Involvement of Drug-Specific T Cells in Acute Drug-Induced Interstitial Nephritis

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Abstract

Drug-induced interstitial nephritis can be caused by a plethora of drugs and is characterized by a sudden impairment of renal function, mild proteinuria, and sterile pyuria. For investigation of the possible pathomechanism of this disease, drug-specific T cells were analyzed, their function was characterized, and these in vitro findings were correlated to histopathologic changes that were observed in kidney biopsy specimens. Peripheral blood mononuclear cells from three patients showed a proliferative response to only one of the administered drugs, namely flucloxacillin, penicillin G, and disulfiram, respectively. The in vitro analysis of the flucloxacillin-reactive cells showed an oligoclonal immune response with an outgrowth of T cells bearing the T cell receptor Vβ9 and Vβ21.3. Moreover, flucloxacillin-specific T cell clones could be generated from peripheral blood, they expressed CD4 and the αβ-T cell receptor, and showed a heterogeneous cytokine secretion pattern with no clear commitment to either a Th1- or Th2-type response. The immunohistochemistry of kidney biopsies of these patients revealed cell infiltrations that consisted mostly of T cells (CD4+ and/or CD8+). An augmented presence of IL-5, eosinophils, neutrophils, CD68+ cells, and IL-12 was observed. In agreement with negative cytotoxicity assays, no cytotoxicity-related molecules such as Fas and perforin were detected by immunohistochemistry. The data indicate that drug-specific T cells are activated locally and orchestrate a local inflammation via secretion of various cytokines, the type of which depends on the cytokine pattern secreted and which probably is responsible for the renal damage.


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Acute interstitial nephritis (AIN) is characterized by a sudden impairment of renal function, mild proteinuria, and sterile pyuria. It is considered to be caused by drugs, toxins, or autoimmune diseases (e.g., systemic lupus erythematosus, Sjögren’s disease, sarcoidosis). Early in the 1980s, Neilson and colleagues (1,2) could already demonstrate that immunization with tubular antigens in adjuvants can induce interstitial nephritis in susceptible mouse strains, which can be transferred by T cells. The effector cells that were detectable in the interstitial lesions could be either CD4+ or CD8+ T cells. The detailed pathogenesis of drug-induced interstitial nephritis (DIN), which represents the most common form of AIN, still is unclear but could—in analogy to drug-induced skin diseases (3)—be related to an immune reaction to drug antigens. A plethora of xenobiotics, such as antibiotics (β-lactams, sulfonamides, aminoglycosides, and quinolones), anti-convulsants, diuretics (thiazide and furosemide), proton pump inhibitors (omeprazole), and nonsteroidal anti-inflammatory drugs, have been described to be responsible for DIN (4).

Histologically, DIN can be discriminated from other types of renal failure by specific morphologic characteristics. In healthy renal tissue, fibroblasts predominate in the interstitial space, whereas biopsies of patients with DIN reveal an excessive interstitial infiltrate that consists mostly of T lymphocytes, macrophage/monocytes, eosinophils, and/or polymorphonuclear neutrophils (PMN) (5). In addition, interstitial edema, disruption of the tubular basement membrane (TBM), and, in some cases, significant changes of the normal interstitial architecture can be found. Extrarenal manifestations that indicate a systemic reaction, such as skin eruptions, eosinophilia, and fever, also may occur.

Although the pathologic features of DIN are well defined, restricted access to renal tissue and the delayed appearance of clinical symptoms complicate the study of its pathomechanism. T cells seem to play a major role in the pathogenesis of the disease, because they are the predominant cell type in the interstitial infiltrate (6). This is comparable to our earlier findings of allergic skin reactions: Drug-specific T cells were found to orchestrate the allergic reactions, inducing cell infiltrations that consisted mainly of eosinophils and occasionally of neutrophils (7–10) and leading to maculopapular, bullous, or pustular exanthems (3).

The immunogenicity of drugs is thought to depend on their
ability to form covalent bonds with larger molecules, in particular proteins. Chemically reactive drugs form so-called hapten-carrier complexes, which are newly immunogenic protein antigens that are able to stimulate both T and B cell immune responses (3,11,12). For example, in methicillin-induced DIN, Border et al. (13) showed that methicillin molecules act as hapten, which bind to the TBM and elicit the production of anti-TBM antibodies. However, many drugs are not chemically reactive but become so after an intermediate metabolism step, which transforms these so-called pro-haptons to haptons. Sulfamethoxazole is a typical example of a drug that acts as pro-hapten, because it is transformed to sulfamethoxazole-hydroxylamine and further oxidized to sulfamethoxazole-nitroso (14-16). Such transformations from pro-haptons to haptons occur mainly in the liver but also might occur in the kidney, because tubuloendothelial cells produce various cytochrome P450-associated metabolizing enzymes (17). Alternatively, the “immunogenicity” of drugs relies on a direct interaction of the drug with immune receptors such as the T cell receptor (TCR) for antigen, as postulated by the concept of pharmacologic interaction with immune receptors such as the T cell receptor (TCR) for antigen, (18,19). Under certain circumstances, this “pharmacologic” interaction can lead to T cell activation and expansion.

In this study, we analyzed the role of drug-specific responses in patients with a histologic diagnosis of DIN. We identified drug-specific T cells, characterized them phenotypically and functionally in vitro, and supplemented this in vitro analysis by immunohistochemistry of kidney biopsies. Our data support the concept that drug-specific T cells are important in the development of DIN because they can coordinate the local inflammation that affects kidney function.

Materials and Methods

Patient Characteristics

Lymphocyte transformation tests (LTT) were performed in 12 patients with DIN presumed upon histologic analysis. The involvement of a drug-specific immune response in the pathogenesis of the disease was confirmed in three of the patients by positive LTT (20). Table 1 summarizes the clinical characteristics of these three patients, who were included in the study. As controls, LTT also were performed in five healthy individuals without medical history of drug allergy. The study was approved by the ethical committee of the University of Bern.

Culture Medium

Culture medium (CM) consisted of RPMI-1640 (52400-25; Life Technologies/Invitrogen, Basel, Switzerland) supplemented with 10% pooled heat-inactivated human AB serum (Swiss Red Cross, Bern, Switzerland), 2 mmol/L l-glutamine (K0202; Biochrom, Berlin, Germany), 25 μg/ml holotransferrin (Sigma-Aldrich, Buchs, Switzerland), 100 μg/ml streptomycin, and 100 IU/ml penicillin (4–01F00-H; Amimed, Allschwil, Switzerland). For the culture of T cell lines (TCL) or T cell clones (TCC), either 180 or 300 IU/ml human recombinant IL-2 (Prolutexin; Roche Pharma, Reinach, Switzerland) was added, respectively.

LTT and Generation of Drug-Specific TCL and TCC

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll/Hypaque gradient. For LTT, 2×10⁶ cells were cultured in 0.2 ml of CM in 96-well plates together with different drugs at various concentrations. After 6 d, 0.5 μCi of ³H-thymidine was added for 8 to 14 h. Cells were harvested (96-well Cell Harvester; Inotech, Dottikon, Switzerland), and the incorporated radioactivity as an indicator of proliferation was measured with a β-counter (Trace 96, Filter Counting; Inotech). Toxicity of the used drug concentrations for PBMC was excluded previously, and proliferative responses were induced in sensitized individuals but not in healthy individuals (20). Stimulation indexes were calculated as counts per minute (cpm); in the presence of drug (antigen) divided by cpm the absence of drug.

TCL were generated by stimulation of PBMC in CM with the respective drug and 180 IU/ml human recombinant IL-2. After 14 d, restimulation and further expansion of the reactive cells was induced by adding irradiated (45 Gy), autologous PBMC and the respective drug (namely fluclaxacillin, penicillin G, and disulfiram). TCC were generated using the limiting dilution technique as described (14). Briefly, drug-specific TCL were diluted to a cell density of 0.3 or 1 cell/well and cultured in 96-well round-bottom culture plates together with 5×10⁶ irradiated autologous PBMC/well and 1 μg/ml

Table 1. Characteristics of patients with DIN

<table>
<thead>
<tr>
<th>Parameter</th>
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<tr>
<td></td>
<td>P1</td>
</tr>
<tr>
<td></td>
<td>67/m</td>
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<tr>
<td>Drug intake</td>
<td>Fluclaxacillin, gentamicin, rifampicin</td>
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<tr>
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<tr>
<td>skin eruptions</td>
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<tr>
<td>increased liver enzymes</td>
<td>+</td>
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<tr>
<td>proteinuria</td>
<td>+</td>
</tr>
<tr>
<td>leukocyturia</td>
<td>+</td>
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<tr>
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<td></td>
<td>P2</td>
</tr>
<tr>
<td></td>
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<td>CRP (mg/L)</td>
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<td>CRP (mg/L)</td>
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</table>

CRP, C-reactive protein; DIN, drug-induced interstitial nephritis.
phytohemagglutinin (Sigma-Aldrich). IL-2–containing medium was added the next day. TCC were restimulated after 14 d.

**Specificity Assays with Drug-Specific T Cells**

Antigen specificity of TCL and TCC was tested by incubating $5 \times 10^4$ T cells (days 10 to 16 after restimulation) with either $2.5 \times 10^4$ irradiated (45 Gy), autologous PBMC or $1 \times 10^4$ irradiated (60 Gy), autologous Epstein-Barr virus–transformed B cell lines as antigen-presenting cells in the presence or absence of antigen in 200 µl of CM in U-bottom 96-well microplates. After 48 h, 0.5 µCi of $^3$H-thymidine was added for 14 h. Cells were harvested, and incorporated radioactivity was measured with a $\beta$-counter.

**Flow-Cytometric Analysis**

Aliquots that contained $10^5$ cells were stained with fluorochrome-conjugated antibodies in 50 µl of buffer (PBS with 1% FCS and 0.02% NaN$_3$) for 15 to 30 min at 4°C and analyzed on a Coulter EPICS XL-MCL (Beckmann Coulter, Fullerton, CA). TCR-β expression of TCL and TCC was analyzed by TCR-V$\beta$ staining using a panel of 24 mAb against various V$\beta$ gene products (Beckmann Coulter), detecting approximately 75% of all V$\beta$ families (21). Phenotypic characterization and expression of surface markers of TCL and TCC were performed using fluorochrome-labeled anti-CD3, anti-CD4, and anti-CD8 (all from BD Biosciences Pharmingen, San Diego, CA).

**Cytokine and Chemokine Detection in Cell Culture Supernatants**

T cells ($5 \times 10^4$) were stimulated in 200 µl of CM for 48 h in flat-bottom 96-well plates that were coated with anti-CD3 (1 µg/ml, Orthoclone OKT3; Janssen-Cilag AG, Baar, Switzerland) and soluble anti-CD28 mAb (1 µg/ml; BD Biosciences Pharmingen) as co-stimulatory factor in the presence of low amounts of IL-2 (120 IU/ml). Supernatant of unstimulated T cells was taken as control, and proliferation was measured by $^3$H-thymidine incorporation overnight as described. The following cytokine and chemokines ELISA sets were used: IL-5 and CXCL8 (BD Biosciences Pharmingen and Diaclone, Besançon, France); IFN-$\gamma$, TNF-$\alpha$, and IL-4, (Diaclone); and GM-CSF (R&D Systems, Minneapolis, MN). Duplicate samples were diluted (1:10, 1:100, and 1:1000 as indicated) and measured according to the standard protocol of the corresponding ELISA set. The detection limit of the assays was 1 pg/ml for IL-4, 6 pg/ml for IFN-$\gamma$, 8 pg/ml for IL-5, 13 pg/ml for TNF-$\alpha$, and 16 pg/ml for CXCL8 and GM-CSF.

**Staining of Biopsy Specimens**

Renal biopsy specimens from patients with AIN were snap-frozen in tissue-embedding medium (TissueTek O.C.T. Compound; Sakura Finetek, Zoeterwoude, Netherlands) and stored at $-80^\circ$C. Control stainings were performed with a nontumorous specimen from a patient with renal carcinoma. The following antibodies were used for single immunostainings: Anti-CD4 (1:150, clone MT 310; DakoCytomeration AS, Glostrup, Denmark), anti-CD8 (1:30, clone DK25; DakoCytomation), anti-neutrophil elastase (1:300, clone NP57; DakoCytomation), anti-perforin (1:30, clone 8G9; BD Biosciences Pharmingen), anti-Fas (CD95/APO-1 1:30, clone 13; BD Biosciences Pharmingen), anti–IL-5 (1:1200, clone 9906.1; R&D Systems), anti–CD68 (1:30, clone PG-M1; DakoCytomation), and anti–IL-12 (1:30, clone 24910.1; R&D Systems). The TCR-V$\beta$ staining of the tissue section was performed by using anti–TCR-V$\beta$5.1 mAb (1:30, clone IMMUB157; Beckmann Coulter), because the antibody to TCR-V$\beta$1.3 was unsuitable for immunohistochemistry.

CD4, CD8, and neutrophil elastase staining was performed using the chain polymer–conjugated procedure. Briefly, 7-µm cryostat tissue sections were fixed for 8 min with acetone and then incubated for 10 min with peroxidase block (0.3% H$_2$O$_2$ in methanol) to prevent nonspecific binding. Incubation of the slides with the indicated primary antibody was followed by horseradish peroxidase–labeled polymer conjugated to goat anti-mouse immunoglobulins (K4000, DakoCytomation). Horseradish peroxidase activity was detected using diaminobenzidine substrate chromogen (K3467; DakoCytomation), which gave a brown staining. Finally, slides were counterstained with Mayer’s hematoxylin. Immunohistochemistry for the other antibodies was performed using the alkaline phosphatase/anti–alkaline phosphatase complex method (22,23). Cryostat tissue sections were fixed for 8 min with acetone or 4% paraformaldehyde in PBS. Nonspecific binding was blocked by preincubation for 20 min with PBS that contained rabbit serum and with the biotin-avidin blocking system (X0596; DakoCytomation). Slides were incubated with the indicated primary antibodies, followed by biotinylated rabbit anti-mouse antibody [1:30, F(ab')2, E0413; DakoCytomation] and thereafter with alkaline phosphatase/anti–alkaline phosphatase complex mAb antibody (1:50, D0651; DakoCytomation). Finally, sections were developed with fuchsin substrate chromogen (K0597; DakoCytomation) and counterstained with Mayer’s hematoxylin. The esinophilic and neutrophilic granulocytes were identified using eosin-hematoxylin staining.

**Evaluation of Sections**

Slides were analyzed with a Leica microscope DM500 by the same investigator. CD4$^+$ and TCR-V$\beta$5.1$^+$ cells were identified in the interstitial infiltrates by their characteristic morphology and by their membrane and/or cytosolic staining. In each section, staining was assessed by analysis of 16 to 20 fields in the interstitium at $\times 400$ magnification. The eyepiece had a grid of 1 cm$^2$ covering 0.0625 mm$^2$ of the biopsy. The results were calculated as the mean of positive cells per mm$^2$.

**Statistical Analyses**

Statistical analysis for secreted cytokines was performed using the Pearson correlation and the Mann-Whitney U test for CD4+ TCR-V$\beta$5.1 counts in biopsy sections. P < 0.05 was considered statistically significant.

**Results**

**Patient Characteristics**

Table 1 summarizes the clinical characteristics of patients P1, P2, and P3. Patient P1 was treated with flucloxacillin, rifampicin, and gentamicin because of endocarditis. Within the first 3 wk of treatment with this triple antibiotic therapy, a reduction of creatinine clearance was found, and an interstitial nephritis was diagnosed subsequently. Patient P2 was treated with penicillin G and azithromycin because of a suspected endocarditis. After 8 d of drug intake, the first symptoms of decreased kidney function appeared, accompanied by a pruritic exanthem. In patient P3, disulfiram, diclofenac, and irbesartan were administrated, and a renal impairment was found approximately 3 wk later.

**Drug-Specific Proliferation of Patients’ PBMC**

The drug-specific proliferation of patients’ PBMC was analyzed in LTT using all of the drugs that were given at the time of nephritis development. The tetanus toxoid–specific response served as a positive control in all three (previously tetanus toxoid–immunized) patients. Specific proliferative responses of PBMC were observed in all three patients: Patient P1 showed a positive response to flucloxacillin (Figure 1A), the PBMC of patient P2 proliferated to penicillin G (Figure 1B), and patient
P3 reacted to disulfiram (Figure 1C). Note that all of LTT were positive for one drug but negative for the other drugs to which the patients had been exposed as well. All drugs that were used for LTT were unable to induce proliferation in a control group of five healthy individuals (data not shown).

**Generation of Flucloxacillin-Specific TCL**

For further investigation of whether drug-specific T cells are involved in the interstitial nephritis of patient P1, PBMC of this patient were incubated with flucloxacillin and IL-2 for up to 4 wk to generate a drug-specific TCL. After 10 d of stimulation, the TCR-Vβ distribution was analyzed by flow cytometry and compared with the TCR-Vβ distribution of unstimulated PBMC. CD3+ T cells bearing the Vβ9 (TRBV3-1) and Vβ21.3 (TRBV11-2) were enriched in the TCL, with up to 28 and 31% of cell expansion (Figure 2A). Normally, these TCR-Vβ have been expressed dominantly in the TCL together with TCR-β21.3. At 21 d, a flucloxacillin-specific proliferation confirmed an additional enrichment of drug-specific T cells in the generated TCL (Figure 2B) compared with the PBMC in the LTT (Figure 1A). This indicates the presence of drug-specific T cells in patient P1 that are capable of a highly specific immune response to flucloxacillin.

**Generation and Characterization of Flucloxacillin-Specific TCC**

By limiting dilution, 23 flucloxacillin-specific TCC were generated and 13 of them analyzed further (Table 2). All were CD4+, expressed αβ-TCR, and showed a restricted TCR-Vβ usage, confirming the oligoclonality of the drug-specific immune response. Ten of 13 TCC were positive for Vβ21.3, and two were positive for Vβ5.1 (TRBV5-1). TCR-Vβ9, which had been expressed dominantly in the TCL together with TCR-Vβ21.3, was not found in these TCC.

For analysis of cytokine and chemokine secretion, eight TCC were stimulated with anti-CD3 and anti-CD28 antibodies, and secreted cytokines/chemokines were determined in the supernatant by ELISA (Figure 3). Overall, we could detect a mixed cytokine secretion pattern with no clear commitment to either Th1 or Th2 phenotype. Two TCC expressed high amounts of Th1-type cytokines, such as IFN-γ and TNF-α (TCC 3F and 8F), whereas three TCC (TCC 18F, 9F, and 12F) expressed mainly Th2-type cytokines, such as IL-4 and IL-5 and only low amounts of IFN-γ. The other TCC secreted intermediate amounts of both Th1 and Th2 cytokines with no clear commitment. The neutrophil-attracting chemokine CXCL8 was produced by two TCC in very high amounts (>8000 pg/ml), and other clones secreted this chemokine in considerable amounts (400 to 2000 pg/ml) as well. Statistical analysis of cytokine secretion revealed significant correlations between IFN-γ and TNF-α (P < 0.001) and between IL-4 and IL-5 (P < 0.01).

**Immunohistochemistry of Kidney Biopsy Specimens**

For correlation of the in vitro analysis of PBMC with the type of renal inflammation, biopsies of all patients were analyzed by immunohistochemistry and compared with a nontumorous specimen of renal carcinoma. Because of lack of tissue, we could not perform all of the necessary immunohistologic stainings in the biopsy of patient P3. In all three patient specimens, the morphology of the glomeruli on the light microscope seemed to be normal. Both immunofluorescence and electron microscopy showed no IgG or complement deposits in the mesangial area or capillary loop (data not shown).

The immunohistochemical analysis of the biopsy specimens of patients P1 and P2 showed an augmented presence of T cells in the interstitial infiltrations. This T cell infiltration in patient P1 was composed mainly of CD4+ T cells (Figure 4A), but a substantial amount of CD8+ T cells (Figure 4C) also could be detected. Patient P2 showed a CD4+ T cell infiltration (Figure 4B) as well, whereas CD8+ cells were not detected (Figure 4D).

PMN and eosinophilic leukocytes also were present. Some PMN could be identified in the interstitial areas of all of the biopsies, and others also were found in the lumen of the tubuli. In patient P1, an augmented presence of PMN (Figure 4E) was observed in comparison with patient P2 (Figure 4F). Furthermore, eosinophils were present in the interstitial area of patient P1 (Figure 5A), probably in response to the enhanced presence

![Figure 1](image). Lymphocyte transformation test (LTT) of patients P1, P2, and P3. Drug-specific proliferation was detected for flucloxacillin (A), penicillin G (B), and disulfiram (C) in patients P1, P2, and P3, respectively. Tetanus toxoid (TT) was used as positive control. Mean of triplicate cultures and SE are shown.
of T cells that were producing the eosinophil-stimulating cytokine IL-5 in the interstitial cell infiltrate (Figure 5C). Patient P2 had fewer T cells expressing IL-5 and only very few eosinophils present (Figure 5, B and D). A comparably low number of monocytes/macrophages (CD68<sup>+</sup>/H11001) were found in the biopsies of patients P1 (Figure 5E) and P3 (data not shown). Consequently, the monocytes/macrophage-derived cytokine IL-12 was barely detectable (Figure 5G). Patient P2 differed because local granulomas were found in the interstitial area. Here, a higher number of CD68<sup>+</sup>/H11001 cells (Figure 5F) and expression of IL-12 (Figure 5H) suggested a stronger involvement and activation of monocyte-macrophage cells in his form of DIN.

To determine whether cell-mediated cytotoxicity plays a detectable role in the pathogenesis of DIN, we stained for perforin and Fas, two key molecules that are involved in different pathways of cell-mediated cytotoxicity (25). However, both perforin (Figure 6, A and B) and Fas (Figure 6, C and D) were barely detectable in either patient P1 or P2.

In the comparison biopsies, only very few CD4<sup>+</sup>/H11001, CD8<sup>+</sup>/H11001, and neutrophil-elastase positive cells were found in the interstitial area (supplementary data). There also was no IL-5 secretion found, and both Fas and perforin molecules were hardly detectable (supplementary data).

**TCR-V<sup>β</sup> Staining in Kidney Biopsy Specimens**

To determine whether the drug-specific T cells that were isolated from the peripheral blood also might be present in the kidney, we took advantage of the predominant TCR-V<sup>β</sup> usage of the flucloxacillin-specific T cells and stained the biopsy of patient P1 for TCR-V<sup>β</sup>5.1, whereas a biopsy of another patient who had interstitial nephritis with abundant T cell infiltration served as a control. TCR-V<sup>β</sup>5.1 was one of the TCR used by the flucloxacillin-specific TCC but is found on 4 to 7% of circulating T cells only (24). If the TCR-V<sup>β</sup>5.1 also were detected in substantial numbers in the kidney biopsy, then one might assume that such an accumulation of TCR-V<sup>β</sup>5.1<sup>+</sup> T cells represents the recruitment of the same drug-specific T cells. Indeed, an accumulation of TCR-V<sup>β</sup>5.1<sup>+</sup> T cells was observed in patient P1: The mean of TCR-V<sup>β</sup>5.1<sup>+</sup> T cells was 120/mm<sup>2</sup> (Figure 7A) but only 27.2/mm<sup>2</sup> in the control patient (<i>P</i> < 0.05; Figure 7B), whereas the total CD4 T cell count was comparable (mean CD4 272/mm<sup>2</sup> in patient P1 and 316.8/mm<sup>2</sup> in control patient). The

![Figure 2](image-url)
ratio of the mean TCR-V \( \beta 5.1^+ \) versus total CD4\(^+\) cells in patient P1 was 0.441 and 0.085 in the control biopsy, indicating a 5.2-fold higher incidence of TCR-V \( \beta 5.1^+ \) cells in patient P1.

**Discussion**

This study supports the concept that a T cell–mediated drug hypersensitivity reaction is responsible for DIN. We validated this concept by demonstrating that drug-specific T cells can be found in the peripheral blood from patients with DIN, that they react strongly to the drug, and that they are capable of orchestrating an inflammatory reaction on the basis of secretion of various cytokines. These in vitro findings are supplemented by data that were obtained from renal biopsies in which a massive infiltration of T cells that secrete cytokines and chemokines locally seem to coordinate an either predominantly granulocyte-rich (patient P1) or monocyte-rich (patient P2) inflammation.

The detection of drug-specific T cells in the peripheral blood of patients with DIN, as shown by positive proliferation assays of PBMC of three patients and confirmed by generating drug-specific TCL and 23 TCC of one patient, has major implications for our understanding of this disease. First, in vitro proliferation assays might be helpful to identify the drug that is responsible for the hypersensitivity reaction. In patients who were exposed to more than one drug that potentially could have caused a DIN, the relevant compound could be identified in the LTT. The incriminated drugs can be identified long after the reaction, because drug-reactive cells seem to persist for months to 12 yr after a severe drug hypersensitivity reaction (26).

Second, it is likely that the T cell infiltration into the kidney is due to drug-specific T cells, which then might coordinate the local inflammatory reaction. This conclusion is supported by the relatively high expression of TCR-V\( \beta 5.1 \) in the kidney biopsy of patient P1, because the same TCR-V\( \beta \) also was expressed by the drug-specific TCC. This suggests that the drug is presented in the kidney in an immunogenic way, thereby triggering an immune response or reactivating immigrating T cells.

Third, our data suggest a certain similarity to other drug hypersensitivity reactions (e.g., to various drug-induced exanthems) in which functionally different T cells can lead to distinct clinical
pictures, causing skin symptoms such as maculopapular, bullous, or pustular exanthems (3,14). In these diseases, drug-specific T cells cause distinct inflammatory responses by secreting different cytokines, namely IFN-\(\gamma\)-activating monocytes/macrophages, IL-5 for eosinophil activation, and CXCL8 together with GM-CSF for PMN recruitment and activation (23,27,28). In addition, some T cells (CD4\(^+\) and CD8\(^+\)) are able to kill other cells (25,29,30).

Delayed-type hypersensitivity reactions of type IV (31) are heterogeneous themselves and recently were subdivided further into four subtypes (IVa through d) (3). Type IVa reactions correspond to Th1-like, IL-12/IFN-\(\gamma\)-driven reactions with monocyte activation; type IVb reactions correspond to Th2 responses with activation and recruitment of eosinophils; type IVc reactions correspond to cytotoxic T cell reactions; and type IVd reactions correspond to activation and recruitment of PMN, leading to sterile PMN-rich inflammations. Although these reactions may occur together, a clinically characteristic picture (e.g., pustules) may arise nevertheless, because one reaction often may dominate.

Because drug-specific immune reactions are systemic reactions, we assumed that this subclassification may be applicable not only to the skin but also to DIN. Patient P1 showed histologically an eosinophil-rich reaction with an abundance of IL-5\(^+\) cells, suggesting that a type IVb reaction is dominating locally. The in vitro analysis of the cytokine secretion patterns of stimulated T cells was more heterogeneous because a Th2-like response (IL-4 and IL-5) was found together with IFN-\(\gamma\)- and CXCL8-producing T cells. It is interesting that despite an eosinophil-rich reaction in the
that are suitable for immunohistochemistry seem to be available. nately, staining of IFN- was abundant staining for CD68 and IL-12, respectively. Unfortu- as the activation of monocytes/macrophages was confirmed by representing a predominant IVa reaction. His histology already tular psoriasis, and Behçet’s disease (8,27).

In comparison, patient P2 showed a different picture, possibly a predominant IVa reaction. His histology already revealed a granulomatous reaction, and the accumulation as well as the activation of monocytes/macrophages was confirmed by abundant staining for CD68 and IL-12, respectively. Unfortunately, staining of IFN-γ was not possible, because no antibodies that are suitable for immunohistochemistry seem to be available.

Cytotoxic CD8⁺ and CD4⁺ T cells play a dominant role in most forms of drug-induced exanthemas (30). However, this mechanism may be less important in the three patients analyzed, because stainings did not reveal the presence of typical cytotoxicity markers such as perforin or Fas and the TCC were not cytotoxic in in vitro tests (data not shown). Nevertheless, these findings do not rule out that in other forms of DIN, cytotoxicity that is directed, for example, against tubulop epithelial cells or other antigens may be important. Although the reason for the absence of T cell–mediated cytotoxicity in the three patients is not clear, it should be kept in mind that cytotoxic T cells may not proliferate well in proliferation tests. The selection of the three patients, which was based on a positive LTT to the drug antigens, therefore may have been biased against detecting such reactions (30).

The accumulation of T cells that express a particular TCR-Vβ that is involved in drug recognition in the kidney suggests that drug-specific T cells are recruited from the peripheral blood to the kidney. The drugs gain immunogenicity by being metabolized locally (e.g., in epithelial cells of the tubuli) and by forming drug–carrier complexes, which might be transported to the lymph nodes but also might be presented locally. The drug-specific T cells would be activated and expanded in the lymph node, circulate, and home to the kidney, where they are restimulated by local drug presentation. Because an interstitial nephritis is a rare event, it may occur only when certain co-factors facilitate an immune response to the drug. This could be high reabsorption of the drug in the Henle’s loop and/or kidney damage by simultaneously applied aminoglycosides, or unknown genetic factors that affect drug transport in tubulop epithelial cells, etc. If disulfiram and β-lactams were able to form the same antigenic determinants also outside the kidney and probably also during the in vitro culture of the LTT, the specific T cells could be detected. However, if the drug that presumably caused the DIN requires metabolism in the kidney to gain its hapten-like features and immunogenicity, then the antigenic determinant might not be formed during cell culture and an LTT would remain negative. Such a selective metabolism of the relevant antigenic determinant in the kidney also may be an explanation for the localization of the hypersensitivity reaction to the kidney because the antigenic de- terminant would be expressed only there.

Although the list of drugs that cause DIN is long, we could confirm the prominent role of β-lactams in the pathogenesis of DIN. All β-lactams are known to be able to act as haptons and bind to amino groups of amino acids, such as lysine (33). Disulfiram, which caused the reaction in the third patient, is a pro-hapten, which quickly degrades in vivo to diethylthiocarbamate. Further metabolism leads to free carbon disulfide that may bind to free amino groups in peptides.

Conclusion
The combined evaluation of drug-specific T cells in vitro and the phenotypic analysis of the cellular infiltrate in the kidneys have revealed many similarities between DIN and drug- induced cutaneous hypersensitivity reactions. DIN is a T cell–medi- ated drug hypersensitivity reaction whereby drug-specific T cells can be detected and can elicit various forms of local inflammations that depend on the preferential cytokine produced. Our findings have implications for the understanding, clinical features, and diagnostic possibilities of DIN and therefore may contribute to treating and avoiding these important drug-induced adverse effects.

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References

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