T Cell Recognition of Hapten

ANATOMY OF T CELL RECEPTOR BINDING OF A H-2K\(^d\)-ASSOCIATED PHOTOREACTIVE PEPTIDE DERIVATIVE*

(Received for publication, September 8, 1998, and in revised form, November 2, 1998)

Benedikt Kessler‡, Olivier Michelini‡, Christopher L. Blanchard‡, Irina Apostolou‡, Christaiane Delarbre‡, Gabriel Gachelin‡, Claude Grégoire‡, Bernard Malissen‡, Jean-Charles Cerottini‡, Florian Wurm**, Martin Karpluss‡, and Immanuel F. Luescher‡‡

From the ‡Ludwig Institute for Cancer Research, Lausanne Branch, University of Lausanne, 1066 Epalinges, Switzerland, the Department of Immunology, INSERM U277, Pasteur Institute, 75015 Paris, France, ‡‡Centre d’Immunologie, INSERM-CNRS, 13288 Marseille-Luminy, France, ‡‡‡Institut Le Bel, Louis Pasteur University, 67404 Strasbourg, France, and **Centre for Biotechnology, Department of Chemistry EPFL, 1015 Lausanne, Switzerland

To elucidate the structural basis of T cell recognition of hapten-modified antigenic peptides, we studied the interaction of the T1 T cell antigen receptor (TCR) with its ligand, the H-2K\(^d\)-bound Plasmodium berghei circumsporozoite peptide 252–260 (SYIPSAEKI) containing photoreactive 4-azidobenzoic acid (ABA) on P. berghei circumsporozoite Lys259. The photoaffinity-labeled TCR residue(s) were mapped as Tyr\(^{48}\) and/or Tyr\(^{50}\) of complementary determining region 2β (CDR2β). Other TCR-ligand contacts were identified by mutational analysis. Molecular modeling, based on crystallographic coordinates of closely related TCR and major histocompatibility complex I molecules, indicated that ABA binds strongly and specifically in a cavity between CDR3\(^a\) and CDR3\(^b\). We conclude that TCR expressing selective V\(^\beta\) and CDR\(\alpha\) sequences form a binding domain between CDR3\(^a\) and CDR2β that can accommodate nonpeptidic moieties conjugated at the C-terminal portion of peptides binding to major histocompatibility complex (MHC) encoded proteins.

CD8\(^+\) cytotoxic T lymphocytes (CTL)\(^1\) recognize by means of their T cell antigen receptor (TCR) antigenic peptides, usually 8–10 amino acids long, bound to major histocompatibility complex (MHC) class I molecules on target cells (1–4). However, CD8\(^+\) and CD4\(^+\) T cells can also recognize antigenic peptides containing nonpeptidic moieties, such as carbohydrates or haptens, like trinitrophenyl, azobenzenearsonate, fluorescein, or phenylazides (5–15). Such T cells can be readily elicited and play a role in diseases, e.g. allergies, contact dermatitis, and eczema (16). The recognition of modified peptides is highly specific, and even small changes in the hapten or carbohydrate moiety can dramatically affect antigen recognition (13–18). This is reminiscent of immunoglobulins, which can be raised against and specifically bind such structures (19, 20). While x-ray crystallographic studies have revealed how antibodies bind hapten, little is known about how TCR do this. This is of particular interest, because TCR genes, unlike immunoglobulin genes, have no somatic mutations allowing affinity maturation. Moreover, TCR need to recognize hapten or carbohydrate moieties in the context of an MHC-peptide complex in a predefined orientation (21–25).

Available three-dimensional structures of TCR-ligand complexes revealed a consensus “diagonal” TCR-ligand orientation, in which the MHC-bound peptide runs diagonally between the CDR3 loops, extending from CDR1\(\alpha\) to CDR1\(\beta\) (21, 23–25). In this orientation, the CDR3 loops can interact extensively with peptide side chains, which are mainly located in the center of MHC molecules, as well as with residues of the MHC \(\alpha\)-helices. The \(\alpha\)-helices of MHC class I molecules are elevated at the N-terminal portions; therefore, the approximately planar surface of the TCR ligand binding site can realize the best contact with the ligand in a diagonal orientation (21).

Hapten or carbohydrates conjugated with antigenic peptides are part of the epitope recognized by TCR (5, 8–15). TCR specific for hapten-modified antigenic peptides typically exhibit preferential usage of certain V\(^\beta\)\(\alpha\) and/or specific junctional sequences (13, 15, 18). We used as hapten photoreactive 4-azidobenzoic acid (ABA). This allowed assessment of TCR-ligand binding by TCR photoaffinity labeling and identification of the photoaffinity-labeled site(s), i.e. the contact(s) of the hapten with the TCR (13, 15).

We have previously generated and characterized two families of H-2K\(^d\)-restricted CTL clones, specific for two different photoreactive derivatives of the Plasmodium berghei circumsporozoite peptide PbCS–252–260 (SYIPSAEKI) (13, 15). In one peptide derivative, ABA was conjugated with PbCS Lys259, whereas P-255 was replaced by Lys(ABA) in the other. In addition, to prevent K\(^d\)-peptide derivative complex dissociation, PbCS Ser252 was replaced with iodo-4-azidosalicylic acid (ISA), which upon selective photoactivation permitted covalent attachment of the peptide derivative to K\(^d\) (26). The ABA, but not the ISA group, was part of the epitope recognized by these CTL. The two families of CTL clones were non-cross-reactive, and exhibited different TCR sequences (13, 15).

In this study, we describe the interaction of the TCR of the T1 CTL clones with its ligand, K\(^d\)-bound IASA-YIPSAEK (ABA1). Using mutational analysis, mapping of the photoaffinity-labeled site(s) and molecular modeling, we identified a specific binding mode, how the T1 TCR binds the ABA group. We propose that this binding principle has universal aspects.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** To whom correspondence should be addressed: Ludwig Institute of Cancer Research, Chemin des Boveresses 155, 1066 Epalinges, Switzerland. Tel.: 41 21 692 59 88; Fax: 41 21 653 44 74; E-mail: iluesche@eliot.unil.ch.

† The abbreviations used are: CTL, cytotoxic T lymphocyte(s); ABA, 4-azidobenzoic acid; CDR, complementary determining region; MHC, major histocompatibility complex; TCR, T cell antigen receptor(s); PbCS, P. berghei circumsporozoite; HPLC, high pressure liquid chromatography; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; LZ, leucine zipper; IASA, iodo-4-azidosalicylic acid.

‡‡‡ To whom correspondence should be addressed: Ludwig Institute of Cancer Research, Lausanne Branch, University of Lausanne, 1066 Epalinges, Switzerland, the Department of Immunology, INSERM U277, Pasteur Institute, 75015 Paris, France, Centre d’Immunologie, INSERM-CNRS, 13288 Marseille-Luminy, France, Institut Le Bel, Louis Pasteur University, 67404 Strasbourg, France, and Centre for Biotechnology, Department of Chemistry EPFL, 1015 Lausanne, Switzerland.

 petrolatum, which is used as a topical ointment in human medicine.

This paper is available online at http://www.jbc.org.

Printed in U.S.A.
**TCR photoaffinity labeling, 10^7 cpm of K\(^d\)-125IASA-YIPSAEK(ABA)I procedures were performed as described previously (15, 17). In brief, for labeling observed for K\(^d\)-125IASA-YIPSAEK-icity labeling on cloned T1 CTL. The label-complexes were used for TCR photoaffinity labeling and antigen recogni-
tion by T1 CTL. The antigenic activities are expressed rel-
tive to SYIPSAEK(ABA)I and normalized with the relative K\(^d\) competitor activities; thus, by definition the normalized relative antigenic activity of SYIPSAEK(ABA)I was 1 (see "Experimental Procedures"). Each experiment was performed in triplicate and repeated at least once. The mean values and S.D. values were calculated from all experiments.

**EXPERIMENTAL PROCEDURES**

**Peptide and Peptide Derivative Synthesis**—Amino acids and other chemicals were obtained from Bachem Finechemicals AG (Bubendorf, Switzerland), Sigma Chemie (Buchs, Switzerland), and Neosystems (Strasbourg, France). Synthesis and characterization of peptide derivatives was performed as described previously (15, 17, 26). HPLC-purified peptide derivatives were reconstituted in PBS at 1 mM. The specific radioactivity of 125I conjugates was approximately 2000 Ci/mmol.

**Cellular Assays**—For the cytolytic assay, 35Cr-labeled P815 cells (5 \times 10^4 cells/well) were incubated for 1 h at 37 °C in medium containing 10-fold dilutions of peptide derivatives, followed by UV irradiation at 350 nm. For peptide mapping, T1 CTL (4 \times 10^4 cells/well) were incubated likewise with K\(^d\)-SYIPSAEK(ABA)I (1 mCi) in 2 ml of medium containing 20% FCS, 10% heat-inactivated fetal calf serum, 2 mM HEPES, 1 mM phenylmethylsulfonyl fluoride, leupeptin, and iodoacetamide. The detergent-soluble fractions were subjected to immunoprecipitation with anti-TCR C\(^\gamma\) monoclonal antibody H57–597. The immunoprecipitates were analyzed by SDS-PAGE (10%, reducing conditions) and quantified using a PhosphorImager and the ImageQuant software (Molecular Dynamics, Inc., Sunnyvale, CA). S.D. values were calculated from 2–4 experiments.

**T Cell Recognition of Hapten**

**Fig. 1.** Effect of PbCS peptide derivative mutations on T1 TCR photoaffinity labeling and antigen recognition by T1 CTL. A, the indicated radiolabeled peptide derivatives were photo-cross-linked to soluble K\(^d\), and the resulting covalent K\(^d\)-peptide derivative complexes were used for TCR photoaffinity labeling on cloned T1 CTL. The labeling observed for K\(^d\)-125IASA-YIPSAEK(ABA)I was defined as 1, and labeling values for the ligand variants are expressed relative to this value. B, alternatively, the specific lysis of 35Cr-labeled P815 cells was assessed in the presence of the indicated PbCS peptide derivatives. The antigenic activities are expressed relative to SYIPSAEK(ABA)I and normalized with the relative K\(^d\) competitor activities; thus, by definition the normalized relative antigenic activity of SYIPSAEK(ABA)I was 1 (see "Experimental Procedures"). Each experiment was performed in triplicate and repeated at least once. The mean values and S.D. values were calculated from all experiments.

**K\(^d\)** and TCR Photoaffinity Labeling—All photoaffinity labeling procedures were performed as described previously (15, 17). In brief, for TCR photoaffinity labeling, 10^6 cpm of K\(^d\)-125IASA-YIPSAEK(ABA)I were incubated with 10^5 T1 CTL on ice for 3 h, followed by UV irradiation at 312 ± 40 nm. For peptide mapping, T1 CTL (4 \times 10^5) were incubated likewise with K\(^d\)-SYIPSAEK(ABA)I (1 mCi) in 2 ml of medium containing 20% FCS, 10% heat-inactivated fetal calf serum, 2 mM HEPES, 1 mM phenylmethylsulfonyl fluoride, leupeptin, and iodoacetamide. The detergent-soluble fractions were subjected to immunoprecipitation with anti-TCR C\(^\gamma\) monoclonal antibody H57–597. The immunoprecipitates were analyzed by SDS-PAGE (10%, reducing conditions) and quantified using a PhosphorImager and the ImageQuant software (Molecular Dynamics, Inc., Sunnyvale, CA). S.D. values were calculated from 2–4 experiments.

**Soluble T1 TCR and Mutants**—T1 and \(\alpha\) and \(\beta\) DNAs extending from the 5′ terminus up to, but not including, bases encoding the extracellular membrane-proximal cysteine residues were generated by reverse transcription on total T1 CTL RNA followed by polymerase chain reaction (PCR) amplification. The DNA fragments encoding a linker sequence and leucine zipper (LZ) components were generated by using oligonucleotides and PCR on templates pACID and pBASE (27). The T1 TCR-leucine zipper cDNAs were prepared by using recombinant PCR on these templates. The T1\(\alpha\)LZ and T1\(\beta\)LZ cDNA containing basic and acidic LZ, respectively, were cloned into pCR-script (Stratagene) and subcloned into the EcoRI site of the mammalian expression vector pCI-neo (Promega). All PCR amplifications were performed using Pfu DNA polymerase (Stratagene), and both strands of cloned inserts were sequenced and found to be error-free. TCR mutants were generated using the QuickChange site-directed mutagenesis kit (Stratagene) following the supplier's instructions. 293T cells (ATCC) were transfected with pT1aLZ and pT1bLZ DNA (1:2 ratio) for transient expression of soluble \(\alpha\)\(\beta\)T1 TCR following published procedures (28). After 2 days, supernatants were harvested, and T1 TCR concentrations were equalized. Preparation of T1 single chain Fv DNA constructs and protein expression were performed as described previously (29).

**Soluble K\(^d\)** and Mutants of Soluble K\(^d\)**—A full-length K\(^d\) cDNA cloned in pKC expression vector (promotor SV40, Hanahan) was double-di-
A distance restraint was introduced initially between the phenyl rings of complex was built using MODELLER by satisfaction of spatial re-
chains were aligned using a dynamic programming method imple-
ments were assessed by SDS-PAGE as described (32).

radioactivity of 20 Ci/mmol). The 

M products; specific activity of 142 Ci/mmol) at 37 °C for 20 h. The washed 

Peptide Mapping—All procedures have been described previously (13, 15). Enzymes were obtained from Boehringer Mannheim (Rot-

krenz, Switzerland) and used as recommended (31). In brief, photoaf-
finity-labeled T1 TCR was reduced, alkylated, and reconstituted in 500 

m particle size, Vydac, Hис-
peria, CA). The column was eluted with a linear gradient of acetoni-
trol. Aliquots of enzymes (10 μg) were added in 12-h intervals, and after 48 h of incu-

bation at 37 °C, the digests were subjected to reverse phase HPLC on an 
analytical C-18 column (4 x 250 mm, 5-μm particle size, Vydac, His-
peria, CA). Radioactivity was monitored by γ-counting of 1-ml fractions. For de-

constructive digestion the double-labeled V8 and Asp-N digest fragment 

were assessed by SDS-PAGE as described (32).

Molecular Modeling—A homology model of the T1 TCR and the 

Kd α1

Kd α2

wt

ES2A

G65A

S69A

Q72A

R75A

G149A

E154A

Y155A

E163A

E166A

Fig. 2. Effect of Kd mutations on T1 TCR photoaffinity labeling. Soluble Kd molecules containing single alanine substitutions in the 
indicated position were photo-cross-linked with 125IASA-YIPSAEK(ABA)I, and their ability to photoaffinity-label T1 TCR was assessed as 
described for Fig. 2. Each experiment was performed 2–4 times.

Effect of Kd Mutations on T1 TCR Photoaffinity Labeling—To define the interaction of ABA with T1 TCR, PbCS(ABA) 
variants with modified ABA were examined. These nonphoto-
reactive compounds were assessed in a cytolytic assay as derivatives of SYIPSAEK(ABA)I (Fig. 1B). Cloned T1 CTL killed 
target cells sensitized with SYIPSAEK(benzoic acid)I approximately 100-fold less efficiently than those sensitized with SY-
IPSAEK(ABA)I. Replacement of the phénylazole by a methyl 

V123, J32; HLA-A2-Tax peptide complex (21), TCR 2C (Vα3, Jα58; 

Vβ8.2, Jβ2.4) (22, 23), TCR 14.3 β-chain (Vβ8.2, Jβ2.1) (34), TCR 1934.4 

Va (Vα2) (35), and H2-Kβ (3). The related sequences of corresponding 
chains were aligned using a dynamic programming method imple-
mented in the MODELLER program (33). An all atom model of 
the complex was built using MODELLER by satisfaction of spatial re-

straints obtained from the alignment and parameters in the program. 
A distance restraint was introduced initially between the phenyl rings of 

ABA and β-Tyr and β-Tyr , respectively. Side chain orientations 

were optimized using a backbone-dependent rotamer library (36, 37). 

CDR1 loops and CDR2 loops were not subsequently refined, since their 

conformation was modeled from the TCR 2C, which has the same Vβ8.2 
as TCR T1. For the other CDR loops, the conformations with the low 

energies were identified by simulated annealing with the rest of the 
structure fixed. From these, the final loop orientations were selected by 

using data from the mutation experiments. The resulting structure was 
refined with 500 steps of steepest descent energy minimization using 

the CHARMM (version 25) program (38) with the all-atom PARAM 22 

parameter set (39). No significant violation of spatial restraints was 
found for β-Tyr  and β-Tyr , and K/ABA/ after optimization, indicating 
that the imposed distance restraint does not imply a distortion of the 
structure. Details concerning the modeling will be presented separately.2

RESULTS

Effect of PbCS Peptide Derivative Mutations on T1 TCR-
Ligand Binding and Antigen Recognition by T1 CTL—To obtain 
information on PbCS/(ABA) contacts with T1 TCR, several 
PbCS/(ABA) variants were assessed by TCR photoaffinity labeling 
with soluble Kd,125IASA-YIPSAEK(ABA)I complexes (Fig. 1A). 
The replacement of PbCS Pro by Ala, Asp, or Ser re-
tuced T1 TCR labeling by 10-, 100-, and 17-fold, respectively, 
whereas replacement by Leu increased it 2-fold, suggesting 
that voluminous aliphatic residues in this position stabilize, 
and polar ones destabilize, T1 TCR-ligand binding. Alanine 
substitution of PbCS Ser impaired T1 TCR photoaffinity labeling 
by 95%. Substitution of PbCS Glu with alanine or glutamine 
obliterated detectable T1 TCR labeling, indicating that Glu 
forms a polar contact with T1 TCR. Shortening of PbCS Lys by 
one methylene group (YIPSAEOrn(ABA)I) also abolished T1 TCR labeling, indicating that the full spacer 
length was required.

To define the interaction of ABA with T1 TCR, PbCS/(ABA) 
variants with modified ABA were examined. These nonphoto-
reactive compounds were assessed in a cytolytic assay as derivatives of SYIPSAEK(ABA)I (Fig. 1B). Cloned T1 CTL killed 
target cells sensitized with SYIPSAEK(benzoic acid)I approximately 100-fold less efficiently than those sensitized with SY-
IPSAEK(ABA)I. Replacement of the phénylazole by a methyl 

group (SYIPSAEK(ABA)I) abolished detectable antigen recog-
nition, while introduction of an iodine and hydroxy substituent in 

ABA (YIPSAEK(ABA)I) reduced the efficiency of antigen recognition by 8-fold. These results indicate that the phényl-
azole of the ABA moiety was essential for antigen recognition 
and that changes of substituents predictably affected the effi-
ciency of recognition.

Effect of Kd Mutations on T1 TCR Photoaffinity Labeling—To identify Kd-TCR contacts, we prepared soluble Kd and 12 Kd 
mutants containing single alanine substitutions on the surface of the α1 or α2 helices (Fig. 2). After photo-cross-linking with 
radiolabeled 125IASA-YIPSAEK(ABA)I, TCR-ligand binding 
was assessed by T1 TCR photoaffinity labeling, as described above. Six of the Kd mutations impaired T1 TCR-ligand binding 
by ≥50%. Two were on Kdα1 (E62A and Q72A), and four were on 
Kdα2 (Q149A, D152A, Y155A, and E166A). Some Kd mutations 
increased T1 TCR photoaffinity labeling by up to 20% (S69A, R79A, and E163A).

Effect of T1 TCR Mutations on T1 TCR Photoaffinity Label-
ning—To define ligand contact residues, soluble T1 TCR and 31 
mutants were prepared and tested by T1 TCR photoaffinity labeling with soluble Kd,125IASA-YIPSAEK(ABA)I (Fig. 3). 
Seven of these mutations reduced TCR photoaffinity labeling by ≥90%. Three of these were in CDR3 loops, three others were 

in CDR1, and one was in CDR2α. In addition, six other mutations 
reduced T1 TCR photoaffinity labeling by ≥50%. Four of 
these were in CDR2α, one was in CDR2β, and one was in CDR3α. 
Several mutations increased TCR photoaffinity labeling by up to 60% (e.g. αN27A, βY50F, and βT55A). Mutants 

labeled with an asterisk were T1 TCR Fv single chain con-
structs (29).

Localization of the Photoaffinity-labeled Site(s) on T1 
TCR—To localize the photoaffinity-labeled site(s), T1 TCR was 

2 O. Michielin and M. Karplus, manuscript in preparation.
photoaffinity-labeled with SYIPSAEK\(_{125}\)IASA\(_{YIPSAEK}ABA\_I\), a derivative that was efficiently recognized by T1 CTL (Fig. 1B) and, being monovalent, precluded cross-linking with \(K_d\). Since T1 TCR was photoaffinity-labeled exclusively at the \(\beta\)-chain (15), the photoaffinity-labeled TCR was directly subjected to peptide mapping. After extensive digestion with trypsin, the resulting digest fragments were separated by reverse phase HPLC. The major labeled digest fragment eluted from the C-18 column after 30 min and according to SDS-PAGE was homogenous and had an apparent \(M_r\) of approximately 3000 (Fig. 4, lane 2).

When protease V8 was used instead of trypsin, a major labeled material eluted from the column after 32–33 min, which migrated on SDS-PAGE with an apparent \(M_r\) of approximately 8300 (Fig. 4, lane 1). The size of this fragment was bigger than any theoretical V8 fragment of the variable domain of the T1 TCR \(\beta\)-chain (Fig. 5B), suggesting that protease V8, even after extensive digestion, omitted a cleavage site. Since this enzyme primarily cleaves C-terminal to Glu and hence may fail to cleave after Asp (31), the labeled V8 digest product was digested with protease Asp-N. Essentially the same HPLC profile was observed; however, on SDS-PAGE this material migrated with an apparent \(M_r\) of approximately 2000 (Fig. 4, lane 3), indicating that V8 failed to cleave at an aspartic acid. This residue probably was \(\beta\)-Asp\(_{38}\), because the big reduction in size (approximately 6300 Da) may correspond to the size of the segment 2–37, which contains the glycosylation site \(\beta\)-Asn\(_{24}\) (Fig. 5B and Ref. 34). Accordingly, the labeled V-8 digest fragment would be 2–56 (Fig. 5B).

To verify this, it was treated with CNBr, which cleaves at methionine residues. This resulted in the formation of a new labeled fragment, which eluted from the C18 column after 34–35 min and migrated on SDS-PAGE with an apparent \(M_r\) of approximately 2500 Da (Fig. 4, lane 4). Since the variable domain of the T1 TCR \(\beta\)-chain contains only one methionine, this confirmed that the labeled V-8 digest fragment consisted of residues 2–56 (Fig. 5B). The observed shift in \(M_r\) suggested that the labeled site(s) was located C-terminal to \(\beta\)-Met\(_{32}\), i.e. in the segment 33–56. Moreover, the predicted labeled V8

---

**Fig. 3. Effect of T1 TCR mutations on TCR photoaffinity labeling.** Soluble T1 TCR comprising the variable and constant domains of both chains and mutants containing single alanine substitutions in the indicated positions were incubated with soluble covalent K\(^{125}\)IASA-YIPSAEKABA\_I complexes at 0–4 °C for 30 min. After UV irradiation, TCR photoaffinity labeling was assessed as described for Fig. 2. The mutants labeled with an asterisk were single chain Fv T1 TCR constructs. The mean values and S.D. values were calculated from two experiments.
digest fragment contains three arginine residues (Arg³, Arg¹⁶, and Arg⁴⁴). To assess their presence, it was partially digested (6 h) with Arg-C. As assessed by HPLC, this treatment produced earlier eluting radioactive materials, probably of mixed composition, as suggested by the broad range of elution (29–31 min). SDS-PAGE analysis of fractions 29–31 showed three new labeled species, migrating with apparent Mₚ of approximately 1800, 2600, and 7000, respectively, with the smaller ones eluting earlier from the HPLC column (Fig. 4, lanes 5–7). Upon extensive digestion with Arg-C, only the smallest fragment was observed (data not shown). These findings confirm that the labeled primary V8 digest fragment was residues 2–56 and suggest that the labeled site was contained in the segment 45–56.

To substantiate this, we tested whether the labeled digest fragment resulting after digestion with V8 and Asp-N contained an arginine, by extensively digesting it with Arg-C. This treatment resulted in a new labeled fragment that according to HPLC and SDS-PAGE was indistinguishable from the smallest fragment obtained after digestion of the labeled V8 digest fragment with Arg-C (Fig. 4, lane 8). By contrast, no change was observed when protease Lys-C was used (data not shown). Together, these findings demonstrate that the photoaffinity-labeled site(s) was contained in the uncharged sequence 45–56 (LIHYSYGAGSTE).

T1 TCR Photoaffinity Labeling Involved Tyrosine Residues—Because this sequence contains two tyrosines and ABA and IASA have low, but measurable affinity for tyrosine (14, 15), we examined whether the T1 TCR photoaffinity labeling involved tyrosine. To this end, T1 TCR was biosynthetically labeled with [³H]tyrosine and photoaffinity-labeled with Kd-SYIPSAEK(125IASA)I. Following digestion with V8 and Asp-N, HPLC showed the same ¹²⁵I profile as observed previously and various ³H-labeled materials (Fig. 6A). The ¹²⁵I-labeled material was extensively digested with cathepsin C and carboxypeptidase P. HPLC showed that the major ¹²⁵I-labeled material also contained ³H (Fig. 6B). On SDS-PAGE, this material migrated at the gel front, i.e. had a Mₚ of less than 1000–1200, confirming extensive, probably complete degradation (data not shown). Tyrosine and monoiodotyrosine eluted from the HPLC column in fractions 6, 7, and 19, respectively, i.e. both earlier than the double-labeled digest fragment (Fig. 6C). These results indicate that the T1 TCR 𝛽-chain was photoaffinity-labeled at tyrosine residue(s) and demonstrate that this tyrosine modification was not a UV irradiation mediated trans-iodination.

Model of the T1 TCR-Kd-SYIPSAEK(ABA)I Complex—To evaluate our data in structural terms, we built a model of the T1 TCR-Kd-SYIPSAEK(ABA)I complex. Accurate homology modeling of the T1 TCR and Kd was made possible by the

![Fig. 4. Localization of photoaffinity-labeled site(s) on T1 TCR 𝛽-chain.](http://www.jbc.org/)

**Fig. 4.** Localization of photoaffinity-labeled site(s) on T1 TCR 𝛽-chain. T1 TCR was photoaffinity labeled with soluble Kd-SYIPSAEK(125IASA)I and digested with different proteases. The resulting fragments were separated by C-18 reverse phase HPLC, and the ¹²⁵I-containing materials were analyzed by SDS-PAGE. Lane 1, V8 digest; lane 2, trypsic digest; lane 3, digest with V8 and Asp-N; lane 4, digest with V8 and CNBr cleavage; lanes 5, 6, and 7, HPLC fractions 31, 30, and 29, respectively, of V8 and Arg-C digest; lane 8, digest with V8, Asp-N, and Arg-C. Representative experiments are shown. Each experiment was repeated 1–5 times.

![Fig. 5. Amino acid sequence of T1 TCR 𝛼- and 𝛽-chain.](http://www.jbc.org/)

**Fig. 5.** Amino acid sequence of T1 TCR 𝛼- and 𝛽-chain. For the 𝛽-chain, which contains the labeled site(s), the cleavage sites for protease V8 (E and D) and for trypsin (R and K) are marked. Cysteines and methionines are printed in boldface type. The digest fragment 45–56 containing the photoaffinity-labeled site(s) is shown in gray. For both TCR chains, the CDR sequences are shown in boxes, and the residues subjected to mutational analysis are marked by triangles; open triangles indicate residues sensitive to mutation. The constant domains on both TCR chains start at position 112.
availability of crystal coordinates of closely related TCR and Kb
(TCR 2C and TCR 14.3 express V\text{\textsubscript{b}}\text{8.2 as TCR T1 and TCR}
1934.4 expresses a V\text{\textsubscript{a}} of the same subfamily). The C\text{\textsubscript{a}} root
mean square deviation between our model and (i) the V\text{\textsubscript{b}} of the
2C TCR was 0.35 Å, (ii) the V\text{\textsubscript{a}} of the 1934.4 TCR was 1.68 Å,
(iii) the variable domain of K\text{\textsubscript{b}} was 0.53 Å, and (iv) the A6
TCR-HLA-A2-Tax peptide complexes was 1.83 Å (CDR3 includ-
ated). The diagonal orientation of the T1 TCR relative to the
Kd-PbCS(ABA) complex and the positioning of the CDR loops
resulted in a pocket between CDR1\text{\textsubscript{b}}, CDR2\text{\textsubscript{b}}, and CDR3\text{\textsubscript{a}}. The
bottom of the pocket is formed by
\alpha-Arg99 and \beta-Glu95, one side
by CDR3\text{\textsubscript{a}} residues Asn\text{\textsubscript{96}} and Asn\text{\textsubscript{97}} and the other side by
CDR2\beta residues Tyr\text{\textsubscript{48}}, Tyr\text{\textsubscript{50}}, and Asn\text{\textsubscript{31}} of CDR1\beta (Fig. 7). The
Lys\text{\textsubscript{259}}(ABA) side chain was orientated such that it inserts into
this cavity between the side chains of \beta-Tyr\text{\textsubscript{48}} and \beta-Tyr\text{\textsubscript{50}}. The
first two methylene groups of K259(ABA) have van der Waals
contacts with K\text{\textsubscript{d}} Trp\text{\textsubscript{73}} and the ABA carbonyl forms a hydro-
gen bond with its indol nitrogen. These interactions restrict
the mobility of the K(ABA) side chain and keep it in a slanted
orientation.

The phenyl rings of \beta-Tyr\text{\textsubscript{48}}, \beta-Tyr\text{\textsubscript{50}}, and ABA are nearly
parallel and equally spaced at a distance of approximately 3.0
Å. This is in agreement with the finding that \beta-Tyr\text{\textsubscript{48}} and/or
\beta-Tyr\text{\textsubscript{50}} were photoaffinity-labeled sites (Figs. 4 and 6). Five
other TCR residues, \alpha-Asn\text{\textsubscript{96}}, \alpha-Asn\text{\textsubscript{97}, \alpha-Arg\text{\textsubscript{99}}, \beta-Asn\text{\textsubscript{31}, and
\beta-Glu\text{\textsubscript{95}, also interact with the ABA moiety. The \delta-amide nitro-
gen (ND2) of \alpha-Asn\text{\textsubscript{96}} forms a hydrogen bond to the ABA car-
bony l OF (see Table I for atom labels); the Ca of \alpha-Asn\text{\textsubscript{97}} makes
a van der Waals contact with its CE2; OD1 of \beta-Asn\text{\textsubscript{31}} makes a
polar interaction of the C-H...X type with its CD1 and CE1
and OE1 of \beta-Glu\text{\textsubscript{95}}, a weak hydrogen bond between the OH group of \beta-Tyr\text{\textsubscript{50}} and ABA CE2
as well as a polar contact between the OH group of \beta-Tyr\text{\textsubscript{48}} and
N2 of ABA. Finally, there is a hydrogen bond between NH1 and
NH2 of \alpha-Arg\text{\textsubscript{99}} and N3 of ABA. These interactions are consist-
ent with the data from the mutational analysis (Figs. 1–3),
although there was no quantitative evaluation of the free en-
ergies involved.

The K\text{\textsuperscript{d}}-bound peptide runs diagonally between the CDR3
loops, and its termini are located underneath the CDR1 loops

Fig. 6. T1 TCR is photoaffinity-la-
beled at tyrosine residue(s). A, soluble
T1 TCR, biosynthetically labeled with
[\textsuperscript{3}H]tyrosine, was photoaffinity-labeled
with soluble K\text{\textsuperscript{d}}-SYIPSAEK(125IASA)i.
Following extensive digestion with prote-
ase V8 and Asp-N, the digest fragments
were separated by C-18 reverse phase
HPLC, and 0.75-ml fractions were
counted for \textsuperscript{125}I (diamonds) and \textsuperscript{3}H
(squares). B, the major \textsuperscript{125}I-labeled mate-
rial (fractions 42–44) was treated with
cathepsin C and carboxypeptidase P, and
the resulting fragments were analyzed
likewise. The \textsuperscript{125}I spill-over was sub-
tracted from the \textsuperscript{3}H cpm. C, as references,
tyrosine (squares) and iodotyrosine (dia-
monds) were subjected to HPLC, and the
OD was measured at 275 nm.
FIG. 7. Molecular modeling of the T1 TCR-K\textsuperscript{d}-SYIPSAEK(ABA)I complex. A, top view of the surface of the T1 TCR ligand binding site with bound SYIPSAEK(ABA)I in the bound state (\(\alpha\)-chain on the left and \(\beta\)-chain on the right). Peptide residues are shown in yellow, surface-exposed acidic TCR residues are shown in red, and basic ones are shown in blue. The Ca backbones of the underlying CDR1 loops are shown in light yellow, those of the CDR2 loops are shown in light green, and those of the CDR3 loops are shown in purple. B, stereo view of K259(ABA) (green) and
T Cell Recognition of Hapten

TABLE I

T1 TCR-ligand contacts predicted by model

<table>
<thead>
<tr>
<th>Contact No.</th>
<th>Location</th>
<th>TCR</th>
<th>Ligand</th>
<th>Distancea</th>
<th>Inhibitionb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CDR1α</td>
<td>Thr26</td>
<td>OGl1</td>
<td>Kd Glu58</td>
<td>OE1</td>
</tr>
<tr>
<td>2</td>
<td>CDR1α</td>
<td>Thr27</td>
<td>OGl1d</td>
<td>Kd Glu58</td>
<td>OE1</td>
</tr>
<tr>
<td>3</td>
<td>CDR1α</td>
<td>Tyr29</td>
<td>OHδ</td>
<td>Kd Glu58</td>
<td>OE1</td>
</tr>
<tr>
<td>4</td>
<td>CDR2α</td>
<td>Lys48</td>
<td>NZδ</td>
<td>Kd Glu154</td>
<td>OE1</td>
</tr>
<tr>
<td>5</td>
<td>CDR2α</td>
<td>Lys48</td>
<td>NZδ</td>
<td>Kd Tyr153</td>
<td>OH</td>
</tr>
<tr>
<td>6</td>
<td>CDR2α</td>
<td>Thr31</td>
<td>OGl1d</td>
<td>Kd Ala158</td>
<td>CB</td>
</tr>
<tr>
<td>7</td>
<td>CDR2α</td>
<td>Asn33</td>
<td>ND2</td>
<td>Kd Glu166</td>
<td>OE2</td>
</tr>
<tr>
<td>8</td>
<td>CDR3α</td>
<td>Arg34</td>
<td>O</td>
<td>PbCS Pro55</td>
<td>CG</td>
</tr>
<tr>
<td>9</td>
<td>CDR3α</td>
<td>Gly56</td>
<td>CA</td>
<td>PbCS Pro55</td>
<td>CB</td>
</tr>
<tr>
<td>10</td>
<td>CDR3α</td>
<td>Asn56</td>
<td>ND2</td>
<td>ABA*</td>
<td>OF</td>
</tr>
<tr>
<td>11</td>
<td>CDR3α</td>
<td>Asn56</td>
<td>N</td>
<td>PbCS Pro55</td>
<td>O</td>
</tr>
<tr>
<td>12</td>
<td>CDR3α</td>
<td>Asn56</td>
<td>CA</td>
<td>ABA</td>
<td>CE2</td>
</tr>
<tr>
<td>13</td>
<td>CDR3α</td>
<td>Arg56</td>
<td>NH1</td>
<td>ABA</td>
<td>N3</td>
</tr>
<tr>
<td>14</td>
<td>CDR3α</td>
<td>Arg56</td>
<td>NH2</td>
<td>ABA</td>
<td>N3</td>
</tr>
<tr>
<td>15</td>
<td>βN terminus</td>
<td>Glu1</td>
<td>OE2</td>
<td>Kd Gln149</td>
<td>NE2</td>
</tr>
<tr>
<td>16</td>
<td>CDR1β</td>
<td>Asn31</td>
<td>OD1</td>
<td>ABA</td>
<td>CE1</td>
</tr>
<tr>
<td>17</td>
<td>CDR1β</td>
<td>Asn31</td>
<td>OD1</td>
<td>ABA</td>
<td>CD1</td>
</tr>
<tr>
<td>18</td>
<td>CDR2β</td>
<td>Tyr49</td>
<td>OH</td>
<td>ABA</td>
<td>N2</td>
</tr>
<tr>
<td>19</td>
<td>CDR2β</td>
<td>Tyr49</td>
<td>OH</td>
<td>ABA</td>
<td>CE2</td>
</tr>
<tr>
<td>20</td>
<td>CDR2β</td>
<td>Tyr49</td>
<td>OH</td>
<td>ABA</td>
<td>CE2</td>
</tr>
<tr>
<td>21</td>
<td>CDR3β</td>
<td>Glu52</td>
<td>OE1</td>
<td>ABA</td>
<td>N2</td>
</tr>
<tr>
<td>22</td>
<td>CDR3β</td>
<td>Glu52</td>
<td>OE2</td>
<td>PbCS Glu158</td>
<td>OE1</td>
</tr>
<tr>
<td>23</td>
<td>CDR3β</td>
<td>Glu52</td>
<td>N</td>
<td>PbCS Glu158</td>
<td>OE1</td>
</tr>
<tr>
<td>24</td>
<td>CDR3β</td>
<td>Glu52</td>
<td>NE2</td>
<td>PbCS Glu158</td>
<td>OE1</td>
</tr>
<tr>
<td>25</td>
<td>CDR3β</td>
<td>Glu52</td>
<td>NE2</td>
<td>PbCS Glu158</td>
<td>OE1</td>
</tr>
<tr>
<td>26</td>
<td>CDR3β</td>
<td>Ser58</td>
<td>OG</td>
<td>Kd Tyr155</td>
<td>OH</td>
</tr>
<tr>
<td>27</td>
<td>CDR3β</td>
<td>Ser58</td>
<td>OG</td>
<td>Kd Tyr155</td>
<td>OH</td>
</tr>
</tbody>
</table>

a Asterisks denote hydrogen bonds; no asterisk signifies van der Waals or polar contacts.

b As observed for A6 TCR (21).
c As observed for 2C TCR (23).
d As observed for 2C TCR (23).

c,d Nonstandard atom designation used for ABA:

![Diagram of T Cell Recognition of Hapten](image)

According to the model, residues Kd Glu58, Glu62, Glu72, Gln149, Asp152, Glu154, Ala158, Tyr155, Tyr159, and Glu166 form hydrogen bonds with T1 TCR (Table I). Alanine substitution of the underlined residues significantly reduced T1 TCR photoaffinity labeling (Fig. 2). The predicted contacts with Kd Glu58 and Glu62 are in accordance with TCR mutational analysis (Fig. 3). TCR-MHC contacts 1, 6, and 7 (Table I) were also observed in the A6 TCR-HLA-A2-Tax complex (21), and the contacts 2–4 and 6 were observed in the 2C TCR-Kb-dEV8 complex (23). According to the model, the PbCS residues Pro255, Ser256, Glu258, and Lys259(ABA) interact with T1 TCR (Table I). Indeed, mutation of these residues, especially of Glu258 and Lys259(ABA), substantially impaired T1 TCR photoaffinity labeling or antigen recognition by T1 CTL (Fig. 1). For Pro255, the model shows that the upper part of the nonpolar proline ring makes a van der Waals contact with the methylene group of α-Gly255 and a hydrogen bond with O of α-Arg94, which is consistent with the observation that replacement of Pro255 with Ala, Ser, Asn, or Asp decreased T1 TCR photoaffinity labeling (Fig. 1). For PbCS Ser256, the model predicts a hydrogen bond between its OH group and OD1 of αN96, and for PbCS Glu258 contacts with β-Gln97 and Kd Lys146. Alanine substitution of β-Gln97 dramatically reduced T1 TCR photoaffinity labeling (Fig. 3) and of Kd Lys146 peptide binding to Kd (data not shown), since this residue also interacts with the C-terminal carboxyl group of the peptide (3, 4).

**DISCUSSION**

The present study uses peptide mapping, mutational analysis, and molecular modeling to describe in structural terms how TCR can avidly and specifically bind a hapten-modified peptide in the context of a MHC class I molecule. The hapten, photo-reactive ABA, conjugated at the penultimate residue of the PbCS peptide SYIPSAEKI, constituted an essential part of the epitope recognized by T1 CTL (Figs. 1 and 2). The fact that peptide mapping allowed localization of the photoaffinity-labeled site(s) on T1 TCR (Figs. 4 and 6) indicates that this labeling was site-specific. In view of the high chemical reactivity of the radicals produced by UV irradiation (14, 15) of phenylazides and the flexible nature of the lysine side chain, this implies that the ABA group associated with the T1 TCR in a well defined orientation. If this were not so, heterogeneous photo-cross-linking would occur to preclude reproducible and resolved peptide mapping (40).

Contacting T1 TCR residues. CDR3α residues are shown in light blue, CDR2β residues are shown in dark yellow, CDR1β and CDR3β residues are shown in white, and Kd Trp73 is shown in light yellow. The dotted lines indicate hydrogen bonds. The images were produced with the MOLMOL program (42).
To better understand in structural terms the interaction of the photoactive ligand side chain with the T1 TCR, we modeled the T1 TCR-I\(^3\)-SIPS\(\text{A}\)E\(\text{K}\)A\(\text{B}\)\(\text{A}\)I complex. While available crystal coordinates of related TCR and MHC class I molecules permitted homology modeling of most of the system with good accuracy, the main problems concerned the docking the TCR to the ligand and the positioning of certain CDR loops. The docking was based on the diagonal orientation observed for all TCR-ligand complexes whose three-dimensional structures have been elucidated (21, 23–25). This orientation involves conserved TCR-MHC class I contacts, mainly between TCR V\(\alpha\) and residues of the MHC a1 and a2 helices (21, 23, 24). Our model is in accordance with these contacts. Moreover, we made use of the interaction of ABA with \(\beta\)-Tyr\(^{48}\) and \(\beta\)-Tyr\(^{50}\), as indicated by the observations that (i) the T1 TCR was photoaffinity-labeled at tyrosines of the CDR2\(\beta\) segment 45–56 (LI-HYSYGAGSTET) (Figs. 4 and 6); (ii) TCR of the same specificity, which expressed \(\beta\)-Tyr\(^{48}\), but not \(\beta\)-Tyr\(^{50}\), were photoaffinity-labeled also at the a-chain (15); (iii) ABA and IASA have low affinities for Tyr and Trp (14); and (iv) TCR-ligand binding, as assessed by inhibition of T1 TCR photoaffinity labeling by the soluble Fv T1 TCR, correlated with TCR photoaffinity labeling, except for the mutants \(\beta\)Y48F and \(\beta\)Y50F.\(^3\)

According to the model, ABA inserts between the side chains of \(\beta\)-Tyr\(^{48}\) and \(\beta\)-Tyr\(^{50}\), which are part of a pocket between CDR2\(\beta\) and CDR3\(\alpha\) (Fig. 7). This position is energetically favorable and provides extensive \(\pi\)-\(\pi\) interactions and hydrogen bond formation (Table I). The ABA group was placed initially with a constraining potential between these tyrosine side chains, but it moved very little in subsequent energy minimization. The model shows that nine contacts, involving seven different TCR residues, further stabilize the interaction of the Lys(ABA) side chain with T1 TCR (Table I). This is a consequence of a specific three-dimensional arrangement of CDR3\(\alpha\), CDR3\(\beta\), CDR2\(\beta\), and CDR1\(\beta\) residues around the Lys(ABA) side chain. Mutation of these TCR residues, as well as modification of the Lys(ABA) side chain significantly affected T1 TCR photoaffinity labeling and antigen recognition by T1 CTL (Figs. 1 and 3). Similarly, TCR specific for peptides conjugated with benzozarsonate or fluorescein also utilize residues from different CDR loops for hapten binding (6, 41).

While our model is in good agreement with most of the mutational data, it failed to account for the effects of three TCR mutations (aV49A, aT50A, and \(\beta\)E56A) (Fig. 3 and Table I). Possible explanations include the following. (i) Certain mutations may result in conformational changes of CDR loops (e.g. the side chain of a-Val\(^{49}\)) is in the center of CDR2\(\alpha\) and forms various contacts with the peptide backbone; therefore, the aV49A mutation probably affects the CDR2\(\alpha\) loop conformation). (ii) Some mutations may affect intramolecular amino acid interactions, resulting in reorientation of side chains engaged in TCR-ligand contacts (e.g. \(\beta\)-Glu\(^{26}\)) forms a hydrogen bond with \(\alpha\)-Arg\(^{27}\), which interacts with N3 of ABA; thus, the \(\beta\)E56A mutation may be explained by reorientation of the \(\alpha\)-Arg\(^{27}\) side chain). (iii) Water was not included in the model, which may account for some of these divergences.

Interestingly, the majority of CTL clones obtained from mice immunized with IASA-YIK(ABA)EK(A)I expressed V\(\beta\)1- and J\(\alpha\)T2A28-encoded TCR, which were photoaffinity-labeled at both chains (15). For one of them, the photoaffinity labeled sites were identified as J\(\alpha\)T2A28-encoded tryptophan 97 and a residue of the V\(\beta\)1 segment 46–51, which contains \(\beta\)-Tyr\(^{48}\), but not \(\beta\)-Tyr\(^{50}\) (15). It thus appears that for these TCR, the ABA group was inserted between \(\alpha\)-Trp\(^{97}\) (CDR3\(\alpha\)) and \(\beta\)-Trp\(^{48}\) (CDR2\(\beta\)), rather than two V\(\beta\) encoded tyrosines.

Although the interaction of ABA with T1 TCR constituted an important part of T1 TCR-ligand binding, free peptide derivative, even at high concentration (10 \(\mu\)M), was unable to photoaffinity-label T1 TCR.\(^3\) However, it has been reported that in other systems hapten in polymeric form or hapten-conjugated peptides at high concentrations can directly bind to TCR, i.e. that some hapten-reactive T cells may have promiscuous MHC restriction (7, 10). This is reminiscent of antibodies that can strongly bind small organic molecules. As shown by x-ray crystallography, such antibodies utilize residues from different CDR loops (19, 20). Comparison of the three-dimensional structures of the complexes of fluorescein with a monoclonal anti-fluorescein antibody and a fluorescein-specific TCR showed that both molecules use very similar principles in binding hapten (19, 41). Although TCR specific for hapten-modified peptides are unlikely to achieve the high affinities by which antibodies can bind hapten, they may reach higher affinities than TCR specific for conventional peptides, since cells expressing such high affinity TCR are prone to be eliminated by negative selection.

The present study suggests that TCR are able to bind specifically hapten-conjugated peptides by selection of particular V\(\beta\) and CDR3\(\alpha\) sequences. From the data and the model, V\(\beta\) encoded residues of CDR1 and mainly CDR2 play a key role in binding the Lys(ABA) side chain; however, residues of CDR3\(\beta\) and CDR3\(\alpha\) spatially and electronically complement these interactions to an integral and sophisticated binding mode (Fig. 7, Table I). We suggest that this binding principle has universal aspects, i.e. that TCR expressing selected V\(\beta\) and CDR3\(\alpha\) sequences are able to specifically bind hapten conjugated at the penultimate residue of MHC binding peptides by means of a pocket between CDR2\(\beta\) and CDR3\(\alpha\). As far as is known, and presumably for reasons of chemical reactivity, most “hapten-reactive” TCR recognize antigenic peptides containing a hapten-conjugated lysine (5, 11, 12). The long and flexible nature of the lysine side chain enables hapten to bind in this cavity, in the framework of the canonical diagonal orientation of TCR-ligand binding.

We and others have previously observed that CTL can also be readily elicited and specifically recognize hapten conjugated in position 4 or 5 of the peptide (11, 13). In a previous study, we mapped the photoaffinity labeled site(s) for a TCR specific for IASA-YIK(ABA)EK(A)I (13). The labeled site(s) was located in the V\(\alpha\)-encoded C-strand segment 33–39, and computer modeling suggested that the Lys(ABA) side chain is inserted into a pocket between the two CDR3 loops (13). Three-dimensional structure analysis showed that TCR indeed have a cavity between the CDR3 loops and that it can accommodate side chains of MHC-bound peptides (21, 22, 24).

These results provide significant insights into the structural basis of T cell recognition of hapten-conjugated MHC binding peptides. TCR expressing selected V\(\beta\) and CDR3\(\alpha\) sequences can form cavities between CDR loops that can accommodate peptide-conjugated hapten in a highly specific manner (Fig. 7 and Refs. 13 and 15). The structural variability required for this is based mainly on junctional diversity, but V-encoded TCR sequences, in some cases even framework residues, play a role as well. This is consistent with the observation that hapten-reactive T cells typically express different TCR sequences than those recognizing the parental epitope (13–15).

Acknowledgments—We thank Drs. E. Bernasconi and M. Jordan for excellent technical assistance; Drs. D. Kuznetsov and V. Jongeneel for computational work; Drs. R. Stote, D. York, and X. Lopez for aid in determining force field parameters for the ABA group; and J. Muller and K. Roy for preparing the manuscript.
Additions and Corrections


T cell recognition of hapten. Anatomy of T cell receptor binding of a H-2K\textsuperscript{d}-associated photoreactive peptide derivative.

Benedikt Kessler, Olivier Michielin, Christopher L. Blanchard, Irina Apostolou, Christiane Delarbre, Gabriel Gachelin, Claude Grégoire, Bernard Malissen, Jean-Charles Cerotini, Florian Wurm, Martin Karplus, and Immanuel F. Leuscher

Page 3629, Table I: Table I is missing a line between contact 11 and 12. Also, contact 23 is a hydrogen bond and should have an asterisk in the “Distance” column. The correct table is printed below.

<table>
<thead>
<tr>
<th>Contact No.</th>
<th>Location</th>
<th>TCR</th>
<th>Ligand</th>
<th>Distance(^a)</th>
<th>Inhibition(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CDR1(\alpha)</td>
<td>Thr\textsuperscript{26}</td>
<td>OG\textsubscript{1}'</td>
<td>K\textsuperscript{2} Glu\textsuperscript{28}</td>
<td>OE1</td>
</tr>
<tr>
<td>2</td>
<td>CDR1(\alpha)</td>
<td>Thr\textsuperscript{27}</td>
<td>OG\textsubscript{1}'</td>
<td>K\textsuperscript{2} Glu\textsuperscript{28}</td>
<td>OE1</td>
</tr>
<tr>
<td>3</td>
<td>CDR1(\alpha)</td>
<td>Tyr\textsuperscript{29}</td>
<td>OE\textsubscript{2}</td>
<td>K\textsuperscript{2} Glu\textsuperscript{52}</td>
<td>OE1</td>
</tr>
<tr>
<td>4</td>
<td>CDR2(\alpha)</td>
<td>Lys\textsuperscript{48}</td>
<td>NZ(^d)</td>
<td>K\textsuperscript{2} Glu\textsuperscript{45}</td>
<td>OE1</td>
</tr>
<tr>
<td>5</td>
<td>CDR2(\alpha)</td>
<td>Lys\textsuperscript{48}</td>
<td>NZ</td>
<td>K\textsuperscript{4} Tyr\textsuperscript{45}</td>
<td>OH</td>
</tr>
<tr>
<td>6</td>
<td>CDR2(\alpha)</td>
<td>Thr\textsuperscript{21}</td>
<td>OG\textsubscript{1}'(^d)</td>
<td>K\textsuperscript{2} Ala\textsuperscript{148}</td>
<td>CB</td>
</tr>
<tr>
<td>7</td>
<td>CDR2(\alpha)</td>
<td>Asn\textsuperscript{53}</td>
<td>N92(^d)</td>
<td>K\textsuperscript{2} Glu\textsuperscript{146}</td>
<td>OE2</td>
</tr>
<tr>
<td>8</td>
<td>CDR3(\alpha)</td>
<td>Arg\textsuperscript{94}</td>
<td>O</td>
<td>PbCS Pro\textsuperscript{255}</td>
<td>CG</td>
</tr>
<tr>
<td>9</td>
<td>CDR3(\alpha)</td>
<td>Gly\textsuperscript{95}</td>
<td>CA</td>
<td>PbCS Pro\textsuperscript{255}</td>
<td>CB</td>
</tr>
<tr>
<td>11</td>
<td>CDR3(\alpha)</td>
<td>Asn\textsuperscript{96}</td>
<td>ND2</td>
<td>ABA(^a)</td>
<td>OF</td>
</tr>
<tr>
<td>13</td>
<td>CDR3(\alpha)</td>
<td>Arg\textsuperscript{99}</td>
<td>NH\textsubscript{1}</td>
<td>ABA</td>
<td>N3</td>
</tr>
<tr>
<td>15</td>
<td>CDR3(\alpha)</td>
<td>Arg\textsuperscript{99}</td>
<td>NH\textsubscript{2}</td>
<td>ABA</td>
<td>N3</td>
</tr>
<tr>
<td>16</td>
<td>CDR1(\beta)</td>
<td>Asn\textsuperscript{31}</td>
<td>OE2</td>
<td>Glu\textsuperscript{149}</td>
<td>NE2</td>
</tr>
<tr>
<td>17</td>
<td>CDR1(\beta)</td>
<td>Asn\textsuperscript{31}</td>
<td>OD1</td>
<td>ABA</td>
<td>CE1</td>
</tr>
<tr>
<td>18</td>
<td>CDR2(\beta)</td>
<td>Tyr\textsuperscript{48}</td>
<td>OH</td>
<td>ABA</td>
<td>N2</td>
</tr>
<tr>
<td>19</td>
<td>CDR2(\beta)</td>
<td>Tyr\textsuperscript{50}</td>
<td>OH</td>
<td>ABA</td>
<td>CE2</td>
</tr>
<tr>
<td>20</td>
<td>CDR2(\beta)</td>
<td>Tyr\textsuperscript{50}</td>
<td>OH</td>
<td>K\textsuperscript{2} Glu\textsuperscript{72}</td>
<td>OE1</td>
</tr>
<tr>
<td>21</td>
<td>CDR3(\beta)</td>
<td>Gln\textsuperscript{97}</td>
<td>OE1</td>
<td>ABA</td>
<td>NZ</td>
</tr>
<tr>
<td>22</td>
<td>CDR3(\beta)</td>
<td>Gln\textsuperscript{97}</td>
<td>NE2</td>
<td>PbCS Glu\textsuperscript{258}</td>
<td>OE1</td>
</tr>
<tr>
<td>23</td>
<td>CDR3(\beta)</td>
<td>Gln\textsuperscript{97}</td>
<td>N</td>
<td>PbCS Glu\textsuperscript{258}</td>
<td>OE1</td>
</tr>
<tr>
<td>24</td>
<td>CDR3(\beta)</td>
<td>Gln\textsuperscript{97}</td>
<td>NE2</td>
<td>K\textsuperscript{2} Asp\textsuperscript{152}</td>
<td>OD1</td>
</tr>
<tr>
<td>25</td>
<td>CDR3(\beta)</td>
<td>Gln\textsuperscript{97}</td>
<td>OE1</td>
<td>K\textsuperscript{2} Asp\textsuperscript{122}</td>
<td>N</td>
</tr>
<tr>
<td>26</td>
<td>CDR3(\beta)</td>
<td>Ser\textsuperscript{98}</td>
<td>OG</td>
<td>K\textsuperscript{2} Tyr\textsuperscript{109}</td>
<td>OH</td>
</tr>
<tr>
<td>27</td>
<td>CDR3(\beta)</td>
<td>Tyr\textsuperscript{99}</td>
<td>OH</td>
<td>K\textsuperscript{2} Tyr\textsuperscript{109}</td>
<td>OH</td>
</tr>
</tbody>
</table>

\(^a\) Asterisks denote hydrogen bonds; no asterisk signifies van der Waals or polar contacts.

\(^b\) Inhibition of T1 TCR photoaffinity labeling upon mutation.

\(^d\) As observed for A6 TCR (21).

\(^d\) As observed for 2C TCR (23).

\(^*\) Nonstandard atom designation used for ABA.

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.
T Cell Recognition of Hapten: ANATOMY OF T CELL RECEPTOR BINDING OF A H-2Kd-ASSOCIATED PHOTOREACTIVE PEPTIDE DERIVATIVE

Benedikt Kessler, Olivier Michielin, Christopher L. Blanchard, Irina Apostolou, Christiane Delarbre, Gabriel Gachelin, Claude Grégoire, Bernard Malissen, Jean-Charles Cerottini, Florian Wurm, Martin Karplus and Immanuel F. Luescher

doi: 10.1074/jbc.274.6.3622

Access the most updated version of this article at http://www.jbc.org/content/274/6/3622

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 41 references, 22 of which can be accessed free at http://www.jbc.org/content/274/6/3622.full.html#ref-list-1