HLA-restricted, Processing- and Metabolism-independent Pathway of Drug Recognition by Human αβ T Lymphocytes

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Abstract

T cell recognition of drugs is explained by the hapten-carrier model, implying covalent binding of chemically reactive drugs to carrier proteins. However, most drugs are nonreactive and their recognition by T cells is unclear. We generated T cell clones from allergic individuals specific to sulfamethoxazole, lidocaine (nonreactive drugs), and cef-triaxone (per se reactive β-lactam antibiotic) and compared the increase of intracellular free calcium concentration ($[Ca^{2+}]_i$) and the kinetics of T cell receptor (TCR) downregulation of these clones by drug-specific stimulations. All drugs tested induced an MHC-restricted, dose- and antigen-presenting cell (APC)-dependent TCR downregulation on specific $CD4^+$ and $CD8^+$ T cell clones. Chemically nonreactive drugs elicited an immediate and sustained $[Ca^{2+}]_i$ increase and a rapid TCR downregulation, but only when these drugs were added in solution to APC and clone. In contrast, the chemically reactive hapten ceftriaxone added in solution needed > 6 h to induce TCR downregulation. When APC were preincubated with ceftriaxone, a rapid downregulation of the TCR and cytokine secretion was observed, suggesting a stable presentation of a covalently modified peptide. Our data demonstrate two distinct pathways of drug presentation to activated specific T cells. The per se reactive ceftriaxone is presented after covalent binding to carrier peptides. Nonreactive drugs can be recognized by specific $\alpha\beta^+$ T cells via a nonconventional presentation pathway based on a labile binding of the drug to MHC–peptide complexes. (*J. Clin. Invest.* 1998. 102:1591–1598.) Key words: drug hypersensitivity • T cell receptor downregulation • $\alpha\beta^+$ T lymphocytes • nonpeptide T cell antigens • drug presentation

Introduction

Nonpeptide antigens comprise a heterogeneous group of molecules which are able to stimulate $\alpha\beta^+$ and $\gamma\delta^+$ T cells (1). A first category of nonpeptide antigens is represented by lipids or glycolipids such as mycolic acids and lipoarabinomannan (2, 3). These are presented to $\alpha\beta^+$ T cells by CD1 molecules which display a specificity for hydrophobic antigens (4, 5). A second class of nonpeptide antigens includes phosphorylated molecules such as isopentenyl pyrophosphate and phosphorylated sugars (6, 7). Their presentation to $\gamma\delta^+$ T cells is independent from uptake and processing and does not require MHC or CD1 molecules (8). A third group of nonpeptide antigens comprise metals, which are recognized by $\alpha\beta^+$ T cells without need of prior uptake and processing (9, 10). Recently, Corinti et al. described a distinctive pollen antigen, most likely a carbohydrate, which does not require MHC or CD1 presentation and is recognized by $CD8^+$ $\alpha\beta^+$ T cells exclusively (11).

An additional group of nonpeptide antigens is represented by drugs, such as penicillins, sulfonamides, or local anesthetics, that are often responsible for allergic reactions. As far as analyzed, most drug-specific T cell clones (TCC)[1] obtained from allergic individuals are MHC-restricted $CD4^+$ or $CD8^+$, $\alpha\beta^+$ T cells, although two $\gamma\delta^+$ lidocaine-specific clones were described as well (12–16). In contrast to the above-mentioned nonpeptide antigens, they trigger $\alpha\beta^+$ T cells in an MHC-restricted way. Previously it was postulated that the immunogenicity of drugs is due to their hapten feature. β-lactam antibiotics such as penicillins or cephalosporins represent per se reactive drugs; their core structure, the β-lactam ring, opens spontaneously under physiological conditions. The formed penicilloyl can covalently bind to lysine residues of proteins (17, 18). Modification of soluble (i.e., penicilloyl-modified human serum albumin) or membrane-bound proteins leads to presentation of β-lactam–modified peptides to T cells (processing-dependent pathway) (14, 15). Alternatively, β-lactams appear to be able to bind directly to immunogenic peptides within MHC molecules (processing-independent pathway) (14, 15).

However, most drugs are not chemically reactive per se. It was postulated that they gain chemical reactivity upon drug metabolism (19). However, we showed recently that fixed antigen-presenting cells (APC) were still capable of presenting nonreactive drugs like sulfamethoxazole (SMX) or lidocaine to drug-specific TCC. Strikingly, APC pretreated with drugs and washed were not stimulatory, suggesting a rather labile drug interaction with the MHC–peptide complex (20). This indicates that nonreactive drugs can be presented in a processing-independent and probably noncovalent way to activated T cells (20).

To better define this new mechanism of drug presentation to specific T cells, we measured early parameters of T cell re-

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1. *Abbreviations used in this paper:* APC, antigen-presenting cell; B-LCL, B lymphoblastoid cell lines; $[Ca^{2+}]_i$, intracellular free calcium concentration; CM, culture media; SEB, staphylococcus enterotoxin B; SMX, sulfamethoxazole; TCC, T cell clone; TCR, T cell receptor; TT, tetanus toxoid.

ceptor (TCR) engagement such as TCR downregulation and intracellular free calcium ($[Ca^{2+}]_i$) mobilization in drug-specific TCC (21, 22). We report that, unlike T cell stimulation by soluble protein antigens, nonreactive drugs stimulate T cells via a rapid and labile binding to MHC molecules, similar to the recognition of peptides, alloantigens, and superantigens. Our data support the concept of a distinct pathway of T cell stimulation by nonreactive drugs which is independent of processing and metabolism.

Methods

Culture media (CM). CM consisted of RPMI 1640 supplemented with 10% pooled heat-inactivated human AB serum (Swiss Red Cross, Bern, Switzerland), 25 mM Hepes buffer, 2 mM L-glutamine, 10 mg/ml streptomycin, and 100 U/ml penicillin. CM^+ used to culture TCC was enriched with 20 U/ml recombinant IL-2 (Dr. A. Cerny, Inselspital, Bern, Switzerland). The medium for culture of EBV-transformed B lymphoblastoid cell lines (B-LCL) was RPMI 1640 supplemented with 10% heat-inactivated FCS (Gibco, Paisley, UK), 25 mM Hepes buffer, but no L-glutamine and no antibiotics. B-LCL were generated as described (23).

Drugs and antigens. Preparation of drug solutions in PBS was always done freshly just before use. Lidocaine and mepivacaine were purchased from Grogg Chemie (Stettlen, Switzerland), ceftriaxone from Sigma Chemie (Buchs, Switzerland), and SMX was obtained from Hofmann La-Roche (Basel, Switzerland). Tetanus toxoid (TT) was kindly provided by Dr. J. Cryz (Serum und Impfinstitut, Bern, Switzerland). The TT-derived peptides p2 (amino acids 830–847) and p30 (amino acids 947–967) were obtained from Multiple Peptide Systems (San Diego, CA).

TCC and APC. All drug-specific clones used were isolated from polyclonal cell lines derived from four different donors having suffered from a drug allergic reaction to SMX, lidocaine, mepivacaine, or ceftriaxone, respectively (16, 20, and Hari, Y., K. Frutig, M. Hurni, N. Yawalkar, M.P. Zanni, B. Schnyder, A. Kappeler, S.V. Greyerz, L.R. Braathen, and W.J. Pichler, manuscript in preparation). Selected clones were tested for monoclonality either by staining with a panel of antibodies each recognizing a distinct TCR Vβ family or by using PCR methodology (24). The clones were used 7–18 d after restimulation with allogeneic feeder cells and PHA (1 μg/ml, Bacto; Difco Laboratories, Detroit, MI) as described (24). TCC specific for TT or its peptides (C31, Y14 and KS140) have been described previously (23, 25).

Proliferation assay and cytokine measurement. 2.5×10^4 clone cells were incubated with 5×10^3 autologous irradiated (6,000 rad) B-LCL in the absence or presence of drugs (100 μg/ml) in 200 μl CM in a 96-well round-bottomed plate (No. 3077; Falcon Labware, Cockeysville, MD) and [^{3}H]thymidine incorporation was measured as described (16). Stimulation indices were calculated as cpm in culture with antigen/cpm in culture without antigen. For pulsing experiments B-LCL were incubated with and without SMX or ceftriaxone (100, 500, or 2,000 μg/ml) overnight in CM. The cells were washed three times with HBSS and resuspended in CM and 5.0×10^4 APC were added to 2.5×10^4 clone cells. Culture supernatants were collected after 6 h and TCR expression was measured as described below. Quantification of cytokines (IL-4, IL-5, and IFN-γ) was done as described (16).

TCR downregulation. 2.5×10^4 clone cells were incubated with 5×10^4 autologous, HLA-matched, or HLA-mismatched B-LCL in 200 μl CM in U-bottomed plates in the presence of drugs (100 μg/ml), peptide p30 (25 μg/ml), TT (10 μg/ml), or without antigen. The plates were centrifuged for 2 min to allow conjugate formation and incubated for 6 h at 37°C. Cells were washed with PBS containing 1% FCS and 0.02% NaN_3 and stained with a PE-labeled anti-CD3 and a FITC-labeled anti-CD19 or anti-CD20 mAb to exclude B-LCL. APC were gated out using both forward and side scatter and green fluorescence. The CD3 fluorescence was analyzed on an EPICS profile II flow cytometer in triplicates (Coulter Corp., Hialeah, FL). CD25 upregulation after 48 h was determined by using an anti-CD25 mAb instead of an anti-CD3 mAb (all mAb were obtained from DAKO Diagnostics, Zug, Switzerland). The time of incubation, the dose of antigen, and the number of APC used were altered as described. 100% values corresponds to the mean CD3 fluorescence of clones conjugated with APC without antigen.

Flow cytometric determination of $(Ca^{2+})_i$. TCC were loaded with 5 μM Indo-1 (Sigma Chemical Co., St. Louis, MO) for 45 min at 37°C in 5% FCS, Hepes-buffered RPMI, washed twice with the same medium, mixed with B-LCL only, with unpulsed B-LCL and antigen, or with antigen-pulsed B-LCL. The cells were centrifuged at 1,500 rpm for 1 min, incubated for 1 min at 37°C, resuspended, and analyzed on a FACS® flow cytometer (Becton Dickinson, Mountain View, CA). To detect $[Ca^{2+}]_i$ in T cell–APC conjugates, only live, Indo-1–loaded, and conjugated T cells were included in the analysis. The 405/525 nm fluorescence ratio of all the acquired events is reported.

Results

$CD4^+$ and $CD8^+$ drug-specific TCC undergo class II– or class I–restricted TCR downregulation. A panel of SMX-, lidocaine-, mepivacaine-, and ceftriaxone-specific TCC from four different allergic donors was generated (Table I). Since APC pretreated with drugs and washed do not induce a T cell response (20), drugs, APC, and TCC were mixed simultaneously; proliferation and TCR downregulation (6 h) were measured. All clones were specific as shown by their proliferative response (stimulation indices). A significant decrease of TCR expression was observed in the presence of drugs after 6 h of culture compared with cultures without drugs. SMX did not induce a downregulation of the TCR in lidocaine-specific clones, and, vice versa, lidocaine does not affect TCR expression in SMX-specific clones (data not shown). Thus, the effect of these drugs on TCR expression is the result of TCR engagement and not due to a nonspecific side effect of drugs. Moreover, a TCR downregulation was observed only if MHC-matched APC were used, indicating an MHC-restricted antigen presentation to $CD4^+$ and $CD8^+$ T cells. MHC restriction of drug presentation was further confirmed by blocking experiments with anti-MHC class I or class II antibodies (20, and data not shown). This downregulation was dependent on the number of APC added (data not shown). Note that the $CD8^+$ clone S211E recognizes SMX in the context of MHC class II molecules (20).

TCR downregulation is dose dependent. Clones S23E and OFB2 were used to study the relationship between drug concentration and the induction of TCR downregulation. Detectable TCR downregulation could be elicited by 20 μg/ml SMX and 100 μg/ml lidocaine (Fig. 1, *A* and *B*). These concentrations were also stimulatory for T cell proliferation and cytotoxicity (16, 20). In the absence of APC or in the presence of mismatched APC, high doses of drugs (500–1,000 μg/ml) were able to induce a weak (SMX) or strong (lidocaine) TCR downregulation. However, high doses of lidocaine induced also a significant TCR downregulation on a TT-specific TCC (Y14) (data not shown). We interpret this as a toxic effect of high concentrations of lidocaine, since CD25 (IL-2 receptor) upregulation, a marker for T cell activation was only seen with 20–200 μg/ml but not higher concentrations (Fig. 1 *C*). Neither did CD25 upregulation occur when the TT-specific clone Y14 was stimulated with lidocaine (data not shown). Moreover, after a

Table I. Phenotype, Specificity, and HLA Restriction of CD4⁺ and CD8⁺ Drug-specific TCC

Drug	Donor	Clone	Phenotype	TCRVβ	SI*	CD3 staining (% of control)		
						Autologous	Allogeneic matched	Allogeneic mismatched
SMX	UNO‡	S23E	CD4	13.2	43.5	51.3±0.3	53.1±0.9	102.7±2.1
	UNO	S101	CD4	6	113	64.0±1.6	62.2±2.8	108.4±3.2
	UNO	S211E	CD8	16	136	73.3±2.7	80.4±2.0	102.4±5.0
	UNO	P26B	CD8	ND§	213	63.9±7.3	72.1±7.7	99.6±3.7
	UNO	8-21	CD4	9	69.5	75.6±3.3	86.1±1.3	102.2±1.5
Lidocaine	OF‖	OFB2	CD4	17	315.5	73.2±1.6	73.3±0.7	100.4±3.9
	SF¶	SFL22	CD4	ND	9.6	84.8±3.0	81.7±7.0	98.8±1.0
	SF	SFL30	CD4	ND	4.6	71.3±2.3	73.1±8.5	98.5±3.2
Mepivacaine	SF	SFM8	CD4	ND	50.6	70.7±4.0	76.6±4.3	94.8±0.9
	SF	SFM10	CD4	ND	15.3	82.9±1.1	76.6±4.3	104±5.9
Ceftriaxone	MBZ**	DB3	CD4	7	18.7	66.2±1.1	77.4±2.4	98.8±2.4

‡HLA phenotype: A2/A26, B44/B60, DRB1*0101/DRB1*1001; restriction element DRB1*1001, B44, respectively. ‖HLA phenotype: A2/A26, B7/B7, DRB1*1501/DRB1*1501; restriction element DRB1*1501. ¶HLA phenotype: A3/A29, B7/B7, DRB1*1501/DRB1*1501; restriction element DRB1*1501. **HLA phenotype: A2/A29, B7/B7, DRB1*1101/DRB1*1501; restriction element DRB1*1101. §Not done. *Stimulation index of the proliferative response with autologous B-LCL as APC, cpm of control cultures were always < 200 cpm.

48-h culture with 500 and 1,000 μg/ml of lidocaine, only a low viability in both clones (OFB2 and Y14) was observed as determined by forward scatter analysis and trypan blue exclusion (data not shown). Thus, lidocaine in low concentrations results in a specific TCR downregulation, whereas high concentrations induce an unspecific, most likely toxic, TCR downregulation.

Downregulation of the TCR by drugs can be observed within 20 min. An efficient presentation of protein antigens requires antigen uptake, processing, and subsequent presentation on MHC molecules. It is assumed that this process takes place within 4–6 h. Since we observed that glutaraldehyde-fixed APC are still capable of presenting SMX to specific clones, we concluded that certain drugs are presented without need of processing (20). To verify whether a specific TCR engagement by drugs does require processing, we compared the time needed to achieve significant TCR downregulation in

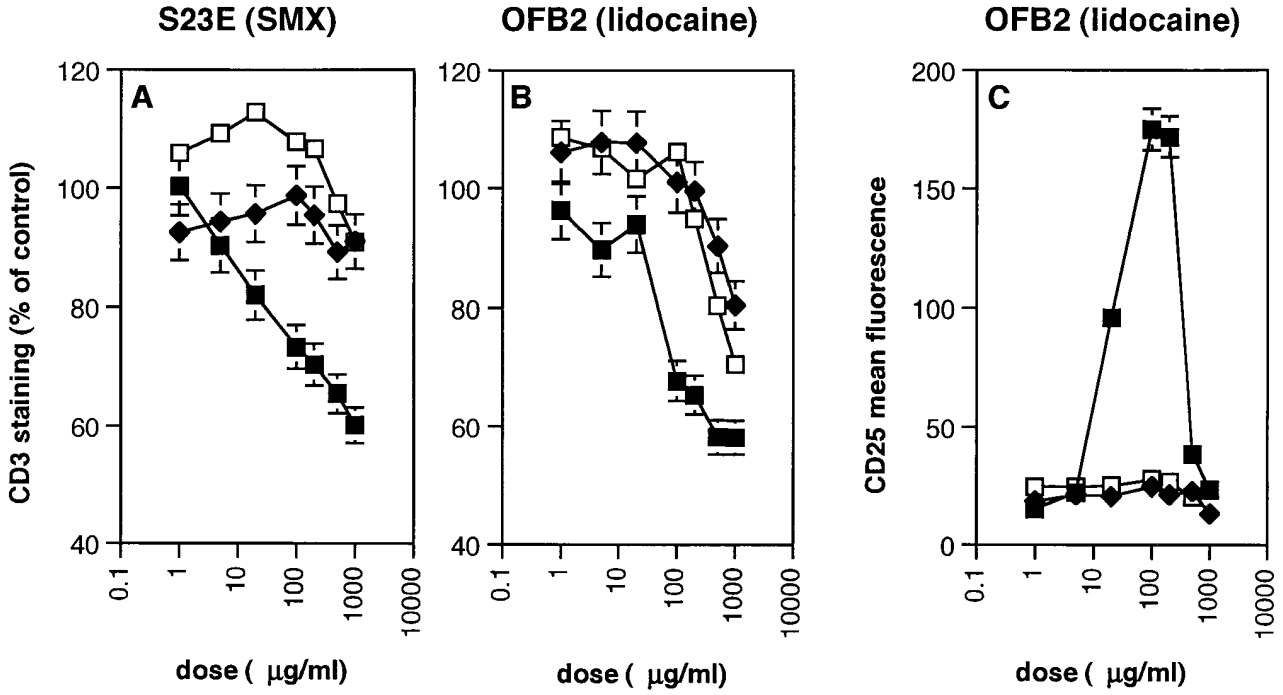

Figure 1. Drug-induced TCR downregulation is dose dependent. The SMX specific clone (S23E) and the lidocaine-specific clone OFB2 were incubated with various amounts of SMX or lidocaine in the presence of either autologous (*filled boxes*) or allogeneic HLA-mismatched (*filled diamonds*) or without APC (*open boxes*). Downregulation of TCR was determined after 6 h as described in Methods. Results indicate the percentage of CD3 mean fluorescence calculated from values without drugs (triplicate determinations±SE) (*A* and *B*) or absolute CD25 mean fluorescence values (*C*).

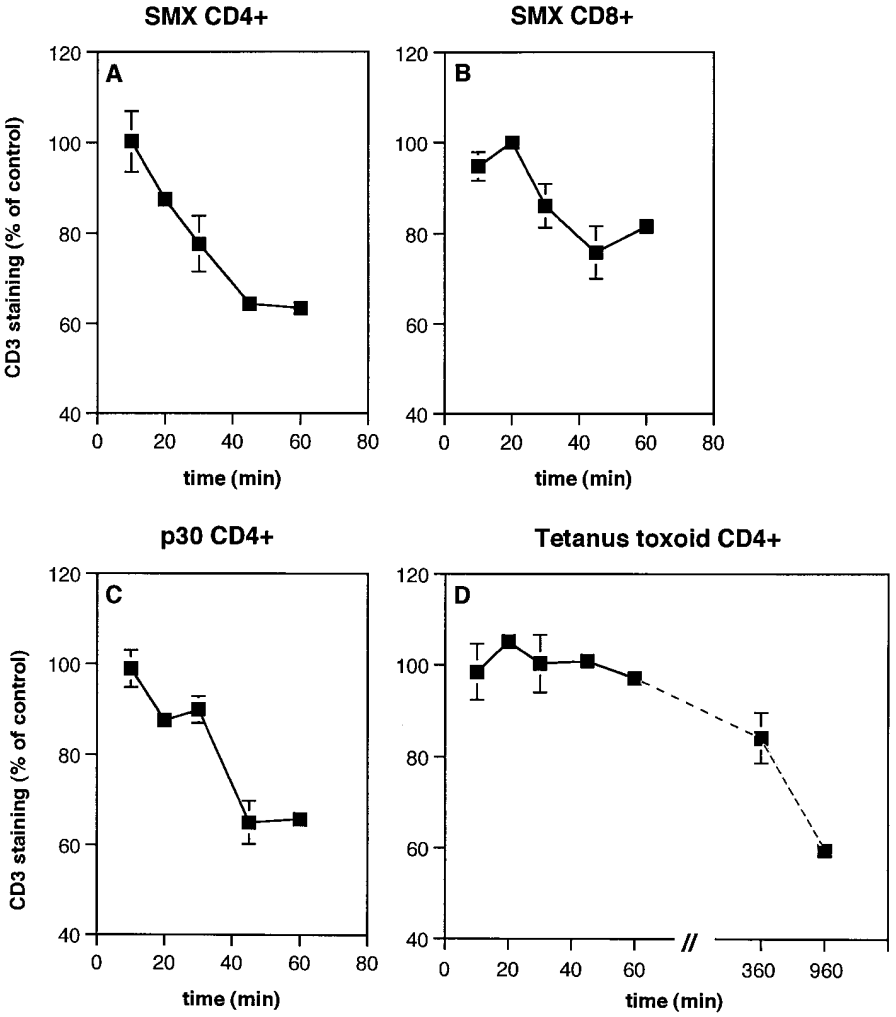

Figure 2. Chemically nonreactive drugs induce a TCR downregulation within 20 min. Two SMX-specific clones (S101 $CD4^+$, *A*, and P26B $CD8^+$, *B*), one peptide p30-specific clone (C31, *C*), and one protein-specific clone (Y14, *D*) were incubated with or without appropriate antigens. TCR downregulation was determined at indicated time points as described in Methods.

drug-, peptide- and protein-specific clones. Fig. 2 shows the kinetics of TCR downregulation of two representative SMX-specific (S101, $CD4^+$; P26B, $CD8^+$) TCC. Moreover, a peptide-specific clone (p30; C31) and a protein-specific (TT; Y14) clone were investigated as well. All antigens were added to the TCC and B-LCL without prior pulsing of APC. As shown in Fig. 2 *A*, a significant TCR downregulation was detected by 20 to 30 min with SMX specific $CD4^+$ and $CD8^+$ clones. TCR downregulation reached a plateau already after 1 h. Similar kinetics of TCR downregulation were seen with the p30-specific TCC C31, which recognized a preprocessed peptide (Fig. 2 *B*). In contrast, addition of TT was unable to elicit an effect on the TT-specific clone Y14 within 1 h (Fig. 2 *C*). As expected, TCR downregulation occurred after 6–12 h, due to the time required for processing.

The time kinetics of TCR downregulation with drugs are similar to the one observed with superantigen and "pulsed" peptide antigens. The lidocaine-specific clone OFB2 expresses TCR Vβ17 and therefore binds staphylococcus enterotoxin B (SEB). In addition, this clone is also alloreactive. In addition to the autologous restriction element HLA-DRB1*15 plus lidocaine, it also reacts with APC bearing the HLA-DRB1*08 allele (16). In Fig. 3, a comparison of these three, presumably processing-independent stimuli for the time kinetics of TCR downregulation, is shown. All three stimuli showed the same time kinetics, indicating that lidocaine binds rapidly to the

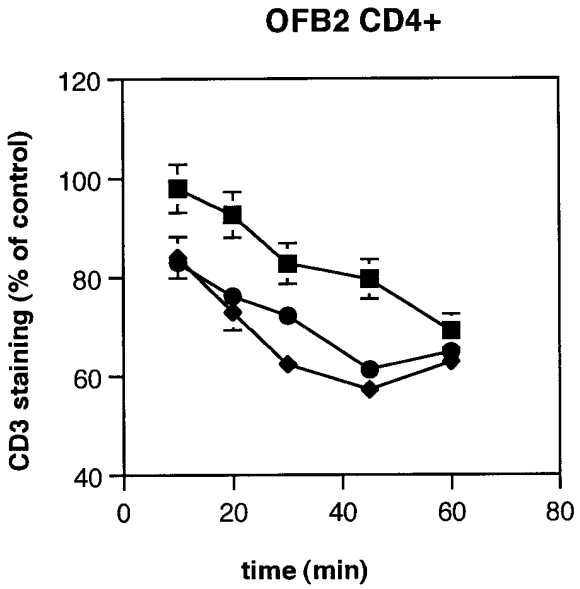

Figure 3. The kinetics of TCR downregulation are similar after drug (lidocaine, *boxes*), SEB (*diamonds*), and allostimulation (*circles*). Clone OFB2 was used to compare the kinetics of TCR downregulation with these three different types of antigens. All three stimuli revealed the same kinetics of TCR triggering. TCR downregulation was determined as described in Fig. 1.

MHC–peptide complex and the TCR, as superantigens or alloantigens do.

Drug-specific clones show a sustained $[Ca^{2+}]_i$ increase when clones and APC where cocultured with drug in solution. To further confirm the processing-independent presentation of nonreactive drugs, we measured the calcium $[Ca^{2+}]_i$ increase, which is known to occur within seconds after TCR engagement (21). Clone KS140, specific for the peptide p2 and TT (25), was used as a control. Stimulation of this clone with p2 in solution or prepulsed on autologous APC resulted in a strong and sustained $[Ca^{2+}]_i$ mobilization (Fig. 4, *B* and *C*). An increase in $[Ca^{2+}]_i$ after TT addition was only visible when the B-LCL were pulsed previously overnight with TT but not when TT was added in solution to T cell–APC conjugates, demonstrating the need for protein processing (Fig. 4, *D* and *E*). As expected, SMX did not induce a $[Ca^{2+}]_i$ increase in the TT-specific TCC KS140 (Fig. 4 *F*). Fig. 4, *G–L*, shows the $[Ca^{2+}]_i$ levels of the SMX-specific clone S23E after different stimulation procedures. As shown in Fig. 4 *H*, the clone responds to the drug when conjugated with autologous APC with a rapid and prolonged $[Ca^{2+}]_i$ increase. No response is seen when the clone is incubated with mismatched APC with

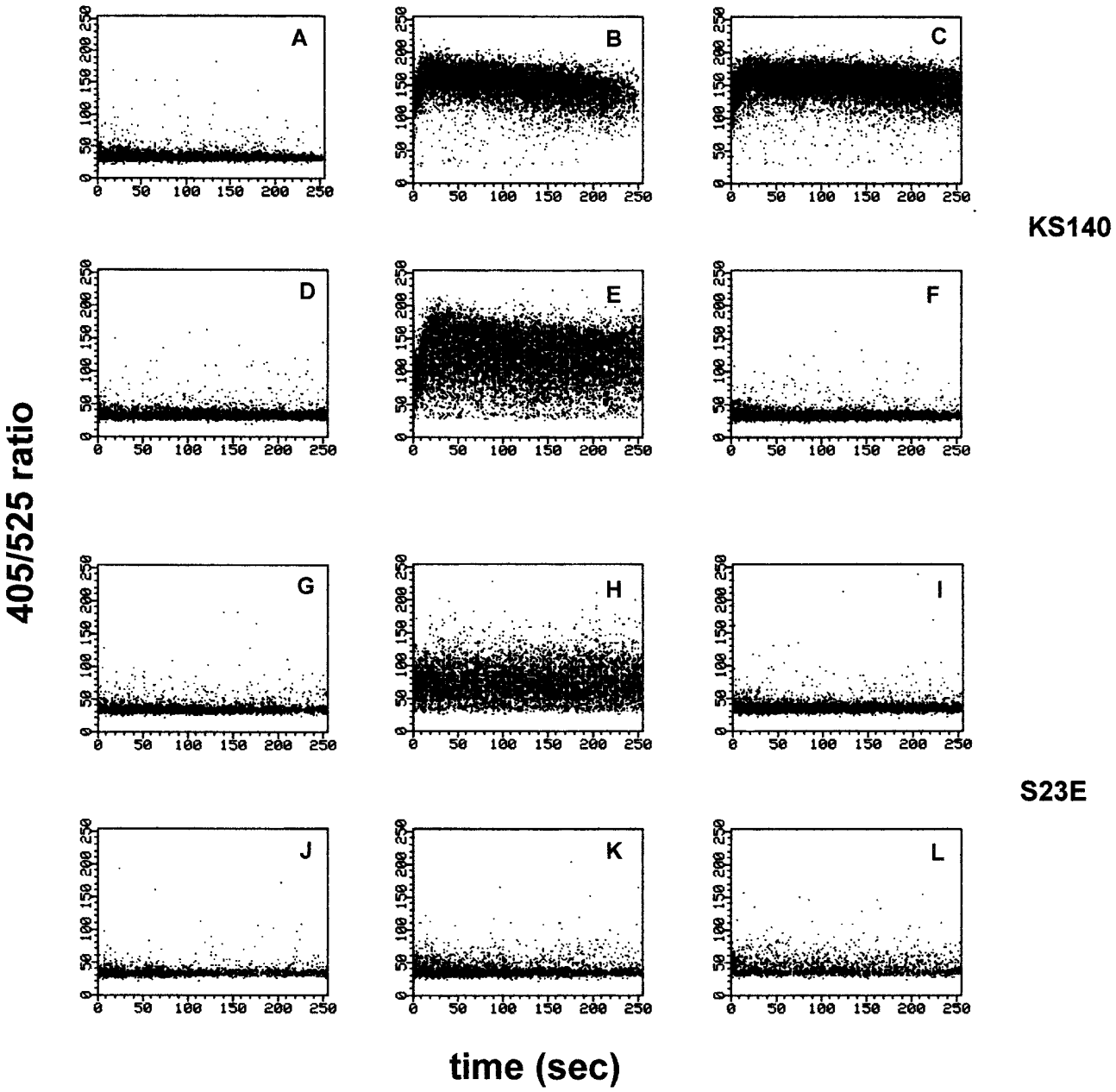

Figure 4. Sustained $[Ca^{2+}]_i$ increase can be observed only when SMX is present in the culture. The p2- and TT-specific clone KS140 (*A–F*) and the SMX-specific clone S23E (*G–L*) were tested for $[Ca^{2+}]_i$ increase under the following conditions. Clone KS140 was incubated with: (*A*) autologous APC; (*B*) APC preincubated with p2; (*C*) APC and p2 in solution; (*D*) APC and TT in solution; (*E*) APC preincubated for 16 h with TT; and (*F*) SMX in solution. Clone S23E was incubated with: (*G*) autologous APC; (*H*) autologous APC and SMX in solution; (*I*) autologous APC pulsed with SMX and washed; (*J*) mismatched APC; (*K*) mismatched APC and SMX in solution; and (*L*) SMX in solution. $[Ca^{2+}]_i$ was determined as described in Methods.

or without SMX (Fig. 4, *J* and *K*), or with APC pulsed overnight with SMX and washed (Fig. 4 *I*) or in the absence of APC but in the presence of SMX (Fig. 4 *L*). These results demonstrate that this category of drugs binds directly to MHC–peptide complexes without prior metabolism. They also demonstrate that, in order to induce the sustained $[Ca^{2+}]_i$ increase that is required for T cell activation, nonreactive drugs need to be present in culture throughout the assay.

Preincubation of APCs with nonreactive drugs does not induce a T cell stimulation. To compare the above-described mechanism of direct drug presentation and T cell stimulation with the hapten model, we generated TCC from a ceftriaxone-sensitized patient. Ceftriaxone is a third generation cephalosporin, and is, like other β-lactams, able to covalently modify proteins. It may either bind directly to the immunogenic peptide embedded in the MHC (26) or to proteins, which need processing before the hapten-modified peptides are presented (14, 15). The ceftriaxone-specific clone described in Fig. 5 *A* showed no rapid TCR downregulation if the drug was added in solution. However, APC prepulsed with ceftriaxone for 16 h could induce a rapid TCR downregulation. These data were further confirmed by measuring cytokine secretion (IL-4, IL-5) after a 6-h culture of the T helper cell type 2 cytokine-secreting clone (Table II).

In contrast, the SMX-specific clone S23E is unable to undergo TCR downregulation when incubated with APC pretreated with SMX and washed (Fig. 5 *B*). In addition, cytokine secretion (Th0 clone) can be measured only when the T cells and APCs are coincubated with the drug but not when APC preincubated with SMX were used and the drug was removed from the culture (Table II). These results demonstrate that per se reactive drugs such as β-lactams are recognized only when they are covalently bound to the MHC–peptide complex, whereas a stable binding is not required for nonreactive drugs.

Table II. Cytokine Production after 6 h of Stimulation

	IL-4‡			IL-5			IFN-γ		
Clone*	Control	Solution	Pulsed	Control	Solution	Pulsed	Control	Solution	Pulsed
DB1	< 16	< 16	26	< 16	< 16	190	< 16	< 16	< 16
S23E	< 16	117	< 16	< 16	145	< 16	< 16	378	< 16

*Clone DB1 was stimulated either with 500 μg/ml ceftriaxone in solution in the presence of autologous B-LCL or with B-LCL cells preincubated overnight with the same drug concentration. Clone S23E was stimulated similarly with 500 μg/ml SMX. Cytokines were measured in culture supernatant collected after 6 h of stimulation. ‡Detection limit was 16 pg/ml for all cytokines.

Discussion

Drug recognition by T cells has hitherto relied on the carrier hapten model implying covalent binding of the drug or drug metabolite to carrier molecules, which are processed and presented as hapten-modified immunogenic peptides. In this study we extend this concept since we show that nonreactive drugs such as SMX or lidocaine are recognized very rapidly in a processing- and metabolism-independent but still MHC-restricted way. In contrast to classical haptens like β-lactams, nonreactive drugs such as SMX or lidocaine directly bind in an unstable way to the MHC–peptide complex and the TCR, resulting in T cell activation.

Nonreactive drugs such as sulfonamides were thought to be metabolized to chemically reactive metabolites such as hydroxylamines or nitroso structures to become immunogenic (19, 27). The reactive intermediates would then bind to proteins, leading to neoantigens, which are processed by APC and presented to T cells. In support of this hypothesis, the pharmacogenetic background of an individual is important for the severity of SMX-induced side effects, as severe side effects occur more frequently in persons having the slow acetylator phenotype (28). Moreover, phagocytes have been shown to express functional drug-metabolizing enzyme systems, which may facilitate drug presentation to T cells (29, 30). Thus, it has been suggested that processing and covalent binding of the drug can play an important role for primary sensitization of T cells by professional APC. Here we report that the activation of bio-

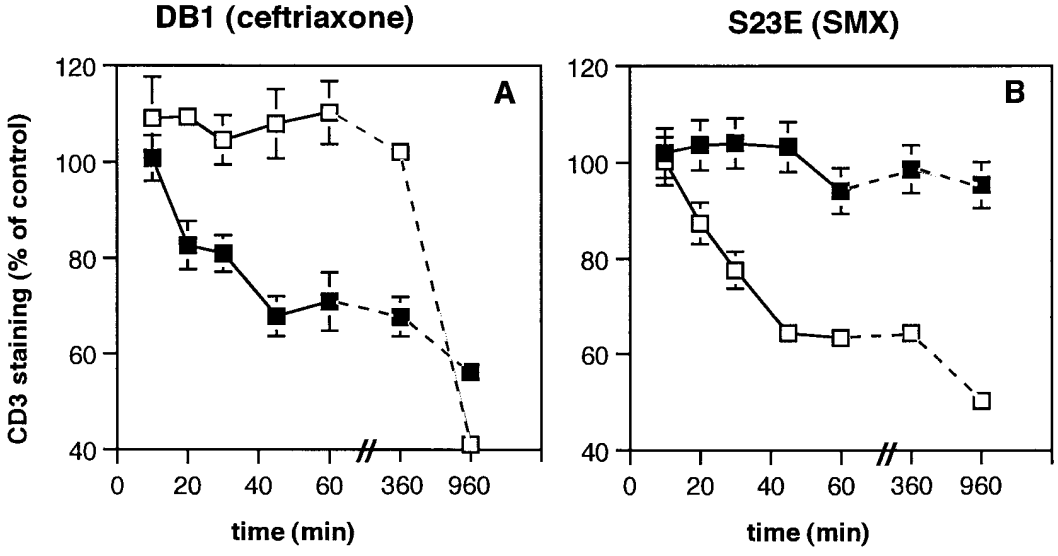

Figure 5. Per se reactive and nonreactive drugs differ in the stability of drug binding to MHC–peptide complexes. TCC DB1 (ceftriaxone-specific) (*A*) and S23E (SMX-specific) (*B*) were tested for their ability to downregulate the TCR either in the presence of drug-pulsed APC (*filled boxes*) or APC and drug in solution (*open boxes*). The drug concentration used was 500 μg/ml ceftriaxone or SMX, respectively. Results indicate the percentage of CD3 mean fluorescence obtained from values without drugs (triplicates±SE).

logical response in preactivated T cells has a different requirement. Indeed, TCC recognize SMX and lidocaine in a direct, processing- and metabolism-independent way. Recognition by specific TCC occurred within seconds as revealed by $[Ca^{2+}]_i$ increase and the immediate TCR downregulation in conditions in which the drugs were clearly not permanently bound to the MHC–peptide complex. These results are incompatible with intracellular drug metabolism, covalent protein binding, and processing.

The kinetics of TCR engagement and downregulation observed with clone OFB2 when stimulated with a drug (lidocaine), superantigen (SEB), or an alloantigen (HLA-DR8) were similar. This underlines the concept that lidocaine or SMX binds directly from the outside to the MHC–peptide complex. Additional features of drug stimulation are also reminiscent of superantigen stimulations: like superantigens, drugs activate both $CD4^+$ and $CD8^+$ $\alpha\beta^+$ T cells (12, 16). We repeatedly observed the expansion of T cells bearing certain TCR Vβ chains in several drug-specific T cell lines (12, 16, 24), and we found in a recent study that 5% of drug-specific clones recognize drugs in an HLA-DR–restricted, but allele-independent way (31). Last but not least, even some clinical features of drug allergy and superantigen stimulations are similar (12). However, the size of drugs is far lower than the one of superantigens and drug-specific clones have been so far only established from allergic donors. This implies that a certain expansion of drug-specific T cells must have occurred in vivo in allergic patients.

This new model of drug presentation to $\alpha\beta^+$ T cells extends the classical hapten model, which, as we show here, is still relevant for certain drugs such as β-lactams. The main difference is that nonreactive drugs such as SMX or lidocaine do not need to covalently modify proteins to become immunogenic. Fig. 6 summarizes the three mechanisms of drug presentation that have been described.

β-lactams are presented on immunogenic peptides after having bound covalently to proteins. These covalently modified peptides might derive from membrane or soluble proteins, which were processed and presented on MHC class I and/or II (Fig. 6 *A*) (14, 15). There is also the possibility that the β-lactam directly modifies the immunogenic peptide (processing-independent pathway) (Fig. 6 *B*) (14, 26). In both cases, pulsing of APC for > 5 h results in drug presentation and rapid TCR downregulation, cytokine production, and proliferation of specific TCC. A new, third pathway implies a labile and therefore noncovalent drug binding to the MHC–peptide complex, which is nevertheless sufficient to trigger TCC (Fig. 6 *C*). This new pathway of drug presentation relies on the finding that fixed APC can present the drugs (20) and on the rapid kinetics of TCR engagement as measured by TCR downregulation and $[Ca^{2+}]_i$ increase. This novel pathway of nonpeptide ligand presentation may be relevant for many chemically nonreactive xenobiotics and may explain the manifestation of drug allergy in tissues without known drug-metabolizing capacities.

Acknowledgments

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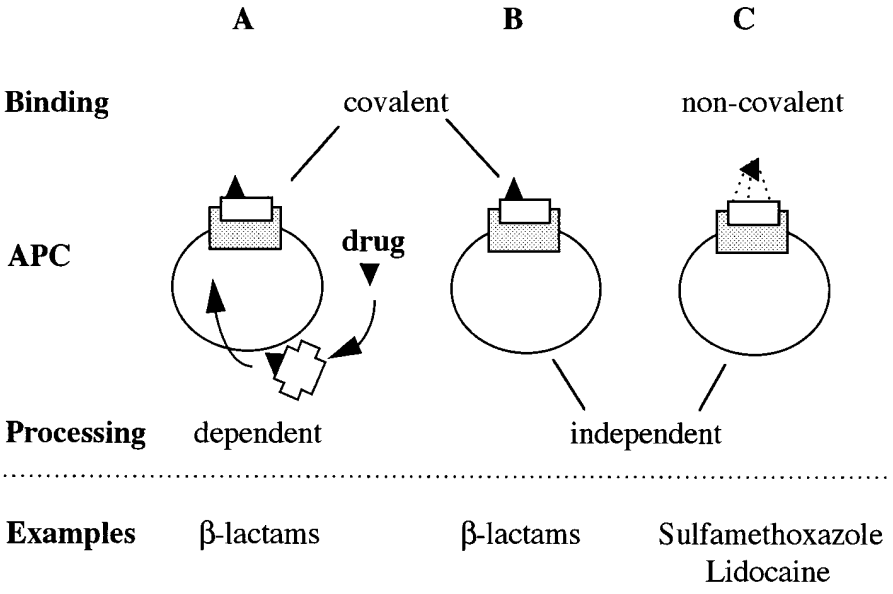

Figure 6. Mechanisms of drug presentation. Three mechanism of drug presentation are outlined. Classical, per se reactive haptens such as β-lactam antibiotics can be presented via two pathways. (*A*) The drug covalently modifies soluble or membrane-bound proteins, which are taken up by APC and presented as modified self peptides on MHC molecules to T cells (covalent, processing-dependent pathway). (*B*) Alternatively, β-lactams can directly bind to the peptide in the MHC molecule (covalent, processing-independent pathway). (*C*) Nonreactive drugs such as SMX or lidocaine bind directly to the MHC–peptide complex (noncovalent, processing-independent pathway).

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