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Degeneracy and additional alloreactivity of drug-specific human $\alpha\beta^+$ T cell clones

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Abstract

It has been well established that T cells can recognize small mol. wt compounds such as drugs. Results from previous studies revealing a high heterogeneity and cross-reactivity of drug-specific T cell clones (TCC) in individual patients prompted us to analyze the degeneracy of drug-reactive TCR in detail. Hence, we analyzed the MHC restriction pattern of a panel of 100 drug-specific TCC isolated from different drug-allergic donors. We found that 28 of the tested clones showed an MHC allele-unrestricted drug recognition. Most of these clones were at the same time highly drug specific, i.e. they could only be stimulated by the original drug and not by any drug derivatives. In contrast, TCC with the ability to interact with different drug derivatives displayed a clearly MHC allele-restricted drug recognition. Therefore, we concluded that the TCR of these clones is mainly interacting with side chains of the appropriate drug molecules and hence able to tolerate alterations in the MHC molecule. Moreover, we tested all clones for additional alloreactivity and found that 27 clones could be stimulated by a self-MHC–peptide–drug complex as well as by a non-self-MHC–peptide complex. This cross-reactivity with allogeneic MHC molecules was substantially higher in drug-specific TCC compared to tetanus toxoid-specific clones from the same donors. This suggests that from the point of view of drug-specific TCR, non-self-MHC–peptide complexes have a higher incidence to mimic the 'original' self-MHC–peptide–drug complex and this may occur for TCR recognizing self-MHC–pathogen-derived peptide complexes. Finally, the biological functions of bispecific TCC were not influenced by the nature of the stimulating ligand. Both drug as well as allogeneic stimulation led to similar reaction patterns in the analyzed TCC.

Introduction

In addition to the recognition of pathogen-derived peptides embedded in the groove of self-MHC molecules, T cells can also recognize non-peptide antigens (1). In addition to lipids, glycolipids (2) and carbohydrates (3), T cells can also be triggered by small mol. wt compounds such as drugs (4) or contact sensitizers like TNP or metal salts (5).

Drugs like β-lactam antibiotics, sulfonamides or local anesthetics are often responsible for allergic reactions in humans. The T cell involvement in such drug allergic reactions is well established (for review, see 6). However, several molecular aspects of the recognition of drugs by the TCR of drug-reactive T cell clones (TCC) remain still unclear. In principle two groups of drugs can be distinguished: (i) chemically reactive compounds with the ability to covalently modify side chains of amino acids such as β-lactam antibiotics or reactive drug metabolites and (ii) drugs like lidocaine or sulfamethoxazole (SMX) which are not *per se* chemically reactive. Covalent modification of serum or cellular proteins can lead to presentation of modified self structures (haptenated peptides) by MHC class I or class II molecules to reactive T cells (7–9). However, recent work has elucidated that covalent binding is not a necessary prerequisite for drug presentation: preincubation and subsequent washing of antigen-presenting cells (APC) with chemically inert drugs like lidocaine or SMX did not lead to any detectable T cell responses (10,11). We postulated, therefore, that these compounds can associate with MHC and/or embedded peptides in a non-covalent way, forming rather unstable trimolecular MHC–peptide–drug complexes.

Although self-MHC-restricted T cell recognition has sufficient specificity to discriminate between an enormous variety

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of antigenic peptides, recent studies on TCR recognition have begun to emphasize the importance of a significant level of cross-reactivity on different peptides, which is necessary to produce an adequate TCR repertoire capable of responding to the universe of pathogens (reviewed in 12). Several studies of the cross-reactivity and fine specificity pattern of drug-specific TCC revealed highly heterogeneous T cell responses in the analyzed donors (13–16).

It is generally agreed that alloreactivity is the result of cross-reactive recognition by self-MHC-restricted T cells and is not a function of a particular population of T cells that has escaped the constraints of the thymic selection (17). A recent study form Daniel *et al*. proposes that the majority of the alloreactivity represents a variation of self-restricted responses in which there is a shift in the relative contribution of the MHC and peptide residues toward the overall strength of binding between the TCR and the MHC–peptide ligand. More energy is provided by the TCR–MHC interactions and less from the TCR–peptide interactions; however, the latter are still necessary to reach the threshold required for T cell activation (18).

The aim of this study was to study the degeneracy of the TCR of drug-specific TCC isolated from patients with hypersensitivity reactions to different drugs. We analyzed the MHC restriction pattern of the different clones as well as a possible cross-recognition of allogeneic MHC alleles. In addition we compared the activation mechanisms in bispecific clones.

Methods

Culture media (CM)

CM consisted of RPMI 1640 supplemented with 10% pooled heat-inactivated human antibody serum (Swiss Red Cross, Bern, Switzerland), 25 mM HEPES buffer, 2 mM L-glutamine (Seromed, Fakola, Basel, Switzerland), 25 μg/ml transferrin (Biotest, Dreieich, Germany), 100 μg/ml streptomycin and 100 U/ml penicillin. The culture medium CM+, used to culture TCC, was additionally enriched with 50 U/ml recombinant IL-2 (obtained from Dr D. Wrann, Novartis Research Institute, Vienna, Austria).

Drugs used for stimulation

Amoxicillin, ceftriaxone, penicillin G (Pen G) and sulfonamide derivatives were purchased from Sigma (St Louis, MO), and lidocaine from Grogg Chemie (Stetteln, Switzerland). All drugs used have been tested previously for their inhibitory activity on the proliferative response to mitogens in non-allergic individuals. Stock solutions of each drug were always freshly prepared just before use. Sulfonamide derivatives were dissolved in RPMI/0.05 M NaOH, all other drugs in RPMI. Tetanus toxoid (TT) was kindly provided by Dr J. Cryz (Serum und Impfinstitut, Bern, Switzerland).

Generation of drug- or TT-specific TCC

TCC from peripheral blood mononuclear cells (PBMC). Freshly isolated PBMC from donors allergic to lidocaine (19), β-lactam antibiotics (13) and SMX (10) were stimulated with the corresponding drug in CM at a cell density of 2×10^6 cells/well in 24-well plates. The following drug concentrations were used: amoxicillin (500 μg/ml), Pen G (500 μg/ml), lidocaine (100 μg/ml) and SMX (200 μg/ml). CM+ was added after 7 days of culture. After 14 days, bulk cultures were re-stimulated with autologous irradiated (4000 rad) PBMC and the respective antigen. Specific T cell lines (TCL) were cloned by limiting dilution as described previously (20). Growing TCC were expanded in CM+ and re-stimulated every 14 days with allogeneic irradiated (6000 rad) PBMC plus phytohemagglutinin (PHA, 1 μg/ml, Bacto; Difco, Detroit, MI). From three donors (KB, SE and SF; see Table 5), freshly isolated PBMC were additionally simulated with TT (5 μg/ml) and TT-specific TCC were generated after cloning by limiting dilution as described above.

TCC from skin biopsies. Skin punch biopsies were taken from dermal reaction centers from a patient allergic to amoxicillin and a patient allergic to ceftriaxone (21). T cells were isolated as described (21). Briefly dermal T cells were cultured with 25,000 irradiated allogeneic PBMC plus PHA (2 μg/ml) in 100 μl CM in 96-well round-bottomed plates. Growing T cell lines were expanded in CM+ and subsequently cloned by limiting dilution as described (20).

Immunofluorescence and PCR-based TCR V_β analysis

Monoclonality of the TCC used was proven either by staining with a panel of 22 different mAb recognizing different V_β gene products (Immunotech, Marseilles, France) or by RT-PCR-based TCR oligotyping as described previously (8). Additionally, cross-reactive clones were tested for TCR V_α expression by RT-PCR-based TCR oligotyping as described (8).

Proliferation assay

In order to reveal the MHC restriction pattern as well as to identify the alloreactive potential of the drug- or TT-specific TCC we used a panel of allogeneic HLA-typed PBMC from healthy donors (see Tables 2 and 3). HLA class II phenotyping was performed by PCR amplification using sequence-specific primers (Protrans Endotell, Allschwil, Switzerland). To determine the proliferation of the TCC to the different allogeneic PBMC, 5×10^4 TCC were incubated either with 5×10^4 of the respective irradiated PBMC or with 5×10^3 autologous B-LCL in the presence or absence of the indicated drug or TT (5 μg/ml) in 200 μl CM in round-bottomed microplates (Falcon no. 3077) for 48 h. Cultures were pulsed with [^{3}H]thymidine (0.5 μCi) for the last 8 h, and cells were then harvested onto glass fiber disks and counted in a microplate β-counter (Inotech filter counting system INB 384; Dottikon, Switzerland).

Proliferation assay with TCC of multiple specificity

TCC cells (5×10^4) were incubated in round-bottomed microplates (Falcon no. 3077) with different concentrations of drugs or allogeneic B-LCL. Irradiated (6000 rad) HLA-matched B-LCL (2×10^4) were added to drug-stimulated cultures. Cultures were pulsed with [^{3}H]thymidine (0.5 μCi) for the last 8 h to determine proliferation as described above.

FACS analysis of CD25, CD69 expression

For immunofluorescence analysis of CD25, CD69 expression, 5×10^4 TCC cells were incubated with different concentrations

of the antigens; again, 2×10^4 irradiated (6000 rad) HLA-matched B-LCL were added to drug-stimulated cultures. After 12 h, the supernatant was harvested for cytokine measurement and cells were washed in PBS containing 0.5 mM EDTA to break the conjugates. Cells were then stained either with anti-CD25–FITC (Dako Research, Besancon, France) or anti-CD69–FITC (PharMingen, San Diego, CA). Analysis was performed on a Coulter Epics XL-MCL flow cytometer (Beckman Coulter, Nyon, Switzerland).

Cytokine measurement

To detect cytokines produced after stimulation with the different antigens, supernatants of cells stimulated as described above were collected after 12 or 48 h and cytokines were quantified by a sandwich ELISA according to standard protocols (PharMingen) (22). Detection limits were 10 pg/ml.

Measurement of TCR down-regulation

For immunofluorescence analysis of CD25, CD69 expression, TCR down-regulation induced by different compounds was determined as described previously (10,16). TCC cells (5×10^4) were incubated with different concentrations of the antigens in 200 μl CM in U-bottom microtiter plates; again, 2×10^4 irradiated (6000 rad) HLA-matched B-LCL were added to drug-stimulated cultures. The plates were centrifuged at 1200 r.p.m. for 2 min to allow conjugate formation and incubated at 37°C. After 12 h, cells were resuspended, washed in PBS containing 0.5 mM EDTA to break the conjugates and stained with anti-CD3 (UCHT-1; Dako Diagnostics, Zug, Switzerland) followed by an FITC-labeled goat anti-mouse Ig (Dako Diagnostics). Analysis was performed on a Coulter Epics XL-MCL flow cytometer (Beckman Coulter). The absolute number of CD3 molecules per cell was estimated by reference to a standard curve of beads coated with known amounts of mouse Ig (Qifikit; Dako Diagnostics). Cytokine production was measured in the culture supernatant using an ELISA as described above.

Results

Heterogeneous MHC restriction pattern of drug-specific TCC

In previous studies we showed that drug-specific TCC have a high heterogeneity in their ability to respond to different drugs with the same core structure (13,15,16). Table 1 gives a summary of the observed cross-reactivity pattern of SMX-specific TCC to a panel of different sulfonamide derivatives. On one hand, nine TCC were isolated, which were highly specific as they could be stimulated by the parent drug SMX only and did non-tolerate even slight modifications of the drug molecule. On the other hand, several clones, such as H9, showed a broad cross-reactivity pattern in as much as they could recognize up to nine different compounds sharing only minor structural similarities (16). Interestingly, for several cross-reactive clones, such as 8.15, the different compounds stimulating these clones differed in their capacity to induce proliferation (data not shown and 16). Few compounds had the same full agonistic properties as the parent drug SMX, whereas other compounds needed a 10- to 100-fold higher concentration to achieve half-maximal proliferation and could therefore be classified as weak agonists.

We concluded that the drug-specific T cell responses in most of the analyzed patients were polyclonal and heterogeneous. In addition, another preceding analysis revealed that certain drug-specific TCC displayed a MHC allele unrestricted recognition pattern, implicating that they recognize non-polymorphic residues of the MHC molecules in addition to certain side chains of the drug molecule (23). The aim of this study was to characterize the degeneracy of drug-specific TCC in detail. In order to do that we analyzed a total number of 100 drug-specific TCC isolated either from PBMC or from skin biopsies of seven different drug-allergic donors (see Table 2). To compare drug-specific TCC with clones specific for pathogen-derived peptides we tested in addition a panel of 58 TT-specific TCC obtained from three different TT-immunized and drug-allergic patients (donors KB, SE and SF). In a first set of experiments, we analyzed the MHC restriction pattern of both groups of clones. For that purpose we used a panel of 10 different allogeneic PBMC expressing a wide variety of different HLA-DRB1 molecules. Most of the drug-specific clones (72 out of 100 tested clones) recognized the respective antigen only in the presence of HLA-matched allogeneic APC as shown for clone DB3 in Table 3. For this clone the ceftriaxone recognition is clearly HLA-DRB1*1101-1104 restricted. However, 28 of the tested clones showed a drug-specific proliferative response irrespective of the HLA phenotype of the APC used. As shown in Table 3, clone ABH47 could be stimulated in the presence of amoxicillin by any of the used allogeneic feeder cells. This phenomenon could also be observed in TCC specific for non-covalently binding drugs such as lidocaine and SMX (see Table 5 and data not shown). The drug-specific response of all these HLA allele-non-restricted clones could easily be blocked by addition of anti-DR mAb (data not shown). Thereby we could exclude that in these clones the drug molecules were presented by HLA-DP or HLA-DQ, or by non-classical antigen-presenting molecules such as CD1. None of the 58 tested TT-specific TCC showed an MHC allele-unrestricted antigen recognition.

Furthermore, a third group of clones could be detected where the need of professional APC was not required: clone H4 had the ability to recognize the drug SMX presented on MHC molecules expressed on the surface of the T cells themselves (see Table 3). Twelve other clones from three different donors showed the same 'T cell responder' features (data not shown and 20).

Alloreactivity of drug-specific TCC

In addition to the above described clones, we could detect in total 27 clones which reacted in the absence of stimulatory drug molecules in an alloreactive manner to a certain non-self-MHC allele. By RT-PCR oligotyping of the TCR V_α chains we could exclude that the additional alloreactivity of the drug-specific clones was due to expression of two different V_α chains. Table 4 shows the representative reactivity pattern of three alloreactive drug-specific TCC from three different donors. Clone OFB12 showed lidocaine recognition in the context of HLA-DRB1*1501-03 (donors WP and autologous B-LCL) and an additional alloreactivity to the HLA-DRB1*0801

Table 1. Cross-reactivity-pattern of 21 different SMX-specific TCC[a]

	Group									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Total no. of clones[b]	9	1	2	1	2	2	1	1	1	1
No. of MHC allele-unrestricted TCC[c]	5	1	1	1	1	0	0	0	0	0
Representative TCC	9.18	H1	H4	H5	9.3	H6	9.5	8.15	H18	H9
Phenotype	CD4	CD4	CD4	CD4	CD4	CD4	CD4	CD4	CD4	CD4
V_β gene usage	V_β17	V_β2	V_β6	V_β12	V_β5.2	V_β17	V_β5.2	V_β16	V_β1	V_β16
Control	420 [d]	314	240	208	511	660	473	344	212	380
SMX	**15448**	**4103**	**11351**	**9035**	**32261**	**14378**	**54607**	**23207**	**14143**	**7812**
Sulfamethizole	315	285	**17181**	**10184**	**5237**	**8608**	**33172**	**11526**	**11457**	**4559**
Sulfathiazole	417	319	313	303	**8563**	**8544**	**8815**	**2381**	**5085**	427
Sulfamoxole	240	266	280	214	476	297	365	269	136	**5319**
Sulfapyridine	447	444	340	245	624	494	**23190**	491	266	352
Sulfadiazine	332	**1221**	226	213	482	**3104**	455	519	**3761**	372
Sulfamerazine	311	**1037**	291	186	534	457	519	255	**10507**	**2948**
Sulfamethazine	419	414	311	151	458	483	499	549	**11221**	**9639**
Sulfadimethoxidiazine	433	298	291	201	502	496	557	519	920	**1756**
Sulfamethoxipyridazine	328	426	**9369**	228	**32988**	590	585	316	291	**6278**
Sulfisomidine	375	430	367	323	616	702	625	**20009**	195	**5892**
Sulfadimethoxine	329	557	349	**1249**	532	535	559	**12376**	251	**2216**
Sulfadoxine	509	343	367	208	497	567	552	**10839**	188	482

[a]TCC were analyzed for reactivity with a panel of 13 different sulfonamide derivatives by incubating the T cells in the continuous presence of 200 μg/ml of the indicated compounds and autologous irradiated B-LCL as APC (according to 16). Proliferation was determined after 48 h by measurement of [^{3}H]thymidine incorporation.
[b]Number of clones with the same cross-reactivity pattern.
[c]Number of clones which show an MHC allele-unrestricted drug recognition (for experimental procedures, see Table 3).
[d]Values: c.p.m. of [^{3}H]thymidine incorporation (the experiments were repeated at least 3 times).

Table 2. Origin of drug-specific TCC

Donor	MHC phenotype	Drug	Origin of TCC
KB (male)	A2, A16, B44, B60 DRB1*0101-02, 1001	SMX	PBMC
SE (female)	A1, A2, B8, B44 DRB1*1003, 0401	Pen G	PBMC
SF (female)	A3, A29, B7, Bx DRB1*1501-03, x	lidocaine	PBMC
OF (male)	A2, A26, B7, Bx DRB1*1501-03, x	lidocaine	PBMC
PK (male)	A1, A2, B7, B8 DRB1*0301, 1501-1503	penicillin G amoxicillin	PBMC PBMC
BM (female)	A2, A29, B7, Bx DRB1*1501-03, 1101-04	ceftriaxone	skin biopsy
BA (female)	A2, A68, B35, B44 DRB1*0701-04, 1201	amoxicillin	skin biopsy

allele (donors SK and CB). A similar pattern could be observed for clone H13 which recognized the stimulating drug SMX in the context of HLA-DRB1*1001 (donor KV and autologous B-LCL) and revealed alloreactivity to the HLA-DRB1*1303-04 allele (donors MA, YH and SK). The same cross-reactivity could also be observed in TCC reactive to covalently binding drugs such as Pen G and other β-lactam antibiotics: clone KP26 could be stimulated by Pen G presented in the context of HLA-DRB1*1501-03 (donor WP and autologous B-LCL) and showed an additional allospecific response to the HLA-DRB1*1101-04 allele (donors SK and BL). In all allospecific TCC the alloreactive MHC allele could be clearly evaluated (data not shown). Additionally, in only one female donor (with two children) five TT-specific TCC with an additional alloreactivity could be detected. Therefore, we concluded that the ability to cross-recognize non-self-MHC–peptide complexes must be substantially higher for drug-specific TCC than for T cells initially primed by pathogen-derived peptides (unpaired Student's *t*-test: $P = 0.05$).

Comparison of drug and allo-stimulation

It has been shown for peptide antigens, that the recognition of slightly altered ligands by $\alpha\beta^+$ TCR can have dramatic functional consequences for the T cells (24). The responses to such altered peptide ligands (APL) include cytokine production in the absence of proliferation, differential cytokine production, anergy and antagonism of the response to the wild-type antigen (25–28). We therefore asked if the different antigenic structures (self-MHC–peptide–drug complexes versus non-self-MHC–peptide complexes) may elicit a similar reaction pattern in bispecific TCC. Two TCC, OFB12 and H13 (see Table 4), were analyzed in detail. As previously shown, the kinetics of T cell triggering after drug stimulation resembled the ones observed after activation

Table 3. MHC restriction pattern of selected drug-specific TCC[a]

Donor (HLA-DRB1* phenotype)	HLA-DRB1*	Clone					
		DB3 BM (DRB1*1101-04/1501-03)		ABH47 BA (DRB1*0701-04/1201)		H4 KB (DRB1*0101/1001)	
APC		Control	Ceftriaxone	Control	Amoxicillin	Control	SMX
No APC		72 [b]	53	30	39	169	**5121**
MA	0701-04/1303-04	45	55	64	**15636**	120	9919
YH	1302/1303-04	54	65	78	**9663**	195	8854
NB	1101-04/1201	72	**3783**	156	**12039**	203	9794
SK	0801-05/1303-04	41	43	138	**11412**	147	7825
MV	0301/0901	131	56	144	**3798**	225	7620
KB	0401-12/1401	63	30	133	**11910**	53	8337
WP	0101-02/1501-03	21	39	76	**6956**	225	9758
KV	0301/1001	70	40	29	**1038**	507	13397
HM	0701-04/1501-03	33	34	58	**2519**	106	8522
FK	0701-04/1101-04	50	**4879**	45	**1362**	108	8967
Autologous B-LCL		208	**4000**	178	**10977**	115	6874

[a]TCC were analyzed for MHC restriction by incubating the T cells in the continuous presence of the respective drugs (ceftriaxone 200 μg/ml, amoxicillin 500 μg/ml or SMX 200 μg/ml). A panel of PBMC from 10 different HLA-typed allogeneic donors or autologous B-LCL was used as APC. Proliferation was determined after 48 h by measurement of [^{3}H]thymidine incorporation.
[b]Values: c.p.m. of [^{3}H]thymidine incorporation (the experiments were repeated at least 3 times).

Table 4. Representative drug-specific TCC with additional alloreactivity[a]

Donor (HLA-DRB1* phenotype)	HLA-DRB1*	Clone					
		OFB12 OF (DRB1*1501-03/x)		H13 KB (DRB1*0101/1001)		KP26 PK (DRB1*0301/1501-03)	
APC		Control	Lidocaine	Control	SMX	Control	Pen G
No APC		99 [b]	100	102	73	67	79
MA	0701-04/1303-04	127	157	**8821**	3670	107	100
YH	1302/1303-04	221	241	**8164**	3214	75	86
NB	1101-04/1201	133	140	156	170	**3595**	3018
SK	0801-05/1303-04	**9980**	10890	**6490**	5237	69	77
MV	0301/0901	101	110	127	72	71	80
KB	0401-12/1401	50	56	235	120	101	44
WP	0101-02/1501-03	29	**5357**	96	97	76	**3184**
KV	0301/1001	101	110	169	**5043**	53	91
CB	0401-12/0801	**7645**	8723	67	115	96	121
BL	0701-04/1101-04	129	150	100	105	**3410**	3442
Autologous B-LCL		576	**8316**	599	**7951**	455	**7297**

[a]TCC were analyzed for additional alloreactivity by incubating the T cells in the continuous presence of the respective drugs (lidocaine 100 μg/ml, SMX 200 μg/ml or penicillin G 500 μg/ml). A panel of PBMC from 10 different HLA-typed allogeneic donors or autologous B-LCL was used as APC. Proliferation was determined after 48 h by measurement of [^{3}H]thymidine incorporation.
[b]Values: c.p.m. of [^{3}H]thymidine incorporation (the experiments were repeated at least 3 times).

by allogeneic B-LCL (11). In addition, the comparison of the dose–response curves in different readout systems, i.e. up-regulation of activation markers, proliferation and cytokine production, after drug and allogeneic stimulation did not show any significant differences (Fig. 1). As the ligand density of APC presenting unstably associated drug molecules and APC bearing non-self-MHC–peptide complexes cannot be measured up directly, we compared the extent of different biological responses, measurable after TCR triggering by different ligands. Both stimuli elicited production of a similar cytokine pattern and showed similar extents of TCR down-regulation required for the induction of half-maximal effector functions (see Fig. 2). Therefore, we concluded that in the case of the analyzed TCC the activation by different antigens such as self-MHC–peptide–drug complexes and non-self-MHC–peptide complexes did not influence the biochemical events following TCR–ligand interaction.

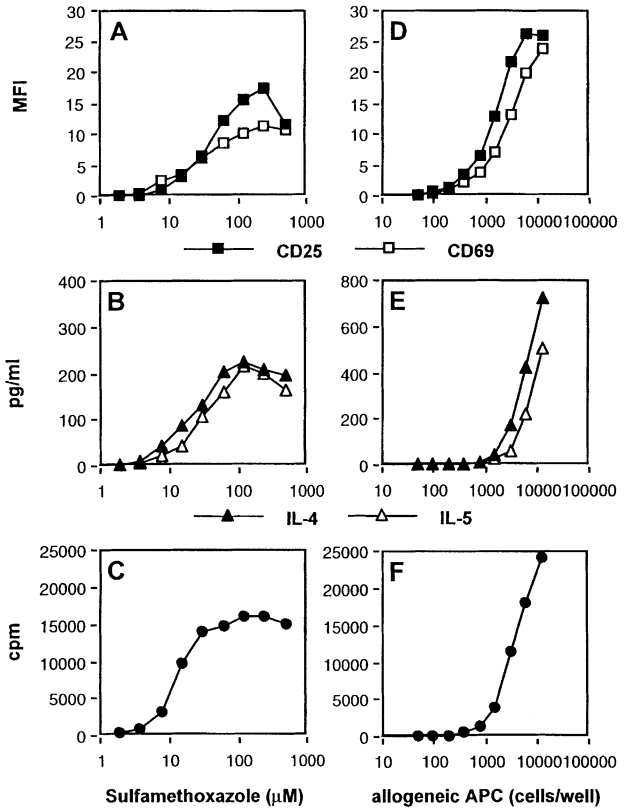

Fig. 1. Dose-dependent reactions of a drug-specific TCC with additional alloreactivity. A representative comparison of the dose-dependent CD25 and CD69 expression (A and D), the IL-4 and IL-5 production (B and E) as well as the proliferative response (C and F) of TCC OFB12 after stimulation with SMX (A–C) or allogeneic APC (D–F) is shown. For experimental details, see Methods. Similar results were obtained with other drug-specific TCC with additional alloreactivity.

Discussion

In this study we analyzed the influence of different HLA-DR alleles on the interaction of a drug-specific TCR with MHC–peptide–drug complexes. We found that 28 out of 100 tested drug-specific clones from different donors showed a proliferative response after drug stimulation irrespective of the presenting HLA-DR allele. Interestingly, this HLA allele-unrestricted drug presentation was found for clones that react to covalently binding drugs such as Pen G or amoxicillin as well as for clones specific for the non-covalently binding drugs lidocaine and SMX. As the responses could easily be blocked by addition of anti-DR mAb, we could exclude that these clones recognize the drug molecules in the context of other presenting molecules such as CD1. Similar observation have been made recently for nickel-specific T cells (29), as well as for peptide-specific clones (30). In addition, murine T cell hybridomas specific for the contact sensitizer TNP showed a similar freedom in their ability to tolerate different presenting MHC alleles (31).

It has been shown by crystallographic analysis of TCR–ligand interactions that the MHC residues that are engaged by the TCR are mostly conserved amino acids, which do not vary between different DR alleles (32,33). The TCR–MHC–peptide structures determined to date (34,35) indicate that the required degree of peptide cross-reactivity can be produced in at least two ways: (i) the TCR 'focus' on only few amino acid side chains of the peptide and can accommodate peptides with other side chains, depending on the size and surface chemistry of the TCR contact surfaces, and (ii) the structural flexibility of the CDR3 loops that contact the peptide allow a degree of accommodation of binding to multiple peptide ligands (33). These findings, together with the data summarized in Table 5, prompted us to establish a model of the molecular basis of the recognition of drug molecules by the TCR of specific T cells (see Fig. 3): allele-restricted and allele-unrestricted clones vary in the degree by which MHC

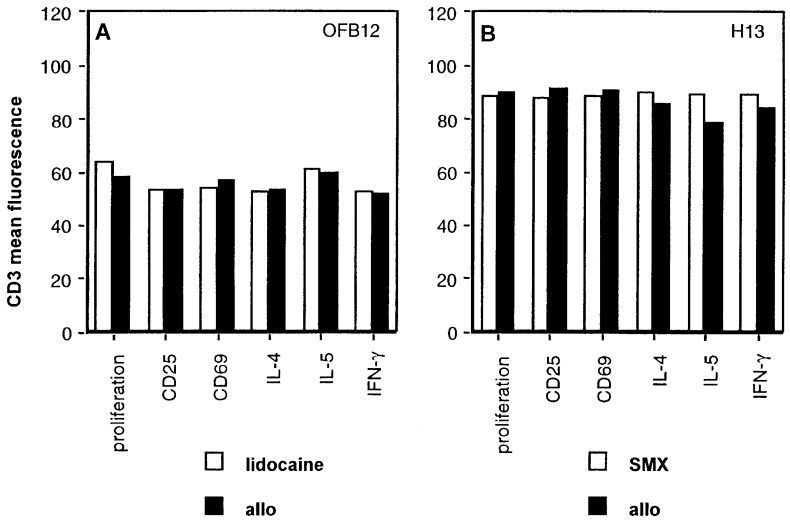

Fig. 2. Comparison of drug and allo-stimulations. A comparison of TCR down-regulation and effector functions (obtained in the same experiment) for TCC OFB12 (A) and TCC H13 (B) was performed. The level of TCR expression required to induce 50% activation of the different effector functions is shown for the two different ligands. The CD3 MFI of unstimulated T cells was 90.0 for OFB12 and 134.7 for H13.

Table 5. Comparison of drug- and TT-specific TCC

Donor	Antigen	No. of tested TCC	No. of allele-unrestricted TCC	No. of alloreactive TCC
KB (M)[a]	SMX	21	**9**	**1**
	TT	10	0	0
SE (F)	Pen G	4	0	**2**
	TT	16	0	0
SF (F)	lidocaine	23	0	**7**
	TT	32	0	**5**
OF (M)	lidocaine	2	0	**1**
PK (M)	Pen G	23	**4**	**9**
	amoxicillin	9	**4**	**5**
BM (F)	ceftriaxone	7	0	**2**
BA (F)	amoxicillin	11	**11**	0
Total	drug	100	**28** (28.0%)	**27** (27.0%)
	TT	58	0 (0.0%)	**5** (8.6%)

[a]Male/female.

residues and peptide–drug side chains contribute to the overall strength of the TCR–ligand interactions. This would imply that in the case of allele-unrestricted clones the main part of the energy required for a productive TCR engagement is provided by the TCR–peptide–drug interaction. Therefore, it is feasible that alteration in the involved MHC residues may be allowed. In addition, such clones are highly sensitive to alteration of the presented drug molecule, as they recognize only the parent drug and do not tolerate even slight modifications of the drug molecules. In contrast, the TCR of allele-restricted TCC interacts predominantly with amino acid residues of the MHC molecule and thus does not allow larger modifications of the presenting MHC molecule (18,36). In consequence, they allow broader alterations of the embedded drug molecule. However, for the activation of these clones the presence of drug molecules in the stimulating MHC–peptide–drug complex is still necessary as they do not proliferate in the absence of the antigenic drug.

The data obtained from the analysis of the MHC restriction pattern of drug-specific TCC revealed an other interesting

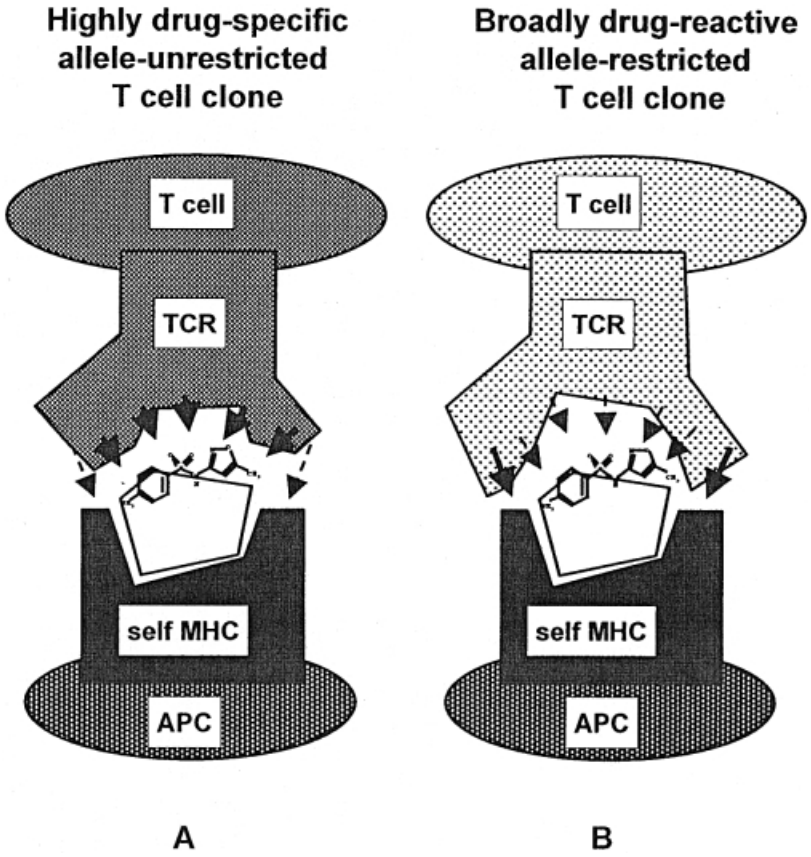

Fig. 3. Models of degenerate TCR–ligand interactions. The scheme delineates a possible explanation for the degeneracy of drug recognition by TCC (see also Table 1). The depicted MHC–peptide–drug complex shows a schematic view of the unstable, non-covalent association of a non-reactive drug (SMX) with a MHC–peptide complex. Notice that the thickness of the arrows corresponds to the energy provided for the overall strength of the TCR–ligand interaction. (A) In the case of highly drug-specific clones the TCR is mainly triggered by interactions with side chains of the drug molecule and therefore even slight alterations of the three-dimensional structure of the ligand surface (i.e. the drug molecule) are not tolerated. If the main part of the energy required for a productive TCR engagement is provided by the interaction of the TCR with the peptide–drug complex, then it seems feasible that alteration in the involved MHC residues may also be allowed. Therefore, highly specific TCC show mainly a MHC allele-unrestricted recognition. (B) Broadly drug-reactive TCC interact predominantly with amino acid residues of the MHC molecule and thus allow larger modifications of the embedded drug molecule. As a consequence, these clones show a clearly MHC allele-restricted recognition, as modifications of the relevant MHC residues abrogate the interaction.

phenomenon: 12 clones, isolated from three different donors, did not need the presence of professional APC for the induction of a productive drug-specific response. These clones had the ability to recognize the drug molecules presented on MHC molecules expressed on the surface of the T cells themselves. It is known from studies with peptide-specific TCC that the ability to react to T cells as APC is a feature of the responding T cells and not of the presenting T cells. In analogy, these drug-specific clones can also be classified as 'T cell responder clones' (20). The functional differences between 'responder' and 'non-responder' T cells have not yet been fully elucidated; however, the data obtained with peptide-specific clones indicate that these two groups of clones differ in their expression of co-stimulatory ligands, mainly CD28 (20).

Under normal conditions, T cells will not encounter allo-MHC molecules during their maturation. This fact has led to the hypothesis that alloreactivity is the result of cross-reactivity of T cells selected for self-restricted responses (37). This also implies that alloreactive T cells are not a particular population of the mature T cell repertoire that has escaped the constraints of the positive selection process in the thymus (17). This directs us to the interesting phenomenon that, although TCR-mediated recognition appears to be sensitive to minute changes in the peptide or the MHC molecule, interaction of the TCR with MHC–peptide complexes is permissive to various combinations of peptides and MHC molecules (37). From the point of view of bispecific TCR, self-MHC–peptide–drug complexes must show a similar three-dimensional structure as non-self-MHC–peptide complexes. The data shown in Table 5 indicate that the degeneracy of drug-specific TCR might be higher compared to peptide-specific TCR. As a consequence, a much higher proportion of drug-specific clones (27% compared to 8.6% in peptide-specific clones) has the ability to recognize a non-self-MHC molecule in addition to the original self-MHC–peptide drug complex. The data could also be interpreted in the way that self-MHC–peptide–drug complexes can more easily mimic non-self-MHC–peptide complexes than self-MHC–pathogenic peptide complexes. Interestingly the two different antigenic stimuli lead to similar T cell activation pattern as no altered functions of drug-specific TCC after simulation with allogeneic B-LCL could be observed.

In summary, the obtained data indicate that drug-specific T cells isolated from allergic donors comprise a heterogeneous population of highly degenerate T cells. This finding leads to the assumption that drugs can be presented to specific T cells in a multitude of ways. Therefore, a high predominance of MHC allele-unrestricted drug recognition as well as additional alloreactivity in drug-specific TCC might be a peculiar feature of the activation of the specific immune system by drug antigens.

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Abbreviations

APC	antigen-presenting cell
APL	altered peptide ligand
B-LCL	B lymphoblastoid cell line
CM	culture media
PBMC	peripheral blood mononuclear cell
Pen G	penicillin G
PHA	phytohemagglutinin
SMX	sulfamethoxazole
TCC	T cell clone
TCL	T cell line
TT	tetanus toxoid

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