Virchows Arch (1996) 428:5–12


ORIGINAL ARTICLE

Béla Iványi · Stephen J. Hamilton-Dutoit
Hans E. Hansen · Steen Olsen

# Acute tubulointerstitial nephritis: phenotype of infiltrating cells and prognostic impact of tubulitis

Received: 14 November 1995 / Accepted: 4 December 1995

**Abstract** The prognostic impact of tubulitis and the phenotype of the infiltrating cells in the tubules were studied in ten percutaneous renal biopsies from six patients with acute tubulointerstitial nephritis (ATIN). The inflammatory cell subsets in the tubules and interstitium (CD3+, CD4+, CD8+, CD20+, CD45RO+, CD56+, CD57+, CD68+ and TIA-1+ cells), the expression of vimentin and the proliferation-associated antigen Ki-67 by cortical tubular cells, and the grade of tubulitis, interstitial infiltration and fibrosis were analysed. Cytotoxic injury to tubular cells in the vicinity of tubular-wall-localized lymphocytes was studied ultrastructurally. ATIN was drug-induced in three patients, related to *Legionella* infection in two and idiopathic in one patient. Four patients recovered, one with reduced renal function. Two patients developed end-stage renal disease. CD8+ and CD4+ lymphocytes, and a smaller number of macrophages, infiltrated the tubules. The predominant lymphocyte subset in the tubules was the same as in the interstitium. Cytotoxic injury to tubular cells was not seen electron microscopically. The tubular cells exhibited increased proliferative activity and expressed vimentin, indicating non-specific tubular damage. The cell subset, the severity of tubulitis, and the tubular expression of vimentin were not related to outcome. The main prognostic factor was the severity of the interstitial fibrosis. Tubulitis in ATIN may be a harmless non-immune reaction, mediated by tubular expression of cytokines, together with adhesion and other molecules.

**Key words** Interstitial nephritis · Tubulitis · Phenotype · Immunohistochemistry · Prognosis

B. Iványi (✉)
Department of Pathology,
Albert Szent-Györgyi Medical University, Kossuth L. sgt. 40,
pf 401, H-6701 Szeged, Hungary

B. Iványi · S.J. Hamilton-Dutoit · S. Olsen
University Institute of Pathology, Kommunehospitalet,
Aarhus, Denmark

H.E. Hansen
Department of Internal Medicine C, Skejby Sygehus,
University of Aarhus, Aarhus, Denmark

## Introduction

Acute tubulointerstitial nephritis (ATIN) is characterized morphologically by interstitial mononuclear cell infiltrates composed mainly of T lymphocytes, tubulitis (infiltration of tubules by mononuclear cells) and tubular epithelial damage [5, 6, 10, 12, 14, 18, 23, 30, 31, 40, 48, 49, 52, 55]. The majority of cases are drug-induced, infection-related or idiopathic. Cell-mediated hypersensitivity reactions have been proposed in the pathogenesis of most cases [11]. The clinical presentation varies from mild, transient renal insufficiency to severe oliguric acute renal failure. The patient may recover with normal renal function or with a variable degree of renal dysfunction, occasionally leading to end-stage renal failure.

No data have been published on the cell subsets in tubulitis in ATIN and it is not clear whether the tubulitis influences the outcome of the disease. The aim of the present study was to determine the phenotype of the cells infiltrating the tubules and to investigate whether the inflammatory and degenerative tubular changes are predictive of prognosis.

## Materials and methods

Cases were selected from the files of the University Institute of Pathology, Aarhus. In a preliminary study, paraffin section immunophenotyping of inflammatory cells in tubulitis was found to give inconsistent results in spite of optimal antigen retrieval using microwave and enzymatic pretreatments (data not shown). Thus, the main study was confined to cases in which both paraffin-embedded and fresh frozen biopsy material were available. Ten renal biopsies from six patients with ATIN were studied.

### Case reports

*Patient 1*

A 26-year-old man was treated with penicillin in October 1993 for an acute upper respiratory tract infection. On day 7, monospot test was positive and there were 10% atypical lymphocytes in the blood. Serum creatinine was 400 μmol/l with decreased urinary

output. The penicillin was stopped. On day 12, there were uraemic symptoms (serum creatinine: 544 μmol/l). On day 13, Epstein-Barr virus (EBV) serology was strongly positive for early antigen-IgM, weakly positive for early antigen-IgG and negative for nuclear antigen-IgG, suggesting current late primary infectious mononucleosis [45]. Renal biopsy revealed ATIN. The outcome was complete recovery.

*Patient 2*

A 39-year-old woman with multiple sclerosis was treated with amitryptyline and paracetamol in October 1991. Two weeks later, she was admitted with confusion and malaise. Evidence of renal failure (serum creatinine 282 μmol/l), severely affected liver function and thrombocytopenia were found. Renal biopsy demonstrated ATIN. She was on dialysis for 1 week with complete recovery.

The ATIN was thought to be associated with the intake of paracetamol.

*Patient 3*

A 46-year-old woman treated periodically with non-steroidal anti-inflammatory drugs for osteoarthrosis developed mechanical ileus. On admission in April 1993, a serum creatinine level of 160 μmol/l was recorded. Her ileus was resolved by uncomplicated abdominal surgery. In the early postoperative period acute oliguric renal failure developed. Renal biopsy exhibited ATIN. Dialysis was started from the 6th postoperative day. End-stage renal disease resulted.

The intake of non-steroidal anti-inflammatory drugs was thought to be the causative factor.

*Patient 4*

A 46-year-old man was admitted in June 1990 with fever, bilateral pneumonia, haematuria and oliguric acute renal failure. *Legionella* infection was demonstrated, with growth of the organism from the tracheal secretions and identification of the antigen in the urine. He was dialysed for 2 weeks. Renal biopsy revealed ATIN and steroid treatment was started, resulting in incomplete recovery.

*Patient 5*

A 48-year-old woman was admitted in July 1990 with acute respiratory and renal insufficiency. *Legionella* infection was verified by serology and renal biopsy showed ATIN. She was on dialysis for 83 days. Renal function was only partly restored, and end-stage kidney disease developed.

*Patient 6*

A 54-year-old man presented in September 1992 with low-grade fever, anaemia, elevated serum creatinine, proteinuria and microhaematuria. Echocardiography, chest radiography, and bone scintigraphy were unremarkable. No evidence of infective agents, collagen-vascular disease or Bence-Jones protein was found. In October, serum creatinine was 360 μmol/l. Renal biopsy demonstrated ATIN and steroid treatment was initiated. There was complete recovery.

Biopsy material

The percutaneous renal biopsies were fixed for light microscopy in buffered 4% formalin, embedded in paraffin wax and stained with haematoxylin and eosin, periodic acid-Schiff (PAS), Masson's trichrome, silver methenamine and Congo red. Part of the specimen was snap frozen and stored at −80° C, and used for direct immunofluorescence (IgG, IgA, IgM, C3, albumin and fibrinogen) and for immunophenotyping. A third part of the biopsy was double fixed in glutaraldehyde and osmium tetroxide, and embedded in resin for electron microscopy.

Tubulitis and interstitial infiltrates were assessed semiquantitatively on paraffin sections, without knowledge of the clinical data. The severity (S) of the tubulitis was scored on PAS-stained slides as described earlier [28]. In brief, tubular profiles with 1–4 leucocytes, 5–10 leucocytes, and more than 10 leucocytes were scored as $S_1$, $S_2$ and $S_3$ tubulitis, respectively. The extent (E) of tubulitis was assessed in a 2 mm$^2$ area of the biopsy, including the most severe focus of tubular wall inflammation. Four categories were established: $E_1$, 1–10; $E_2$, 11–20; $E_3$, 21–30; $E_4$, more than 30 tubular profiles with tubulitis. Cases scored as $E_1S_{2-3}$ and $E_2S_1$ were regarded as mild, $E_2S_{2-3}$ and $E_{3-4}S_1$ as moderate, and $E_{3-4}S_2$ and $E_{3-4}S_3$ as severe tubulitis. Interstitial infiltrates present in 1%–30%, 31%–50%, and more than 51% of the cortex, respectively, corresponded to mild, moderate and severe grades of interstitial inflammation. The severity of interstitial fibrosis was measured with the point count method, using an image analyser and trichrome-stained slides. Points hitting blue peritubular interstitial areas were counted as fibrosis; points hitting the tubules (including the tubular basement membrane), intertubular capillaries and peritubular interstitium constituted the reference space. Points situated on the large vessels, their perivascular connective tissue sheath, the arterioles, and the glomeruli with their capsular basement membrane were not counted. The stage of the microscope was moved in one direction only and with the 20× objective the entire length of the cortex was sampled in one row. Slides from eight psoriatic patients prior to cyclosporin treatment served as controls.

For immunophenotyping, serial frozen sections were cut at 3–4 μm, mounted consecutively (one section/slide), fixed in acetone (10 min), air-dried and stored at −20° C until use. Standard three-stage immunoperoxidase staining was performed using second and third stage antibodies from Dakopatts (Glostrup, Denmark) with intervening washes in phosphate buffered saline (pH 7.1). Briefly, after inhibition of endogenous peroxidase activity (0.6% hydrogen peroxide in 50 mmol/l TRIS-hydrochloric acid, pH 7.6 for 20 min), sections were blocked with 5% fetal bovine serum (20 min). They were then incubated with primary antibody (Table 1, 60 min), peroxidase-conjugated rabbit anti-mouse immunoglobulin (30 min), and peroxidase-conjugated swine anti-rabbit immunoglobulin (30 min), followed by diaminobenzidine visualization (5–6 min). The slides were weakly stained with PAS and counterstained in Mayers' haematoxylin. Frozen sections of a human tonsil and a lymph node served as positive controls.

Cells showing appropriate staining patterns were scored positive. Interstitial infiltrates included the mononuclear cells in peritubular capillaries. The whole cortex was read. The photographic field of an Olympus AHBS microscope was used as a sampling frame (0.104 mm$^2$, measured with a graticule) and the results were expressed as number of inflammatory cells/mm$^2$.

Tubular damage was assessed in paraffin sections by evaluating the immunohistochemical expression of vimentin and proliferation associated Ki-67 antigen in the cortical tubules. Slides of the ten biopsies with ATIN and controls were deparaffinized. Antigen retrieval was achieved by microwave exposure (5×5 min, maximum power 800 W, in 10 mmol/l citrate buffer, pH 6.0). After preincubation in fetal bovine serum, the slides were incubated with the primary antibody overnight at 4° C (for characteristics of antibodies see Table 1). Standard alkaline phosphatase-antialkaline phosphatase with fast red visualization and streptavidin-biotin complex/horseradish peroxidase with nickel-diaminobenzidine chromogen methods were used for the detection of vimentin and Ki-67, respectively. Slides were counterstained with Mayers' haematoxylin. Omission of the primary antibody served as negative control. Staining of glomerular podocytes acted as an internal positive control for vimentin, and germinal centre cells in a lymph node as positive controls for the Ki-67 staining.

**Table 1** Monoclonal antibodies used in the present study (*CD* cluster of differentiation)

| Clone | Antigen CD | Specificity and reference | Source | Concentration |
|---|---|---|---|---|
| T3-4B5 | CD3 | Pan-T cells | Dakopatts | 1/100 |
| MT310 | CD4 | T helper/inducer cells | Dakopatts | 1/10 |
| C8/144 | CD8 | T cytotoxic/suppressor cells | J. Askaa, Dakopatts | 1/20 |
| L26 | CD20 | Pan-B cells | Dakopatts | 1/200 |
| UCHL-1 | CD45RO | Memory CD4 and CD8 cells [1, 2, 24] | Dakopatts | 1/100 |
| NKH-1 | CD56 | Natural killer cells | Immunotech | 1/50 |
| Leu-7 | CD57 | Large granular lymphocytes | Becton Dickinson | 1/20 |
| KP1 | CD68 | Macrophages and myeloid cells | Dakopatts | 1/230 |
| TIA-1 | Cytolytic granule protein | Cytotoxic cells [4, 43] | Coulter | 1/240 |
| Vim 3B4 | Vimentin | Cells of mesenchymal origin | Dakopatts | 1/60 |
| MIB-1 | Ki-67 nuclear antigen | G1, S, G2 and M phases of cell cycle | Immunotech | 1/100 |

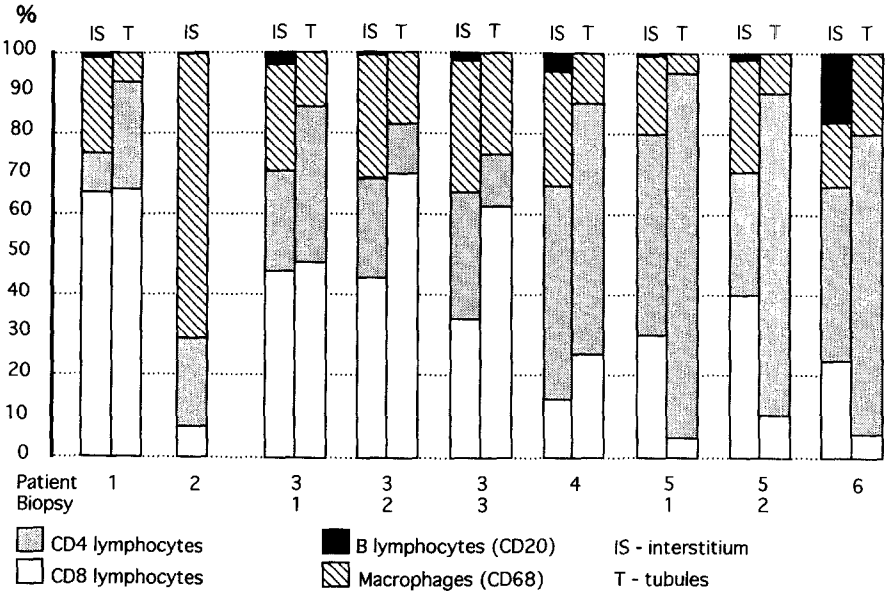



**Fig. 1** The relative percentages of mononuclear cells in the interstitium and tubules in acute tubulointerstitial nephritis (ATIN). The predominant inflammatory cell population in the interstitium tends to be the predominant cell population in the tubules

The numbers of vimentin-positive tubular cross-sections in 100 consecutive cortical tubular profiles were read, without taking into account the intensity of the expression and the number of epithelial cells stained. For Ki-67, tubular profiles with and without positive nuclear staining were read, according to the estimation of the number of objects per area unbiased by edge effects, as described [27]. The photographic field (area: 0.104 $mm^2$) of a photomicroscope and unidirectional movement of the stage were used for sampling. The whole or almost the whole cortex was read in two consecutive rows. The proliferation index was calculated as the number of Ki-67 positive nuclei/all counted tubular profiles ×100.

The presence of EBV was analysed in paraffin sections using immunohistochemistry for EBV-encoded latent membrane protein (LMP)-1 [41] and RNA/RNA in situ hybridization for EBV-encoded small RNAs (EBERs) as described [21]. EBER in situ hybridization is highly sensitive and is the method of choice for the histological detection of EBV.

The ultrastructural appearance of tubulitis was studied in the four first biopsies (one block/case). All tubular profiles with tubulitis were photographed. Since the original electron microscopical investigation was concentrated upon glomerular ultrastructure, only a small amount of tubulointerstitial tissue was available for the present study.

Least square regression analysis was used to test the correlation between lymphocyte subsets in the interstitium and the tubules, and Student's unpaired *t*-test for the analysis of difference between proliferation index of cortical tubular epithelial cells versus controls (significance at 5% level).

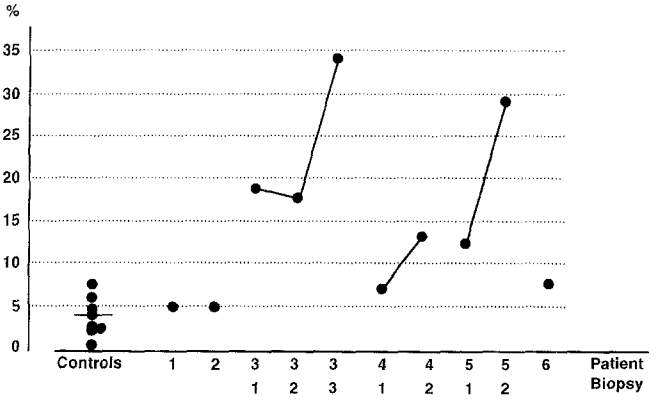



**Fig. 2** Interstitial fibrosis in ATIN. Patients 3 and 5, in whom severe fibrosis was measured by point-counting, developed end-stage renal failure. Those who had normal values, recovered

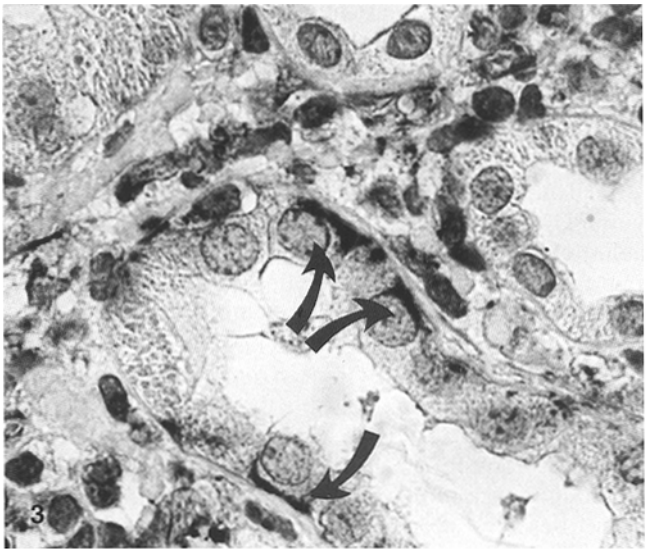
## Results

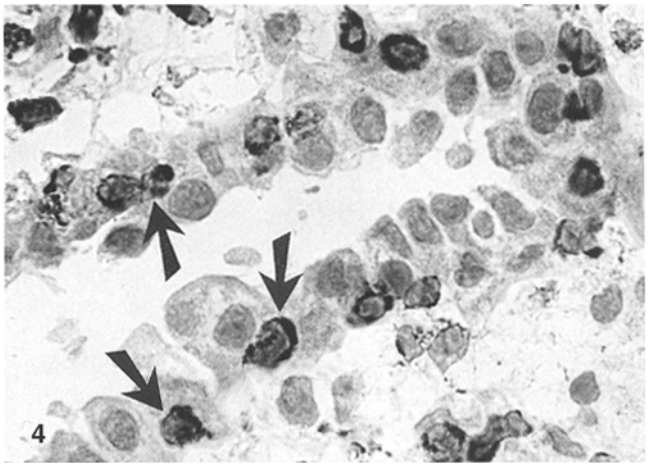
The clinical data, immunohistological and light microscopic findings are summarized in Table 2 and Figs. 1 and 2. All biopsy samples displayed various degrees of basolateral expression of vimentin (Fig. 3). No expres-

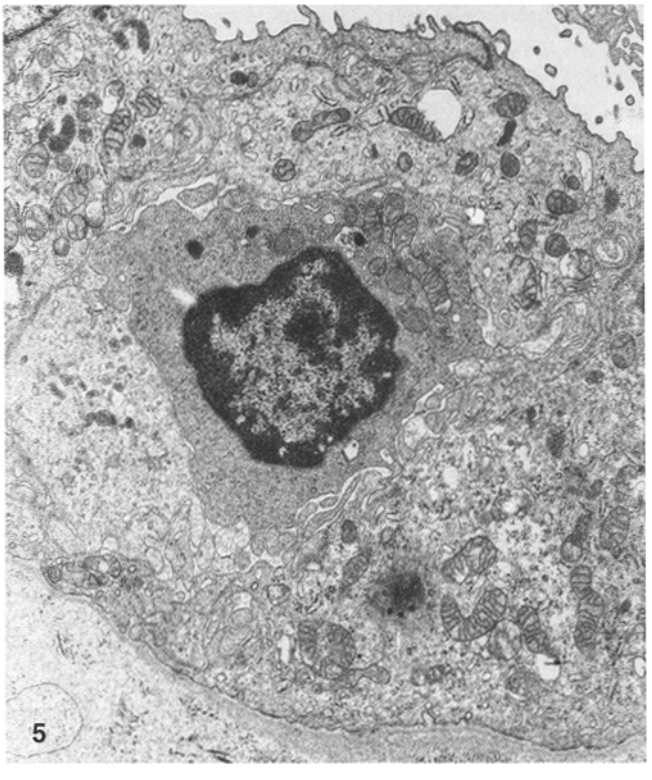
**Table 2** Histology and results of phenotyping (*ESRD* end-stage renal disease)

| | Patient 1 | Patient 2 | Patient 3 | | | Patient 4 | | Patient 5 | | Patient 6 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Biopsy 1 | Biopsy 2 | Biopsy 3 | Biopsy 1 | Biopsy 2 | Biopsy 1 | Biopsy 2 | |
| Etiology | Drug | Drug | Drug | Drug | Drug | Legionella | Legionella | Legionella | Legionella | Idiopathic |
| Biopsy from onset | 11 days | 24 days | 25 days | 62 days | 121 days | 33 days | 10 months | 24 days | 58 days | 56 days |
| Outcome | Recovery | Recovery | ESRD | ESRD | ESRD | Incomplete recovery | Incomplete recovery | ESRD | ESRD | Recovery |
| Tubulitis | Severe | Mild | Severe | Severe | Severe | Severe | Severe | Severe | Severe | Severe |
| Interstitial cell infiltrates | Severe | Mild | Moderate | Mild | Mild | Mild | Mild | Moderate | Moderate | Moderate |
| Interstitial fibrosis (%) | 5.1 | 4.9 | 19 | 18.2 | 34.5 | 6.8 | 13.5 | 11.9 | 29.6 | 7.4 |
| Phenotyping[a] | | | | | | | | | | |
| CD3 | 1793; 67 | 36; 4 | 1256; 28 | 471; 13 | 482; 12 | 244; 19 | | 595; 42 | 356; 21 | 478; 25[b] |
| CD8 | 1368; 44 | 14; 1 | 719; 15 | 318; 12 | 264; 10 | 47; 6 | | 226; 2 | 208; 1 | 180; 2 |
| CD4 | 209; 17 | 34; 1 | 399; 12 | 181; 2 | 271; 2 | 167; 14 | | 382; 38 | 158; 17 | 323; 26 |
| CD68 | 467; 5 | 117; 0 | 391; 4 | 212; 3 | 261; 4 | 88; 3 | | 147; 5 | 138; 1 | 119; 5 |
| CD 57 | 0; 0 | 1; 0 | 0; 0 | 1; 0 | 2; 0 | 1; 0 | | 0; 0 | 7; 0 | 0; 0 |
| CD20 | 12; 0 | 0; 0 | 44; 0 | 7; 0 | 14; 0 | 15; 0 | | 5; 0 | 11; 0 | 131; 0 |
| CD45R0 | 1324; 45 | 89; 5 | 818; 22 | 417; 7 | 461; 12 | 373; 31 | | 489; 35 | 172; 4[b] | 405; 10 |
| Cytotoxic cells (TIA-1) | 1562; 54 | 22; 2 | 785; 15 | 343; 8 | 250; 14 | 132; 5 | | 326; 6 | 216; 5 | 321; 5 |
| CD56 | 0; 0 | 0; 0 | 2; 0 | 0; 0 | 0; 0 | 1; 0 | | 0; 0 | 2; 0 | 0; 0 |
| CD4/CD8 ratio in interstitium and tubules | 0.15; 0.38 | 2.5; 1 | 0.56; 0.8 | 0.56; 0.16 | 1.02; 0.2 | 3.4; 2.3 | | 1.68; 19 | 0.75; 17 | 1.79; 13 |

[a] The first figure in each cell is the number of interstitial leukocytes, the second the number of leukocytes infiltrating in the tubules
[b] Medulla available







sion of vimentin was observed in the control biopsies. The proliferation index in the first biopsies was significantly higher (mean 3.11%, SD 4.12) than in the controls (mean 0.27%, SD 0.43). The proliferation index was lower in the repeat biopsies than in the first biopsies. Expression of vimentin and Ki-67 antigen was observed in tubular profiles without and with tubulitis. In patients 1 and 3, CD8-positive and TIA-1-positive cells were the predominant cell type in the interstitium and tubules (Fig. 4). The majority of cells were CD45RO+. In *Legionella*-related ATIN and idiopathic ATIN, CD4+ cells constituted the major inflammatory cell population in the interstitium and tubules. There was a positive, albeit not significant, correlation between the interstitial and tubular lymphocyte subsets (0.614 for CD8+ and 0.656 for CD4+). In patient 2, macrophages and smaller number of CD4+ cells predominated in the interstitial infiltrates. Marked tubulitis was not present. Electron microscopically, 14 tubular profiles with 24 tubular-wall-localized lymphocytes were found. No lytic injury or apoptosis of adjacent tubular epithelial cells were observed (Fig. 5). The ultrastructural details of tubulitis were identical with those described earlier [26, 39].

In patient 1, EBV-infected cells were not detected using either EBER RNA/RNA in situ hybridization or LMP-1 immunohistochemistry. That the ATIN in this case was not due to direct infection of the kidney with EBV was also shown by the striking CD8+ cell predominance found, which contrasts with the mainly B cell proliferation associated with EBV infection [34].

Patients 1, 2 and 6 recovered completely, and patient 4 partially, suggesting that the outcome was not influenced by the predominant cell subset in the interstitium and tubules, the grade of tubulitis, or vimentin expression in tubules. Taking together the clinical and morphological data, the main determinant for the prognosis of ATIN was the severity of interstitial fibrosis.

## Discussion

Cortical tubular cells are derived from the metanephric blastema. Through embryonic development, a mesenchymal-to-epithelial transition occurs, resulting in tubular cells achieving an epithelial phenotype, without vimentin expression. Various reversible and irreversible pathological processes induce dedifferentiation of the tubular epithelial cells. In such cells, phenotypic transdifferentiation with the expression of vimentin may occur [19, 22, 33,

**Fig. 3** Basolateral expression of vimentin (*arrows*) in a cortical tubule with tubulitis. ×360

**Fig. 4** Penicillin-induced ATIN (outcome: recovery). TIA-1-positive cytotoxic T lymphocytes infiltrate the tubule (*arrows*) and the peritubular interstitium. ×360

**Fig. 5** Penicillin-induced ATIN. The lymphocyte is localized in the lateral intercellular space of the tubular wall. The neighbouring epithelial cells do not exhibit signs of cytotoxic injury, either lysis or apoptosis. ×5600

37, 50, 51, 53]. Since in our material tubular epithelial vimentin display was observed in either patients who recovered or those who did not, the phenomenon cannot be used as a determinant for prognosis in ATIN. The relative number of Ki-67-positive nuclei was very low in the controls. This is in accordance with the findings of Nádasdy et al. [35], who demonstrated that normal tubular epithelial cells have a low proliferation activity. The activity was markedly increased in the first biopsies of ATIN and had a decreasing tendency in the repeat biopsies.

In patients 1 and 3–6, the interstitial infiltrates were dominated by T cells, followed by macrophages. B cells were either absent or rare. These observations are in accordance with the literature [11]. Our drug-induced cases (patients 1 and 3) displayed a CD8+ cell and TIA-1+ cell predominance, pointing to a cytotoxic cell reaction, and our cases with *Legionella* -infection and idiopathic ATIN had a CD4+ predominance, suggesting a delayed-type hypersensitivity response. The majority of the interstitial T cells expressed CD45RO, indicating that these cells were in a late stage of activation. The antigen eliciting the inflammatory response in the interstitium was unknown in our cases, as is the situation in almost all cases of ATIN, and it is unclear why some of our cases exhibited a cytotoxic, and others a delayed-type hypersensitivity cell reaction. Analysis of infiltrating cells in previous reports on drug-induced ATIN has also given conflicting results. Some authors found that the relative numbers of CD4+ and CD8+ cells were close to equality, whereas others reported that either the CD8+ cells or the CD4+ cells were in the majority [5, 6, 10, 12, 49, 52]. We conclude that the present and the previous results do not allow a decision as to whether there is a histocompatibility restriction of the immune response in drug-induced ATIN or not.

Recent studies indicate that the renal tubular epithelium may mediate tubulitis. During inflammatory cell infiltration of the renal interstitium, tumour necrosis factor-alpha, interferon-gamma [36], interleukin-1 (IL-1) [32], and other cytokines are secreted from activated mononuclear cells. These cytokines stimulate the tubular epithelial cells to upregulate the expression of major histocompatibility complex (MHC) class I antigens and intercellular adhesion molecule-1, to express MHC class II antigens, vascular cell adhesion molecule-1 (VCAM-1), IL-6 and IL-8 de novo, and to present antigen to T cells [3, 7, 8, 10, 13, 15–17, 20, 29, 44, 46, 54]. The present study demonstrated that the predominant cell types were identical in the interstitium and the tubules, suggesting similar inflammatory processes in these locations. It is, however, unclear whether the presence of intratubular lymphocytes represents an antigen-initiated immune response acting within the tubules, or whether it is unrelated to antigen recognition.

The following arguments support our view that the tubulitis in ATIN may represent a non-immune inflammatory reaction mediated by cytokines, but not initiated by antigen recognition. Firstly, the capacity of tubular epithelial cells to present antigen is confined to proximal tubules [20, 54]. This segmental feature would favour tubulitis in the proximal tubules. In contrast, the proximal tubules in ATIN and other forms of interstitial nephritis were not found to be the main site of tubulitis [25–27]. Second, if tubular epithelial cells in association with HLA class I molecules can present antigen to CD8+ lymphocytes, these cells in turn will promote tubular epithelial cell death. Lysis of tubular epithelial cells adjacent to tubular wall-localized lymphocytes was, however, not observed in either the present study or in our earlier electron microscopic studies on ATIN [26, 38, 39]. Since no structural evidence of cytotoxic injury to epithelial cells was found, it is conceivable that the tubular-wall-localized lymphocytes had no epithelial targets. We assume, therefore, that the reason for the similar phenotypic predominance in the interstitium and tubules observed in the present study was that following tubular expression of VCAM-1, IL-6, IL-8, and other proinflammatory molecules, the inflammatory cells in the interstitium infiltrated the tubular walls. The predominant cell type in the interstitium, by chance, constituted the predominant cell type within the tubules.

The morphological features of ATIN have many similarities to acute cellular allograft rejection [11]. In the early phase of acute rejection, numerous large granular lymphocytes (CD57+) infiltrate the tubules [47]. In our cases, natural killer cells were practically absent from both the interstitium and the tubules. Since the renal biopsies in patients 1 and 3 were performed relatively early during the course of the disease, and no killer cells were recorded, our cases appear to differ from the tubulitis seen in acute rejection.

In patient 1 CD8+ lymphocytes, and in patient 6 CD4+ lymphocytes were the predominant cell subsets within the tubules. Since both patients recovered, the tubulitis, irrespective of the cell subset predominance, had no impact on the outcome of the disease.

Severe tubular destruction does occur in ATIN [39], with disappearance of tubular basement membranes and disruption of the tubular walls. According to the arguments above, these tubular lesions are not directly caused by the infiltrating cytotoxic T cells. Although the mechanism by which tubular destruction develops is unknown, it is tempting to speculate that delayed-type hypersensitivity may account for the tubular damage. Cytokines, derived from interstitial activated CD4+ cells and macrophages, may lead to microvascular injury, tissue ischaemia, and impaired synthesis of the tubular basement membrane material. Defects in basement membrane synthesis and ischaemia, acting in concert, may lead to tubular destruction. Similar tubular injury ("vanishing tubules") can be seen in acute renal allograft rejection [42], in which delayed hypersensitivity is an essential mechanism of graft destruction.

We analysed the vimentin display of the cortical tubules, the cell subset of the tubulitis, the severity of tubulitis and the severity of interstitial infiltrates and fibrosis in order to determine if one or more of these lesions

had an impact on the prognosis. The results show that interstitial fibrosis is the main determinant for progression to end stage renal disease. Buysen et al. [9] studied clinical and morphological features of ATIN in 27 patients. They had 8 patients with moderately severe interstitial fibrosis, and in 2 of them the renal function did not return to normal in spite of steroid therapy. There was no correlation between the extent of the interstitial infiltrates and the severity of the renal failure. Both their and our observations indicate that interstitial fibrosis is crucial in the prognosis of ATIN. Tubulitis seems to be a harmless epiphenomenon, without clinical consequences.

**Acknowledgements** This study was supported in part by grants to B.I. (OTKA-016525, ETT T-06585, Budapest, Hungary). B.I. was a research fellow at the Institute of Experimental Clinical Research, University of Aarhus, Denmark. The authors thank Jon Askaa (Dakopatts, Glostrup, Denmark) for providing the monoclonal antibody C8/144 and László Krenács (Department of Pathology, Albert Szent-Györgyi Medical University, Szeged, Hungary), for advice on phenotyping.

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