin-conjugating enzyme UbcH5B mRNA in each sample. In kidney cortex, the nephrin mRNA expression levels were three-to-four-fold higher than in the tonsil and adenoid (Fig. 2). The thymus had a fivefold lower nephrin mRNA level expression compared to tonsil and adenoid. In the T lymphocytes, B lymphocytes, monocytes and monocyte-derived dendritic cells and macrophages, very little nephrin mRNA expression was detectable, whereas in the monocyte-derived macrophages, no nephrin could be detected (Fig. 2).

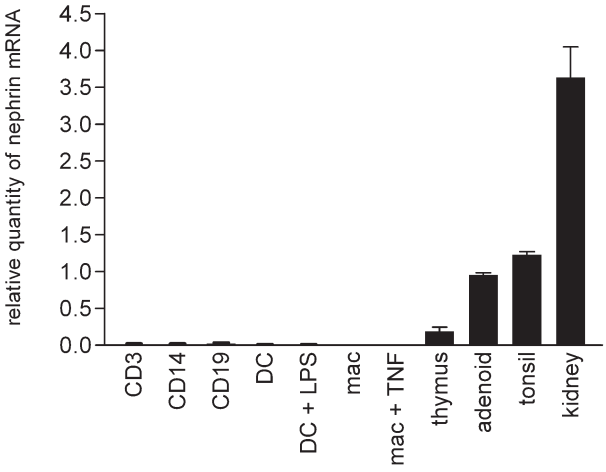

Figure 2. Relative levels of nephrin mRNA in CD3+, CD14+, CD19+, monocyte-derived dendritic cells (DC), monocyte-derived dendritic cells after 48 h LPS stimulation (DC+LPS), monocyte-derived macrophages (mac), monocyte-derived macrophages after 48 h TNF-α stimulation (mac+TNF), thymus, adenoid, tonsil and kidney. The plotted values are relative nephrin mRNA levels normalized to UbcH5B mRNA levels. The values are mean values of five different runs.

Nephrin migrates as a 165-kDa protein. Blotting analysis with the affinity-purified anti-human nephrin antibody identified a 185-kDa as well as a smaller 165-kDa protein band from kidney cortex lysate, and a protein of 165-kDa from human tonsil and adenoid lysates (Fig. 3). No protein band was detected in the lysates from human CD3+, CD14+ or CD19+ cells separated from the periph-

Figure 1. Expression of human nephrin mRNA in lymphoid cells and tissues as detected by RT-PCR. Using the nephrin primer pair flanking the transmembrane portion of *NPHS1*, cDNA from thymus (1), adenoid (2), tonsil (3), monocyte-derived macrophages (4), monocyte-derived macrophages after 48 h TNF-α stimulation (5), CD3+ (6), CD14+ (7), CD19+ (8), human monocyte-derived dendritic cells (9), monocyte-derived dendritic cells after 48h LPS stimulation (10) and human kidney cortex (11) was amplified. Water control in lane 12. The expression levels are compared to the mRNA levels of the housekeeping gene β-actin to verify the viability of the cDNA.

Figure 3. Immunoblot with an anti-nephrin antibody. Lysates of human kidney cortex (1), tonsil (2), adenoid (3), thymus (4), CD3+ (5), CD14+ (6), CD19+ (7), monocyte-derived dendritic cells (8), monocyte-derived dendritic cells after 48 h LPS stimulation (9), monocyte-derived macrophages (10) and monocyte-derived macrophages after 48 h TNF-α stimulation (11) were stained with the affinity-purified anti-human nephrin antibody. The molecular weights of the prestained marker are shown.

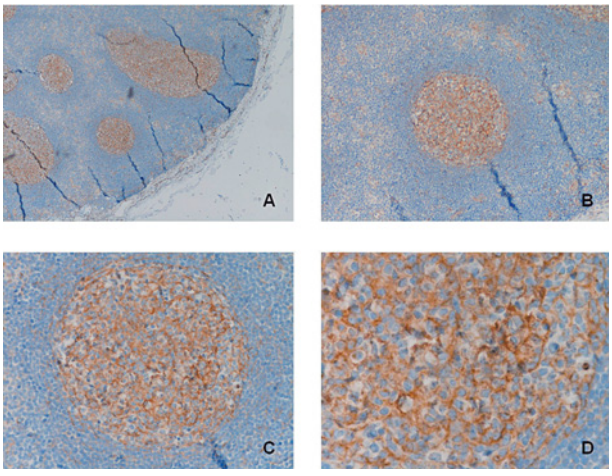

Figure 4. Staining of paraffin sections of human lymph node with the anti-human nephrin antibody shown at different magnifications: ×40 (*A*), ×100 (*B*), ×200 (*C*) and ×400 (*D*). Note concentration of staining in germinal centres.

eral blood (Fig. 3) or from the monocyte-derived dendritic cells or monocyte-derived macrophages (Fig. 3). The signal from the kidney adenoid and tonsil could be blocked with a preincubation of the antibody with the specific recombinant protein used for immunisations (data not shown).

Nephrin localises to the germinal centres of lymphoid follicles. Immunohistochemical staining of lymph node sections with the affinity-purified nephrin antibody showed staining of the germinal centres (Fig. 4). Immunofluorescence staining of frozen sections of human adenoid revealed membrane-type staining of cells in the B lymphocyte (CD19+) areas (Fig. 5d–f). No nephrin-positive cells could be seen in the T lymphocyte region (Fig. 5a–c). The staining pattern is characteristic for interdigitating follicular cells, as verified in the double staining of nephrin and CD21 antibody, a marker diagnostically used for detection of follicular dendritic cells (Fig. 5g–i). The specificity of these stainings was confirmed by performing blocking experiments: preabsorption of the nephrin antibody with the recombinant protein used for immunisations totally blocked these staining patterns (data not shown).

Discussion

Initially, nephrin was proposed to be present exclusively in kidney podocytes [1], but insulin-producing β cells of the pancreas were later also shown to contain this protein [10]. In this study, we analysed nephrin expression in lymphoid cells and tissues and observed nephrin expression specifically in the germinal centres of the lymphoid

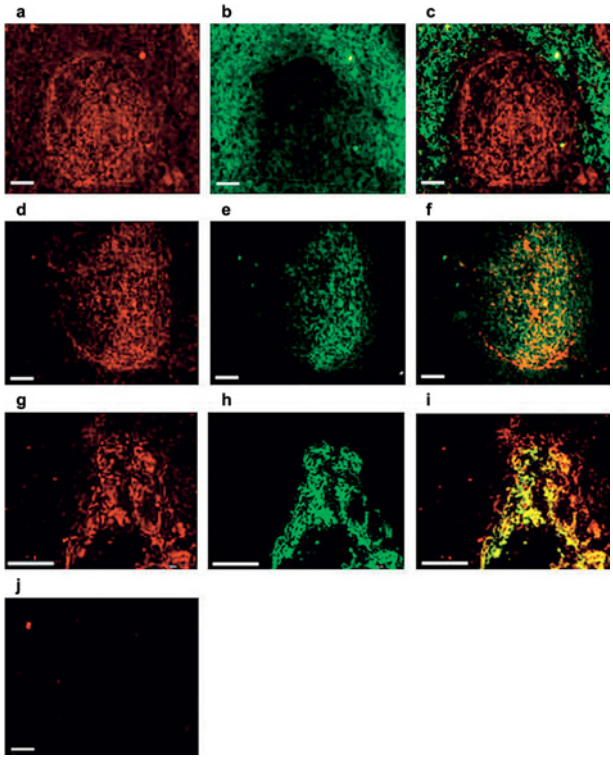

Figure 5. Staining of frozen sections of human adenoid. Adenoid tissue stained with an anti-human nephrin antibody (*a*, *d*, *g*), an anti-human CD3 antibody (*b*), an anti-human CD19 antibody (*e*) and an anti-human CD21 antibody (*h*). Double stainings with overlaid pictures of nephrin and CD3 (*c*), nephrin and CD19 (*f*) and nephrin and CD21 (*i*). An IgG control is also shown (*j*). Scale bars, 500 µm.

follicles in the adenoids and lymph nodes. These results were verified by quantitative RT-PCR of adenoid and tonsil nephrin mRNA, immunoblotting of adenoid and tonsil lysates as well as staining of adenoid and lymph node sections with a nephrin-specific antibody [1].

Our results with the quantitative RT-PCR show at least a three- to fourfold higher expression level of nephrin mRNA in the kidney cortex than in the tonsil or adenoid. A similar difference could also be seen at the protein level. These differences in expression levels may explain why nephrin was originally found only in the kidney glomeruli and thought to be a strictly podocyte-specific protein [1]. Considering its central structural role in the kidney, nephrin may have an important function as a structural protein in the lymphoid tissue as well. No nephrin protein was detected in the cells derived from blood, and the biological significance if any of the low levels of nephrin mRNA in these cells remains to be resolved in detail.

Ten putative N-glycosylation sites have been reported on the nephrin molecule [1]. The molecular weight of nephrin is 135 kDa as predicted by the primary sequence [1], but nephrin in the kidney glomeruli migrates as a 185-kDa [6, 7, 14], and in the pancreas as a 165-kDa [10]

protein in SDS-PAGE. In the adenoid and tonsil, nephrin was detected as a 165-kDa protein corresponding to the size of nephrin found in the pancreas. Deglycosylation studies have shown that the shift in migration in the mouse kidney is due to posttranslational modification [7]. The different migration patterns of the kidney, pancreas and adenoid nephrin can be explained by tissue-specific glycosylation [10] and might also reflect tissue-specific function. Various posttranslational modifications such as differential glycosylation and proteolytic processing patterns are known to suggest functional diversity. For example, podocalyxin found in the kidney podocyte has an anti-adhesive function, whereas in high endothelial venules it functions as a pro-adhesive molecule [30]. Similarly, the diversity in structure and function of the neural cell adhesion molecule is amplified with the existence of various posttranslational glycosylations and splice variants [31]. The use of tissue-specific promoters is known to drive expression of alternatively spliced genes in different tissues, and to contribute to versatility in tissue-specific regulation and function [32]. Interestingly, the alternative use of promoters in different tissues has also been reported for nephrin [13], in addition to the identification of the new mouse brain-specific exon 1B and the existence of specific splicing variants [13], which indicate that nephrin may have distinct functions in different tissues.

A very specific overall staining of the germinal centres of the lymphoid follicle in the adenoid and lymph node sections could be seen with the nephrin-specific antibody. Lymphoid follicles are round or elliptical sites where an intensive B lymphocyte maturation and differentiation as well as T lymphocyte activation occurs [33]. In addition to B and T lymphocytes, the lymphoid follicles contain macrophages, a subset of germinal centre dendritic cells that activate germinal centre T cells [34] as well as a network of follicular dendritic cells, central for the maturation of B lymphocytes [33]. To identify the follicular dendritic cells in the germinal centres, we used an antibody against CD21, the complement receptor 2. This antibody, used diagnostically, is today the most reliable for the identification of follicular dendritic cells. That the nephrin-positive cells in the germinal centres were stained in a manner typical for follicular dendritic cells, as shown by the co-localisation with the CD21-positive cells, and no nephrin protein was detected in the B lymphocytes, T lymphocytes, monocyte-derived dendritic cells and macrophages isolated from peripheral blood, even though the phenotype of these cells in the lymph nodes might change, suggests that nephrin is expressed on the follicular dendritic cells. The partial co-localisation of CD19 and nephrin in the germinal centres can be explained by the presence of B lymphocyte marker CD19 on follicular dendritic cells [35].

Patients born with congenital nephrotic syndrome are seriously ill and show unselective proteinuria already in utero. All groups of circulating proteins are thus lost, including immunoglobulins protecting against infectious inflammatory diseases. Indeed, CNF patients suffer from multiple infections [36]. However, it is difficult to evaluate the role of nephrin or its absence in the follicular dendritic cells of these patients, particularly in the initial steps of immune recognition. Also, such an evaluation of the consequences of the lack of nephrin in follicular dendritic cells is hampered by the general loss of possible modulatory molecules either in the circulation or on the tissues themselves. Furthermore, the general malnutrition starting in utero may lead to severe developmental arrest in response to adequate signals. While, after kidney transplantation, the proteinuria remains under control, the anti-rejection therapy particularly affects the early events in T/B lymphocyte interaction.

What could be the analogy of nephrin expression in the podocyte and in the lymph node and adenoid, if any? In the kidney, nephrin appears to be an important structural and functional adhesion molecule of the interpodocyte slit diaphragm [6, 37]. Co-localisation of nephrin with the cell-junction-associated proteins further implies nephrin as an important adhesion protein maintaining the integrity of the slit diaphragm [18]. In the lymphoid follicles, the follicular dendritic cells have long extending cytoplasmic processes with frequent desmosome-like junctions [38]. The follicular dendritic cells maintain the framework in the lymphoid follicle and offer the surrounding B lymphocytes a stable network for proliferation and differentiation [39]. Nephrin could, similar to its function as a structural adhesion molecule in the slit diaphragm, act in the germinal centres as a cell junction adhesion molecule and structurally support the follicular dendritic cell network.

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