Differential T Cell Receptor Photoaffinity Labeling among H-2K<sup>d</sup> Restricted Cytotoxic T Lymphocyte Clones Specific for a Photoreactive Peptide Derivative. Labeling of the α-Chain Correlates with JoL Segment Usage

By Pedro Romero, Jean-Laurent Casanova, Jean-Charles Cerottini, Janet L. Maryanski, and Immanuel F. Luescher

From the Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland

Summary

Using a direct binding assay based on photoaffinity labeling, we studied the interaction of T cell receptor (TCR) with a K<sup>d</sup>-bound photoreactive peptide derivative on living cells. The K<sup>d</sup>-restricted Plasmodium berghei circumsporozoite (PbCS) peptide 253-260 (YIPSAEKI) was reacted NH<sub>2</sub>-terminally with biotin and at the TCR contact residue Lys<sub>269</sub> with photoreactive iodo, 4-azido salicylic acid (IASA) to make biotin-YIPSAEK(IASA)<sub>I</sub>. Cytotoxic T lymphocyte (CTL) clones derived from mice immunized with this derivative recognized this conjugate, but not a related one lacking the IASA group nor the parental PbCS peptide. The clones were K<sup>d</sup> restricted. Recognition experiments with variant conjugates, lacking substituents from IASA, revealed a diverse fine specificity pattern and indicated that this group interacted directly with the TCR. The TCR of four clones could be photoaffinity labeled by biotin-YIPSAEK(I<sub>259</sub>ASA)<sub>I</sub>. This labeling was dependent on the conjugates binding to the K<sup>d</sup> molecule and was selective for the TCR α (2 clones) or β chain (1 clone), or was common for both chains (1 clone). TCR sequence analysis showed a preferential usage of J<sub>α</sub>T<sub>28</sub> containing α chains that were paired with V<sub>β</sub>1 expressing β chains. The TCR that were photoaffinity labeled at the α chain expressed these J<sub>α</sub> and V<sub>β</sub> segments. The tryptophan encoded by the J<sub>α</sub>T<sub>28</sub> segment is rarely found in other J<sub>α</sub> segments. Moreover, we show that the IASA group interacts preferentially with tryptophan in aqueous solution. We thus propose that for these CTL clones, labeling of the α chain occurs via the J<sub>α</sub>-encoded tryptophan residue.

In general, CD8<sup>+</sup> CTL recognize antigenic peptides bound to MHC class I molecules on the surface of target cells. The specificity of the interaction is determined principally by the TCR-α/β of the CTL that binds the peptide–MHC complex. Recently, the structures of several MHC class I molecules and defined peptide–MHC complexes have been elucidated by x-ray crystallography (2–5). These studies showed that whereas certain amino acid side chains of the bound peptide intruded into allele-specific pockets of the MHC molecule, others were oriented towards the solvent phase, potentially accessible to the TCR.

The precise structure of the TCR is unknown, but the variable domains are presumed to resemble those of Ig based on overall sequence homology and a conservation of certain residues that are critical for the structure of the Ig molecule (6–8). On this basis, a model was proposed for the interaction of the TCR with the peptide–MHC complex in which the most variable, “CDR3-equivalent,” regions of the TCR α and β chains interact with peptide residues, and the less variable regions, CDR1 and CDR2, interact with MHC residues (6). Indirect evidence for a role of the CDR3 region in contacting the peptide comes from studies involving TCR repertoire analysis (7–10), site-directed mutagenesis of the TCR (11) or immunization of single-chain TCR transgenic mice with variant peptides (12). The latter study also suggested an orientation of the peptide with respect to the TCR α and β chains. However, it is not known whether this orientation applies generally.

Our earlier studies on the K<sup>d</sup>-binding antigenic peptide Plasmodium berghei circumsporozoite (PbCS)<sub>I</sub> 253-260 (YIPSAEKI) showed that Tyr<sub>253</sub> and Ile<sub>260</sub> were engaged in peptide binding to the K<sup>d</sup> molecule, whereas Lys<sub>269</sub> appeared to be in contact with the TCR for most CTL clones analyzed (13). Molecular modeling suggested that the side chain of

1 Abbreviations used in this paper: ABA, 4-azido benzoic acid; ASA, 4-azido salicylic acid; BA, benzoic acid; IASA, iodo, 4-azido salicylic acid; PbCS, Plasmodium berghei circumsporozoite; pl, isoelectric point.
the Lys299 residue was indeed oriented away from the Kd molecule (14). Furthermore, we found that a derivative of the PbCS peptide containing an NH2-terminal photoreactive iodo, 4-azido salicylic acid (IASA) moiety, [IASA-YIPSAEK(biotin)] selectively labeled the Kd molecules, whereas an isomeric derivative with the IASA group coupled to the Lys299 side chain, biotin-YIPSAEK(IAASA), could not, even though it bound equally efficiently to Kd molecules (15). Realizing that the IASA group of the latter derivative might be available for TCR interaction, we immunized mice with this material and obtained CTL clones that recognized this photoreactive PbCS derivative. We recently demonstrated that the TCR of one of these clones could be specifically photoaffinity labeled with this antigenic peptide derivative (15). In the present study, eight different Kd-restricted CTL clones that recognize biotin-YIPSAEK(IAASA) were compared in terms of photoaffinity labeling, fine specificity of recognition, and TCR sequences. The results are discussed in the context of current models of antigen recognition by TCR. This approach provides new possibilities for the study of the molecular principles of antigen recognition by T cells.

Materials and Methods

Peptide and Conjugate Synthesis. Reagents for peptide and conjugate synthesis were obtained from Bachem Finechemicals (Buben, Switzerland) and from Sigma Chemie (Buchs, Switzerland). Peptides and NH2-terminal conjugates were synthesized on Wang resin using Fmoc for transient NHz-terminal protection on a manual peptide synthesizer (RAMPS; Du Pont, Regensdorf, Switzerland) and from Sigma Chemie (Buchs, Switzerland). The amino acid compositions of all peptides were assessed according to the DABSYL method and were ± 10% in agreement with the expected ratios.

Photoactive derivatives were prepared under dimmed light as previously described (15, 16). The structures of the different conjugated groups are shown in Figure 1. In brief, the bifunctional conjugates (a) biotin-YIPSAEK(IAASA), (b) biotin-YIPSAEK(4-azido salicylic acid [ASA]), (c) biotin-YIPSAEK(4-azido benzoic acid [ABA]), and (d) biotin-YIPSAEK(benzoic acid [BA]) were obtained by reacting biotin-YIPSAEK(IAASA) with N-hydroxysuccinimidyl iodo, 4-azido salicylic acid (IASA) and, after Fmoc removal, was purified by HPLC. The purified lysates was performed as described (15, 18).

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Radioactive 125I-labeled IASA-YIPSAEK(biotin) and biotin-YIPSAEK(125I)-labeled IASA were prepared by reacting freshly radioiodinated 125I-IASA-ONSu with the corresponding biotin derivatives as described (15). After ethyl acetate precipitation and HPLC purification, the photoprobes were reconstituted in PBS at ~2 x 10^6 cpm/ml. The specific radioactivities were ~2,000 Ci/mm mol. The photoprobes were kept in the dark at 4°C and were used within 4 d.

Photoaffinity Labeling. Cells were washed once in DMEM supplemented with 0.5% FCS and 10 mM HEPES and resuspended in the same medium at 6 x 10^6 cells/ml. Cells (6 x 10^6) were incubated with biotin-YIPSAEK(125I)-labeled IASA (6 x 10^5 cpm in 30-50 μl PBS) and human β2m (2.4 μg/ml; Sigma Chemie) or 125I-labeled IASA-YIPSAEK(biotin) (10^6 cpm in 10-20 μl PBS) in 6-well plates at 37°C, 5% CO2 for 4.5 h and then UV irradiated at 4°C for 5 min (15-W lamp with emission maximum at 312 nm).

The labeled cells were washed once in PBS and solubilized with NP-40 (0.7%) in the presence of leupeptin (10 μg/ml), PMSF (1 mM) and iodoacetamide (10 mM). Kd was immunoprecipitated with mAb 20-8-4S and the TCR-α/β with mAb H57-597 (American Type Culture Collection [ATCC], Rockville, MD) and analyzed on 10% linear SDS-PAGE under reducing conditions as previously described (15). Two-dimensional gel electrophoresis of TCR precipitated with α-CD3 mAb (145-2C11; ATCC) from digitonin lysates was performed as described (15, 18).

The relative extent of photoaffinity labeling of single amino acids by IASA was determined by a solid phase assay. N, Lys IASA was prepared by reacting Fmoc-Lys with iodoacetyl N-hydroxysuccinimidyl ASA and, after Fmoc removal, was purified by HPLC. The purified Ilys(IASA) was reacted with CNBr-activated Sepharose 4B (Sigma Chemie) in 50 mM sodium bicarbonate in water/acetonitrile (1:1). The degree of coupling was 18 μmol IASA per ml sedimented Sepharose. 300 pmol of 3H-labeled amino acids (New England Nuclear, Boston, MA) dissolved in 10 μl PBS were incubated with 20 μl sedimented Iyas(IASA)-Sepharose under agitation at 4°C for 1 h. After 5 min UV irradiation (15-W lamp with emission maximum at 365 nm), the agarose was washed extensively with PBS containing 0.5 M NaCl, 10 mM EDTA, and 0.5% NP-40, with PBS containing 1% SDS, 75% acetonitrile, and DMSO and the bound radioactivity was determined liquid scintillation counting. In parallel, the same experiments were performed in the absence of UV irradiation. After subtraction of the latter from the former radioactivity values, the amount of the photolabeled amino acids was calculated via the specific radioactivity. All experiments were performed at least in triplicates and the SDs were calculated according to the Student's t method.

mAbs. All mAbs were produced by hybridomas obtained from the ATCC and were purified by affinity chromatography on protein A-Sepharose.

Generation of CTL Clones and CTL Assays. The N and O CTL clones were derived from two (BALB/c × C57BL/6)F1 mice immunized with biotin-YIPSAEK(IASA) by a previously described method (13). The mice were injected subcutaneously at the base of the tail with 50 μg of biotin-YIPSAEK(IASA) emulsified in IFA. Cell suspensions from draining LNs were prepared 9 d later.

LN cells (4 x 10^6) were cocultured with 5 x 10^4 peptide biotin-YIPSAEK(IASA) and irradiated (10,000 rads) in a volume of 2 ml in 12-well plates (Costar Corp.). The medium was used was DMEM supplemented with 10% FCS, 10 mM Heps, 1% Gln, 5 x 10^-4 M 2-ME, and EL-4 supernatant as a source of IL-2 (30 U/ml). 7 d later, 5 x 10^4 viable cells were restimulated with 2 x 10^6 peptide pulsed and irradiated P815 and
5 × 10^6 irradiated BALB/c spleen cells in 2 ml of the same culture medium in 12-well plates. The two cultures were cloned by limiting dilution 9 d after the second in vitro stimulation. A total of 11 clones were analyzed for TCR-c~/B expression by cDNA-PCR. Two clones were eliminated as probable sister clones, based on Vβ segments. Clone O8 grew poorly and thus was not included in all of the experiments reported here. Details of the recognition assay are described (12, 19). The specific lysis was calculated as 100 × ([experimental - spontaneous release]/[total - spontaneous release]).

cDNA-PCR and Direct Sequencing. RNA extraction, cDNA synthesis, and PCR with Vα, Vβ, Cα, and Cβ primers were carried out on CTL clones as previously described (20). A detailed description including the nucleotide sequences will be reported elsewhere (Casanova, J.-L., P. Romero, and I. F. Luescher, manuscript in preparation).

Results

CTL Recognition of Biotin-YIPSAEK(IASA)I. Eight independent CTL clones, derived from (BALB/c × C57BL/6)F1 mice immunized with biotin-YIPSAEK(IASA)I, specifically recognized the PbCS derivative conjugate, as illustrated in Fig. 2 for CTL clones N9 and O1. The concentration of biotin-YIPSAEK(IASA)I required for half maximal lysis varied for the different clones in the range of 10^-9-10^-11 M (Table 1). All of the six clones tested were K^d restricted as assessed by recognition of the antigenic peptide derivative on L cell transfectants that expressed cell surface K^d molecules but not on untransfected controls (data not shown). The IASA group appeared to form a critical part of the antigenic epitope, since the CTL clones failed to recognize the peptide derivative lacking the IASA group (biotin-YIPSAEK(IASA)I) (Fig. 1) or the unmodified PbCS peptide (15 and data not shown). None of the clones recognized the isomeric derivative IASA-YIPSAEK(biotin)I (data not shown).

As previously shown (15), deletion of the NH2-terminal biotin or of substituents from the IASA group had no significant effect on the binding efficiency of the peptide derivatives to the K^d molecule. In contrast, these variant peptide derivatives were differentially recognized by the CTL clones. The biotin group that was included in the derivative for technical reasons (ease of synthesis of the derivative and reactivity with streptavidin for biochemical studies) was not required for recognition by most (six out of eight tested) of the CTL clones (Table 1). Removal of the iodine from the IASA group [biotin-YIPSAEK(ASA)]I clearly decreased (by 30-1,000-fold) the efficiency of recognition for three of the clones (N1, N4, and O1), but actually improved recognition for the other four by 6-12-fold. Only two clones (N6 and N9) efficiently recognized a derivative in which both the iodine and the hydroxy residues were deleted [(biotin-YIPSAEK(ABA)]. One of these clones, N9, recognized this particular derivative about 50 times more efficiently than the original photoprobe. It was also the only clone among the eight tested to recognize the variant derivative lacking all three substituents of the IASA group [biotin-YIPSAEK(BA)]I. It

| CTL clone | IASA | ASA | ABA | BA |
|-----------|------|-----|-----|----|
| N1 20*    | 1.2  | 0.02| <10^-5 |
| N4 70     | 1.2  | 0.09| <10^-5 |
| N6 7      | 0.7  | 12  | 0.2 | <10^-5 |
| N9 100    | 1.4  | 8.3 | 50  | 1.1 |
| N11 50    | 2    | 12  | <10^-5 |
| O1 18     | 0.9  | 0.0012| <10^-4 |
| O7 30     | 0.008| 6   | <10^-4 |
| O11 600   | <0.0006| ND | <0.0006| <10^-5 |

* The numeric values represent the concentration (pmol, 10^-12 M) of the photoreactive peptide derivative required for 50% maximal lysis by the corresponding CTL clones in each experiment. This concentration was determined in a 51Cr-release assay (see Fig. 1). The E/T ratio was 3:1. Threefold dilutions of the peptide derivative were made covering a concentration range between 10^-12 and 10^-15 M. The 50% lysis value was used as the reference concentration for calculating the relative antigenic activity of the other peptide derivatives.

† The antigenic activity of the four variant photoreactive peptide derivatives was assayed in a 51Cr-release assay as described above for the reference peptide. The relative antigenic activity is calculated as the concentration of the reference peptide biotin-YIPSAEK(IASA)I required to obtain 50% maximal lysis divided by that of the variant peptide.

‡ The structural formulas of the variant IASA groups are as shown in Fig. 1.
is remarkable that it recognized the latter compound as efficiently as the original biotin-YIPSAEK(IASA)I conjugate.

**TCR Photoaffinity Labeling**  
The eight biotin-YIPSAEK(IASA)I-specific CTL clones were tested for TCR photoaffinity labeling. The different clones were incubated with the radiolabeled photoprobe. After UV irradiation, the immunoprecipitated TCR were analyzed by SDS-PAGE. In addition to the N6 clone that was previously analyzed (15), the clones N4, N9, and N11 showed TCR photoaffinity labeling (Fig. 3). No significant TCR labeling was observed under the same conditions for the other four clones tested (N1, O1, O7, and O11), although clone N1 showed a faint band on overexposure of the gel (data not shown). The labeled immunoprecipitated TCR material migrated with an apparent molecular mass of ~82–92 kD under nonreducing conditions, and 42–47 kD under reducing conditions. This corresponds to the migration pattern of conventional disulfidelinked α/β heterodimer TCR molecules (21). Two labeled species of different molecular weights were apparent for the photoaffinity labeled TCR of clone N9 under reducing conditions, but only one species was observed under nonreducing conditions (Fig. 3).

We verified that the TCR labeling of clones N4, N9, and N11 was dependent on the peptide derivative binding first to the Kd molecules expressed on the surface of the clones, i.e., that the photoprobe per se was unable to label directly these TCR. This has been shown previously for clone N6 (15) and is illustrated for clone N9 in Fig. 4. Thus the TCR labeling was abolished when cells were incubated with biotin-YIPSAEK(IASA)I in the presence of a 300-fold molar excess of the Kd-restricted peptides PbCS 253-260 or P198-14-22 (19), but not of the Db-restricted adenovirus peptide 234-243 (17) (Fig. 4, lanes 2–4). Furthermore, the TCR labeling was also inhibited in the presence of the anti-Kd mAb 20-8-4S, but not by an isotype-matched irrelevant mAb (anti-I-Ak) (Fig. 4, lanes 5 and 6).

Since biotin-YIPSAEK(IASA)I was presented by Kd molecules expressed on the CTL clones, we compared the ability of the different clones to bind peptide by Kd photoaffinity labeling. We previously showed that the isomeric IASA-YIPSAEK(biotin)I selectively photoaffinity labels the Kd molecule on different cell types, but fails to label TCR (15–17). As shown in Fig. 5, the Kd photoaffinity labeling was efficient in the case of the N4, N6, N9, and O1 clones and slightly reduced for the N1 and N11 clones. The Kd labeling on the O7 and O11, however, was considerably weaker (~10-fold). We assessed the level of expression of several cell surface molecules that are involved in CTL target cell interactions by flow cytometric analysis. There was, however, no obvious correlation between the level of expression of Kd, CD3, CD8, LFA-1 or CD44 and TCR photoaffinity labeling (data not shown), suggesting that the lack of TCR photoaffinity labeling observed with some clones could not be accounted for by a low degree of surface expression of these molecules.

**Assessment of Photoaffinity-labeled TCR Chain.**  
We next wished to identify which TCR chain was photoaffinity labeled. The clones N4, N9, and N11 were either lactoperoxidase radioiodinated or photoaffinity labeled with biotin-YIPSAEK(IASA)I and the immunoprecipitated TCR were analyzed by two-dimensional gel electrophoresis (Fig. 6). The lactoperoxidase-labeled TCR of the N4 clone showed a weakly labeled α chain of an ~isoelectric point (pl) of 4.8 and an intensely labeled β chain of a pl of ~6.1, both having a molecular mass of ~43 kD (Fig. 6 A). The photoaffinity-labeled material from the N4 TCR migrated very similarly to the α chain, indicating that this TCR was selectively photoaffinity labeled at the α chain. The lactoperoxidase-labeled N9 TCR showed a strongly labeled β chain of an ~pl of 6 and a molecular mass of ~45 kD and a weakly labeled α chain with a pl of ~4.7 and a molecular mass of 42 kD (Fig. 6 B). The photoaffinity-labeled N9 TCR showed a very similar pattern, with a relatively more intense labeling of the α chain. This indicated that the N9 TCR was photoaffinity labeled on both chains and explains the two labeled species seen in the SDS-PAGE under reducing conditions (Fig. 3). The lactoperoxidase-labeled N11 TCR showed a weakly labeled β chain and an intensely labeled α chain with approximate pl's of 6.6 and 4.9, respectively, both of a molecular mass of ~45 kD (Fig. 6 C). For clone N11, only the
TCR β chain was photoaffinity labeled. The same analysis performed on the N6 clone showed that its TCR was selectively labeled at the α chain (15). Taken together, the data showed that biotin-YIPSAEK(125IASA)I selectively labeled the α chain of the N4 and N6 TCR and the β chain of the N11 TCR, but labeled both chains of the N9 TCR.

**TCR Sequence Analysis of Biotin-YIPSAEK(125IASA)I-specific CTL Clones.** Four out of the eight clones analyzed (N1, N4, N6, and N9) were found to express a Vβ1-containing TCR. Moreover, all four Vβ1-expressing clones display α locus rearrangements that use the same Jα segment (TA28) (Table 2). The same pairing was observed for the additional O8 clone. This clone also recognized biotin-YIPSAEK(1ASA), but not biotin-YIPSAEK1 (data not shown). Because of its slow growth, this clone was not further analyzed. It should be noted that for one of the latter clones (N9), a second in-frame α chain transcript was also found, so that the functional α chain cannot be identified with certainty. The occurrence of two rearranged α loci, each encoding potentially functional chains, is a common finding among T cell clones (20). A different pair of Vβ1α segments (Vβ8.3/JαC7) is used by two other CTL clones, N11 and O1. There is no apparent preferential usage of Vα or Jβ segments among the nine clones studied.

For the β chains, there is no obvious CDR3 sequence similarity, except for the two clones that used the same Jβ element, N11 and O8. The CDR3β lengths are variable, from 6 to 11 amino acid residues long, although those of the five Vβ1 TCR are only six or seven residues long. The CDR3 regions of the α chains show more sequence similarity, in part because of the common Jα usage, and the CDR3α lengths are less variable than those of the β chains.

**Comparison of the Relative Photolabeling of Individual Amino Acids by the IASA Group.** The preferential expression of the Trp containing Jα segment prompted us to assess whether this amino acid preferentially interacts with the IASA group. To test this possibility, we compared the relative photolabeling of various radiolabeled amino acids by Sepharose-coupled IASA in aqueous solution (Fig. 7). The highest degree of labeling was observed for Trp (32%), followed by Cys (17%) and Tyr (13%). The other amino acids tested gave lower values. Thus,
among the 15 amino acids tested, Trp was labeled by the IASA group with a high preference. Since the UV irradiation-induced intermediate nitrenes are short-lived (22, 23) and capable of undergoing diverse chemical reactions with virtually any amino acid side chain (23, 24) the preferential photolabeling of Trp suggests a physical interaction of Trp with the IASA group in aqueous medium.

Discussion

We investigated antigen recognition by CTL using a new approach based on two strategies. The first was to modify a potential TCR contact residue of a MHC-restricted antigenic peptide with a photoreactive group and to generate CTL clones that specifically recognized this peptide derivative. The second strategy was to study the TCR–ligand interactions in such systems by TCR photoaffinity labeling. We have previously demonstrated the feasibility of this approach for one CTL clone that specifically recognized the biotin-YIPSAEK-(IASA)I derivative of the PbCS 253-260 peptide (15). In this report, eight independent biotin-YIPSAEK(IASA)I–specific CTL clones were studied for recognition of their photoreactive ligand, the K\textsuperscript{d} molecule–biotin-YIPSAEK(IASA)I complex.

Our results demonstrate that MHC class I–restricted CTL specific for a chemically modified peptide can be induced by
Nine biotin-YIPSAEK(1ASA)-specific CTL clones are listed on the vertical axis. The TCR-β (left) and α (right) only segments consistent with an open reading frame, encoding for junctional, hypervariable, and putatively CDR3-like regions are shown.

| CTL clone | Vβ | FW | CDR3 | FW | Jβ | Vα | FW | CDR3 | FW | Jα |
|-----------|----|----|------|----|----|----|----|------|----|----|
| N1        | 1  | CAS| SQGNSDY | TFG | 1.2 | 8.3 | F3.4 | CAL | SVPGSWQL | IFG | TA28 |
| N4        | 1  | CAS| SQGGIQ | YFG | 2.7 | 8.3 | F3.3 | BMA | VSWSWQL | IFG | TA28 |
| N6        | 1  | CAS| SSGREV | FFG | 1.1 | 8.3 | BDFLII | CVL | GDSWSWQL | IFG | TA28 |
| N9        | 1  | CAS| SQAPSEQ | YFG | 2.7 | 1.1 | TA65 | CAM | SPDNSNRL | TFG | N9  |
| O8        | 1  | CAS| SGGNTL | YFG | 1.3 | 13.1 | TA65 | CAA | SAPGSWQL | IFG | TA28 |
| N11       | 8.3| CAS| SEVSGLTL | YFG | 1.3 | 4.1 | TA65 | CAL | GITEKTRKL | IFG | C7  |
| O1        | 8.3| CAS| SEGGGERL | FFG | 1.4 | 3.0 | TA65 | CAL | GITGTRKL | IFG | C7  |
| O7        | 10 | CAS| SSNRVSYSNPL | YFA | 1.6 | 8.3 | F3.4 | BMB | RERPTGSNRL | TFG | 07  |
| O11       | 13 | CAS| SPGRYEQ | YFG | 2.7 | 10.0 | FN1-18 | CAL | IHTGNYKY | VFG | TA19 |

**AMINOACIDS**

**Labeling Extent (%)**

Figure 7. IASA labeling of individual amino acids in PBS. The fraction of the IASA photolabeled amino acids is shown as percent of the offered amino acid. The highest degree of labeling was 32% for Trp and the lowest was 0.33% for Ala.

Direct immunization with the peptide derivative, much as has been previously shown for the parental PbCS peptide (13). It is noteworthy that the in vivo induction of T cell reactivities against chemically modified antigenic peptides may play a role in certain diseases. It is known that naturally processed peptides derived from autologous proteins efficiently bind to MHC class I and II histocompatibility molecules (25, 26) and that various chemical agents, including many drugs, can modify autologous proteins (27). It is thus conceivable that the induction of T cells specific for chemically modified self-peptides is a primary event in the development of disorders such as drug allergies or delayed-type hypersensitivities. Indeed, it has been shown recently that in vivo-induced trinitrophenyl-reactive CTLs specifically recognize trinitrobenzene sulfonic acid-modified self-peptides in an MHC-restricted manner (28).

The biotin-YIPSAEK(1ASA) I-specific CTL clones displayed all the hallmarks of conventional antigen recognition by CTL, including specific peptide binding to MHC molecules and involvement of auxiliary molecules (15 and Fig. 4). The photoreactive IASA group was an essential part of this CTL epitope, since its removal abrogated recognition and deletions of its substituents affected recognition, in a clone-specific
manner, but not $K^d$ binding (15 and Table 1). This suggested that the TCR of these clones directly interact with the conjugated photoaffinity group. For some of the clones, this interaction was indeed directly demonstrated by photoaffinity labeling (Fig. 3).

It is not clear why only some of these CTL clones displayed detectable TCR photoaffinity labeling. It is conceivable that TCR labeling requires a certain TCR ligand affinity, which may not be achieved by some of the TCR studied. TCR ligand interactions, where measured, were reported to be of low affinity ($10^{-4}$-$10^{-5}$ M; 29, 30) and thus may not be detectable in the present TCR photoaffinity labeling experiments, in which the photoprobe concentration is low ($\sim 10^{-8}$ M). The photoaffinity-labeled TCR may indeed have a higher affinity than the others, since the recognition by the clones that displayed TCR labeling generally required lower antigen concentrations and/or was less susceptible to deletions of substituents from biotin-YIPSAEK(ASA)I (Table 1).

Other factors, however, may also be important for TCR photoaffinity labeling. For example, the TCR labeling in these experiments depended on the presentation of the photoprobe by the $K^d$ molecules of the CTL clones. The relatively inefficient presentation by the N1, O7, and O11 clones, as reflected by the reduced $K^d$ photoaffinity labeling (Fig. 5) could thus account, at least in part, for the lack of their TCR labeling. Furthermore, the possibility also exists that the photoaffinity labeling of some TCR results in unstable covalent bonds that decay during subsequent procedures (23).

TCR sequence analysis of the biotin-YIPSAEK(ASA)I-specific CTL clones showed a preferential usage of TCR containing the $\beta^b$1 and $\alpha^b$TA28 segments (Table 2). It is remarkable that all the clones, except N11, that displayed TCR labeling, expressed such TCR. Preliminary results from a currently ongoing study showed an even higher preference. In that study, six out of eight independent CTL clones recognizing a PbCS derivative, in which the IASA group was replaced by the related ABA group and that were selected for efficient TCR photoaffinity labeling, also expressed TCR using the $\beta^b$1 and $\alpha^b$TA28 paired elements (Luescher, I. F., P. Romero, and J.-L. Casanova, unpublished results).

A different pairing ($\beta^b$83.3/$\alpha^b$C7) was expressed by two of the biotin-YIPSAEK(ASA)I-specific clones, N11 and O1 (Table 2). A pairing of $\beta\beta$ and $\alpha\alpha$ segments is not unique to these responses against modified peptides since we found that all of 23 $H-2K^d$-restricted CTL specific for the CW3 170-179 antigenic peptide express TCR that use the $\beta^b$10 and $\alpha^b$TA28 segments in combination with a variety of different $\beta$ and $\alpha$ elements (31). It is interesting that a reciprocal pairing in which TCR express the $\alpha^b$10 and $\beta^b$2.6 elements was described for $H-2^b$-restricted TNP-specific CTL clones (32). One possible explanation for this pairing of TCR elements is provided by the molecular model of the TCR proposed by Chothia et al. (7), where the $\alpha\alpha$-encoded residues of the CDR3$\alpha$ would be in close proximity to the CDR2 region of the $\beta$ chain. Similarly, those of the CDR3$\beta$ would be close to the CDR2$\alpha$ region.

The preferential $\beta^b$1/$\alpha^b$TA28 usage by the biotin-YIPSAEK(ASA)I-specific TCR is remarkable when compared with the TCR repertoire found in the response to the parental peptide, PbCS 253-260 (20). In that case, none of the TCR expressed by 28 different CTL clones used the $\beta^b$1 or $\alpha^b$TA28 segments, and no preferential $\beta^b$1/$\alpha^b$ usage was observed. Since all of the $\beta^b$1/$\alpha^b$TA28 expressing CTL clones specific for the biotin-YIPSAEK(ASA)I compound recognized the YIPSAEK(ASA)I derivative but not biotin-YIPSAEKI, it appears that recognition of the IASA-modified Lys259 residue and the preferential usage of $\beta^b$1/$\alpha^b$TA28 expressing TCR are directly related. The mouse genome contains at least 50 distinct J$\alpha$ gene segments (6), and the J$\alpha$TA28 element has thus far not been found in analyses of other $K^d$-restricted CTL clones (Casanova, J.-L., unpublished results). The high occurrence of this particular J$\alpha$ segment among the biotin-YIPSAEK(ASA)I-specific TCR thus argues strongly for its direct involvement in TCR selection and ligand recognition.

It is now generally accepted that the CDR3 loops of TCR-$\alpha/\beta$ interact mainly with amino acid side chains of MHC-bound antigenic peptides (6-12). The J$\alpha$TA28 segment contains Trp, an amino acid rarely found in other CDR3 $\alpha$ sequences. From our analysis of the photolabeling of different amino acids by IASA (Fig. 7), it appears that the IASA group preferentially associates with Trp. It is therefore conceivable that the CDR3$\alpha$ Trp of the J$\alpha$TA28 segment is directly involved in an efficient binding of the photoactive group of the ligand. This interpretation is supported by the finding that all the photoaffinity-labeled TCR expressing the J$\alpha$TA28 segment were labeled at the $\alpha$ chain. The finding that the N9 TCR was labeled at the $\beta$ chain does not discount this interpretation since $\beta$ chain residues, located in close proximity to the J$\alpha$TA28 Trp, could also become photocrosslinked. The specific cross-linking of the IASA group to two distinct residues in a target protein has already been observed (33).

A recent study using immunization of TCR single chain transgenic mice with variants of the I-$\beta^b$-restricted mouse cytochrome peptide 88-103 and TCR analysis indicated that NH2-terminal peptide residues interacted with the CDR3$\alpha$ and COOH-terminal residues with the CDR3$\beta$ (12). In contrast, the present study suggests that a residue next to the COOH-terminal end of the peptide derivative preferentially interacts with the CDR3$\alpha$ region. The possibility has thus to be considered that TCR-ligand interactions follow no simple, general rules and may involve considerable variation in topology and relative orientation. This may be true for TCR recognizing the same MHC-peptide complex. In the present system, for example, the finding that the N11 TCR, which expresses different CDR3$\alpha$ and $\beta$ sequences, was selectively labeled at the $\beta$ chain, suggests that this TCR may interact with the IASA group preferentially via the $\beta$ chain.

Further analysis, including the characterization of additional systems, will be required before the general molecular principles of TCR-ligand interactions become apparent. The approach described here should facilitate such studies and also
provide new possibilities. For example, because of the high chemical reactivity and short life span of phenylnitrenes, photoaffinity labeling often takes place in a site-specific manner (23, 33). In principle, the photoaffinity labeled residue(s) on a target protein can be identified by amino acid sequencing of the labeled digest fragment (33–35). Tryptic peptide maps of the photoaffinity-labeled N6 TCR indeed showed reproducibly one major labeled digest fragment (15). This should now allow us to test directly the hypothesis that the Trp residue of the JotTA28 segment interacts with the IASA group of the antigenic peptide derivative presented by the K\(^d\) molecule.

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Address correspondence to Dr. P. Romero, Ludwig Institute for Cancer Research, Ch. des Boveresses 155, 1066 Epalinges, Switzerland. J.-L. Casanova is currently at Unité de développement normal et pathologique du système immunitaire, INSERM 132, Hôpital des Enfants Malades, 149 rue de Sèvres, 75743 Paris Cedex 15, France.

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