

Long-lasting reactivity and high frequency of drug-specific T cells after severe systemic drug hypersensitivity reactions

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Background: Drug-reactive T cells are involved in most drug-induced hypersensitivity reactions. The frequency of such cells in peripheral blood of patients with drug allergy after remission is unclear.

Objective: We determined the frequency of drug-reactive T cells in the peripheral blood of patients 4 months to 12 years after severe delayed-type drug hypersensitivity reactions, and whether the frequency of these cells differs from the frequency of tetanus toxoid-reactive T cells.

Methods: We analyzed 5 patients with delayed-type drug hypersensitivity reactions, applying 2 methods: quantification of cytokine-secreting T cells by enzyme-linked immunospot (ELISpot), and fluorescent dye 5,6-carboxylfluorescein diacetate succinimidyl ester (CFSE) intensity distribution analysis of drug-reactive T cells.

Results: Frequencies found were between 0.02% and 0.4% of CD4⁺ T cells reacting to the respective drugs measured by CFSE analysis, and between 0.01% and 0.08% of T cells as determined by ELISpot. Reactivity was seen neither to drugs to which the patients were not sensitized nor in healthy individuals after stimulation with any of the drugs used.

Conclusion: About 1:250 to 1:10,000 of T cells of patients with drug allergy are reactive to the relevant drugs. This frequency of drug-reactive T cells is higher than the frequency of T cells able to recognize recall antigens like tetanus toxoid in the same subjects. A substantial frequency could be observed as long as 12 years later in 1 patient even after strict drug avoidance. Patients with severe delayed drug hypersensitivity reactions are therefore potentially prone to react again to the incriminated drug even years after strict drug avoidance. (*J Allergy Clin Immunol* 2006;117:455-62.)

Key words: Frequency, T cells, delayed type drug hypersensitivity, tetanus-toxoid, drug avoidance

Abbreviations used

AGEP:	Generalized exanthematous pustulosis
CFSE:	Fluorescent dye 5, 6-carboxylfluorescein diacetate succinimidyl ester
CM:	Culture medium
cpm:	Counts per minute
DRESS:	Drug rash with eosinophilia and systemic symptoms
LTT:	Lymphocyte transformation test
SI:	Stimulation indices
SJS:	Stevens-Johnson syndrome
TCR:	T-cell receptor
TT:	Tetanus toxoid

Drug-specific T cells are involved in most drug-induced hypersensitivity reactions.¹ They either coordinate the inflammatory response or are involved in the pathogenesis directly by killing tissue cells like keratinocytes, thus causing various forms of exanthema.¹⁻³ Drugs stimulate T cells either by forming hapten-protein complexes presented by MHC or possibly by direct binding to T cell receptors.^{4,5}

It is unclear how many T cells are activated by haptens or via direct T-cell receptor (TCR) stimulation in the various forms of acute drug hypersensitivity. In human beings, the severity of some responses suggests that at least in the acute stage of drug rash with eosinophilia and systemic symptoms (DRESS)/drug hypersensitivity syndrome, generalized exanthematous pustulosis (AGEP), and Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis, a high proportion of cells participates in the reaction, because a massive T-cell activation can be observed in the peripheral blood in some of these reactions.⁶ Some patients respond to minute amounts of the drug with severe reactions even years after the initial hypersensitivity reaction,⁷ and drug reactivity can persist over many years, as evidenced by repeatedly positive *in vitro* and *in vivo* tests.^{8,9} These observations suggest that some individuals preserve a rather constant and substantial repertoire of drug-specific T cells over a long time, comparable to the situation with a recall antigen. Moreover, analysis of drug reactive T cells revealed that the drug-specific response is polyclonal, suggesting that many T cells are able to interact with the drug.¹⁰

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TABLE I. Patient characteristics

	Patients				
	VG	UNO	HH	WZ	CS
Age (y)/sex	29/F	45/M	45/M	62/M	34/F
Drug allergy to	VM	SMX	AX	PY, SMX, AX	CBZ
Disease	AGEP	Exanthema and malaise	SJS	DRESS and erythrodermia	DRESS
Patch test/LTT	+/+	+/+	+/+	-/+	+/+
Interval*	14 mo	12 y	9 mo	19 mo	4 mo

AX, Amoxicillin; CBZ, carbamazepine; PY, phenytoin; SMX, sulfamethoxazole; VM, vancomycin.

*Interval between acute allergy and frequency analysis of drug-specific T cells.

However, only 1 study in a single patient with confirmed sulfamethoxazole allergy has addressed the question of frequency of drug-specific T cells in drug allergy so far, identifying sulfamethoxazole-reactive lymphocytes in the peripheral blood at a frequency of 1/172,000 by using limiting dilution techniques,¹¹ which would be much lower than the known frequencies of a typical recall antigen such as tetanus toxoid (TT; 1/750 to 1/11,500) determined with limiting dilution techniques^{12,13} and confirmed with fluorescent dye 5, 6-carboxylfluorescein diacetate succinimidyl ester (CFSE)/tetramer proliferation assay.¹⁴

The study used limiting dilution assays, an indirect and somewhat cumbersome method susceptible to problems inherent to prolonged cell cultures. To obtain a better understanding of the frequency of drug-reactive cells, we applied 2 methods to determine the frequency of drug-reactive T cells¹⁵⁻¹⁷ and compared the results with the frequency of TT-reactive T cells in the peripheral blood of the patients. These methods allow the detection of cytokine secretion and proliferation of antigen-reacting T cells *in vitro*. Specifically, we measured (1) T_H1 and T_H2 cytokines (IL-2, IFN- γ , IL-4, IL-5, and IL-13) of T cells after drug stimulation for 48 hours by enzyme-linked immunospot (ELISpot), and (2) proliferation of drug-reacting T cells after stimulation for 6 days by CFSE proliferation assay.

We applied these analyses to 5 patients with drug allergy during remission and 5 healthy donors. All patients had well documented forms of drug hypersensitivity reactions like severe maculopapular exanthema, DRESS/drug hypersensitivity syndrome, AGEP, and SJS after administration of various drugs (amoxicillin, vancomycin, sulfamethoxazole, phenytoin, and carbamazepine).

We conclude that the frequency of drug-reactive T cells in remission is much higher than previously thought, and in fact is comparable to TT-specific T-cell frequencies. Furthermore, we show that drug-specific cells persist for as long as 12 years in peripheral blood even after strict avoidance of the drug. Hence, patients with delayed drug hypersensitivity reactions are potentially prone to react again to the incriminated drug even years after strict drug avoidance.

METHODS

Donor characteristics

A summary of the patients' clinical characteristics is given in Table I. The patients were analyzed in clinical remission of their drug

allergy and were healthy at the time of analysis. Healthy blood donors without a history of adverse drug reaction but responsive to TT served as controls. All patients gave informed consent, and the study was approved by the local ethical committee. Epicutaneous patch tests were performed and evaluated according to Barbaud et al.¹⁸

Drugs and antigens

Nontoxic concentrations of the drugs were used for *in vitro* stimulations. The following drugs were used for proliferation assays: amoxicillin (Sigma Chemical Co, Buchs, Switzerland), vancomycin (Sigma Chemical Co), sulfamethoxazole (Hoffmann-La Roche Ltd, Basel, Switzerland), phenytoin (Siegfried Holding AG, Zofingen, Switzerland), and carbamazepine (Sigma Chemical Co). Stock solutions of sulfamethoxazole and phenytoin were dissolved in RPMI-1640 containing 0.05 mol/L NaOH. TT (Berna-Biotech, Bern, Switzerland) 10 μ g/mL was used as a positive control antigen.

Lymphocyte transformation test

Freshly isolated PBMCs (2×10^5 /well) from patients with drug allergy and healthy donors were cultured in triplicate for 6 days (37°C; 5% CO₂) in 96-well U-bottom tissue culture plates in the presence of different drug concentrations or 10 μ g/mL TT as a positive control antigen. After 6 days, 0.5 Ci ³H-thymidine was added to the culture for 14 hours. ³H-thymidine incorporation was measured in a β -counter (Trace 96; Inotech, Wohlen, Switzerland). Stimulation indices (SIs) were calculated as counts per minute (cpm) in culture medium (CM) with antigen divided by cpm in CM without antigen.¹⁹

CFSE-based proliferation assay and cloning

To evaluate the proliferative response to the different antigens, PBMCs were labeled with CFSE (Molecular Probes, Eugene, Ore) for 8 minutes at 37°C in PBS. To stop labeling, an equal volume of heat-inactivated fetal bovine serum (FBS) was added. Cells were then washed in CM. CFSE-labeled cells 5×10^6 were plated into individual wells of a 24-well culture plate, and drugs were added as indicated. Cells were incubated at 37°C in 5% CO₂ for 6 days. Antigen-stimulated CFSE-labeled PBMCs were analyzed with a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif). Phycoerythrin-conjugated antihuman CD3 mAb and allophycocyanin (APC)-conjugated antihuman CD4 mAb were used for flow cytometry. All antibodies were purchased from BD Biosciences (San Jose, Calif). Propidium iodide was used to exclude dead cells. Flow-cytometric data files were analyzed with the Proliferation Wizard module in ModFit LT Macintosh software (Verity Software House, Topsham, Me). The frequency of the antigen-specific CD4⁺ T cells in the original population was determined by back-calculation (ie, 256 CD4⁺ T cells in the 8th peak that have divided 8 times ($2^8 = 256$) originate from 1 CD4⁺ T cell). Cells having undergone 1 to 2 divisions were considered unspecific proliferating

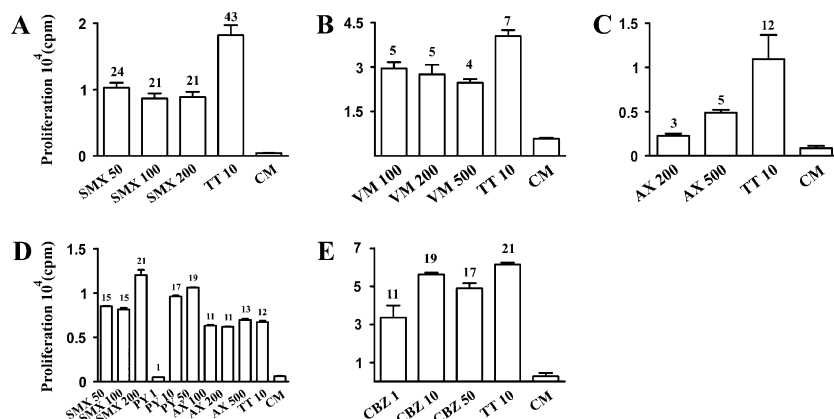


FIG 1. Drug-specific proliferation and SI of patients' PBMCs in a LTT. PBMCs of patients were coincubated with different concentrations ($\mu\text{g/mL}$) of drugs. TT and CM served as positive and negative controls. **A**, Patient UNO, sulfamethoxazole (SMX); **B**, patient VG, vancomycin (VM); **C**, patient HH, amoxicillin (AX); **D**, patient WZ, SMX, phenytoin (PY), and AX; **E**, patient CS, carbamazepin (CBZ).

cells and therefore were not included in the calculation (Fig 2, undivided parental generation P and dividing generations G).²⁰⁻²²

To confirm the drug-specificity of the CFSE^{low} CD4⁺ T cells, cells were sorted by using a FACS Vantage (BD Biosciences) and distributed in 96-well U-bottom culture plates (1 cell/well). T-cell clones were generated and cultured as described previously.^{10,23} To determine the specificity of expanded T-cell clones, 5×10^4 T cells were incubated at days 10 to 16 after restimulation with 10^4 autologous, irradiated (6000 rad) EBV-transformed B-lymphoblastoid cell lines²⁴ in the presence or absence of drug as antigen in 200 μL CM in U-bottom 96-well microplates. After 48 hours, ^3H -thymidine was added to the culture for 14 hours. ^3H -thymidine incorporation was measured in a β -counter (Trace 96).

Enzyme-linked immunospot (ELISpot) assay

Ninety-six-well filtration plates (Millititer, Millipore, Mass) were coated with capture antihuman IL-2, IL-4, IL-5, IL-13, and IFN- γ mAb solution (Diaclone, Besançon, France), washed, and blocked according to the manufacturer's protocol. PBMCs 8×10^5 were added to wells in triplicate, including drug. Plates were incubated for 48 hours at 37°C in 5% CO₂. PBMCs incubated with 10 $\mu\text{g/mL}$ TT or CM alone served as positive and negative controls, respectively. After washing with PBS/0.1% Tween 20, a biotinylated anti-IL-2, IL-4, IL-5, IL-13 and IFN- γ mAb was added for 90 minutes at 37°C. After 3 washes with PBS/0.1% Tween 20, streptavidin-alkaline phosphatase was added for 1 hour at 37°C. Spots were visualized with nitroblue tetrazolium/5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt after extensive washing. Plates were washed with tap water and dried overnight, and spots were analyzed in a Bioreader 3000 CL/PRO (BIO-SYS GmbH, Karben, Germany).

RESULTS

Patients' characteristics

Table I summarizes the clinical characteristics of the patients with drug allergy. All 5 patients showed delayed drug hypersensitivity reactions to different drugs, proven by positive patch tests (except patient WZ) and lymphocyte transformation test (LTT). Patient WZ had a well documented multiple drug hypersensitivity syndrome²⁵

with reactions to phenytoin, sulfamethoxazole, and later to amoxicillin. All 3 reactivities could be documented by LTT. Patient UNO has first been documented more than 12 years ago,²⁶ and many sulfamethoxazole-specific T-cell clones (TCCs) have been generated from his PBMCs over the years.^{10,27-30} PBMCs of all 5 patients also showed a robust *in vitro* proliferation to TT (Fig 1). Healthy blood donors without history of adverse drug reactions but responsive to TT served as controls.

LTT

An LTT was initially performed with nontoxic concentrations of pure substance of the drugs to which the patients were exposed.⁹ Only some drugs reacted in this assay, and these drugs were also positive in epicutaneous patch tests (except for patient WZ), confirming the selective sensitization to the drug in the patients, whereas other drugs were negative in both epicutaneous patch test and LTT. Drugs to which the patients were not exposed were negative as well, and none of the drugs induced a proliferation in 5 healthy individuals. The LTT was repeated at the time point of frequency analysis with different concentrations of drugs together with TT, because the Swiss population is comprehensively vaccinated against it. Proliferative responses of PBMCs were observed in patient UNO to different concentrations of sulfamethoxazole (Fig 1, A); in patient VG to vancomycin (Fig 1, B); in patient HH to amoxicillin (Fig 1, C); in patient WZ to sulfamethoxazole, phenytoin, and amoxicillin (Fig 1, D); and in patient CS to carbamazepin (Fig 1, E). SIs are given as cpm of stimulated culture divided by cpm of unstimulated culture. One representative out of 3 experiments performed is shown.

CFSE-based assay for frequency analysis

Cellular proliferation can be measured readily by flow cytometry, because the color intensity of CFSE-labeled PBMCs is reduced by half with each cell division. Fig 2, A, shows representative dot plots of drug-stimulated or

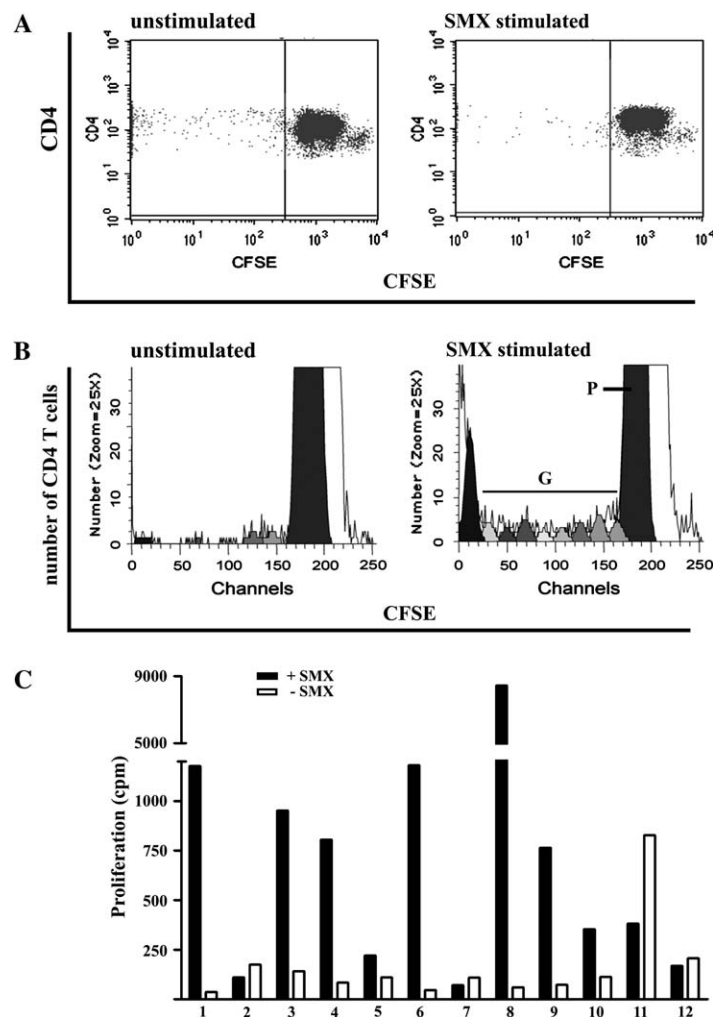


FIG 2. Flow-cytometric analysis of unstimulated or sulfamethoxazole (SMX)-stimulated CFSE-labeled PBMCs. **A**, CFSE^{low}-CD4⁺ T cells were sorted and cloned. **B**, Intensity histograms show CFSE fluorescence on CD3⁺/CD4⁺ gated T cells after 6 days of stimulation or with culture medium alone. *P* indicates the parental generation and *G* the resulting generations. **C**, Specificity assay of FACS-sorted CFSE^{low} SMX-specific CD4⁺ T-cell clones from patient UNO.

unstimulated CFSE-labeled PBMCs for 6 days of a patient with drug allergy (UNO). The CFSE-fluorescence intensity decreased substantially in sulfamethoxazole-stimulated cultures, but not in control cultures without drug addition, where the number of CFSE^{low}-CD4⁺ T cells was very low, reflecting some background proliferation of CD4⁺ T cells (Fig 2, *B*, left blot). Deconvolution of the histogram distributions into generations was performed by ModFit software. Notably, no or only a minimal response was seen in the PBMCs of patient UNO cultured with all drugs not involved in the patients' drug hypersensitivity reaction, and no response occurred in healthy individuals after drug stimulation (Fig 3, *F* and *G*).

In all 5 patients with drug allergy and healthy individuals, a substantial and specific expansion in response to TT was detected. After 6 days of TT stimulation of PBMCs of patients with drug allergy and controls, 0.09% (patient HH) to 0.43% (patient VG) of CD4⁺ T cells

reacted. This corresponds to frequency of about 1:232 to 1:1100 of TT-specific CD4⁺ T cells. In 4 of the 5 individuals with drug allergy, a similar or even higher frequency of drug-specific CD4⁺ T cells could be observed, whereas the amoxicillin-stimulated cells of patient HH yielded a significantly lower frequency (1:5000), as shown in Fig 3. No or only a minimal response was seen in patients' PBMCs cultured with the drug not involved in the drug hypersensitivity reaction, and no response occurred in healthy individuals after stimulation with any of the drugs used. One representative out of 2 experiments performed is shown.

To validate the specificity of the expanding CD4⁺ T cells in response to drugs, CFSE-labeled PBMCs of patient UNO were stimulated for 6 days with sulfamethoxazole. CFSE^{low} CD4⁺ T cells (Fig 2, *B*, generations *G*) were sorted, distributed at 1 cell per well, and restimulated with 5×10^4 allogeneic irradiated PBMCs after

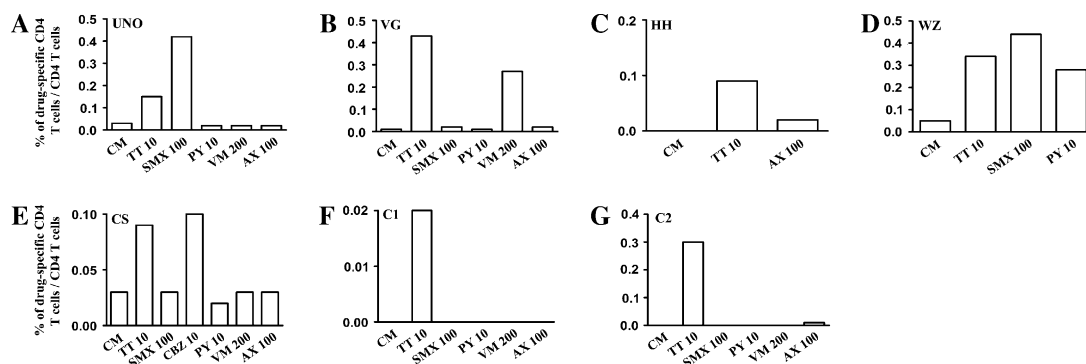


FIG 3. Drug-specific proliferation of CFSE-labeled PBMCs after stimulation. **A**, Patient UNO, sulfamethoxazole (SMX); **B**, patient VG, vancomycin (VM); **C**, patient HH, amoxicillin (AX); **D**, patient WZ, SMX, PT; **E**, patient CS, carbamazepin (CBZ). Cells of 2 healthy donors (**F**, C1, and **G**, C2) were incubated with all drugs. TT and CM served as positive and negative controls.

14 days and 28 days. Sixty-seven percent of the sorted and expanded CD4⁺ T cells proliferated to sulfamethoxazole (Fig 2, C), supporting the notion that the majority of the drug-stimulated CD4⁺ T cells react in a specific way to the drug.

ELISpot determination of IL-2, IL-4, IL-5, IL-13, and IFN- γ

Freshly isolated PBMCs (0.8 million/well) of patients WZ, UNO, HH, VG, and CS and of 3 blood donors without allergy were incubated for 48 hours with different concentrations of the drugs to which the patients were allergic, as well as of control drugs. TT served as positive control and culture medium alone as a negative control. The number of IL-2, IL-4, IL-5, IL-13, and IFN- γ secreting cells after stimulation was measured by ELISpot. The background secretion of IL-2, IL-4, IL-5, and IL-13 cytokines in culture medium was negligibly low. A 2-day culture of 3 healthy blood donors' PBMCs did not feature any cytokine production to any drug, because the values obtained were in the range of the culture medium control.

In all patients with drug allergy, the corresponding drug elicited a specific cytokine production of the stimulated PBMCs (Fig 4). IFN- γ is prominently secreted in patients WZ (DRESS), UNO (exanthema), and VG (AGEP). Only WZ shows a typical T_H1 response, whereas UNO and VG in addition secrete IL-13, which classifies their patterns as T_H0-like. Patients HH (SJS) and CS (DRESS) produce no IFN- γ , whereas HH shows high IL-4 and medium IL-13 production (T_H2). PBMCs of patient CS (DRESS) show a T_H2 response and prominently secrete IL-5, which is typical for the disease DRESS.

After TT stimulation, all individuals (patients with allergy and healthy donors) showed a typical T_H0 cytokine pattern composed of IL-2, T_H2 cytokines (IL-13, IL-4, IL-5), and the T_H1 cytokine IFN- γ .

The ELISpot technique does not discriminate between T cells secreting various cytokines simultaneously and different T cells producing only 1 or 2 cytokines. On the basis of the assumption that a drug-reactive T cell produces

all cytokines, the minimal frequency would correspond to the cytokine with the highest amount of spots. Taking into account that 40% to 48% (patients VG, UNO, WZ, and CS) or 27% (patient HH) of all PBMCs are T cells (data not shown), a frequency of 1:2500 to 1:10,000 is thus the minimal estimate for reactive T cells. If one assumes that each cytokine is produced by a different T cell, the frequency would be calculated by all spots together, which would correspond to a maximal frequency of approximately 1:1250 to 1:10,000. It is likely that IL-4, IL-5, and IL-13 are produced by the same cells, whereas IFN- γ would at least partly be produced by different T cells. For these reasons, the actual frequency may lay between these minimal and maximal frequency values (Table II).

DISCUSSION

In this study, we analyzed patients with well documented drug hypersensitivity reactions. All 5 patients had a clear history of drug hypersensitivity, a positive epicutaneous patch test reactivity after 48 and 72 hours, and positive and specific *in vitro* reactivity of T cells to the drug as shown by LTTs. The interval between drug hypersensitivity and the frequency analyses varied from 4 months to 12 years. Because the symptoms had been severe in all cases, all patients had strictly avoided the incriminated drugs in this period.

To determine the frequency of peptide-specific T cells, fluorochrome-conjugated MHC multimers containing the specific peptide has been used successfully.³¹ However, in drug hypersensitivity, neither the hapten/metabolite nor the precise association with a specific peptide is known. Thus, the identification of drug-specific T cells with the help of multimers is difficult, and preliminary attempts were negative.

Staining by CFSE of PBMCs relies on proliferation induced by a specific antigen and yielded frequencies of drug-reactive CD4⁺ T cells in the range of ~1:250 to 1:5000. Again, drugs and TT elicited similar values. The

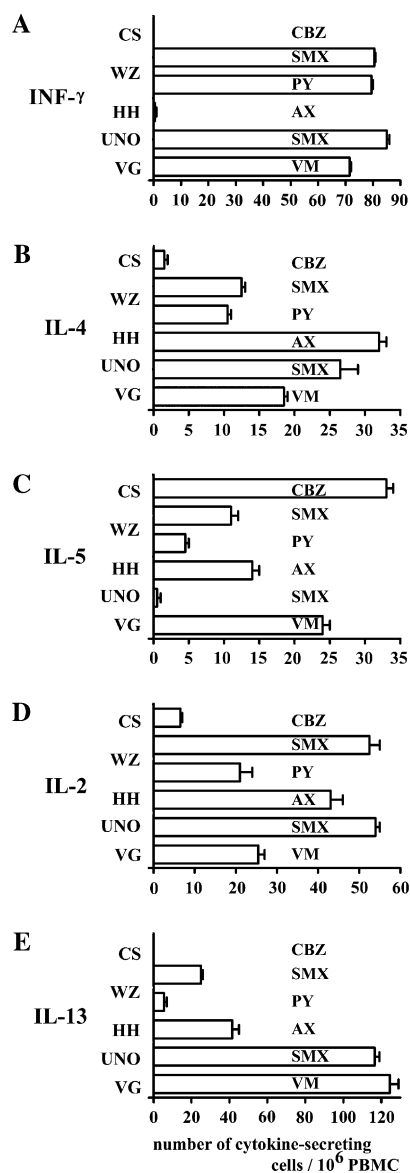


FIG 4. Number of cytokine-secreting cells per 10^6 PBMCs after drug stimulation for 48 hours in patients CS, WZ, HH, UNO, and VG. **A**, IFN- γ ; **B**, IL-4; **C**, IL-5; **D**, IL-2; and **E**, IL-13. One representative out of 2 experiments performed is shown.

specificity of the drug-reactive T cells is documented by cloning the reacting T cells, which had undergone multiple cell divisions. Although this cloning method may not be very sensitive, it still revealed that 2 out of 3 T cells obtained from the cell fraction dividing most strongly were specific to the drug, which supports the usefulness of the CFSE staining for frequency determinations.

The second method is based on the secretion of cytokines by T cells after specific drug stimulation. The determination of cytokines by ELISpot methodology yielded frequencies of drug-specific T cells in the range of 1:2500 to 1:10,000 for the major cytokine produced. However, this frequency may be 2-fold to 3-fold higher if the different cytokines (eg, IL-13 and IFN- γ) are produced

TABLE II. Minimal and maximal frequencies of drug-specific T cells; number of cytokine-secreting cells measured by ELISpot

Patient	Drug	Frequency	
		Minimum	Maximum
VG	TT	1/10,000	1/3000
	Vancomycin	1/2500	1/2500
UNO	TT	1/5000	1/2500
	Sulfamethoxazole	1/3000	1/1250
HH	TT	1/5000	1/1700
	Amoxicillin	1/5000	1/1700
WZ	TT	1/10,000	1/5000
	Sulfamethoxazole	1/5000	1/3000
CS	Phenytoin	1/5000	1/2000
	Carbamazepin	1/50,000	1/50,000

by different T cells, as one would expect. The ELISpot methodology has the advantage that the cytokines measured hint at the type of immune mechanism involved. Indeed, there were clear differences in the cytokine patterns in the different patients. The PBMCs of patients with AGEP and severe exanthema had high IL-13 and IFN- γ values, whereas 1 patient with DRESS (CS) produced high amounts of IL-5, which fits well with her marked eosinophilia during the disease. The other patient (WZ) with DRESS showed low IL-13, IL-4, and IL-5 levels, but a rather selective elevation of IFN- γ and IL-2 to both drugs to which he had reacted previously. This pattern of cytokine production reflects the generally heterogeneous cytokine profile from TCC of patients with DRESS.^{32,33}

In patient HH, the ELISpot method yielded quite a substantial cytokine response (IL-4, IL-5, and IL-13) to amoxicillin, comparable to the TT response (Fig 4), whereas the frequency of drug-specific CD4⁺ T cells determined by CFSE proliferation assays was very low. This might indicate that in certain diseases like SJS and toxic epidermal necrolysis, assays measuring CD4⁺ T-cell proliferation are not very sensitive. Recent data indicate that these diseases are mediated by cytotoxic CD8⁺ T cells, and the ELISpot method may be better suited to detect a sensitization than the CFSE proliferation assays.^{34,35}

Because the data obtained with ELISpot analysis and CFSE staining are similar, and because the specificity of the CFSE assay was confirmed in addition by single-cell cloning, we conclude that 1 in 250 to 5000 CD4⁺ T cells is able to react with the drug in patients in remission after severe drug hypersensitivity reactions. This value is at first sight surprisingly high for a small compound like a drug, which, compared with a large molecule like TT, might seem to contain fewer potential T-cell epitopes. However, a small drug may fit into numerous TCRs, which would explain the great heterogeneity of the T-cell response to drugs.¹⁰ The high frequency is also compatible with the massive immune reactions seen in severe drug hypersensitivity symptoms.⁶ Moreover, peptide-epitopes

with 1 to 2 TCR binding sites may have a more stringent restriction to fit into a particular TCR than a small molecule like a drug.

As outlined, there are 2 distinct models of T-cell activation by drugs—the hapten concept and the p-i (pharmacologic-interaction with immune receptors) concept—which implies a pharmacologic stimulation of T cells via direct stimulation of the TCR.¹ The majority of sulfamethoxazole, lidocaine, carbamazepine, and lamotrigine stimulations appear to occur via the p-i concept,^{23,27,32,33,36} whereas amoxicillin acts via the hapten mechanism. Obviously, both models induce a memory response, because substantial frequencies were measured even years after the acute event. The strong immune response of patient UNO to sulfamethoxazole, which has now been documented 12 years after the hypersensitivity reaction and the last intake of a sulfonamide-containing drug, has been confirmed repeatedly over the years, because this patient was extensively analyzed, involving the generation of many hundreds of TCCs at different time points, and always showed a strong proliferative immune response.^{10,25,36}

The long persistence of memory in spite of strict drug avoidance has actually been observed previously with *in vitro* tests^{8,9} and also *in vivo*, because a rapid appearance of clinical reactions 20 years after an original hypersensitivity reaction has been documented.⁷ It may be related to some cross-reactivity of the drug-reactive T cells, because drug-reactive T cells frequently possess another specificity, as revealed by high frequencies of cross-reactivity with allogeneic MHC-peptide complexes.²⁶ However, the exact nature of these putative cross-reactive antigens, which might maintain the drug-reactive memory pool, is unknown.

In conclusion, about 1:150 to 1:5000 T cells are drug-reactive in patients after remission from different, severe drug hypersensitivity reactions. This frequency of drug-reactive T cells is often higher than the frequency of T cells able to recognize a complex recall antigen like TT with many distinct epitopes. Hence, drug-specific cells can persist for a long period even after strict avoidance, and patients with severe delayed drug hypersensitivity reactions are potentially prone to react again to the incriminated drug even years after strict drug avoidance.

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