Direct, MHC-dependent Presentation of the Drug Sulfamethoxazole to Human αβ T Cell Clones

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Abstract

T cells can recognize small molecular compounds like drugs. It is thought that covalent binding to MHC bound peptides is required for such a hapten stimulation. Sulfamethoxazole, like most drugs, is not chemically reactive per se, but is thought to gain the ability to covalently bind to proteins after intracellular drug metabolism. The purpose of this study was to investigate how sulfamethoxazole is presented in an immunogenic form to sulfamethoxazole-specific T cell clones. The stimulation of four $CD4^+$ and two $CD8^+$ sulfamethoxazole-specific T cell clones by different antigen-presenting cells (APC) was measured both by proliferation and cytolytic assays. The MHC restriction was evaluated, first, by inhibition using anti–class I and anti–class II mAb, and second, by the degree of sulfamethoxazole-induced stimulation by partially matched APC. Fixation of APC was performed with glutaraldehyde 0.05%. The clones were specific for sulfamethoxazole without cross-reaction to other sulfonamides. The continuous presence of sulfamethoxazole was required during the assay period since pulsing of the APC was not sufficient to induce proliferation or cytotoxicity. Stimulation of clones required the addition of MHC compatible APC. The APC could be fixed without impairing their ability to present sulfamethoxazole. Sulfamethoxazole can be presented in an unstable, but MHC-restricted fashion, which is independent of processing. These features are best explained by a direct, noncovalent binding of sulfamethoxazole to the MHC–peptide complex. (*J. Clin. Invest.* 1997. 100:136–141.) Key words: nonpeptide T cell antigens • drug allergy • drug presentation • cytotoxicity • sulfamethoxazole

Introduction

Drug allergy requires recognition of the drug by T and possibly also B cells, followed by an effector phase, which results in the heterogeneous manifestations of drug allergy. Little is known about the pathomechanism of these immunological reactions. In particular, the recognition of haptens (protein-reactive chemicals such as metal, salts, and drugs) by T cells is not yet well-understood.

Various pathways of T cell stimulation by a hapten have been demonstrated: some haptens bind covalently to serum or cellular proteins, which are subsequently processed. As shown in trinitrophenyl sensitized mice, the predominant T cell response is directed to hapten-modified peptides presented in MHC molecules (1, 2). Another consequence of hapten interaction has been described for the reactive gold metabolite Au (III). It can alter antigen processing, which may lead to the presentation of cryptic peptides on the MHC, to which the individual is not tolerant (3).

In contrast to these pathways, which involve stable binding of the hapten to a carrier protein, followed by the uptake and intracellular processing of the hapten–carrier complex, there may be additional ways to stimulate T cells. Isopentenyl-pyrophosphate, a nonpeptidic antigen can be presented to human $\gamma\delta^+$ T cells in a direct, MHC-independent and extracellular way (4). It has been observed also in gold [Au (I)] specific MHC-restricted T cell clones (TCC)[1] that glutaraldehyde fixation had no effect on the ability of antigen-presenting cells (APC) to induce proliferation, indicating that direct extracellular modification of the MHC–peptide complex by gold has taken place (5). Similarly, $NiSO_4$-specific T cell clones can be restimulated by presumably unstable binding of $NiSO_4$ to glutaraldehyde-fixed APC (6). Even a direct interaction of the hapten with the T cell receptor (TCR) has been proposed. Using fluorescein specific T cells, an MHC-independent interaction with fluorescein could be shown, whereby cells transfected with the fluorescein specific αβ TCR specifically interacted with fluorescein coupled to polymers by their specific αβ TCR (7).

The topic of this study is the recognition of sulfamethoxazole (SMX) by T cells. SMX, like most drugs, is chemically stable. To become reactive, it has to be metabolized. In vivo it is metabolized predominantly in the liver by *N*-acetylation, which leads to the formation of nontoxic metabolites. To a limited extent it is also metabolized in a cytochrome P-450 catalyzed reaction to hydroxylamines which are highly reactive intermediates (8, 9). It has been shown that SMX-hydroxylamines in vivo bind covalently to human serum proteins (10). Thus it is usually thought that SMX gains its immunogenicity by metabolism in the liver and consecutive covalent binding to a carrier protein.

Our previous studies, performed with PBMC of drug-allergic human individuals, confirmed the existence of SMX-reactive T cells in the circulating blood of SMX-sensitized individuals (11). Here, we study a panel of SMX-specific T cell clones from a patient who has developed a hypersensitivity reaction to SMX and focus on the mechanism of SMX recognition. Our investigations of restimulations of activated T cells indicate the existence of a processing-independent, but nevertheless MHC-restricted mode of SMX presentation, which is in contrast to processing-dependent concepts. This pathway may allow the

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1. *Abbreviations used in this paper:* APC, antigen-presenting cells; B-LCL, EBV-transformed B-lymphoblastoid B cell line; SMX, sulfamethoxazole; TCC, T cell clones; TCR, T cell receptor; TT, tetanus toxoid.

drug to be recognized even if it is bound unstably to the MHC–peptide complex. Such a recognition is reminiscent of the way metal salts, e.g., $NiSO_4$ and gold, are recognized by TCR-$\alpha\beta^+$ TCC, or of isopentenyl-pyrophosphate recognition by $\gamma\delta^+$ T cells (4–6).

Methods

Culture media

The culture medium used in this study was RPMI 1640 supplemented with 10% pooled heat-inactivated human AB serum (Swiss Red Cross, Bern, Switzerland), 25 mM Hepes buffer, 2 mM L-glutamine (NR K0202; Seromed, Fakola, Basel, Switzerland), 25 μg/ml transferrin (NR 663.710; Biotest, Dreieich, Germany) without addition of any drugs like penicillin or other antibiotics in order not to interfere with the specific drug stimulation. The culture medium, used to culture TCC, was enriched additionally with 20 U/ml recombinant IL-2 (obtained from Dr. D. Wrann, Sandoz Research Institute, Vienna, Austria).

EBV-transformed B cells

EBV-transformed B-lymphoblastoid cell lines (B-LCL) from the drug-allergic patient have been generated by transformation of freshly isolated PBMC with supernatant of the EBV producing cell line B95-8 (obtained from Dr. D. Neumann-Haefelin, University of Freiburg, Freiburg, Germany). The medium used for culture of EBV-transformed B cells was RPMI 1640 supplemented with 10% FCS (Gibco, Paisley, Scotland), 25 mM Hepes buffer, but no L-glutamine, transferrin, or antibiotics. Cyclosporin A 1 μg/ml (Sandoz, Basel, Switzerland) was added in the first 3 wk of culturing to prevent EBV-induced T cell growth.

Drugs used for cell stimulation

All drugs used in these experiments have been tested previously for the inhibitory response to mitogens (PHA) in nonallergic individuals and only nontoxic concentrations of the drugs have been used for in vitro stimulations. Stock solutions of each drug were always freshly prepared just before use. SMX was obtained from Hoffmann La Roche (Basel, Switzerland); sulfapyridine, sulfasalazine, and hydrochlorothiazide from Sigma Chemical Co. (St. Louis, MO); furosemide from Hoechst (Frankfurt am Main, Germany). Tetanus toxoid (TT; kindly provided by Dr. J. Cryz, Serum und Impfinstitut, Bern, Switzerland) was used as a control antigen at concentrations of 5 and 10 μg/ml, respectively.

Generation and characterization of drug-specific TCC and TT-specific T cell lines

Our donor had a history of sulfonamide allergy with the clinical manifestations of malaise and generalized exanthema. The epicutaneous tests to sulfonamide were negative, whereas the lymphocyte transformation test demonstrated a proliferation to SMX.

Freshly isolated PBMC were stimulated with SMX (0.2–0.5 mg/ml) or TT (10 μg/ml) in culture medium at a cell density of 2×10^6 per well of a 24-well culture plate (Falcon 3044). Cells were grown in culture medium, and IL-2 (20 U/ml) was added after 6 and 9 d of culture. After 14 d, bulk cultures were restimulated with autologous irradiated (4,000 rad) PBMC plus SMX (0.2 mg/ml) or TT (10 μg/ml), and tested for their specificity (see proliferation assay below). After 14 d of culture, SMX-activated cells were cloned by limiting dilution as described earlier (11).

Flow cytometry

The phenotype of TCC was identified by immunofluorescence analysis with PE-labeled anti-CD4 and FITC-labeled anti-CD8 mAb from Becton Dickinson, Inc. (Rutherford, NJ). Cells were analyzed on an EPICS profile II flow cytometer (Coulter Immunology, Hialeah, FL) as described previously (10).

PCR-based TCR Vα and Vβ analysis

29 primers for the TCR Vα chain (12) and 25 primers for the TCR Vβ chains (13, 14) were tested by amplification and hybridized with an oligoprimer recognizing the overall amplified constant region by a nonradioactive hybridization method as described previously (11).

Proliferation assays

For measurements of specific proliferation of the TCC or T cell lines to various drugs and TT, 5×10^4 cells of the clone were incubated with the 3×10^4 PBMCs or 5×10^3 B-LCL, respectively, in the presence or absence of drugs, or TT, at the indicated concentrations in 150 μl culture medium in a 96-well round-bottom plate. After 48 h, [^{3}H]thymidine (0.5 μCi) was added, and the cultures were harvested 12 h later.

In APC-pulsing experiments, autologous APC were incubated with and without SMX (0.2 mg/ml) for 3 or 18 h in culture medium. The cells were washed twice with HBSS and resuspended in culture medium. They were then either irradiated or fixed (see below).

Glutaraldehyde fixation

APC were fixed as described by Shimonkevitz et al. (15). Briefly, the APC were resuspended in 0.5 ml medium without serum and fixed by addition of 12.5 μl glutaraldehyde 2% (end concentration 0.05%) for 30 s at room temperature. The reaction was stopped by adding 1 ml of 0.2 M L-glycine for an additional 45 s.

HLA restriction

Antibody blocking experiments. We used the anti–HLA class I mAb W6-32 (American Type Culture Collection, Rockville, MD) and the anti–HLA class II mAb L243 (anti-DR), B7.21 (anti-DP), or SVPL3 (anti-DQ; all obtained from Dr. E. Padovan, Max-Planck-Institut, Freiburg, Germany) at the indicated dilutions to inhibit MHC-restricted stimulations of the $CD8^+$ and $CD4^+$ TCC in proliferation assays. The mAbs were tested in the same concentrations on unrelated clones without showing any toxicity on TCC.

Stimulation by allogenic PBMC. To evaluate the MHC restriction, we used PBMC from various donors, which were partly matched in certain HLA-alleles, as APC in a proliferation assay with or without SMX. The HLA type of the donor was HLA class I: A2, A26, B44, B60, Cw3, Cw5; HLA class II: DRB1*0101/*10**, DQA1 *0101/*0104, DQB1*0501/−, DPB1*0201/*0401. For matching HLA-alleles, see Fig. 4. The HLA typing was performed in our tissue typing laboratory by the Terasaki method using anti–HLA class I antibodies from Biotest, and PCR-based oligotyping for the HLA class II (DR, DP, and DQ).

Assessment of cytotoxic activity

Cytotoxicity of the TCC was tested at days 10–14 after restimulation. To assess cytotoxicity as ^{51}Cr release, autologous B-LCL were labeled at 10^6 with 50 μCi ^{51}Cr for 90 min. These cells were then washed extensively to remove free ^{51}Cr. Then 5×10^4 cells were used as target cells in a standard 4-h cytotoxicity assay (16) in presence or absence of SMX at 0.1 mg/ml. E/T ratios were 10:1, 3:1, and 1:1. Specific lysis was calculated as 100 × (experimental release − spontaneous release)/(maximal release − spontaneous release).

Results

Phenotype and specificity of TCC generated against SMX. From one patient with SMX hypersensitivity, we could generate 54 SMX specific clones with an SI ranging from 3 to 310. Most (46/54) clones were of the $CD4^+$ phenotype, but 5 $CD8^+$ and 3 double positive $CD4^+/CD8^+$ TCC could be generated as well.

To determine, whether the response of TCC was directed to a common structure of sulfonamides, we evaluated the reactivity of eight SMX-generated clones to other sulfonamide-

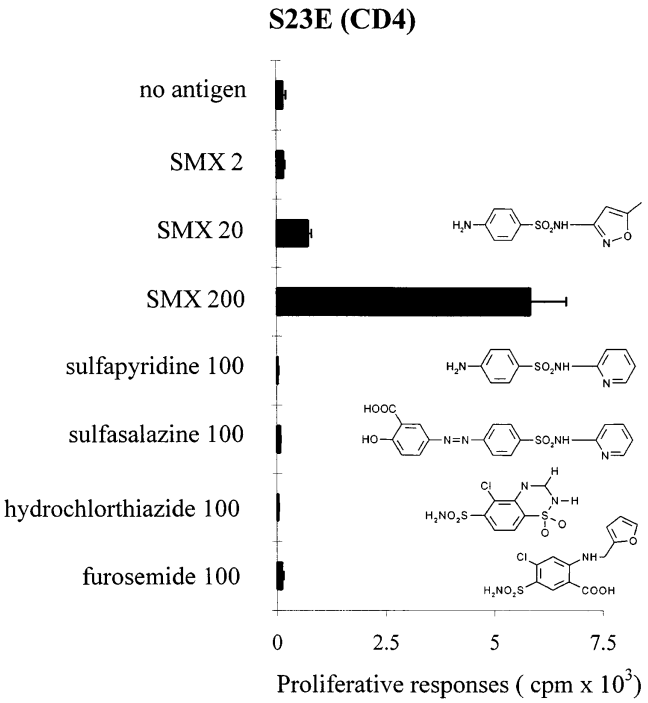

Figure 1. Stimulation of a representative TCC with different drugs at the indicated concentrations (micrograms per milliliter). TCC were incubated for a standard proliferation assay with autologous MNC in presence or absence of the drugs for 48 h as described in Methods. Results are given as mean [^{3}H]thymidine uptake of duplicate cultures; the error bar indicates the deviation of the mean.

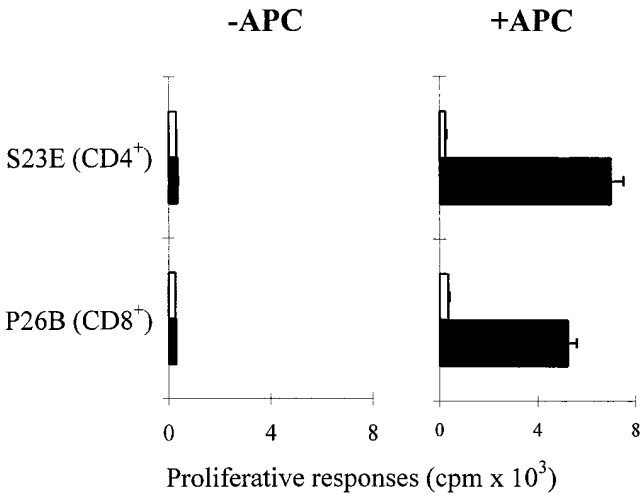

Figure 2. Requirement of APC for SMX presentation. TCC were stimulated by SMX in a proliferation assay with and without addition of APC as described in Methods. Results are given as mean [^{3}H]thymidine uptake of duplicate cultures, the error bar indicates the deviation of the mean. TCC with SMX, *black bars*; TCC without SMX (control), *white bars*.

derived compounds, namely sulfapyridine, sulfasalazine, hydrochlorothiazide, and furosemide tested at 1, 10, and 100 μg/ml. None of the eight clones tested showed any cross-reactivity (Fig. 1 shows representative data for one TCC).

Six well-growing clones were chosen for further characterization (Table I). The Vα, Vβ, and CDR 3 α,β region were sequenced to investigate whether the SMX-induced TCR show certain characteristics. Table I shows a summary of the TCR Vα and Vβ used by those clones. All clones have αβ TCR; no preferential usage of a certain Vα or Vβ by these clones could be seen. Three of six clones tested showed an SMX-specific cytolytic activity.

Requirement of APC for SMX presentation. To investigate the role of APC in the stimulation of SMX specific clones, we stimulated the TCC by SMX in presence or absence of two different types of APC: irradiated autologous PBMCs (containing monocytes, macrophages, resting T, B, and NK cells), or autologous B-LCLs. In experiments repeated three times, five out of six clones recognized SMX, if presented either by MNCs or B-LCL as APC, whereas one clone (S1011E) did not recognize SMX presented by B-LCL, but reacted to SMX presented by PBMC. There was no stimulation by addition of SMX in any of the clones without addition of APC. Fig. 2 shows the specific proliferation of a representative CD4$^+$ and CD8$^+$ TCC in presence or absence of MNCs.

MHC restriction of SMX-reactive TCC. To identify the HLA molecules involved in the presentation of SMX-derived antigenic determinants, blocking experiments with mAb against HLA class I and HLA class II were performed in proliferation assays. We found in all clones a dose-dependent blocking of the SMX-induced stimulation of TCC by mAbs either against MHC class I or HLA-DR. mAbs against DP or DQ did not inhibit SMX-induced stimulation of TCC. Fig. 3 shows the blocking of proliferation in presence of SMX and autologous APC by mAbs against either MHC class I or HLA-DR in the dilution 1:10 in a representative CD4$^+$ and the two CD8$^+$ TCC (representative of several experiments).

In addition, heterologous APC with different HLA types were used to stimulate specific TCC in proliferative and cytolytic assays (Figs. 4 and 5). These APC shared either no or one HLA-allele with the HLA-type of the donor of whom the TCC were generated. Matching in HLA B44 or DRB1*10 leads to a proliferative or cytolytic response, while matching in A2, A26, and DRB1*0101 did not stimulate specific TCC. Thus the APC from allogenic donors were able to present SMX, if their HLA-alleles were matched appropriately. These donors were symptomless if exposed to SMX in vivo, which indicates that nonallergic individuals can present SMX as well.

Surprisingly, both the experiments with blocking mAb and with partially matched APC show an MHC class II restriction

Table I. Phenotype, TCR Analysis, and Cytotoxicity of SMX-specific TCC

TCC	Phenotype	TCR Vα	TCR Vβ	Cytotoxicity
S23E	CD4$^+$	21	13.2	Yes
S29A	CD4$^+$	20	13.2	No
S1010E	CD4$^+$	7	6	nt
S1011E	CD4$^+$	28	5	No
P26B	CD8$^+$	nt	nt	Yes
S211E	CD8$^+$	2	16	Yes

nt, not tested; Cytotoxicity (*Yes*), specific ^{51}Cr release > 40% in E/T ratio 10:1; Cytotoxicity (*No*), specific ^{51}Cr release < 5% in E/T ratio 10:1.

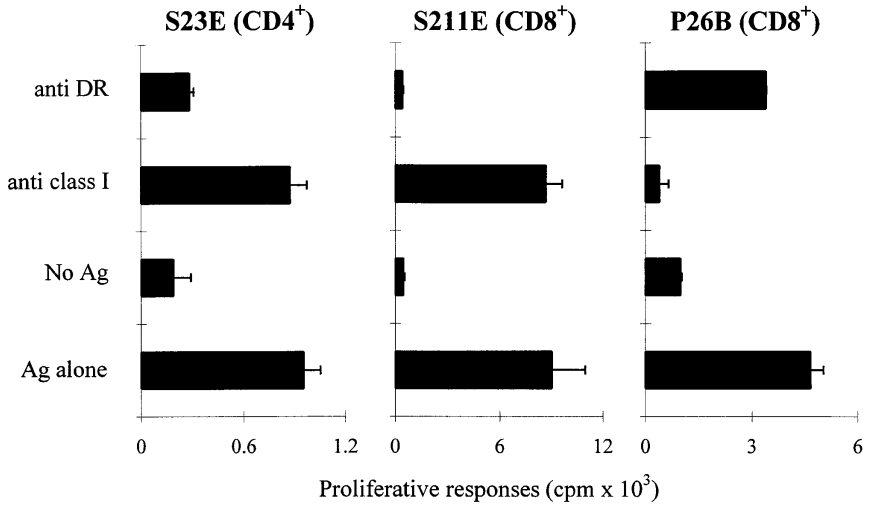

Figure 3. MHC restriction of SMX-specific TCC; mAb blocking experiments. One representative $CD4^+$ clone and two $CD8^+$ clones were stimulated in standard proliferative assays with autologous irradiated MNC as APC in the presence of SMX and mAbs either to MHC class I (W6/32) or MHC class II/HLA-DR (L243), both in a dilution of 1:10. Positive control was the proliferation without any mAbs, negative control the proliferation in absence of SMX. Results are given as mean [^{3}H]thymidine uptake of duplicate cultures, the error bar indicates the deviations of the mean. Note that the $CD8^+$ TCC S211E was MHC class II (DR) restricted.

(DRB1*10) of the $CD8^+$ clone S211E (Figs. 3–5). It has been shown that MHC class II specific $CD8^+$ T cells can develop in CD4-deficient mice and were even observed, to a limited extent, in the presence of $CD4^+$ cells (17). This is consistent with our observation that $CD8^+$ lineage choice is not dependent strictly on coreceptor usage or MHC class specificity.

Failure to pulse APC; processing is not required for presentation of SMX. Pulsing of APC with proteins, or even peptides, normally leads to a sufficiently stable MHC–peptide complex that is able to stimulate specific T cells. In contrast, all SMX specific clones could be stimulated only in the continuous presence of soluble SMX. Pulsing of APC with SMX failed to stimulate the clones, both in proliferative (Fig. 6) and cytolytic assays (data not shown).

We performed several experiments to optimize the pulsing conditions, all without success. Different APC (PBMC, B-LCL, activated T cells, monocytes, and neutrophils) and the TCC themselves were pulsed with SMX at various concentrations and for different incubation times. Neither irradiating nor fixing the APC before or after pulsing with SMX led to a proliferative response.

To determine whether processing is required for SMX recognition, glutaraldehyde-fixed APC were tested for their ability to activate the SMX-specific clones. In these experiments autologous B-LCL were fixed with glutaraldehyde and used to stimulate the different SMX-specific clones with and without addition of soluble SMX. The APC could be fixed in five out of six clones without impairing their ability to present SMX in proliferation assays. In contrast, the clone S1011E could be stimulated neither by fixed B-LCLs nor by fixed PBMC.

Fig. 6 shows the proliferation of a representative $CD4^+$ and $CD8^+$ TCC stimulated by pulsed APC in comparison to the stimulation in the continuous presence of SMX or the control without addition of SMX either with irradiated or fixed APC. Fig. 7 shows that even the cytotoxicity of the TCC P26B (representative for the three TCC) persists with fixed target cells. As in the proliferative assays, cytotoxicity was only observed if SMX was present throughout the assay period.

It is critical for the interpretation of these results that the fixation of the APC is effective in inhibiting the processing and is not altering the surface MHC molecules. We tested the presentation of TT to a TT-specific T cell line. After fixing, the APC were unable to present TT, whereas prior pulsing with TT followed by fixation did not abrogate their ability to present TT (data not shown). Thus, the fixation procedure abolished the ability of APC to take up and/or to process TT,

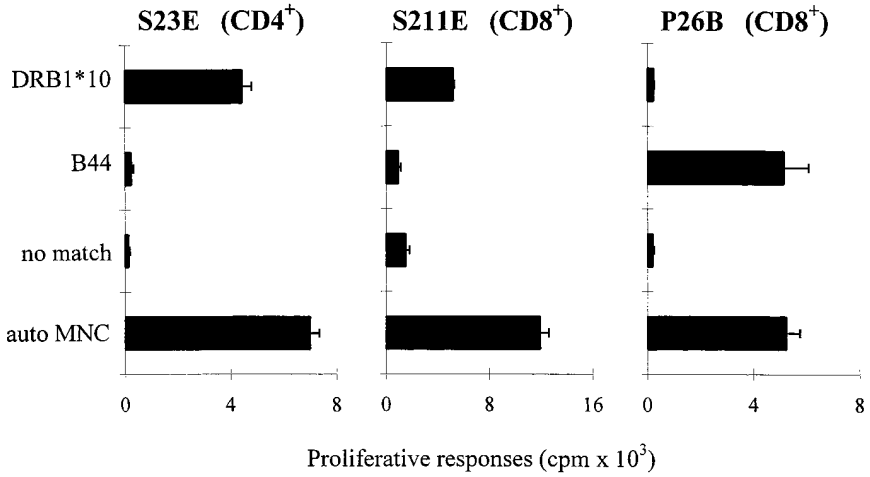

Figure 4. MHC restriction of SMX-specific TCC; presentation by partially matched APC. Stimulation of a representative $CD4^+$ and two $CD8^+$ TCC in proliferative assays for 48 h in presence of SMX. The assays were performed in the presence of 5×10^4 autologous MNC or 5×10^4 heterologous MNC. The heterologous MNC shared either DRB1*10 or HLA-B44 or no HLA-alleles. The cpm values of heterologous MNC without HLA match were in the same range as the controls in the absence of additional MNC (not shown). Results are given as mean [^{3}H]thymidine uptake of duplicates cultures, the error bar indicates the deviation of the mean. In agreement to mAb blocking experiments (Fig. 3) $CD8^+$ TCC S211E could be stimulated only by MNC sharing the HLA class II DRB1*10 allele, whereas the $CD8^+$ clone P26B could be stimulated only by MNC sharing the HLA class I B44 allele.

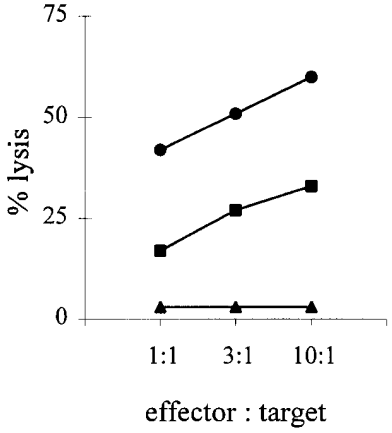

Figure 5. MHC restriction in cytotoxicity assay. Specific cytotoxicity of the $CD8^+$ TCC S211E was evaluated in a 4-h ^{51}Cr release standard assay in presence of SMX with the following targets: autologous EBV-transformed B cells (●), allogeneic B cells sharing either class I B44 (▲), or class II DRB1*10 (■) HLA-allele. Percent lysis was calculated as 100 × (experimental release − spontaneous release)/(maximal release − spontaneous release). The values of spontaneous release from the different targets ranged between 261 and 528 cpm, the ratio of maximal release to spontaneous release ranged between 4.4 and 6.5.

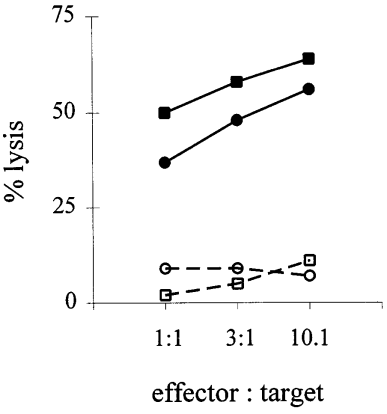

Figure 7. Processing of SMX is not required to induce cytotoxicity. In a 4-h ^{51}Cr release assay, labeled and then fixed cells (■) could still present the SMX to the same extent as unfixed cells (●) to $CD8^+$ TCC P26. No killing occurred in absence of SMX (□, ○). The values of spontaneous release from the unfixed targets ranged between 923 and 1,072 cpm, from the fixed targets ranged between 181 and 201 cpm, the values of maximal release from the unfixed targets between 4,011 and 4,518 cpm, and from the fixed targets between 1,791 and 1,783 cpm.

but had no influence on the ability to present processed antigens by MHC molecules. In our hands glutaraldehyde fixation also eliminates the nucleic acid ([^{3}H]thymidine) incorporation of B-LCL (data not shown).

Discussion

In this study we used a panel of SMX-specific TCC from one SMX allergic donor to better define the way in which T cells recognize SMX. The TCC generated were highly specific for SMX, and failed to cross-react with other sulfonamides or sulfonamide-derived drugs. TCR sequencing confirmed the monoclonality and revealed heterogeneity of the SMX-specific clones with no preferential use of a certain TCR.

All SMX-specific clones needed—both for proliferation or cytolytic assays—the addition of APC, which had to be compatible with the donor in either MHC class I (B44) or MHC class II (DRB1 10). This demonstrates an allele restricted recognition of SMX by the TCC tested.

The ability of fixed APC to present SMX to our clones indicates that antigen uptake and processing is not required for recognition by TCC. This is similar to results on the presentation of isopentenyl-pyrophosphate to specific human γδ cells (4), as neither the SMX specific αβ-TCR^+ TCC nor the γδ-TCR^+ TCC could be stimulated by pulsed APC. All six SMX-specific clones could be stimulated both for proliferation and cytolytic activity only in the continuous presence of soluble SMX in the culture medium. However, there is a decisive difference to the recognition by γδ-T cells: the SMX-specific αβ-TCR^+ TCC were clearly MHC restricted.

Our results of SMX presentation are difficult to reconcile with the usual concept that drug metabolites are the stimulating antigens for all SMX-reactive T cells. It has been proposed that SMX and other drugs are metabolized intracellularly to reactive compounds that covalently bind to a self protein. This self protein, conjugated with the drug metabolite, is then processed into peptides that are presented as altered self peptides by MHC molecules. Although such a pathway might occur for SMX and be important for primary sensitization, the data presented here suggest an additional pathway for SMX presentation. This mode of presentation is independent of intracellular drug metabolism and covalent binding and occurs at SMX concentrations (0.1–0.2 mg/ml) comparable to those normally found in plasma during standard oral therapy.

In support of this direct presentation pathway, we found that SMX can be presented to T cells without processing because fixed cells, which are unable to process antigen, can present SMX in an MHC-restricted way. Since we observed MHC-restricted cytotoxicity, the SMX is not directly interacting with the TCR on the TCC, as such an interaction could not explain the MHC restriction.

Moreover, the recognition of SMX seems to be independent of drug metabolism because (*a*) there is an absence of

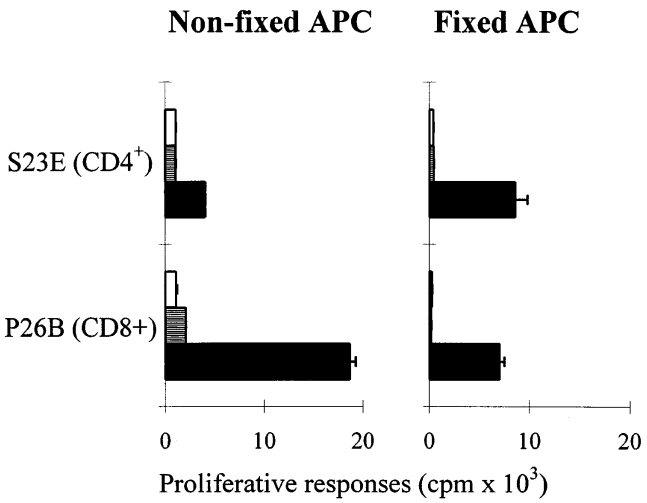

Figure 6. Preincubation of APC with SMX and washing (pulsing) fails to stimulate SMX-specific TCC; processing of SMX is not required to induce proliferation. Representative $CD4^+$ and $CD8^+$ TCC were stimulated in proliferative assays either by addition of soluble SMX and 5×10^3 B-LCLs (■) or 5×10^3 SMX pulsed B-LCLs (▤). Controls were TCC and B-LCLs without SMX (□). For pulsing, autologous B-LCL were incubated for 4 h with SMX and subsequently washed. Results are given as mean [^{3}H]thymidine uptake of duplicate cultures, the error bar indicates the deviation of the mean. In the same experiment, we compared the ability of irradiated and fixed APC to present SMX.

cross-reaction to other sulfonamides, which have at least in part common metabolites; (*b*) APC could not be pulsed with SMX either for proliferation or cytolytic assays; and (*c*) fixed B cells that are unable to process antigen could rapidly serve as targets in cytolytic assays with SMX-reactive T cells.

Covalent binding also does not seem to be required for the recognition of SMX because the binding of SMX to the MHC–peptide complex appears to be unstable, as washing the drug-incubated APC abrogated their presenting capacity in cytolytic assays as well, probably because washing removed the drug from the MHC–peptide complex. The nature of this unstable SMX binding to the MHC–peptide complex is unclear.

The proposed model of drug presentation has some similarities to the way in which gold or $NiSO_4$ may be recognized by T cells (5, 6). These metals are thought to bind directly and noncovalently to the MHC–peptide complex. Some Ni-specific clones react with fixed APC, others appear to need metabolism. The majority of clones were not reacting if the APC were pulsed (6).

The clinical course of SMX allergies is also consistent with such an unstable, but nevertheless, immunogenic presentation. Most SMX allergies recover promptly after discontinuation of the drug application. Such a rapid recovery is hard to explain, if the relevant allergic compound was a covalently haptenated serum protein, most of which have quite a long half-life, e.g., $>$ 20 d for serum albumin. The direct binding of SMX to MHC–peptide complexes may also explain the increased frequency of drug allergies in viral infections like HIV, where an upregulation of MHC-class II molecules occurs, thereby facilitating SMX presentation.

Drug presentation by noncovalent binding may be quite common, as five out of six clones evaluated reacted in a similar, processing independent way. We cannot rule out that the generation of the clones was biased, i.e., by culture of the peripheral blood, which may have allowed only the generation of metabolism-independent TCC. But the evaluation of such a peripheral T cell reactivity correlates to the clinical symptoms (18, 19), suggesting that it indeed reflects a clinically relevant sensitization. This alternative path of drug presentation is not restricted to SMX, since we found the same pattern of presentation for lidocaine-specific TCC as well (data not shown).

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