Antibodies recognizing peptide bound to a major histocompatibility complex (MHC) protein usually have a higher affinity for the composite peptide-MHC (pMHC) ligand than T cell receptors (TCR) with the same specificity. Because the solvent-accessible peptide area constitutes only a small portion of the contacting pMHC surface, we hypothesized that the contribution of the MHC moiety to the TCR-pMHC complex stability is limited, ensuring a small increment of the binding energy delivered by the peptide to be distinguishable by the TCR or the peptide-specific antibody. This suggests that the gain in affinity of the antibody-pMHC interaction can be achieved through an increase in the on-rate without a significant change in the off-rate of the interaction. To test the hypothesis, we have analyzed the binding of an ovalbumin peptide (pOV8) and its variants associated with soluble H-2Kb protein to the 25-D1.16 monoclonal antibody and compared it with the binding of the same pMHC complexes to the OT-1 TCR. This comparison revealed a substantially higher on-rate of the antibody-pMHC interaction compared with the TCR-pMHC interaction. In contrast, both the antibody and the TCR-pMHC complexes exhibited comparably fast off-rates. Sequencing of the 25-D1.16 V_H and V_L genes showed that they have very few somatic mutations and those occur mainly in framework regions. We propose that the above features constitute a signature of the recognition of MHC-bound peptide antigens by TCR and TCR-like antibodies, which could explain why the latter are rarely produced in vivo.

*This work was supported by National Institutes of Health Research Grants AI43254 and AI39966 (to Y. S.). 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.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EBI Data Bank with accession number(s) AY704179 and AY704180.

‡ To whom correspondence should be addressed: Dept. of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107. Tel.: 215-503-4530; Fax: 215-923-0249. E-mail: ysykulev@lac.jci.tju.edu.

† The abbreviations used are: TCR, T cell receptor; MHC, major histocompatibility complex; pMHC, complex of antigenic peptide with MHC protein; mAb, monoclonal antibody; V_H, variable domain of antibody heavy chain; V_L, variable domain of antibody light chain; FR, framework regions; CLIP, class II invariant chain-associated peptide; CDR, complementary determining region.

T cell antigen-specific receptors (TCR) must recognize major histocompatibility complex (MHC) protein and at the same time discriminate between many different peptides bound to that MHC. Approximately 80% of the solvent-accessible area of the peptide is buried in the MHC binding groove (1), limiting the amount of energy that a peptide can contribute to the TCR-pMHC interaction. To ensure TCR specificity for the MHC-bound peptide, the amount of binding energy coming from the MHC moiety must be restricted, allowing the peptide contribution of the composite ligand to be distinguishable by the TCR. In accord with this idea, it has been found that only two to three residues of an MHC class I protein mediate critical TCR-MHC contacts (2, 3). In addition, experimentally measured values of the free energy for various TCR-pMHC interactions (4–8) are significantly lower than the free energy of a typical protein-protein interaction (9, 10). However, it has been shown that the TCR intrinsic affinity can be significantly increased without the loss of its specificity (11). The increase in the TCR affinity was mainly the result of the on-rate, not the off-rate, indicating that limited activation energy of the dissociation phase of the TCR-pMHC reaction is required to preserve the peptide specificity. This led us to suggest that, similar to the TCR with enhanced affinity, the apparent increase in the binding energy of pMHC-specific antibodies is achieved through a faster on-rate, without a significant change in the off-rate of the interaction.

To test this hypothesis we analyzed the binding of an ovalbumin peptide, SIINFEKL (pOV8), and its variants with known biological activities (12) in association with soluble Kb protein to the pOV8-Kb-specific monoclonal antibody (mAb) 25-D1.16 (13). Biosensor technology was used to compare the binding parameters with those measured previously (14, 15) for the interaction of the OT-1 TCR with the same set of pMHC complexes. Comparison of the equilibrium and kinetic constants of OT-1 TCR and 25-D1.16 antibody binding parameters revealed that an increase in intrinsic affinity of antibody interactions with peptide-Kb complexes was mainly the result of changes in the on-rate but not the off-rate. We have also found very few somatic mutations in the variable domain of antibody heavy chain (V_H) and light chain (V_L) 25-D1.16 genes encoding this mAb, which appeared to be very similar to the corresponding germ line genes. Based on these data, we suggest that the above features are essential characteristics of the recognition of short peptides bound to MHC proteins by TCR and TCR-like antibodies. This knowledge improves our understanding of the specificity of recognition of pMHC ligands and may be useful for designing pMHC-specific reagents. It may also help to understand why pMHC-specific antibodies are usually selected from combinatorial libraries in vitro but are very rarely elicited in vivo in response to immunization in vivo.

EXPERIMENTAL PROCEDURES

Peptides—Peptides were synthesized using Fmoc (N-(9-fluorenylethoxycarbonyl) chemistry by BioSynthesis (Lewisville, TX). Purity of the peptides was confirmed by high pressure liquid chromatography and mass spectrometric analyses. Peptides used were SIINFEKL, a chicken ovalbumin peptide (257–264) (pOV8), and its variants RGY-
Comparison of TCR with TCR-like Antibody

NYEKL (V-OVA), IJNIIEFKL (E1), SAJNKFEKL (A2), SHIFKFEKL (R4), and SHINPEFEL (D7); RGYVQYL, a vesicular stomatitis virus nucleocapsid protein (52–59) peptide (VSV); FAPGNYPAL, a Sendai virus nucleocapsid (324–332) peptide (SNV); and KVVRFPEKL, a chicken ovalbumin (55–62) peptide (KVDL).

Soluble pOvK Complexes—The pRHa-3 plasmids coding the H-2Kk extracellular domain of the H-2Kk with His-tag at the C-terminal end and mouse β2-microglobulin were kindly provided by Anders Brunmark. These plasmids and a plasmid containing a neomycin resistance gene (Invitrogen) were co-transfected into Schneider cells (28) by calcium phosphate precipitation, and stable transfectants were selected as described previously (16). The cells were expanded in serum-free medium SF-900 II (Invitrogen) and grown to a density of 1.4–2.0 × 10^5 cells/ml. The expression H-2Kk^* had been induced by 1 μg cupric sulfate for 72 h, and soluble H-2Kk^* molecules were isolated from the culture supernatant essentially as described previously (8, 17).

Soluble Fab fragments in this work were loaded with peptide L-ovalbumin (55–62) peptide (KVDL). Typically, 50 μg of peptide in 5 μl of Me2SO was added to 1.2 mg of H2Kk in 250 μl of phosphate-buffered saline, pH 7.4, and the reaction mixture was incubated at room temperature (22–24 °C) overnight. The peptide-MHC complexes were stored in the presence of peptide excess at 4 °C. Gel filtration of the peptide-MHC complexes on a Sephacryl S200 HiPrep 16/60 column (Amersham Biosciences) did not reveal the presence of aggregates in the samples.

Purification of 25-D1.16 Antibody and Fab Fragment—Murine hybridoma 25-D1.16 secreting an IgG1 mAb specific for the pOvK-H-2Kk complex (13) was kindly provided by Drs. Germain and Porgador. The hybridoma was grown in serum-free high glucose Dulbecco’s modified Eagle’s medium’s Ham’s F-12 medium (1:1) supplemented with L-glutamine, sodium pyruvate, non-essential amino acids, L-ascorbic acid, and SPITE (Sigma) (18).

The monoclonal antibody 25-D1.16 was purified from culture supernatant by affinity chromatography on protein G-agarose. Fab fragment was produced by papain digestion and purified on a MonoQ anion exchange column (Amersham Biosciences). The identity of purified Fab fragment was confirmed by SDS-PAGE and enzyme-linked immunosorbent assay with soluble pOvK complex.

Native and Sequencing of mAb 25-D1.16 Heavy and Light Chains—Partial N-terminal amino acid sequences of mAb 25-D1.16 heavy and light chains were determined by Edman degradation on a PROCISE-C LC (Applied Biosystems) sequencer. The heavy chain of the mAb was produced by papain digestion and purified on a MonoQ anion exchange column (Amersham Biosciences). The identity of purified Fab fragment was confirmed by SDS-PAGE and enzyme-linked immunosorbent assay with soluble pOvK complex.

RESULTS

Analysis of V<sub>H</sub> and V<sub>L</sub> Sequences—The nucleotide sequences obtained for the V region genes encoding 25-D1.16 mAb V domains are shown in Fig. 1. Sequence analysis of the V<sub>H</sub> region (Fig. 1A) (GenBank<sup>TM</sup> accession no. AY704180) shows that it shares 98.6% sequence identity with the germ line gene J585.8. Comparison of the two genes revealed four base pair changes, of which two are silent mutations located in the framework regions (FR)-H1 and FR-H3, and two others are located within FR-H3, resulting in the replacement of Thr-77 with Ala and Leu-82 with Val, respectively. The CDR-H3 com-
prises five codons contributed by the D gene segment D-SP2.7 used in reading frame 3 and the JH3 segment (Fig. 1B). No mutations are present in the sequence contributed by D-SP2.7 and JH3. Alignment of the mature VH gene and corresponding germ line gene segments shows four nucleotide insertions at the V-D junction and two nucleotide insertions at the D-J junction. This results in the appearance of Lys-95 and Phe-100A.

Sequence analysis of the VL region showed that it is a /H9260 gene (GenBankTM accession no. AJ231273). D, FR-L4 is compared with the J5 germ line gene (GenBankTM accession no. V00777).

Fig. 1. Nucleotide and encoded amino acid sequences for the variable domain genes of 25-D1.16. The amino acid residue numbering and the CDR limits are according to Kabat et al. (52). Differences between germ line sequences and the corresponding encoded amino acid substitutions are also shown. A, the VH domain is compared with the VH germ line J558.6 gene (GenBankTM accession no. AF309387). B, CDR-H3 and FR-H4 are compared with the DSP2.7 and JH3 segments (accession nos. J00438 and V00770). C, the VH domain is compared with the germ line V33 gene (GenBankTM accession no. AD231273). D, FR-L4 is compared with the J5 germ line gene (GenBankTM accession no. V00777).
CDRs of the 25-D1.16 mAb and the OT-1 TCR—Based on x-ray crystallographic analysis, CDRs of heavy and light chains of Ig molecules can be described in term of known canonical structures (21, 22). The conformation of a particular canonical structure is determined by the length of the loop and the nature of amino acid residues at key positions. This conformation is conserved despite different positioning of the canonical loops relative to the framework regions. The structure of the hyper-variable regions -H1, -H2, -L1, and -L2 of mAb 25-D1.16 (Fig. 2) suggests that they belong to canonical classes 1, 2A, 2B, and 1, respectively (21). The CDR-H3 that is expected to contact MHC-bound peptide is much more variable and is not included in the canonical structure description (22), whereas CDR-L3 belongs to canonical class 1.

The primary structures of the CDRs of the OT-1 and 25-D1.16 mAb are shown in Fig. 2. They appear to be very different, indicating that CDRs with divergent sequences may determine very similar specificities for pMHC binding (see below).

Binding Interactions of the 25-D1.16 mAb to Various Peptide-Kb Complexes—Fig. 3 shows specific binding of the soluble pOV8-Kb complex to the 25-D1.16 mAb immobilized on a biosensor surface. The binding of two irrelevant peptide-Kb complexes containing either Sendai virus peptide (SV9) or peptide from vesicular stomatitis virus was undetectable (not shown).

The values of kinetic and equilibrium (affinity) constants for the 25-D1.16 mAb binding to pOV8-Kb and variant peptide-Kb complexes with defined biological activities measured at 25 °C are summarized in Table I. Because the mAb was immobilized on the biosensor surface, soluble monovalent pOV8-Kb complexes bound to the antibody-binding sites independently, allowing measurements of intrinsic parameters of the interaction. This was confirmed by measuring binding of the antibody monovalent Fab fragment to pOV8-Kb immobilized on the biosensor surface (data not shown).

Derivation of the $k_\text{on}$ and $k_\text{off}$ values using a 1:1 Langmuir binding model demonstrated a good correlation between the experimental and globally fitted data ($\chi^2 = 0.05$). The equilibrium constant $K_\text{eq}$ was calculated from the ratio $k_\text{on}/k_\text{off}$. For a two-step reaction, $K_\text{eq}$ measured from the maximum (plateau) binding response is expected to be different from the $K_\text{eq}$ value measured by the $k_\text{on}/k_\text{off}$ ratio, suggesting a complex mechanism of the reaction (23, 24). Very often, however, the intermediate complex of the reaction is short lived, and the difference between the two $K_\text{eq}$ values is not significant (25).

We did not investigate this issue in detail here and used the $K_\text{eq}$ value determined from the ratio $k_\text{on}/k_\text{off}$ to compare it with corresponding data measured for the binding of the same pMHC complexes to the OT-1 TCR (14).

Comparison of the Specificity of the OT-1 TCR with the 25-D1.16 mAb—To compare the specificity of 25-D1.16 mAb with OT-1 TCR, we analyzed binding to peptide-Kb complexes in which biological activity (12, 26) and the binding to the OT-1 TCR (14, 15) were determined previously. The $K_\text{eq}$ value of mAb 25-D1.16 binding to pOV8-Kb was $2.9 \times 10^4 \text{RU}^{-1} \text{s}^{-1}$, 1 order of magnitude higher than the $K_\text{eq}$ value for the OT-1 TCR-pOV8-Kb interactions, although the difference in rate constant of dissociation for the two reactions was only approximately 3-fold (Table I). Thus, the gain in the free energy of the 25-D1.16-pOV8-Kb interaction is primarily determined by an increase in on-rate but not by a decrease in off-rate. This suggests that the activation energy of the dissociation phase of the antibody-pMHC interaction is only slightly higher than that of the TCR-pMHC interaction.

Amino acid substitutions in MHC-bound peptide had an effect on the recognition of pOV8-Kb by the OT-1 TCR that was similar to the 25-D1.16 mAb. For instance, the R4-Kb complex, which induces positive selection of immature OT-1” thymocytes (26) and antagonizes cytolytic activity of mature OT-1 cytotoxic T lymphocytes (12), bound to the 25-D1.16 mAb with lower affinity as it did to the OT-1 TCR (Table I). In both cases, the loss of the free binding energy was caused by a faster off-rate, whereas changes in the on-rate were marginal. Similarly, the Kb complex with V-ova peptide, which shares solvent-accessible amino acid residues with P0V8 and has biological activity similar to R4 (12, 26), had a faster rate of dissociation from the antibody and the TCR (Table I). Interestingly, the free energy of V-ova-Kb and pOV8-Kb binding to the 25-D1.16 mAb were very similar because of a faster association rate of the V-ova-Kb-25-D1.16 interaction. The most profound effect resulted from the substitution of a positively charged Lys for a negatively charged Asp at P7 of the peptide (Table I). The
Comparison of TCR with TCR-like Antibody

Table I

| Peptide | pOV8 | SV9 | VSV | E1 | R4 | D7 | V-ova | KVDL | A2 |
|---------|------|-----|-----|----|----|----|-------|------|----|
| 25-D-1.16 Antibody | k_b (s^{-1}) | K_a (M^{-1}) | K_g (cal/mol) | DG (cal/mol) | kd (s^{-1}) | t_{1/2} (s) | t_{1/2} (s) | 
| pOV8 | 2.9 ± 0.1 × 10^{-4} | 2.0 ± 0.1 × 10^{-4} | 3.9 ± 10^6 | 8955 | 91 | 133 | 18 | |
| SV9 | 3.2 ± 0.3 × 10^{-5} | 3.8 ± 0.3 × 10^{-2} | 6.1 ± 10^6 | 7479 | 18 | 53 | 39 | 112 |
| VSV | 1.2 ± 0.2 × 10^{-4} | 1.5 ± 0.1 × 10^{-1} | 0.22 ± 10^6 | <10^6 | ND | 3.9 ± 0.3 × 10^{-2} | 263 | 26.2 |
| E1 | 5.4 ± 0.1 × 10^{-4} | 1.3 ± 0.1 × 10^{-2} | 4.1 ± 10^6 | 4.6 ± 10^6 | 106 | 6.1 | 3.3 | 21.8 |
| R4 | 3.4 ± 0.3 × 10^{-4} | 1.8 ± 0.2 × 10^{-2} | 1.9 ± 10^6 | 24 ± 10^4 | 10^3 | ND | 3.3 | 21.8 |
| D7 | 2.8 ± 0.1 × 10^{-4} | 6.2 ± 0.1 × 10^{-3} | 4.6 ± 10^6 | 26.2 | 10^3 | ND | 3.3 | 21.8 |
| V-ova | 2.5 ± 0.4 × 10^{-4} | 1.8 ± 0.4 × 10^{-2} | 1.8 ± 10^4 | 10^4 | ND | 3.3 | 21.8 |
| KVDL | 10^4 | 10^3 | 10^3 | 10^3 | 10^3 | 10^3 | 10^3 | 10^3 |
| A2 | 10^4 | 10^3 | 10^3 | 10^3 | 10^3 | 10^3 | 10^3 | 10^3 |

* Peptides used were: pOV8, SIINFELK; E1, EIINFELK; R4, SIINFELK; D7, SIINFELK; V-ova, RGYNVKEL; KVDL, KVVRFKDL; A2, SAINFELK; SV9, FAPGNYPAL; VSV, RGGYYQGL.

** Half-life time of the receptor-pMHC complex, i.e. t_{1/2} = 0.693/k_d.

* Reaction equilibrium binding constant, K_eq, was determined as K_g/K_b ratio.

* The free energy of binding (Gibbs energy), ΔG, i.e. ΔG = -RT ln K_g.

* The difference in the free binding energy, i.e. ΔΔG, of receptor-pMHC interactions caused by replacement of K_b-bound pOV8 to indicated peptides.

* NM, not measurable.

* The binding of D7-K_b to 25-D-1.16 immobilized on the biosensor surface was too weak to measure; K_eq of this interaction was estimated to be less than 10^{-4} M^{-1}.

* The binding parameters of OT-1 TCR to indicated peptide-K_b complexes were determined previously by biosensor technology (14) and are presented for comparison.

a ND, not determined.

* These values have been published previously (15).

Comparison of TCR with TCR-like Antibody

Table I

Binding parameters of various peptide-K_b complexes to the OT-1 TCR and to the 25-D-1.16 mAb

The magnitude of the free energy changes (ΔG) resulting from various peptide substitutions was usually higher for OT-1 TCR binding than for 25-D-1.16 mAb binding, suggesting that the peptide energetic contribution to the TCR-pMHC interaction was more significant than its contribution to the antibody-pMHC interaction (Table I). Thus, in the latter case, the MHC moiety probably contributes more significantly, reflecting the 3-fold slower off-rate for the antibody-pOV8-K_b reaction. However, this increase in MHC energy contribution is still small enough to ensure sufficient peptide contribution and the ability to distinguish the MHC-bound peptide.

To further dissect the mechanism used by 25-D-1.16 mAb to recognize K_b-bound peptides, we compared the energy contributed by the association and dissociation phases of OT-1-antibody-K_b with those of 25-D-1.16-antibody-K_b interactions. Comparison was made using the Eyring transition state theory, which allows determination of quasi-thermodynamic parameters of the reaction association and dissociation phases based on respective rate constants (20). The ΔG^a and ΔG^d values reflect the amounts of energy contributed by the association and dissociation phases to the free energy of interaction. The difference in these contributions (Table II) to the antibody and the TCR binding reflects changes in the amount of energy coming from the association and the dissociation phases of 25-D-1.16-antibody-K_b interactions compared with corresponding OT-1-antibody-K_b interactions. With only one exception, the changes in the energy contributions of the dissociation phase (ΔΔG_d) were smaller than those of the association phase (ΔΔG_a). Mutation of Ser to Glu (E1 peptide) had a different effect on the E1-K_b interaction with the TCR and the antibody; it did not affect antibody binding but did decrease the stability of the OT-1-E1-K_b complex (Table I). This explains an unusually high ΔΔG_a value for antibody binding to E1-K_b (Table II). These data provide evidence that the affinity gain for 25-D-1.16 to its natural ligand and other peptide-K_b complexes was caused mainly by the lower energy activation of the association phase, whereas changes in the energy of the dissociation phase were limited to preserve the antibody specificity for the MHC-bound peptide.

**DISCUSSION**

Although recognition of pMHC complexes on the cell surface is a normal function of the TCR, it has been shown that B cells can produce antibodies with TCR-like specificity in vivo as well (13, 28, 29). In addition, the technology of phage display librar-
TABLE II
Comparison of TCR with TCR-like Antibody

| Energy$^{a}$ | Peptide$^{b}$ | pOV8 | E1 | R4 | V-OVA | A2 |
|------------|-------------|------|----|----|-------|----|
| cal/mol    | $\Delta G^a$ |      |    |    |       |    |
| $\Delta G^d$ | $\Delta G^d$ | $-$1320 | $-$1400 | $-$912 | $-$2200 | $-$1040 |
| $\Delta G^k$ | $\Delta G^k$ | 570  | 1517 | 810 | 648 | 691 |

$^{a}$The $\Delta G^a$ values were determined from the difference between $\Delta G^a$ (25-D1.16) and $\Delta G^a$(OT-1), and values of $\Delta G^d$ were calculated from the difference between $\Delta G^d$(25-D1.16) and $\Delta G^d$(OT-1). See “Experimental Procedures” for other details.

$^{b}$Structure and biological activity of peptides are given in the footnotes of Table I.

In this study we determined the intrinsic equilibrium and kinetic constants for the binding of mAb 25-D1.16 to various peptide-K$^b$ complexes with known biological activity and compared these parameters with parameters for the interaction between the OT-1 TCR and the same set of peptide-K$^b$ complexes (Table I). The comparison yielded two major findings. The gain in affinity of the antibody for the peptide-K$^b$ ligands was mainly the result of an increase in the rate constant of association, and changes of the dissociation rate constant were significantly smaller. Differences between the binding of pOV8 and its variants associated with K$^b$ to the antibody and binding to the OT-1 TCR were mainly caused by significant changes in on-rate, whereas changes in off-rate were moderate. This pattern of changes suggests that the preservation of specificity for MHC-bound peptide limits variation in the off-rate but does not restrict changes in on-rate. In accord with these findings, quasi-thermodynamic parameters of the association and dissociation phases have shown that the gain in the free energy of 25-D1.16 binding is determined by a lower energy barrier of the association phase, whereas energy changes of the dissociation phase are similar for both the TCR and the antibody (Table II and Fig. 4).

Kranz and colleagues (11) produced soluble TCR encoded by mutated genes that bound cognate pMHC ligand with an intrinsic affinity of 3.6–6.0 × 10$^7$ M$^{-1}$. The increase in the TCR affinity relative to natural TCR was caused mainly by an increase in the on-rate not the off-rate, suggesting again that limited activation energy of the dissociation phase of the TCR-pMHC reaction is required to preserve peptide specificity. Although this TCR with enhanced affinity clearly discriminates cognate pMHC on the cell surface, T cell hybridomas carrying this TCR respond not only to the cognate pMHC on target cells but also to target cells of the same haplotype that do not display the cognate pMHC complexes. This response is apparently mediated by a prohibitively high level of energy contribution of the MHC moiety, causing an autoimmune reaction. Thus, energy input that is limited by the MHC-TCR contact in recognition of syngeneic pMHC ligands is an important condition by which the MHC restriction and the peptide specificity are met and the reactivity against self-pMHC is avoided. The same group has also investigated the energy map of the interaction between the same TCR and its allogeneic pMHC ligand (37). Site-directed mutagenesis of the TCR showed that CDR1 and CDR2 residues contacting the MHC moiety collectively contribute more binding energy than the CDR3 residues that are responsible for contact with the peptide. This indicates that it is the MHC energy contribution of the allogeneic ligand that dominates, as opposed to that of the syngeneic pMHC. Other analyses (38) of the binding of some recombinant TCRs with enhanced affinity to a syngeneic or to an allogeneic pMHC revealed similar contributions of the MHC and the peptide moieties. However, in this particular system the recognition of allogeneic ligand is strongly peptide-dependent (39, 40). In addition, the peptide induces conformational changes in the MHC protein that are detectable by antibodies (41, 42). In these circumstances, the peptide may contribute significantly to the amount of binding energy delivered indirectly by the allogeneic MHC through conformational changes of the MHC moiety. This may also explain why the affinity of the TCR recognizing this allogeneic pMHC could be increased by significant changes in the off-rate of the interaction (43). Not surprisingly, T cells expressing this high affinity TCR did not respond to target cells bearing irrelevant pMHC complexes (43). In another example (44), the equilibrium binding constant for a TCR interaction with class II invariant chain-associated peptide (CLIP) bound to I-A$^a$ MHC class II protein was measured at 1.6 × 10$^7$ M$^{-1}$. Because CLIP is thought to cause conformational changes of MHC class II molecules (45), this TCR may recognize a unique conformation of the MHC bound to CLIP, and a substantial energy contribution from the MHC moiety may account for high TCR affinity in this case. The increase of the $k_a$ value reflects more precise docking of interacting proteins and a higher probability of productive

---

L. Teyton and A. Y. Rudensky, personal communication.
protein-protein encounters. Usually, $k_b$ depends on long range electrostatic interactions (25, 46). These interactions generate an “energy funnel” between the two molecules, facilitating proper orientation that increases the probability of specific complex formation (47). Higher stability and lower flexibility of the interacting surfaces (48) are thought to facilitate long range electrostatic interactions (49). The latter may not necessarily become a short term interaction contributing to the stability of the complex, measured by the reaction off-rate. Most likely, enhanced long range interactions lead to an increase of the on-rate of 25-D1.16 antibody binding to pOVS-Kβ but do not change the balance of energy contributions made by the peptide and the MHC, ensuring the peptide specificity of the antibody.

Analysis of the primary structure shows that the 25-D1.16 V genes encoding $V_H$ and $V_L$ domains have very few mutations, all of which are in the framework regions, whereas the CDRs appear to be in germ line configuration. This suggests that the $V_H$ and $V_L$ genes of 25-D1.16 have not completed affinity maturation. It has been suggested (50) that the affinity maturation of antibodies is driven initially by an increased on-rate and that this process may be mediated by mutations in framework regions. Subsequent somatic mutations in CDRs lead to a lower off-rate of antibody binding and a further increase of the binding affinity (50). Davis and colleagues (51) found that further maturation of an antibody specific for moth cytochrome $c$ peptide in association with I-Ek MHC class II led to a higher affinity antibody that could no longer discriminate bound I-Ek moth cytochrome $c$ peptide from other self-I-Ek complexes on the surface of live cells. Thus, it appears that the limited affinity of pMHC-specific antibodies and the absence of extensive somatic mutations in the CDRs of these antibodies may be linked and are similar to those properties of TCR in which genes are not a subject for somatic mutations. Normally, a B cell response to protein antigens is to produce IgG antibody with high affinity through extensive somatic hypermutation and clonal selection, which are manifested in the germinal center. The antibody studied here does not appear to fall into this conventional category, providing a possible explanation for the rare occurrence of pMHC-specific antibodies elicited in vivo.

Acknowledgments—We thank Drs. Angel Porgador and Ronald Germain for providing hybridoma 25-D1.16. We also thank Dr. Steve Jameson for critical reading of the manuscript.

REFERENCES
1. Wood, P. G., Krogsgaard, M., Tetin, S. Y., Martinez-Hackert, E., Kalams, S. A., Davis, M. M., and Sykulev, Y. (2003) J. Immunol. Methods 277, 75–86
2. Markvicheva, E. A., Kuptsova, S. V., Mareeva, T. Y., Vikhrov, A. A., Dugina, T. N., Strukova, S. M., Belokon, S. Y., Kochetkov, K. A., Baranova, E. N., Zabov, V. P., Ponec, D., and Ruma, L. D. (2000) Appl. Biochem. Biotechnol. 88, 145–157
3. Mozdzianowski, J., Bongers, J., and Anumula, K. (1998) Anal. Biochem. 260, 185–187
4. Eisenberg, D., and Crothers, D. (1979) Physical Chemistry with Applications to the Life Sciences, pp. 242–243, Benjamin/Cummings Publishing Co., Menlo Park, CA
5. Al-Lazikani, B., Lesk, A. M., and Chothia, C. (1997) J. Mol. Biol. 273, 927–948
6. Jameson, S. C., Carbone, F. R., and Bevan, M. J. (1993) J. Mol. Biol. 225, 269–294
7. Wang, Z., Turner, R., Baker, B. M., and Biddison, W. E. (2002) J. Exp. Med. 193, 451–462
8. Wu, L. C., Tsau, D. S., Lyons, D. S., Garcia, K. C., and Davis, M. M. (2002) J. Biol. Chem. 277, 55–62
9. Al-Lazikani, B., Lesk, A. M., and Chothia, C. (1997) J. Mol. Biol. 273, 927–948
10. Jameson, S. C., Carbone, F. R., and Bevan, M. J. (1993) J. Mol. Biol. 257, 269–294
11. Holler, P. D., Chlewicki, L. K., and Kranz, D. M. (2003) Immunity 6, 715–726
12. Alam, S. M., Travers, P. J., Wung, J. L., Nasholds, W., Redpath, S., Jameson, S. C., and Gascogne, N. R. (1996) Nature 381, 616–620
13. Alam, S. M., Travers, P. J., Nasholds, W., Redpath, S., Jameson, S. C., and Gascogne, N. R. (1996) Nature 381, 616–620
14. Al-Lazikani, B., Lesk, A. M., and Chothia, C. (1997) J. Mol. Biol. 273, 927–948
15. marking, A., and Jackson, M. (1998) in MHC (Fernandez, N., and Butcher, G., eds) Vol. 2, pp. 53–78, Oxford University Press, Oxford
16. Anikeeva, N., Lebedeva, T., Sumarska, M., Kalams, S. A., and Sykulev, Y. (2003) J. Immunol. Methods 277, 75–86
17. Janin, J., Chothia, C. (1978) Biochemistry 17, 2843–2848
18. Stites, W. E. (1997) Chem. Rev. 97, 1233–1250
19. Clackson, T., Hoogenboom, H. R., Griffiths, A. D., and Winter, G. (1991) Nature 351, 640–642
20. Morea, V., Tramontano, A., Rustici, M., Chothia, C., and Lesk, A. M. (1998) J. Mol. Biol. 275, 269–294
Antibody Specific for the Peptide-Major Histocompatibility Complex: IS IT T CELL RECEPTOR-LIKE?
Tatiana Mareeva, Tatiana Lebedeva, Nadia Anikeeva, Tim Manser and Yuri Sykulev

J. Biol. Chem. 2004, 279:44243-44249.
doi: 10.1074/jbc.M407021200 originally published online August 9, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M407021200

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 50 references, 18 of which can be accessed free at http://www.jbc.org/content/279/43/44243.full.html#ref-list-1