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Allele-unrestricted presentation of lidocaine by HLA-DR molecules to specific $\alpha\beta^+$ T cell clones

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Abstract

T cells recognize peptide and non-peptide antigens. Drugs represent typical examples of non-peptide antigens. The majority of drug-specific T cells are $\alpha\beta^+$ TCR T cells and are MHC class I or II restricted. Here we show the existence of drug (lidocaine)-specific T cell clones which proliferate in the presence of antigen-presenting cells (APC) with different HLA alleles. Two clones (SFT24 and E20) were analyzed in detail. They show a narrow dose-dependent proliferation to lidocaine, but not to procaine. With the use of a panel of HLA-typed allogeneic APC, we observed that certain allogeneic APC plus lidocaine lead to a similar, others to partial and some to no proliferation of the lidocaine-specific T cell clones. An APC-independent proliferation could be excluded since both clones proliferated only marginally without APC and increasing the number of APC resulted in a higher proliferation. Blocking experiments with anti-DP, -DQ and -DR antibodies showed that lidocaine is presented in a HLA-DR-restricted way both with autologous or allogeneic APC. Mouse fibroblasts transfected with an allogeneic HLA-DRB1*01 but not HLA-DR-negative mouse fibroblasts could serve as presenting cells. Fixation of APC did not hamper drug presentation, but pulsing of APC with the drug was not possible, indicating that processing is not required and that lidocaine binds in an unstable way to the MHC–peptide complex. This degenerate drug recognition has certain features of superantigen recognition, such as the ability of drugs to bind from the outside to multiple HLA-DR alleles. Such features of drug recognition may open new therapeutic possibilities to intervene with TCR–MHC interactions in a selective way.

Introduction

T cells predominantly recognize foreign and self-peptides presented by MHC class I or class II molecules (1). In addition, a T cell-mediated immune response to non-peptide antigens has been shown for $\gamma\delta^+$ and $\alpha\beta^+$ T cells. Recent studies of the nature of γδ antigens have identified a number of non-peptide compounds which are in general presented without MHC molecules to $\gamma\delta^+$ T cells. Lipid antigens from mycobacteria, such as mycolic acid, are presented via non-polymorphic CD1 molecules to $CD4^-CD8^-$ $\gamma\delta^+$ (or $\alpha\beta^+$) T cells (2,3). Phosphorylated sugars, i.e. xylose-1-phosphate, or prenyl pyrophosphate antigens, such as isopentenyl pyrophosphate, are recognized HLA independently by $\gamma\delta^+$ T cells via an unknown extracellular pathway (4–6).

In contrast to these natural, non-peptide antigens which are mostly derived from mycobacteria, environmental or synthetic non-peptide antigens, so-called xenobiotica like drugs, are preferentially recognized by $\alpha\beta^+$ T cells. Specific T cell clones (TCC) for drugs (i.e. penicillins, sulfonamides, lidocaine, etc.) or trinitrophenol (TNP), a contact sensitizer as well as for metals (i.e. nickel, gold, etc.) were mainly of the CD4 and partly of the CD8 phenotype (7–11). These xenobiotic-specific $CD4^+$ or $CD8^+$ $\alpha\beta^+$ T cells respond to their antigens in a HLA allele-restricted way (12). The only example of MHC-independent recognition by $\alpha\beta^+$ T cells was found with lipids, which are recognized as CD1 restricted (2).

Recognition of antigens in association with multiple HLA alleles by one TCC, termed as *promiscuous*, was reported for peptide-specific TCC. This phenomenon was proposed for MHC class I- and class II-restricted clones in humans and in mice (13–21). In this study, we report that selected HLA-

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DR-restricted clones specific for lidocaine proliferate with mismatched allogeneic antigen-presenting cell (APC). This also indicates that drugs can be recognized in association with multiple HLA-DR alleles by HLA-DR-restricted human TCC.

Methods

Patients

Patient OF, a 39-year-old male, developed pruritus and erythema after topical, perianal application and a pustulous reaction after intracutaneous application of lidocaine. Patient SF, a 36-year-old female, developed angioedema after dental surgery and eczematous eruptions after excision of nevi under lidocaine.

Culture medium (CM) and cell lines

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 μg/ml streptomycin and 100 U/ml penicillin. The 'CM^+' used to culture T cell lines and TCC was enriched with 20 U/ml natural IL-2 (a kind gift from Dr U. Schwulèra, Biotest, Frankfurt/Main, Germany) and 20 U/ml recombinant IL-2 (Dr A. Cerny, Inselspital, Switzerland). The medium for culture of Epstein–Barr virus (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 by transformation of freshly isolated peripheral blood mononuclear cells (PBMC) with supernatant of the EBV-producing cell line B95-8. Cyclosporin A (1 μg/ml; Sandoz, Basel, Switzerland) was added to prevent EBV-induced T cell growth. The HLA-DRB1*0101-expressing fibroblast cell line LC01 (derived from the HLA workshop) and the HLA-DR-negative fibroblast cell line DAP.3 (kindly provided by Dr R. Lechler, London, UK) were used as control APC (22). Preparation of drug solutions in CM was always done freshly just before use. The local anesthetics (LA) lidocaine (Grogg Chemie, Stettlen, Switzerland) and procaine (Siegfried, Zofingen, Switzerland) were used (see Fig. 1A). Tetanus toxoid (TT) was kindly provided by Dr J. Cryz (Serum und Impfinstitut, Bern, Switzerland).

Generation of drug-specific TCC

Freshly isolated PBMC of drug-allergic patients were stimulated with lidocaine (100 μg/ml) in CM at a cell density of 2×10^6/well in a 24-well plate (Falcon 3047). To generate TCC, CM^+ was added after 7 days of culture. After 11 days, 8×10^5 bulk cells were re-stimulated with 5×10^5 autologous irradiated (3000 rad) PBMC, 3×10^5 autologous irradiated (6000 rad) B-LCL and the same concentration of antigen used in primary stimulations. Specific T cell lines were cloned by limiting dilution as described earlier (23). Briefly, blast cells from bulk cultures were seeded at 0.3, 1 or 3 cells/well with 2.5×10^4 allogeneic irradiated PBMC into a 96-well round-bottom plate (Falcon 3077) plus phytohemagglutinin (PHA; 0.5 μg/ml) (Bacto; Difco, Detroit, MI) in 150 μl/well of CM. Growing TCC were expanded in CM^+ and re-stimulated every 14 days with allogeneic irradiated PBMC plus PHA (0.5 μg/ml). The phenotypes of the TCC were identified by immunofluorescence analysis with anti-CD4, anti-CD8, anti-CD3 and anti-TCR αβ or γδ mAb (Becton Dickinson, Rutherford, NJ).

Proliferation assay

To measure the antigen-specific proliferation of TCC, 3×10^4 cells were incubated with the same number of irradiated (3000 rad) or fixed PBMC, 5×10^3 irradiated (6000 rad) or fixed B-LCL or 3×10^4 irradiated (6000 rad) or fixed transfectants in the presence or absence of lidocaine at indicated concentrations in 200 μl CM in a 96-well round-bottom plate (Falcon 3077). In pulsing experiments, autologous APC were incubated with and without lidocaine for 4 or 16 h in culture medium. The cells were washed twice with HBSS, resuspended in culture medium and irradiated as described above. To determine processing requirements, APC were fixed as described by Shimonkevitz *et al.* (24). Briefly, APC were resuspended in serum-free medium and fixed with 0.05% glutaraldehyde for 30 s at room temperature. The reaction was stopped by adding 0.2 M L-glycine for an additional 45 s. After 48 h, [^{3}H]thymidine (0.5 μCi) was added for 14 h, cells were harvested on glass fiber disks and counted in a microplate β-counter (Inotech Filter Counting System INB 384; Inotech, Dottikon, Switzerland). Stimulation indices (SI) were calculated as 'c.p.m. in culture with antigen/c.p.m. in culture without antigen'. Fixation and pulsing experiments were performed simultaneously with a TT-specific T cell line.

Cytotoxicity assay

To assess cytotoxicity, 1×10^6 autologous B-LCL were labeled at with 50 μCi ^{51}Cr for 90 min. These cells were then extensively washed to remove free ^{51}Cr. Cells (5×10^4) were used as target cells in a standard 4 h cytotoxicity assay in the presence or absence of lidocaine (100 μg/ml). E:T ratio were 30:1, 10:1, 3:1 and 1:1. Specific lysis was calculated as 100×(experimental release – spontaneous release)/(maximal release – spontaneous release).

Determination of T cell restriction pattern

To identify elements for presentation we used a panel of allogeneic HLA-typed PBMC or B-LCL of healthy donors (Table 1). TCC were cultured with autologous or allogeneic feeder cells and antigen or medium alone for 48 h and proliferation assayed as described above. HLA class II typing was done by PCR amplification using sequence-specific primers (25). The HLA-DR of patient SF was: DRB1*1501 or 03/-, DRB5*0101.

The restricting class II isotype was determined by blocking with monomorphic anti-class II mAb. T cells were cultured with autologous or allogeneic PBMC, antigen and either anti-DR (L234), anti-DP (B7.21) or anti-DQ (SVPL3) (a kind gift from Dr E. Padovan, Freiburg, Germany) at the indicated dilutions. Proliferation was assayed as described above. Anti-class-II antibodies added to $CD8^+$ TCC did not show an inhibitory effect on the T cell proliferation (data not shown).

Immunofluorescence (TCR V_β distribution)

For direct immunofluorescence, 1×10^6 cells were washed once with PBS, 1% FCS and 0.02% NaN_3 at 4°C and resuspended in 1 ml of the same buffer. A 50 μl aliquot of cell suspension was then incubated with an anti-CD3 antibody

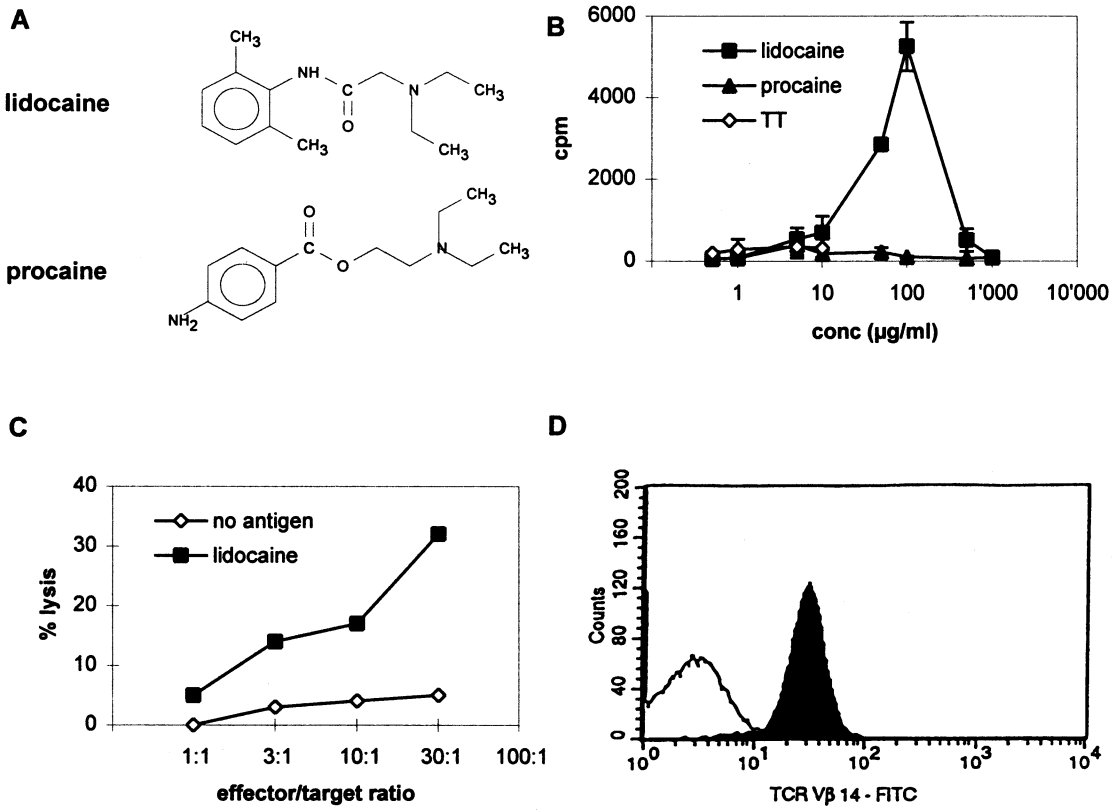

Fig. 1. Dose–response curve and monoclonality. (A) Chemical structure of LA. Lidocaine belongs to the amide family and procaine is an ester-type LA. (B) Dose-dependent proliferation of clone SFT24 to lidocaine. No proliferative response occurs with procaine, another structurally related LA and with TT. Clone cells (3×10^4) were incubated with the same number of autologous PBMC with or without drugs or TT. [^{3}H]Thymidine incorporation was measured after 48 h. One of three representative experiments is shown. (C) Clone SFT24 shows a specific lysis to lidocaine. Specific cytotoxicity was evaluated in a 4 h ^{51}Cr-release assay in the presence or absence of lidocaine (100 µg/ml). Target cells (5×10^4 autologous B-LCL) were incubated with the indicated amounts (1- to 30-fold) of TCC. (D) Monoclonality of clone SFT24. The expression of TCR $V_\beta14$ confirms the monoclonality of the T cell population analyzed in this study. The clone was stained with a panel of 17 FITC-labeled mouse mAb specific for defined human V_β. Fluorescence was analyzed on a FACScan instrument. The result from the staining with an isotype control mAb and the mAb to TCR $V_\beta14$ is shown. No other mAb gave a positive staining with the clone.

(Becton Dickinson) and 17 different anti-TCR V_β antibodies obtained from Immunotech (Marseille, France). Cells were incubated for 30 min at 4°C. After two washings, cells were fixed with the above mentioned buffer supplemented with 1% paraformaldehyde. Analysis was done on an FACScan (Becton Dickinson, Mountain View, CA).

Results

Restriction motifs for drug-specific T cells

We investigated the MHC restriction elements of a panel of drug-specific TCC to various drugs [lidocaine, sulfamethoxazole (SMX) and penicillin G]. Proliferation experiments were performed with a panel of HLA-typed allogeneic APC (n = 11). In general, CD4$^+$ and CD8$^+$ TCC recognize their antigen only in context with HLA-matched allogeneic feeder cells, as shown for the lidocaine-specific CD4$^+$ clone OFB12 in Table 1. Note that proliferation of the clone with feeder cells 'NKa' is not lidocaine specific, as a proliferation is achieved also in the absence of the drug, thus reflecting an alloreactivity of this clone. In striking contrast, a drug-specific proliferation with multiple HLA alleles was observed with one of 13 penicillin G-, one of seven SMX- and two of 17 lidocaine-specific TCC tested. As shown in Table 1, the lidocaine-specific TCC SFT24 and E20 proliferated to lidocaine presented by various allogenic APC.

Specificity and monoclonality

From a limiting dilution of a lidocaine-specific T cell line of donor SE, 80 clones were expanded and tested for specificity. Only one CD4$^+$ clone (SFT24) was indeed drug-specific. Figure 1(B) shows a dose–response curve to the parent drug and the non-responsiveness to procaine, another LA and TT. Note that only a narrow range of concentration (10–100 µg/ml) results in a significant response. A drug-specific killing by this CD4$^+$ TCC could be observed in the presence of autologous target cells (B-LCL) (Fig. 1C).

To evaluate critically the degenerate antigen recognition, proof of clonality is essential. Our evidence for clonality is 2-fold. First, cells were cloned at a statistical concentration of 1.0 cells per culture well and from the plate of which clone SFT24 was harvested, only 12 of 96 wells were growing.

Table 1. HLA-DR unrestricted recognition of lidocaine

APC[a]	HLA[b]						Clone OFB12[c]		Clone SFT24		Clone SFE20	
		DRB1*		DRB3*	DRB4*	DRB5*	c.p.m. lidocaine (no antigen)	SI	c.p.m. lidocaine (no antigen)	SI	c.p.m. lidocaine (no antigen)	SI
Auto	**1501 or 03**	/	X			0101	8316 (576)	**14.4**	11675 (36)	**324.3**	10621 (61)	**174.1**
BC	0401	/	1104	0202	0101 or 0103		377 (400)	0.9	3622 (66)	**54.9**	2944 (55)	**53.5**
WT	1302	/	X	0101–0301			42 (53)	0.8	682 (60)	11.3	66 (61)	1.1
BCo	1101	/	**1502**	0202		0101	6836 (366)	**18.8**	7422 (41)	**181**	6131 (156)	**39.3**
GT	0101 or 02	/	1101–04	0101–0301			71 (149)	0.5	8108 (55)	**147.4**	7525 (35)	**215**
FK	0701	/	1103	0101–0301	0101		24 (40)	0.6	1878 (31)	**60.5**	3261 (35)	**93.2**
VK	0301	/	1001	0101			24 (31)	0.8	856 (54)	15.8	38 (41)	0.9
PW	0101	/	**1502**			0102	1357 (29)	**46.8**	4411 (41)	**107.5**	2987 (73)	**40.9**
ZM	0101 or 02	/	0701		0101		77 (64)	1.2	8579 (58)	**147.9**	4776 (96)	**49.8**
NKa[d]	0701	/	0801		0101		12128 (5669)	2.1	6319 (94)	**67.2**	5402 (193)	**28**
SM	1303 or 04	/	**1501–04**	0101–0301		0101–0202	489 (32)	**15.3**	3696 (85)	**43.5**	8194 (91)	**90**
ZMa	04	/	0701		0101	0102	251 (98)	2.3	595 (48)	12.4	1532 (40)	**38.3**

Clones SFT24 and E20 proliferate to a variety of mismatched APC. Conventional drug-specific clones, i.e. clone OFB12, are HLA-DRB1-restricted and proliferate only in the presence of matched APC (here HLA-DRB1*15). In contrast clones SFT24 and E20 proliferate to lidocaine presented by different HLA-DRB1*15 mismatched alleles. One of three representative experiments is shown.

[a]3×10^4 irradiated PBMC or 5×10^3 irradiated B-LCL were used as APC.

[b]HLA-typing was performed by PCR amplification using sequence-specific primers.

[c]3×10^4 clone cells were cultured in the presence of APC with or without lidocaine (100 μg/ml) in a 48 h proliferation assay.

[d]The high proliferation to feeder cells NKa stands for a HLA-DRB1*08-restricted alloreactivity.

Second, when stained with a panel of 17 defined V_β elements, the clone gave positive staining only with $V_\beta14$ (see Fig. 1D), but negative staining with all other V_β-specific mAb (data not shown). The CD4 clone E20 has been described previously ($V_\beta8^+$) (23).

Lidocaine-specific proliferation is APC dependent

A non-MHC-restricted proliferation is known for $\gamma\delta^+$ T cells and these cells show an almost equal proliferation without addition of APC (5). In contrast, both clones SFT24 and E20 required APC for an optimal T cell proliferation. As shown for clone SFT24 in Fig. 2, increasing the number of APC is accompanied by a higher proliferation. A marginal proliferation in the absence of APC can be observed with most of our drug-specific TCC (23).

Autologous and allogeneic recognition is HLA-DR-restricted

To determine the restricting class II isotype on the APC, we performed blocking assays with anti-DP, -DQ and -DR mAb. As shown in Fig. 3(A) for clone SFT24, addition of anti-DR antibodies inhibited the drug-specific proliferation in the presence of autologous APC in a dose-dependent manner. The anti-DP and anti-DQ mAb had no effect. Presentation by allogeneic APC was blocked similarly (Fig. 3B).

Full, partial or no recognition in the context of different HLA alleles

Figure 4 shows the dose-dependent recognition of lidocaine by clones SFT24 and E20 in the context of a panel of 12 HLA-phenotyped APC in a proliferation assay. Based on the intensity of stimulation, three types of responses can be detected. A proliferative response similar to that with autologous APC was detected with four different allogeneic APC. A partial response was seen with four others. No proliferation above background was seen with the remaining three APC. Positive responses did not correlate with the HLA-DR alleles of the APC. Proliferation in the absence of lidocaine was <200 c.p.m. for all APC used. HLA-DR, CD80 and CD86 expression of APC determined by immunofluorescence was similar for all used APC, demonstrating functionality of APC (data not shown). Since some HLA-DR molecules were able to present while others were not, we attempted to correlate the presenting capacity to DRB1 sequences (Table 2). The

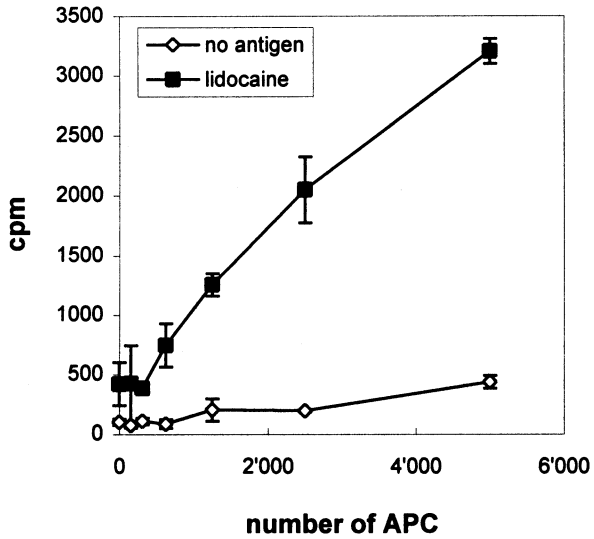

Fig. 2. Proliferation of clone SFT24 is APC-dependent. Clone SFT24 shows only a marginal proliferation to lidocaine in the absence of APC. Clone cells (3×10^4) were incubated with the indicated number of irradiated B-LCL in the presence or absence of lidocaine (100 μg/ml).

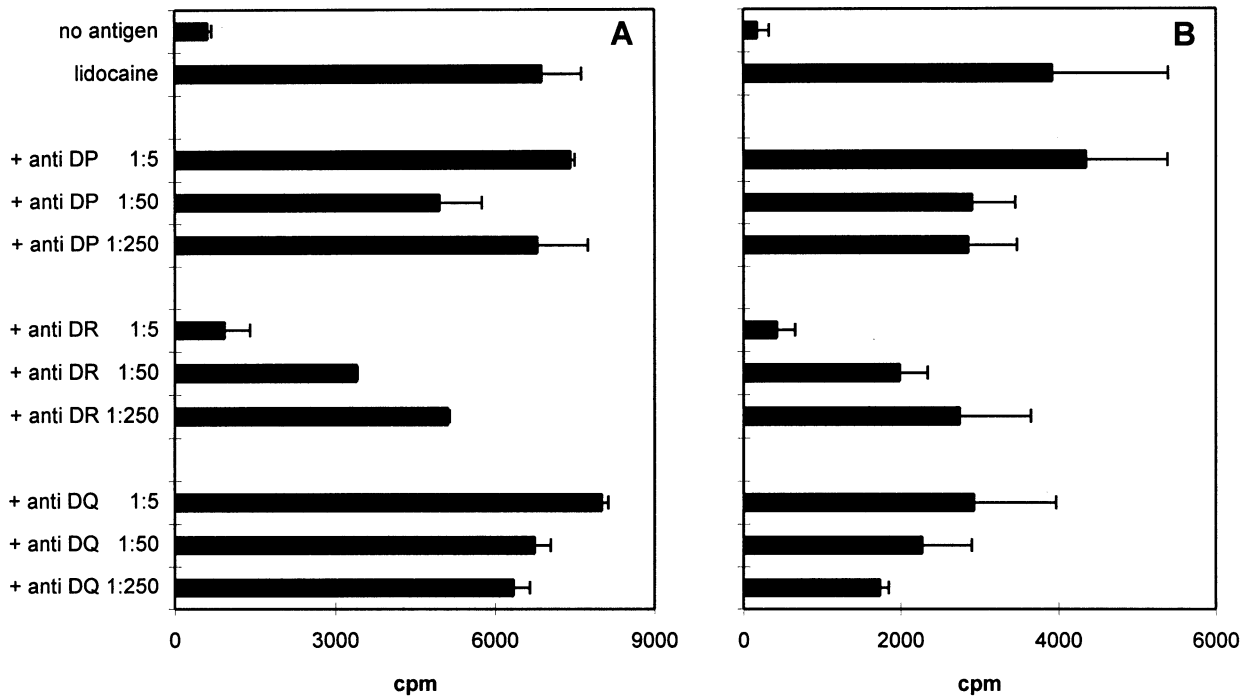

Fig. 3. Promiscuous recognition is HLA-DR-restricted. The proliferative response of TCC SFT24 was analyzed in the presence of different anti-MHC class II antibodies: L234 (anti-DR), B7.21 (anti-DP) and SVPL3 (anti-DQ). Clone cells (3×10^4) were incubated with 5×10^3 B-LCL and the indicated dilutions of mAb in the presence or absence of lidocaine (100 μg/ml). One of three representative experiments is shown. (A) Autologous APC, HLA-DRB1*1501 or 03. (B) Allogeneic APC, HLA-DRB1*0701/1103.

amino acid at position 32 differed between presenting and non-presenting alleles as a tyrosine is found in presenting alleles (DRB1*0101, 0801, 1103, 1104 and 1501), whereas four of five non presenting alleles (DRB1*1302, B1*301, B1*1001 and B1*04) have a histidine in position 32 respectively 33. The allele DRB1*0701, which did not have a histidine in this position, had actually some minor stimulatory capacity (see also Table 1, donor ZMa). Other positions of the DRB1 sequence did not show major differences in presenting and non-presenting alleles.

Presentation of drugs does not need processing, but is HLA-DR dependent

To further confirm the drug-specific proliferation in association with mismatched APC, we used fibroblast cell lines which were untransfected (DAP.3) or transfected with the human HLA-DRB1* 01 allele (LC01). Figure 5(A) shows that a lidocaine-specific proliferation can be observed with HLA-DRB1*01-transfected cells. Thus even mouse cell transfectants with a mismatched HLA-DR allele can present lidocaine to the drug-specific TCC. As the LC01 cell line is only weakly positive for CD80 and negative for CD86, one can rule out a promiscuous recognition of HLA-DR allele restricted clones because of an overexpression of adhesion molecules on APC (26). The HLA-DR negative cell line DAP.3 did not induce a T cell proliferation, confirming the need of MHC molecules for drug presentation.

For peptide presentation by professional APC, antigen uptake, processing and assembly with the MHC in the cell is required (1). Thus, APC can be pulsed with the antigen but fixed APC are unable to present TT to a TT-specific T cell line (TCL) (Fig. 5B and D). However, we showed recently in a study with SMX-specific TCC that drug uptake and processing is not required for class I and class II presentation (27). In agreement with this, Fig. 5(A) demonstrates that APC pulsed with the drug for 4 or 16 h and washed elicited no detectable T cell proliferation. Moreover, as shown in Fig. 5(C), the presentation of lidocaine is processing-independent as fixed autologous APC or fixed allogeneic fibroblasts transfected with HLA-DRB1*01 could still present it. This demonstrates that processing of lidocaine is not required for an immunogenic presentation of this drug to clone SFT24.

Discussion

Our data indicate that some lidocaine-specific TCC recognize the drug in the context of HLA-DR, but in an allele-unrestricted way. Permissive recognition in the context of several class II alleles was first described by Panina-Bordignon *et al.* for peptide-specific TCC. They proposed for p2, a TT epitope, that HLA-DR allele-restricted clones may contact p2 and polymorphic MHC residues, while the TCR of promiscuous TCC may recognize p2 and public or monomorphic MHC residues, i.e. the monomorphic α chain (14). This model served also as explanation for promiscuous recognition of peptides by H-2 I-E-restricted murine clones (15). Recent publications, however, describe recognition of one peptide presented by H-2 I-A molecules which display polymorphism in *both* the α and β chain (16–19).

Here we describe that synthetic drugs can be recognized

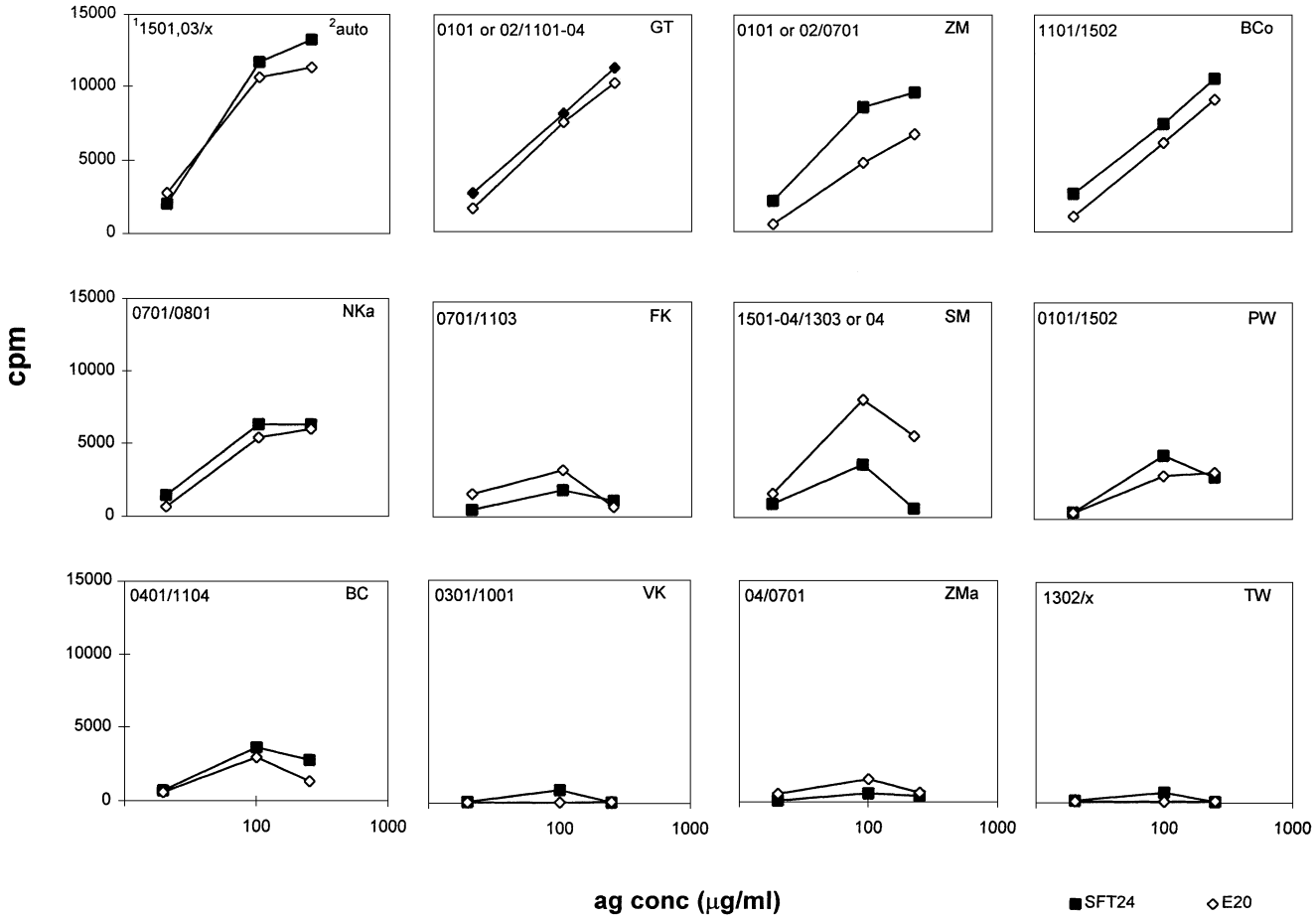

Fig. 4. Clone SFT24 and E20 proliferate in the context of multiple APC. The use of either matched and mismatched APC results in an equal (donor BCo, GT, ZM and NKa), partial (BC, FK, WP and SM) or no T cell response (donor TW, VK and ZMa) to lidocaine. In the absence of APC, c.p.m. <200 was observed for all tested APC. Clone cells (3×10^4) were incubated with the same number of irradiated PBMC in the presence or absence of lidocaine (100 μg/ml). One of three representative experiments is shown. [1]HLA-DRB1 alleles. [2]Type of donor cells used.

by one TCR in the context of multiple HLA alleles. A direct stimulation of the TCC (i.e. by T cell presentation itself in the absence of APC) could be ruled out, since the clones were clearly dependent on the presence of APC, did not proliferate to all tested APC and clone SFT24 was cytotoxic for target cells (B-LCL), indicating the recognition of the drug on the target cell. This recognition is highly specific, as ester LA like procaine and even small alterations in the lidocaine structure, i.e. addition of a hydroxyl group on the benzoic ring of the parent drug (metabolite), abrogated a proliferative response (M. Zanni *et al.*, submitted). A presentation via CD1 or another surface molecule could be excluded, since both autologous and allogeneic presentations are inhibited by addition of anti-DR mAb. The TCR and not other surface molecules such as CD2 or CD28 are involved in the recognition of drugs, as a TCR down-regulation occurs within 20 min after contact with the specific drug presented by APC (M. P. Zanni *et al.*, submitted).

How low mol. wt compounds like drugs are presented by APC and recognized by T cells is still controversial. In a recent study we proposed a direct, drug metabolism and processing-independent recognition of SMX by T cells (27). Addition of SMX to glutaraldehyde-fixed APC results in a proliferation of SMX specific TCC. Pulsed APC (incubation of APC with the drug followed by a washing step) are not recognized (27). As shown in Fig. 5, these results could also be seen with lidocaine, a classical contact-sensitizing agent in humans. Fixed allogeneic transfectants could serve as APC for clone SFT24 and pulsing was not possible, indicating an unstable binding of lidocaine to the MHC–peptide complex. The antigenic determinant recognized by T cells, at least if they are in an activated stage, does not require internalization or processing. This suggests that drugs like lidocaine and SMX are not necessarily real haptens, which are solely immunogenic by *covalent* binding to proteins or peptides, but may become immunogenic by simply interacting with the MHC–peptide complex. Such a presentation is similar to the way nickel salts are supposed to be presented (28).

This new concept of drug presentation has implications for both, the interaction of the drug with the peptide–MHC complex, as well as the drug–peptide–MHC interaction with the TCR. On the APC level, the drug interacts in an unstable way with the MHC–peptide complex. Since different alleles with different peptides could present lidocaine, the presenta-

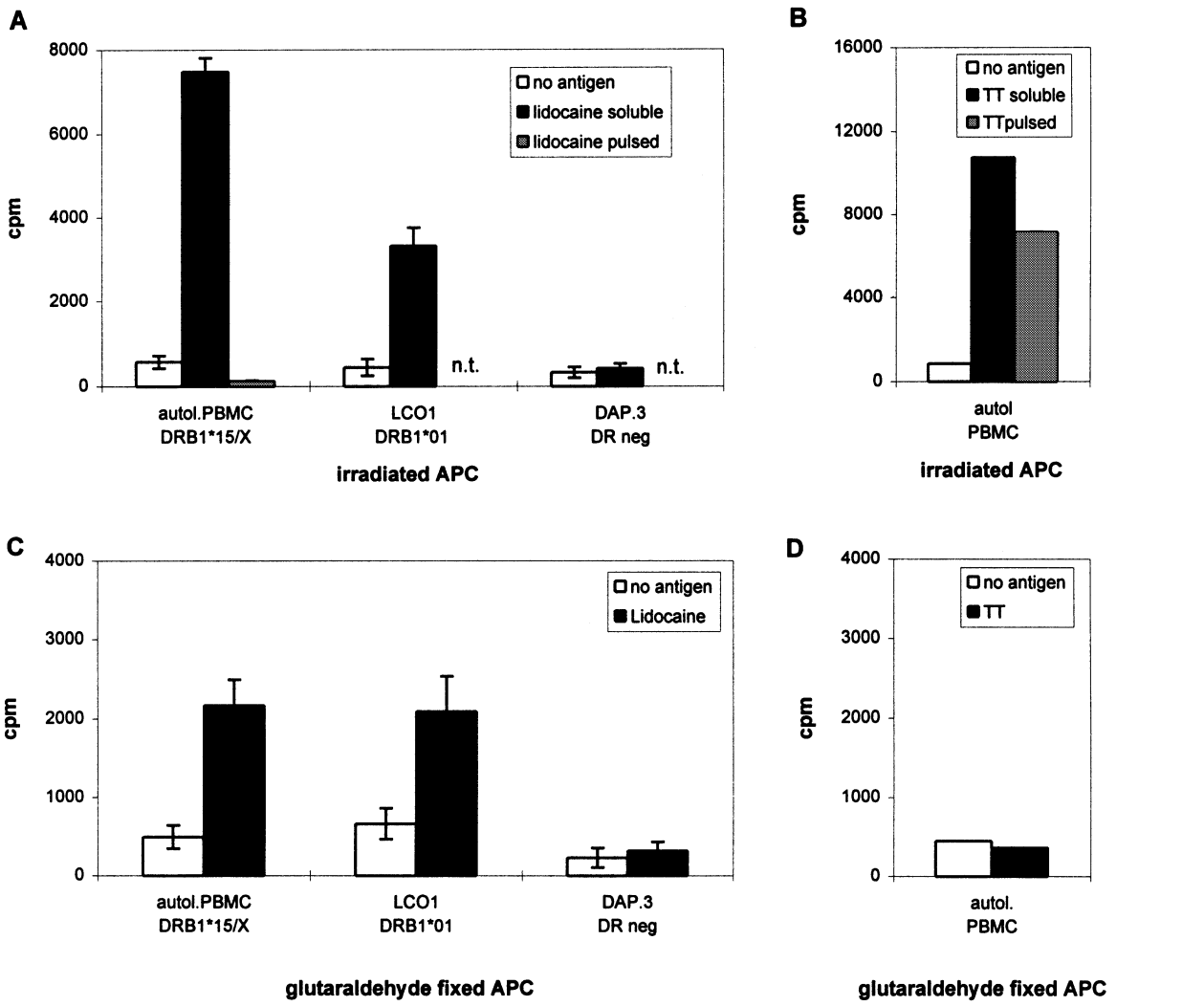

Fig. 5. No requirement of processing for lidocaine recognition. (A) Clone SFT24 proliferates in the presence of mouse fibroblasts transfected with allogeneic HLA-DRB1*01 (LC01), but not with HLA-DR untransfected fibroblasts (DAP.3). Pulsing of APC does not induce a T cell proliferation. (B) As a control, a TT-specific TCL proliferates after pulsing of APC. (C) Proliferation of clone SFT24 was not inhibited by fixing the autologous APC or the allogeneic transfectants with glutaraldehyde. (D) T cell proliferation of the TT-specific TCL is abrogated when APC were fixed. Then, 3×10^4 autologous PBMC or fibroblast cells (LC01 or DAP.3) were irradiated (PBMC 3000 rad, transfectants 6000 rad), pulsed for 4 h or fixed with 0.5% glutaraldehyde and the reaction stopped with 0.2 M L-glycine. Proliferation of clone cells was assayed in the presence or absence of lidocaine (100 μg/ml). As a control, the same procedure was performed with TT as antigen and a TT-specific TCL.

tion appears to be independent of the amino acid sequences presented in the groove of the MHC molecule. Interestingly both clones analyzed in detail showed the same pattern of promiscuity as HLA-DRB1*1302, B1*301, B1*1001, B1*04 and B1*0701 alleles from donors VK, TW and ZMa did not induce a proliferative response. The sequence analysis of the alleles suggest that a histidine at position 32 respectively 33 of the DRB1 sequence may abrogate the presentation of lidocaine in an immunogeneic form to the two specific TCC. A tyrosine at position 32 or 33 in the DRB1 sequence may represent a binding site for the drug itself or favor binding of peptides able to present the drug.

Earlier studies with TNP-modified peptides postulated a binding of the hapten to the immunogenic peptide itself. For T cell recognition the position where TNP is bound to the peptide, was clearly more important than the presented peptide itself (29). Binding of TNP in a central peptide position resulted in a carrier-independent T cell response. TNP modification at the border of the peptide resulted in two contact points for the TCR, i.e. TNP and parts of the peptide. In a recent study, Kohler *et al.* described a $CD4^+$ mouse hybridoma specific for TNP which exhibited a striking promiscuity of MHC restriction, as it proliferated to TNP in association of H-2 haplotypes d, k, or s and moreover even with human HLA-DR4 as APC (20). This model is, however, different from our finding, as TNP is covalently coupled to designer peptides. In our system, we most likely do not have covalent binding of the drug to the MHC–peptide complex. In analogy to the TNP model, one might postulate a rather dominant, central positioning of lidocaine in the MHC–peptide complex which

Table 2. Correlation between the known amino acid sequences of DRB1-encoded chains and promiscuous recognition

HLA alleles	Position	32
Presenting	DRB1*15011	R F L D R Y F Y N
	DRB1*0101	- L - E - C I - -
	DRB1*11041	- - - - - - - - -
	DRB1*1103	- - - - - - - - -
	DRB1*0801	- - - - - - - - -
Non-presenting	DRB1*1302	- - - - - - - **H** -
	DRB1*03011	- - - - - - - **H** -
	DRB1*1001	- L - E - R V **H** -
	DRB1*04	- - - - - - - - **H**
	DRB1*0701	Q - - E - L - - -

A dominant difference between presenting and non-presenting DRB1 alleles was only found in position 32. While all presenting alleles have a tyrosine in this position, four of five non-presenting alleles show a histidine at position 32 or 33. Note that HLA-DRB1*15011 is identical in this part of the DRB1 sequence to DRB1*15012 and DRB1*1503; DRB1*11041 is identical to HLA-DRB1*11042; DRB1*03011 is identical to DRB1*03012; and all DRB1*04 are identical in this positions. DRB1*0701 takes an intermediate position: it has only a limited capacity to present the drug to one clone (see Table 1).

then allows a partly HLA-DR allele-independent recognition by the specific TCR. To what extent a dominant peptide itself (i.e. derived from CLIP) is an important determinant for T cell recognition is unclear (30). Since the anchor motifs of the presenting alleles are quite different, it is likely that the allele independent drug recognition occurs independently of the peptide sequence (31).

In this context, it is of interest that lidocaine and other drugs can lead to an oligoclonal outgrowth of T cells bearing a certain TCR V_β chain (10,23,32). Such an oligoclonal T cell recognition is also observed with bacterial superantigens (33). The crystal structure of superantigen interaction with the TCR–MHC–peptide complex revealed that the superantigen has extensive contact sites to the MHC and the TCR, but that the MHC–peptide complex is only partially engaged by the TCR (34). This leads to the recognition of the superantigen in an HLA-DR-restricted, but allele-independent way (35). Recognition of some drugs such as lidocaine or SMX by certain TCC may be similar, as the drug itself interacts with the TCR and the MHC–peptide complex, but, due to its size and/or its orientation in between the two binding components, simultaneously hampers TCR binding to the MHC molecule.

Our observations that TCR can recognize non-peptide antigens in an HLA allele-independent manner may also be relevant for drug-induced diseases. The ability of the drug to bind to different HLA alleles and to be recognized increases the density of antigenic determinants for such a clone. This may facilitate T cell stimulation, which is clearly dependent on the density of antigenic epitopes (36).

In addition the allele-independent recognition may open new therapeutic possibilities for the treatment of T cell-induced diseases. In a sense, drugs, which cause drug allergies, can be seen as receptor-binding compounds, which are able to interact with various MHC–peptide complexes *and* certain TCR. Based on this finding, drugs may be designed which interact with TCR on T cells with an allospecific, autoimmunogenic or allergic potential. If such drugs could, by analogy to the altered peptide model (37), alter the reactivity of the T cell, they might be valuable and highly specific tools in autoimmunity or transplantation.

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Abbreviations

APC	antigen-presenting cell
CM	culture medium
B-LCL	B lymphoblastoid cell line
EBV	Epstein–Barr virus
LA	local anesthetics
PBMC	peripheral blood mononuclear cells
PHA	phytohemagglutinin
SI	stimulation index
SMX	sulfamethoxazole
TCL	T cell line
TCC	T cell clone
TNP	trinitrophenol
TT	tetanus toxoid

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