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T cell-mediated hypersensitivity to quinolones: mechanisms and cross-reactivity

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Clinical and Experimental Allergy

Summary

Background Quinolones are widely used, broad spectrum antibiotics that can induce immediate- and delayed-type hypersensitivity reactions, presumably either IgE or T cell mediated, in about 2–3% of treated patients.

Objective To better understand how T cells interact with quinolones, we analysed six patients with delayed hypersensitivity reactions to ciprofloxacin (CPFX), norfloxacin (NRFX) or moxifloxacin (MXFX).

Methods We confirmed the involvement of T cells *in vivo* by patch test and *in vitro* by means of the lymphocyte proliferation test (LTT). The nature of the drug–T cell interaction as well as the cross-reactivity with other quinolones were investigated through the generation and analysis (flow cytometry and proliferation assays) of quinolone-specific T cell clones (TCC).

Results The LTT confirmed the involvement of T cells because peripheral blood mononuclear cells (PBMC) mounted an enhanced *in vitro* proliferative response to CPFX and/or NRFX or MXFX in all patients. Patch tests were positive after 24 and 48 h in three out of the six patients. From two patients, CPFX- and MXFX-specific CD4⁺/CD8⁺ T cell receptor (TCR) $\alpha\beta$ ⁺ TCC were generated to investigate the nature of the drug–T cell interaction as well as the cross-reactivity with other quinolones. The use of eight different quinolones as antigens (Ag) revealed three patterns of cross-reactivity: clones exclusively reacting with the eliciting drug, clones with a limited cross-reactivity and clones showing a broad cross-reactivity. The TCC recognized quinolones directly without need of processing and without covalent association with the major histocompatibility complex (MHC)–peptide complex, as glutaraldehyde-fixed Ag-presenting cells (APC) could present the drug and washing quinolone-pulsed APC removed the drug, abrogating the reactivity of quinolone-specific TCC.

Conclusion Our data show that T cells are involved in delayed immune reactions to quinolones and that cross-reactivity among the different quinolones is frequent.

Keywords cross-reactivity, delayed hypersensitivity drug reaction, drug-reactive T cells, p–i concept, quinolone

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Introduction

Quinolones are broad spectrum synthetic antibiotics of increasing importance in the treatment of various bacterial infections [1]. The first quinolone, nalidixic acid (NLDXA), was already introduced in 1964 and was followed in the 1980s by the fluoroquinolones, which show an expanded antibacterial spectrum [2]. Currently, various new quinolones are under evaluation, as they have a broad activity and are even effective in the treatment of multi-resistant organisms [3]. Moreover, they are used in

patients with hypersensitivities to other antibiotics (e.g. penicillin) as they have a structure unrelated to them and hence show no cross-reactivity.

However, quinolones themselves can induce adverse drug reactions as well. Ciprofloxacin (CPFX), the most frequently used fluoroquinolone, induces adverse cutaneous reactions in about 1–2% of treated patients [4]. The most frequently described reactions are immediate – causing urticaria, angio-oedema, anaphylaxis [5] – suggesting a type I IgE-mediated allergic response. Indeed, Manfredi et al. [6] established a serological test capable of

detecting IgE antibodies to CPFX and other quinolones. Delayed-type hypersensitivity reactions, in particular, different forms of exanthema, can also occur in the context of quinolone treatments and are thought to be T cell-mediated. However, the immunogenicity of quinolones for T cells and their apparent cross-reactivity have not yet been studied in detail [7–11].

T cell-mediated drug allergies are examples of immune reactions to small molecular compounds (≤ 1000 Da), indicating that drugs can be stimulatory for T cells by reacting with their T cell receptor (TCR) [12, 13]. T cell reactions can develop if the drug acts as a hapten, i.e. by forming a stable covalent bond with a macromolecular carrier such as a larger endogenous peptide or protein. However, we recently showed that even a chemically inert drug can elicit a T cell stimulation if its structural features allow it to interact with TCR and if antigen (Ag)-presenting cells (APC) with major histocompatibility complex (MHC) molecules are present [14–16]. The stimulated T cells proliferate, are cytotoxic and produce cytokines and chemokines [17, 18]. To account for these observations, which are not compatible with the classical hapten mechanism, we have proposed the concept of pharmacological (p) interaction with immune (i) receptors, or p-i concept [16] in abridged form.

In this study, we analyse how T cells can react with various quinolones by investigating T cells from patients with quinolone hypersensitivity. Both patch (epicutaneous) and *in vitro* lymphocyte proliferation tests (LTT) indicated the presence of quinolone-specific T cells in patients with a history of hypersensitivity to quinolones [19, 20]. We further generated T cell clones (TCC) from one person allergic to CPFX and from a second patient allergic to moxifloxacin (MXFX), and evaluated how these T cells reacted with CPFX/MXFX and other quinolones. Our data show that cross-reactivity of T cells within the quinolone family exists as mentioned previously in clinical studies [4, 9, 21]. On the other hand, the reactivity of quinolones with the TCR may also be very specific, as only one particular quinolone stimulated certain TCC. Moreover, CPFX and MXFX appear to belong to those drugs that can interact directly with TCR [16] without previous need of covalent hapten-carrier formation.

Material and methods

Patients

According to the history of the patients, none of them was exposed to any quinolones before the treatment that caused the allergic reaction.

Patient 1 was a 61-year-old female who developed an exanthema on the trunk and extremities 3 days after starting Ciproxin[®] (Bayer AG, Zurich, Switzerland) (CPFX) for cystitis. Six months later, she was given Noroxin[®] (Merck

Sharp, Glattburg, Switzerland) (norfloxacin, NRFX) as treatment to a recurrent cystitis and, within hours after drug exposure, developed a generalized exanthema. Simultaneously, a slight transient elevation of γ -glutamine transferase and alanine amino transferase was observed.

Patient 2 was a 22-year-old female given CPFX as treatment for pyelonephritis. Within 3 days after the first intake, she developed an itching, maculopapular drug eruption on the back. One year later she was treated with NRFX against a recurrent pyelonephritis. Two to 3 days after the onset of the NRFX treatment, a generalized flush reaction appeared.

Patient 3 was a 22-year-old female who suffered from perinasal skin infections. A skin smear showed the presence of *Staphylococcus aureus* and streptococcus group B. CPFX treatment attenuated the infection but, a few days after the first intake, induced a maculopapular skin eruption around the mouth, on the inside of the arms and on the lower part of the legs, which was diagnosed as an acute generalized exanthematous pustulosis (AGEP).

Patient 4 was a 72-year-old female patient, treated with a 2-week course of CPFX for cystitis. Nineteen days after the beginning of treatment, she developed a maculopapular exanthema on the trunk and upper and lower extremities.

Patient 5 was an 80-year-old female, with gastroenteritis treated for 7 days with CPFX. At this time-point, she developed AGEP on her whole body with the exception of her face.

Patient 6 was a 79-year-old female treated with a 5-week course of Avalox[®] (Bayer AG, Zurich, Switzerland) (MXFX) for a lung infection. On week 5, she developed a general exanthema.

This study was approved by the ethics committee of the university and an informed consent was received from all patients.

Patch tests

The patch tests were performed, as described [22], on the upper back of the patients at 6–8 weeks after complete healing of the cutaneous adverse drug reaction. Briefly, the commercialized form of the drugs (pills) used by the patients were ground to a very fine powder, diluted to 10 or 25% in white petrolatum and applied using plasters. The patch test reactions were read at 20 min, 24 and 48 h. Results were reported according to the International Contact Dermatitis Research Group criteria for patch test reading. [23]

Culture media

Culture medium (CM) used to culture T cell lines (TCL) and TCC was RPMI 1640 (Invitrogen AG, Basel, Switzerland) supplemented with 10% pooled, heat-inactivated human

AB serum (Swiss Red Cross, Bern, Switzerland), 25 mM Hepes buffer (Biochrom AG, Rupperts, Switzerland), 2 mM L-glutamine (Biochrom AG), 10 mg/mL streptomycin (Amimed Products, Allschwil, Switzerland), 100 U/mL penicillin (Amimed Products) and 25 µg/mL transferrin (Sigma Chemicals Co., Buchs, Switzerland). TCC were cultured in CM supplemented with 40–100 U/mL human recombinant IL-2 (rIL-2, Roche Pharma AG, Basel, Switzerland). EBV-transformed B-lymphoblastoid cell lines (B-LCL), generated as described [24], were cultured in RPMI 1640 supplemented with 10% pooled heat-inactivated fetal calf serum (FCS, Invitrogen), 25 mM Hepes buffer, 2 mM L-glutamine, 10 mg/mL streptomycin and 100 U/mL penicillin.

Drugs and antigens

All drugs (Fig. 1) used for the experiments, except for CPFX (Bayer AG, Wuppertal, Germany), MXFX (Bayer AG) and Levofloxacin (LVFX, Aventis Pharma, Frankfurt, Germany), were purchased from Sigma. Before each experiment, stock solutions of each drug in CM with 0.1 M NaOH were freshly prepared and used at 10 µg/mL if not specified otherwise. Tetanus toxoid (TT; Serum and

Vaccine Institute, Bern, Switzerland) was used as a control Ag at 10 µg/mL.

Proliferation assays

Proliferations of T cells to drugs (lymphocyte transformation test (LTT)) were evaluated with 2×10^5 freshly isolated peripheral blood mononuclear cells (PBMC) from healthy or allergic donors. They were cultivated for 6 days in 200 µL CM in U-bottom 96-well microplates (Falcon, Franklin Lakes, NJ, USA) in the presence of drugs at a concentration of 0.1–100 µg/mL. [^{3} H]-thymidine (0.5 µCi; Amersham Pharmacia Biotech, Dübendorf, Switzerland) was added to the culture for 8–14 h. The cells were harvested (96-well Cell Harvester; Inotech, Wohlen, Switzerland) and the incorporated radioactivity was measured by a scintillation, liquid-free β -counter (Trace96; Inotech). Stimulation indices (SIs) were calculated as the ratio between the count/minute (c.p.m.) in culture with Ag and the c.p.m. in culture without Ag.

To test the specificity of TCL and TCC, 5×10^4 T cells were incubated at days 10–16 after restimulation with either 5×10^4 autologous irradiated PBMC or with 10^4 autologous irradiated B-LCL as APC in the presence or

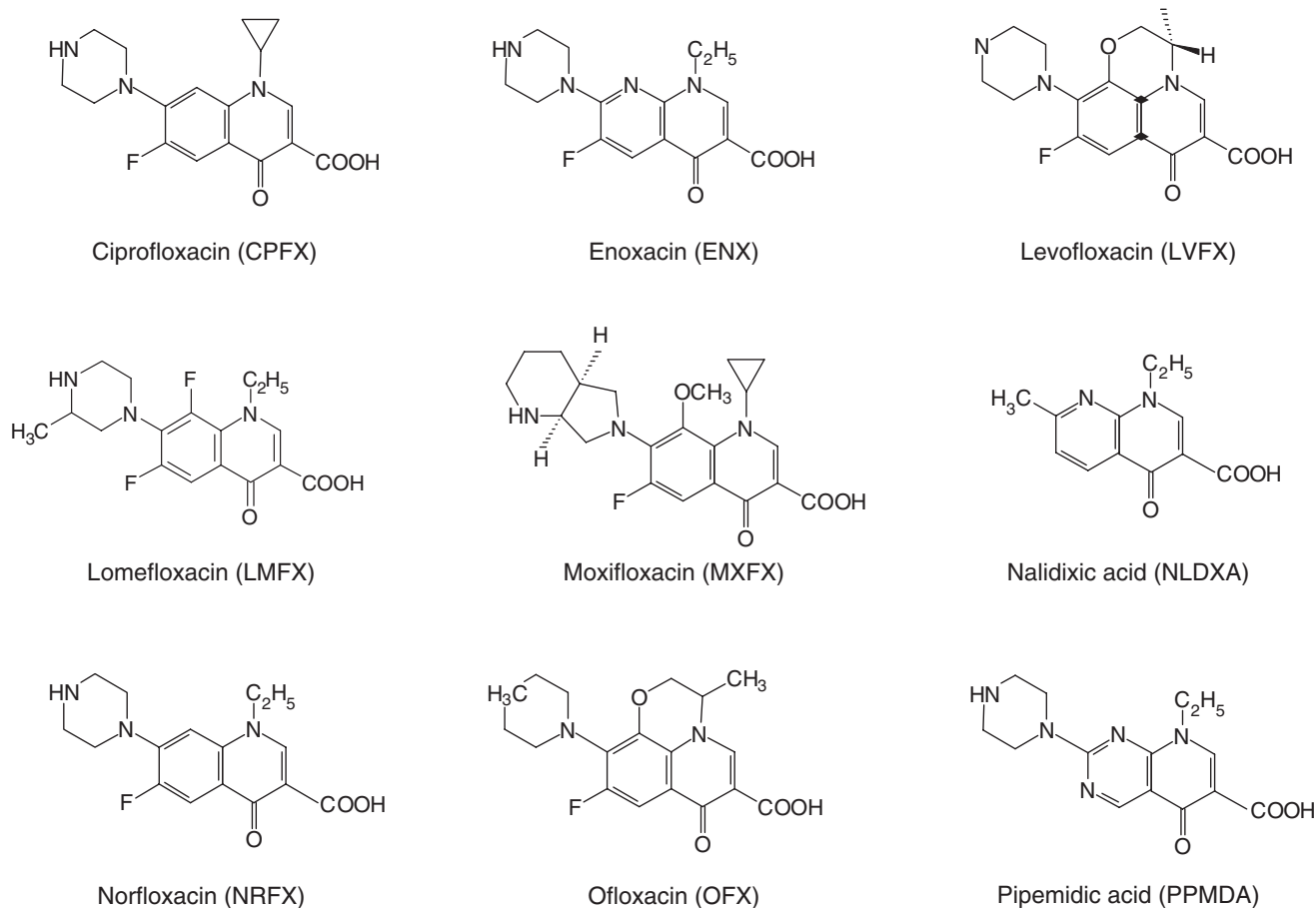


Fig. 1. Chemical structures of the used quinolones.

absence of the indicated drug in 200 µL CM in U-bottom 96-well microplates. Proliferation was detected after 48 h by [³H]-thymidine incorporation as described above. The TCL/TCC with an SI ≥ 2 were further expanded.

In APC-pulsing experiments, autologous B-LCL were incubated with or without the quinolone (10 µg/mL) for 2 or 4 h in Hank's balanced salt solution (HBSS). Cells were washed twice with HBSS and re-suspended in CM before being either irradiated or fixed.

Generation of drug-specific T cell clones

Freshly isolated PBMCs from a NRFX- and CPFX-allergic donor (patient 1) and a MXFX-allergic donor (patient 6) were stimulated with CPFX and MXFX, respectively (0.1–50 µg/mL) in CM at a cell density of 2×10^6 cells per well in a 24-well plate (no. 3047; Falcon). CM with 40 µg/mL rIL-2 was added after 7 days of culture. The bulk culture was restimulated after 14 days with autologous-irradiated (4500 rad) PBMC and CPFX or MXFX (10 µg/mL) and tested for specificity (see proliferation assay). Fourteen days later, CPFX/MXFX-activated cells were cloned by limiting dilution as described earlier [25]. Growing T cells were expanded in CM with 100 µg/mL rIL-2 and restimulated every 14 days with allogeneic, irradiated (4500 rad) PBMC and 1 µg/mL phytohemagglutinin (PHA; Difco, Detroit, MI, USA).

Characterization of drug-specific T cell clones

The phenotype of drug-specific TCC was determined by flow cytometry and RT-PCR. For flow cytometry, aliquots of 10^5 cells were stained with fluorochrome-conjugated monoclonal antibodies (mAb) in 50 µL buffer (PBS 1% FCS 0.02% Na-N₃) for 30 min at 4 °C and then analysed on a Coulter EPICS XL-MCL Flow Cytometer (Beckman Coulter, Nyon, Switzerland). Monoclonality of TCC was proven by staining with fluorochrome-labelled anti-CD3 mAb (Pharmingen/BD, Basel, Switzerland) and a panel of 22 mAb recognizing different Vβ gene products as described [25], or by RT-PCR. The phenotypes of the TCC were determined by labelling with anti-CD3, anti-CD4 and anti-CD8 mAb (Pharmingen/BD).

For RT-PCR, 25 primers for the TCR Vβ-chain [26] were amplified together with an oligoprimers recognizing the constant region as described before [27].

Glutaraldehyde fixation

An aliquot of 2×10^6 APC in 0.5 mL HBSS was fixed by addition of 0.05% glutaraldehyde for 30 s at room temperature as described [28]. The reaction was stopped by adding 500 µL of 0.2 M L-glycine for 45 s. The cells were then washed twice with CM before being added to TCC in the presence or absence of Ag (10 µg/mL). After 48 h, proliferation was determined as described above.

Results

Patients' characteristics and patch test

Six patients with macular or maculopapular exanthema after CPFX, NRFX or MXFX treatment were evaluated by patch tests and LTT to confirm CPFX/NRFX/MXFX-mediated delayed-type hypersensitivity (Table 1 and Fig. 2). Patch tests gave rise to a clearly positive reaction in three (patients 1, 2 and 5) out of the six patients after 24 and 48 h. Patient 1 showed a positive reaction to CPFX and NRFX while the tests of patients 2 and 5 were only positive for CPFX. Patients 3, 4 and 6 were only tested for CPFX or MXFX, and the results were negative. Negative epicutaneous patch tests with quinolones have been reported previously in cases of confirmed quinolone allergy [4, 9, 11]. The patch tests were also performed in five non-allergic subjects and found to be negative.

Drug-induced lymphocyte proliferation

To further confirm the involvement of T cells, LTT was performed [20] (Fig. 2). To determine the non-toxic concentration of quinolones in this assay, the inhibitory effect of quinolones on PHA stimulation was assessed. The different quinolones revealed to be toxic at concentrations above 20 µg/mL and 10 µg/mL was chosen as the optimal concentration for further proliferation experiments (data not shown and Fig. 2). Our results confirm the findings of

Table 1. Patients' characteristics and results of the patch tests

	Patients					
	1	2	3	4	5	6
Diagnosis	Cystitis	Recurrent pyelonephritis	<i>Staphylococcus aureus</i> and <i>Streptococcus</i> Group B skin infection	Cystitis	Gastroenteritis	Lung infection
Treatment	CPFX and NRFX	CPFX and NRFX	CPFX	CPFX	CPFX	MXFX
Patch test (24 and 48 h)						
CPFX	+	+	–	–	+	ND
NRFX	+	–	ND	ND	–	ND
MXFX	ND	ND	ND	ND	ND	–

CPFX, ciprofloxacin; NRFX, norfloxacin; MXFX, moxifloxacin; ND, not done.

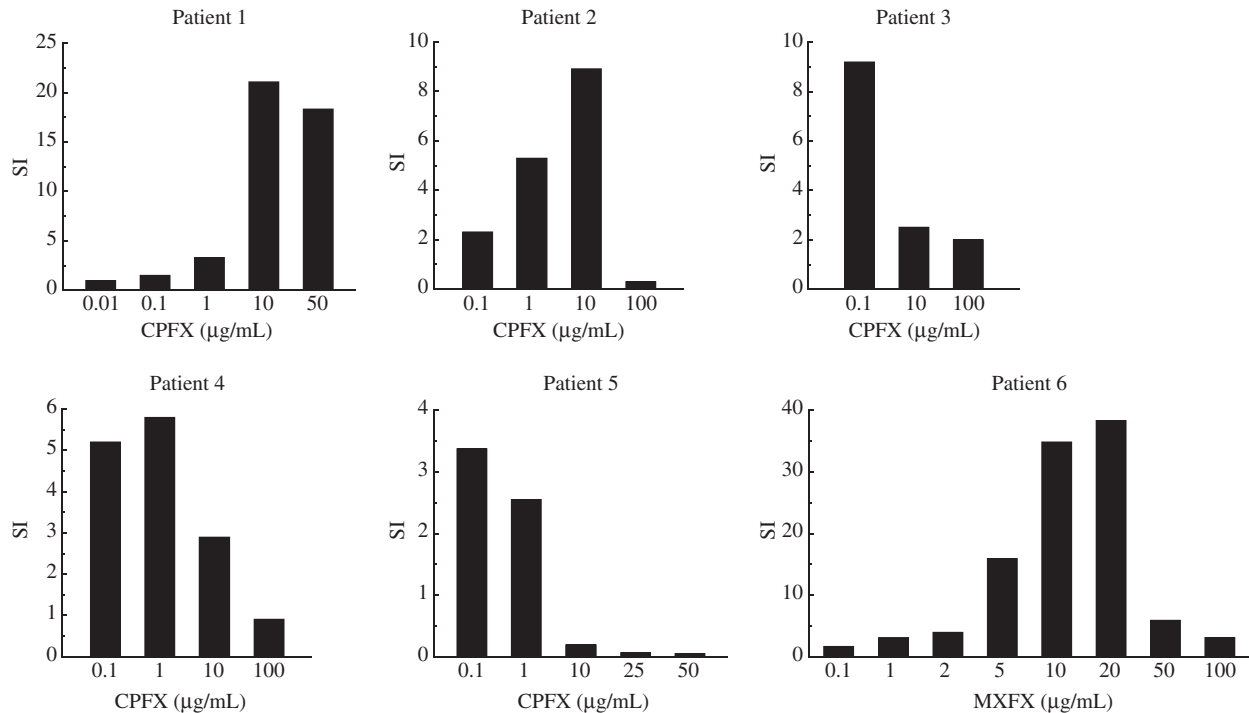


Fig. 2. Lymphocyte proliferation test to the incriminated quinolone. Peripheral blood mononuclear cells (PBMC) were incubated 6 days with the drug and proliferation was measured by ^3H -thymidine incorporation. Background values of control cultures were between 330 and 668 count/minute (c.p.m.). Results are expressed in SI calculated as the ratio between the c.p.m. in culture with antigen (Ag) and the c.p.m. in culture without Ag. Note that different concentrations are needed to achieve optimal proliferation. PBMC of patients 1, 3 and 5 reacted also to NFRX with a maximal SI of 3.9, 2.6 and 2.8, respectively.

Forsgren et al. [29] that CPF and other quinolones inhibit peripheral blood lymphocyte cell growth at concentrations higher than 20 µg/mL.

Stimulation of PBMC of patients 1–5 with CPF and of PBMC of patient 6 with MXF resulted in significant proliferation of PBMC after a 6-day co-incubation with the drug, while PBMC from exposed but non-allergic individuals ($n=3$) and non-exposed individuals ($n=3$) did not show enhanced proliferation (Fig. 2 and data not shown). Quite interestingly, different donors showed differences in the optimal concentration able to stimulate quinolone-specific T cells. This emphasizes the need of dose-response curves in evaluating *in vitro* reactivity [20].

Cross-reactivity of T cells within PBMC to CPF and NFRX was positive in patient 1 (who also had a reaction after NFRX intake), patients 3 and 5 (see legend of Fig. 2). The PBMC of patient 2 showed a proliferative response to CPF, but not to NFRX. The PBMC of patient 4 were tested to CPF only, and were positive. PBMC of patient 6 proliferated in response to MXF. Other quinolones were also tested on the PBMC of patients 1 and 6, but none of them induced an enhanced proliferation (Figs 3a and b).

Generation and analysis of a T cell line

The PBMC of patients 1 and 6 were kept in culture for 4 weeks in the presence of CPF or MXF and the

resulting TCL was tested for specificity to several different quinolones (Fig. 3). The TCL of patient 1 responded to all the quinolones tested, although to a different extent, even though the patient had only been in contact with CPF and NFRX (Fig. 3a). The TCL of patient 6 responded to MXF but did not proliferate in response to any other quinolone. However, the proliferation to MXF was low in those experiments as well (Fig. 3b).

Phenotypic analysis of ciprofloxacin-specific T cell clones

To investigate the involvement of T cells in adverse reactions to quinolones, we generated 45 CPF-specific TCC out of PBMC from sensitized donors 1 and 18 MXF-specific TCC out of PBMC of sensitized donor 6. Their SI ranged from 2 to 210. Figure 4 shows the proliferative response to CPF or MXF of 10 TCC of patients 1 and 6 obtained from 0.3 cells/well cultures after the cloning procedure: they all showed a proliferation to 10 µg/mL (obtained as optimal concentration in dose-response experiments), but only in the presence of APC, excluding APC-independent presentation and presentation by the TCC themselves (data not shown) [13, 30]. Most of the TCC were of the CD4^+ phenotype, which is in agreement with immunohistological analysis of maculopapular exanthema where mainly or exclusively CD4^+ T cells emigrate into the dermis and epidermis [31, 32]. Only one CD8^+ TCC and

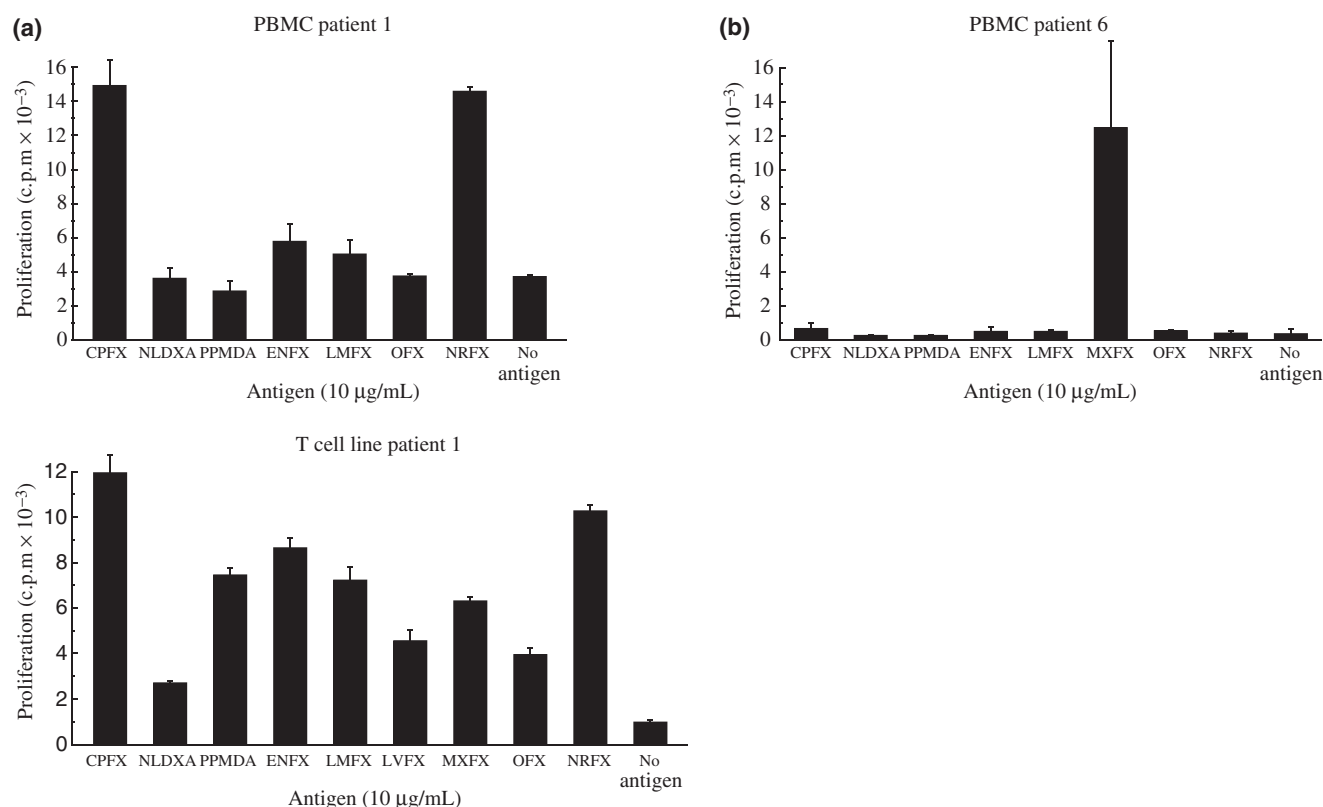


Fig. 3. Proliferative response of peripheral blood mononuclear cells (PBMC) and T cell lines (TCL) of patients 1 (3a) and 6 (b). PBMC were cultured with the different drugs (10 µg/mL) for 6 days and proliferation was measured by [³H]-thymidine incorporation. TCL aliquots were co-cultured with autologous B-lymphoblastoid cell lines cells as antigen-presenting cells in the presence or absence of the drug (10 µg/mL) for 48 h and proliferation was measured by [³H]-thymidine incorporation. Results are given as mean ± SD of duplicate cultures. One representative experiment out of three is shown. TCL of the moxifloxacin (MXFX)-sensitized donor was generated three times but did not reveal a substantial proliferation to any quinolone ($n = 8$) and only once to MXFX.

one CD4/CD8 double positive TCC were generated from the PBMC of patient 6 (Fig. 4 TCC 8 and TCC 3, respectively). They all expressed the $\alpha\beta$ -TCR without a preferential use of certain V β (Fig. 4). To detect preferentially cytotoxic CD8+TCC, a different cell culture technique might be required [31].

Stimulation by ciprofloxacin neither requires covalent binding to the major histocompatibility complex-peptide complex nor processing

In order to determine how quinolones are recognized by quinolone-specific TCC and whether the drug itself or a potential metabolite is responsible for the stimulation, we investigated the response of the TCC to APC pre-pulsed with CPFX (patient 1) or MXFX (patient 6) and then washed. The drug would thereby be removed if the binding to the MHC-peptide complex was labile, while covalently bound drugs are not washed away [32]. Figure 5 shows that pre-incubation of APC with CPFX or MXFX and subsequent washing fails to stimulate the quinolone-specific TCC. This is in favour of a mechanism where the drug is recognized without covalent association with the

MHC-peptide complex. Furthermore, fixation of APC with glutaraldehyde, which abolishes their ability to take up and present the drug on the surface, did not interfere with their capacity to present CPFX or MXFX to specific TCC. Indeed, glutaraldehyde fixed APC induce proliferation up to the same extent as unfixed APC co-incubated with the drug (Fig. 5). Taken together, our results suggest that quinolones bind directly and in a labile manner to the TCR and MHC-peptide complex, and that neither metabolism nor processing is necessary for quinolone presentation and T cell proliferation.

Cross-reactivity of the ciprofloxacin specific T cell clones to other quinolones

The immunological cross-reactivity of the quinolone-specific TCC with other drugs of the same family is of scientific and clinical interest. To investigate this, we used different quinolones (Fig. 1) at concentrations of 10 µg/mL together with autologous B-LCL as APC to stimulate eight different TCC of patients 1 and 6. Within the TCC of patient 1, we could distinguish three patterns of cross-reactivity. One group of clones (two out of eight) showed

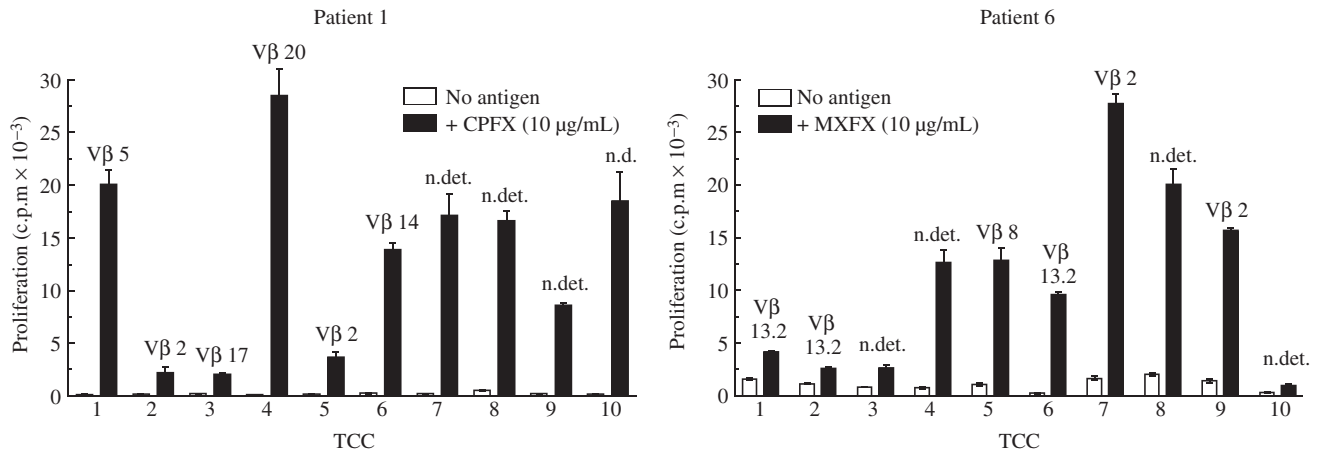


Fig. 4 V β analysis and proliferative response of ciprofloxacin (CPFX)- and moxifloxacin (MXFX)-specific T cell clones (TCC) of patients 1 and 6. TCC were co-cultured with autologous B-lymphoblastoid cell lines (B-LCL) cells as antigen-presenting cells in the presence or absence of the drug (10 μ g/mL) for 48 h. Results are given as mean [3H]-thymidine incorporation $\pm$ SD of duplicate cultures. The results of the T cell receptor-V β analysis are shown (n.det., not detected; nd, not done). One representative experiment out of three performed is shown.

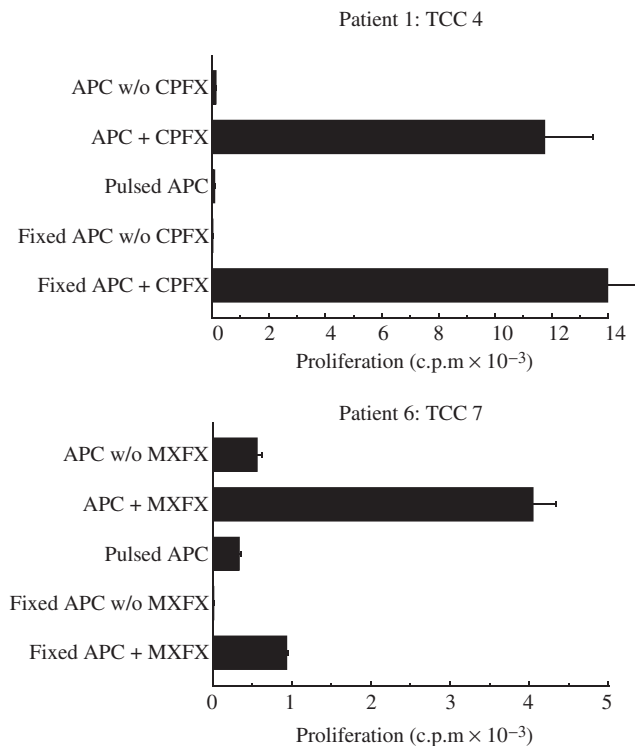


Fig. 5. Lability of the drug binding to the major histocompatibility complex (MHC). Quinolone-pulsed antigen-presenting cells (APC) fail to stimulate quinolone-specific T cell clones (TCC) of patients 1 and 6. Co-incubation of the drug (10 μ g/mL) with APC and TCC was required to elicit proliferation. Glutaraldehyde fixation of APC did not interfere with the presentation of quinolones to quinolone-specific TCC. Mean $\pm$ SD of duplicate cultures are given. Two representative experiments of six TCC tested.

no cross-reactivity as no other compound than CPFX showed a stimulatory effect (represented by TCC 2, Fig. 6). A second group (three out of eight) showed a

restricted cross-reactivity because they only responded to CPFX and NRFX (represented by TCC 1, Fig. 6). A third group of clones (three out of eight) showed a broad cross-reactivity and could be stimulated by as many as five compounds (CPFX, enoxacin (ENX), lomefloxacin (LMFX), LVFX, MXFX, ofloxacin (OFX) and NRFX) (represented by TCC 6 and 7, Fig. 6). In patient 6, all the TCC tested (eight out of eight) were specific to MXFX and did not react to any other quinolone (Fig. 6).

Discussion

Quinolones are widely used antibiotics, which quite frequently elicit adverse drug reactions. Some of them are due to an immune reaction [33]. A better comprehension of the pathomechanism underlying these side-effects is crucial to recognize and avoid them and to prevent cross-reactivity with other quinolones. This understanding is also important for the pharmaceutical industry, as it might contribute to the development of quinolones with lower 'allergenic potential'.

Here, we demonstrate that delayed drug hypersensitivity reactions to quinolones can be associated with a strong T cell immune response against the relevant quinolone. We further give evidence that the parent, chemically inert compound is recognized by T cells without the necessity of metabolism or processing.

Thus, most likely, the parent compound represents the relevant Ag, at least for the T cell reactions causing exanthema, as observed in the six patients of this study. However, the possibility that quinolone metabolites could stimulate T cells as well cannot be excluded. We assume that the drug interacts directly and in a rather labile way with the TCR and MHC-peptide complex, as incubating APC with the drug, followed by washing, removes it [14]. This is different from haptens, like sulphamethoxazole

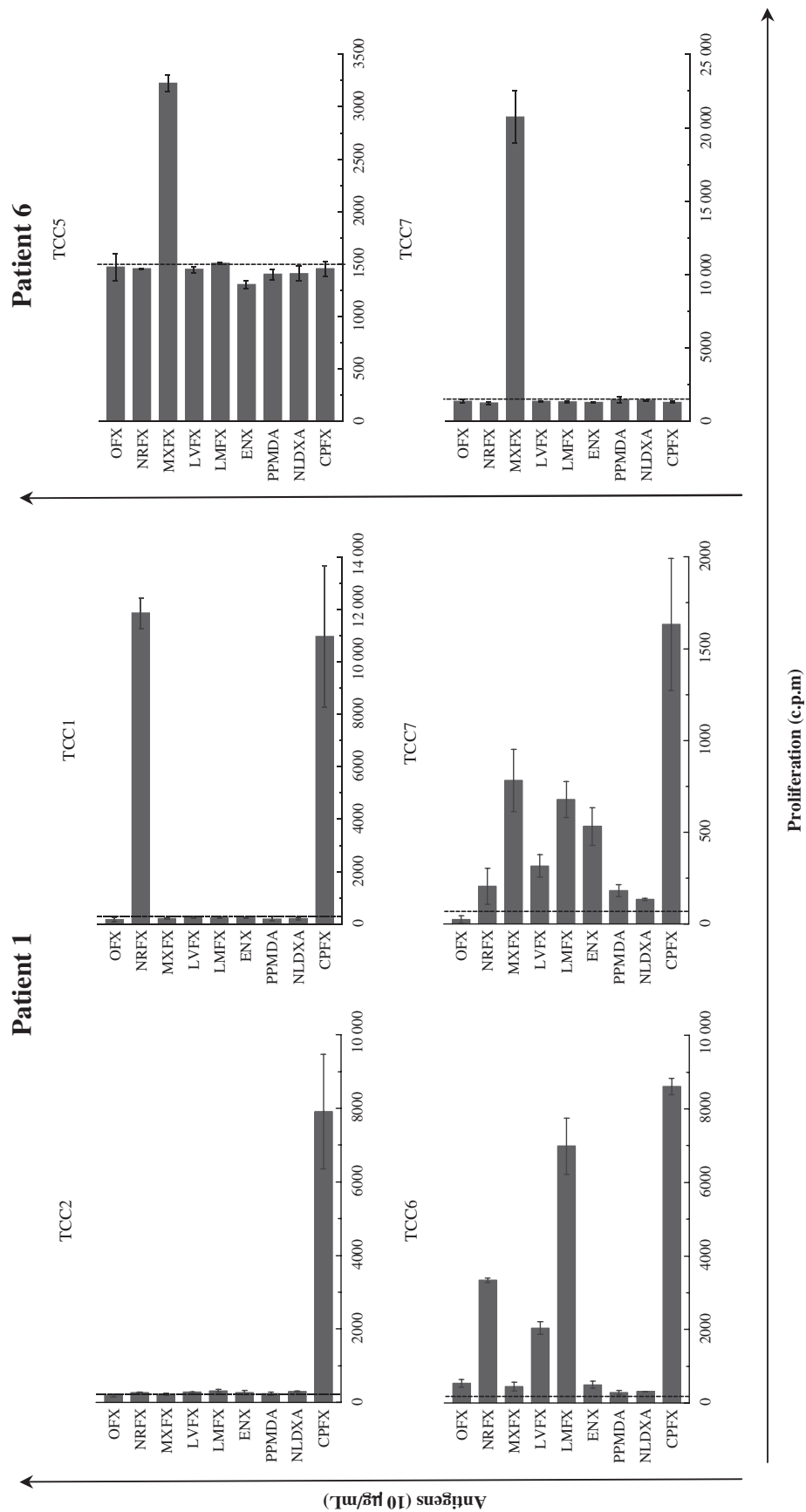


Fig. 6. Ciprofloxacin (CPFX)-specific T cell clones (TCC) of patient 1 show three different patterns of cross-reactivity while moxifloxacin (MXFX)-specific TCC of patient 6 are only specific for MXFX. Eight TCC of each patients 1 and 6 were tested and incubated with autologous Ag-presenting cells and the different antigens (10 µg/mL) for 48 h. Proliferation was inferred from the [³H]-thymidine uptake (mean ± SD) of duplicate cultures. The dotted line represents the proliferation in absence of drug. One representative experiment is shown (each TCC was tested two to three times).

(SMX)-nitroso or penicillin G, which are stimulatory after pulsing, as they bind covalently to MHC-peptide complexes [34, 35]. Thus, our data suggest that a substantial amount of side-effects to quinolones is induced by pathomechanisms similar to the ones already shown for other drugs (SMX, lidocaine, carbamazepine, lamotrigine, etc.) [25, 36–39], and compatible with the 'p-i' concept [16]. This finding has major implications for predictive allergy testing during quinolone development, which focuses almost exclusively on hapten-dependent immunogenicity.

Nevertheless, other side effects of quinolones, for example hepatitis or anaphylaxis [6], may be due to the formation of active intermediates, which act as haptens forming neo-Ags by covalently binding to proteins/peptides and then initiate an immunological reaction [40]. Thus, probably both, hapten (relevant for IgE reactions) and p-i mechanisms (relevant for delayed T cell reactions), can occur with quinolones and may – similar to the sulphamethoxazole model – even occur simultaneously [32].

Some of our patients showed positive patch tests after 24 and 48 h. An immune reaction to the inert drug can explain those skin reactions. Alternatively, chemically reactive metabolites might also be responsible if metabolism of quinolones can occur in the skin, as has been shown for SMX [41]. Other patients showed a negative patch test after 24 and 48 h despite a positive LTT. False-negative drug patch tests can be attributed to various causes. First, the sensitivity of patch test is lower than that of LTT [42], as the read out system is actually a complex inflammatory response in the skin. Second, the drug tested may penetrate insufficiently through the epidermis, or the concentration used in patch testing may be too low to elicit a positive reaction in a particular patient. Finally, the culprit source of the cutaneous reaction may not be the native drug itself, but a metabolite.

Cross-reactivity is a well-known problem in quinolone hypersensitivity. Our data suggest that, similar to the IgE-mediated reactions [6], T cell-mediated reactions can also show certain levels of cross-reactivity. Of note, even patch tests can be positive to related quinolones in one individual. The observed cross-reactivity seen with PBMC (patients 1 and 3) might be of clinical relevance, as patient 1 reacted with an exanthema to both CPFX and NRFX at different time-points. The analysis of TCL gave a substantially broader spectrum of cross-reactivity than the analysis of PBMC. This observation can be explained by the fact that the frequency of drug-cross-reactive lymphocytes is below the level detectable with proliferation assays of PBMC. Specific cells present at very low frequencies may be obscured by background proliferation. Longer lasting cultures may allow the expansion of these rather rare T cells.

As patient 1 had never been in contact with ENX, LMFX, LVFX, MXFX and OFX before, this may reflect a true cross-reactivity at the T cell level. The analysis of TCC

showed that actually one specific TCR is able to recognize and react to more than one quinolone, as TCC specific for one quinolone, for two quinolones or for a battery of different quinolones were found. These TCC to quinolones bear different V β TCR indicating that – at least in the two patients analysed in depth – the immune response to quinolones is quite heterogeneous and may not be restricted to an oligoclonal expansion of certain T cells. Thus, quinolones appear to be able to interact with a great variety of T cells bearing distinct TCR, much like sulphonamide antibiotics, local anaesthetics and penicillins [25, 36, 37].

Looking at the structure of the different quinolones (Fig. 1) and the cross-reactivity experiments (Fig. 6), one can infer an important role for the ring substituent at position 7. When it was missing, like in NLDXA, no recognition by the TCC tested occurred, and when the substituent at position 7 was rather large as in MXFX, a rather selective response developed (patient 6). The fluoride at position 5 might also be important, but this is less consistent (some recognition of pipemidic acid (PPMDA) was seen, and the presence of a fluoride was surely not sufficient for recognition).

For the clinical situation, our findings imply that an allergy to one quinolone may bear the risk of a cross-reactivity to other quinolones, including those which are new on the market. Thus, like in IgE-mediated reactions [6], it might be prudent to avoid the whole class of quinolones in a quinolone-sensitized patient, particularly after a severe, generalized delayed reaction to a quinolone [43]. However, if the reaction was rather mild, re-exposure to another quinolone might be possible, as well documented with the new quinolone 'gemifloxacin', where only 10% of patients who had developed an exanthema during a 10-day treatment with gemifloxacin reacted to CPFX upon re-exposure [44].

In conclusion, quinolones can elicit delayed-type hypersensitivity reactions and are able to stimulate T cells in a specific, TCR- and MHC-dependent way. Quinolones can interact with a broad variety of human TCR. Some of these are highly specific for one compound while others show a broader cross-reactivity, which might be the basis for the frequently observed cross-reactivity of different quinolones in the clinic.

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