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Synthetic Peptides Derived from the Variable Regions of an Anti-CD4 Monoclonal Antibody Bind to CD4 and Inhibit HIV-1 Promoter Activation in Virus-infected Cells*

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The monoclonal antibody (mAb) ST40, specific for the immunoglobulin complementarity-determining region (CDR) 3-like loop in domain 1 of the CD4 molecule, inhibits human immunodeficiency virus type 1 (HIV-1) promoter activity and viral transcription in HIV-infected cells. To design synthetic peptides from the ST40 paratope that could mimic these biological properties, a set of 220 overlapping 12-mer peptides frameshifted by one residue, corresponding to the deduced ST40 amino acid sequence, was synthesized by the Spot method and tested for binding to recombinant soluble CD4 antigen. Several peptides that included in their sequences amino acids from the CDRs of the antibody and framework residues flanking the CDRs were found to bind soluble CD4. Eleven paratope-derived peptides (termed CM1–CM11) were synthesized in a cyclic and soluble form. All the synthetic peptides showed CD4 binding capacity with affinities ranging from 1.6 to 86.4 nM. Moreover, peptides CM2, CM6, CM7, CM9, and CM11 were able to bind a cyclic peptide corresponding to the CDR3-like loop in domain 1 of CD4 (amino acids 81–92 of CD4). Peptide CM9 from the light chain variable region of mAb ST40 and, to a lesser extent, peptides CM2 and CM11 were able to inhibit HIV-1 promoter long terminal repeat-driven β-galactosidase gene expression in the HeLa P4 HIV-1 long terminal repeat β-galactosidase indicator cell line infected with HIV-1. The binding of mAb ST40 to CD4 was also efficiently displaced by peptides CM2, CM9, and CM11. Our results indicate that the information gained from a systematic exploration of the antigen binding capacity of synthetic peptides from immunoglobulin variable sequences can lead to the identification of bioactive paratope-derived peptides of potential pharmacological interest.

The CD4 molecule is a transmembrane glycoprotein (58 kDa) found on thymocytes, mature T-cells, macrophages, monocytes, and Langerhans' cells (1). This surface protein is required to shape the T-cell repertoire during thymic development (2) and to permit appropriate activation of mature T-cells through adhesion with class II major histocompatibility complex molecules and the T-cell receptor (3). Engaged CD4 subsequently plays a role in signal transduction by association with the protein-tyrosine kinase p56 

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‡ The abbreviations used are: HIV-1, human immunodeficiency virus type 1; D1, domain 1; CDR, complementarity-determining region; mAb, monoclonal antibody; VH, variable region of the heavy chain; VL, variable region of the light chain; PDP, paratope-derived peptide; sCD4, soluble CD4; PBS, phosphate-buffered saline; Fmoc, N-(9-fluorenlyl)carbonyl; HPLC, high pressure liquid chromatography; LTR, long terminal repeat.
The of the V_{H} and V_{L} regions of mAb ST40. A set of immobilized overlapping dodecapeptides covering the deduced amino acid sequences of mAb ST40 variable regions was prepared by the Spot method (40, 41). The ability of biotinylated soluble CD4 (sCD4) to bind these peptides was then investigated and led to the selection of peptides with CD4 binding activity. All the selected PDPs prepared in a soluble cyclic form showed CD4 binding capacity, and three of them blocked HIV-1 promoter activity and efficiently competed with mAb ST40 for binding to CD4.

**Experimental Procedures**

**Soluble CD4**—Recombinant purified sCD4, kindly provided by Dr. D. Carrière (Sanofi Recherche, Montpellier, France) (43). Total RNA was extracted from 3 × 10^6 hybridoma cells using the TRIzol™ technique (Life Technologies Inc., Paisley, United Kingdom). The V_{L} gene of the ST40 antibody was obtained by polymerase chain reaction reamplification. Briefly, reverse transcription was performed with 2 μg of total RNA, the reverse transcriptase Superscript (Life Technologies Inc.), and the primer OPP-SoC3 (5'-CCGGGCAATCTACACTCTCCTC-GTGGAAGC-3'), which contains the reverse complement of codons 208–214 of Cx. One μl of first-strand cDNA was used as matrix for the polymerase chain reaction to amplify the ST40 V_{L} gene using Vent DNA polymerase (New England Biolabs, Hitchin, UK) and the primers OPP-SoCe3 and OPP-SoVe5 (5'-GA/CAT/AATGAGGCTC/CAICAG/G/TAICTCCA-3'). These primers contained restriction sites (underlined) for cloning. The degenerate primer OPP-SoVe5 was chosen as the consensus sequence of codons 5–8 in murine FR1 Vx. The polymerase chain reaction-amplified DNA product was digested sequentially with RflII and SacI (New England Biolabs) and purified on a 1.5% low-melting temperature agarose gel (Life Technologies Inc.). This digested DNA was ligated into pUC19 that was prepared in a similar manner. The V_{H} cDNA sequence was determined by double-stranded sequencing using the dye deoxy chain termination method with the T7 sequencing kit (Amersham Pharmacia Biotech, Uppsala). The V_{H} gene of the ST40 antibody was isolated from a cDNA library. Briefly, poly(A)^+ RNA were magnetically separated from total RNA by hybridization with a biotinylated oligo(dT) primer and then captured by streptavidin coupled to paramagnetic beads as described by the manufacturer (Polytract™, Promega, Madison, WI). A cDNA library was constructed from 10 μg of ST40 poly(A)^+ RNA in the pSPORT1 vector (Life Technologies Inc., Paisley, United Kingdom). The VL gene of the ST40 antibody was isolated from a cDNA library. The pH of the ST40 antibody was determined by injecting sCD4 (20 μg/ml) or biotinylated CD4-like peptide (100 μg/ml) into 100 μl of 10 mM sodium carbonate buffer, pH 9.6. Three replicates were tested for each dilution with an initial peptide concentration of 100 μg/ml. An irrelevant peptide, 97026c (CKSSQSLDSDDGKYTNWLC), derived from the heavy chain CDR2 of an anti-p53 antibody was included as a control to verify that binding was sequence-specific. Two cyclic peptides, DiGCHel (KKGWIDGGYGGGKG) and DiGCHe (KKGWIDGGYYGKG), derived from the heavy chain CDR2 and the light chain CDR3 of anti-digoxin mAb 1C10, respectively were used as controls to verify the effect on antigen binding of adding Lys-Cys residues to the peptide sequence. After four washes in 160 μl PBS, pH 7.2, containing 0.1% Tween 20 (PBS-T), plates were saturated with a 1% nonfat powdered milk in PBS-T for 30 min at 37 °C. Biotinylated sCD4 (1 μg/ml) or biotinylated CDR3-like peptide (100 μg/ml) were incubated with 100 μl of each washes in PBS-T, and plates were incubated at 37 °C for 2 h. Following four washes in PBS-T, 100 μl of an alkaline phosphate-streptavidin conjugate was added to each well. The conjugate was used at a 1:3000 dilution in PBS-T. The plates were incubated at 37 °C for 30 min and then washed three times in PBS-T. Finally, a 1 mg/ml 4-nitrophenyl phosphate disodium (Sigma) solution in 1 M diethanolamine, pH 9.8, was added for 20 min at 37 °C, and the absorbance was measured at 405 nm.

**Peptide Synthesis on Cellulose Membranes**—The general protocol has been described previously (45). Membranes were obtained from Abimed (Langenfeld, Germany). Fmoc amino acids and N-hydroxybenzotriazole were obtained from Novabiochem (Laufelfingen, Switzerland). The ASP222 robot (Abimed) was used for the coupling steps. Two-hundred microliters of peptide hydrolysis buffer (running buffer) at a flow rate of 30 μl/min. For the inhibition

**Enzyme-linked Immunosorbent Assay Monitoring of sCD4 and CDR3-like Peptide Interactions with Cyclic PDPs—Enzyme immunoassay plates (96-well; Nunc, Paisley) were coated overnight at 4 °C with 10-fold serial dilutions of the 11 cyclic PDPs (CM1–CM11) in 100 mM sodium carbonate buffer, pH 9.6. Three replicates were tested for each dilution with an initial peptide concentration of 100 μg/ml. An irrelevant peptide, 97026c (CKSSQSLDSDDGKYTNWLC), derived from the heavy chain CDR2 of an anti-p53 antibody was included as a control to verify that binding was sequence-specific. Two cyclic peptides, DiGCHel (KKGWIDGGYGGGKG) and DiGCHe (KKGWIDGGYYGKG), derived from the heavy chain CDR2 and the light chain CDR3 of anti-digoxin mAb 1C10, respectively were used as controls to verify the effect on antigen binding of adding Lys-Cys residues to the peptide sequence. After four washes in 160 μl PBS, pH 7.2, containing 0.1% Tween 20 (PBS-T), plates were saturated with a 1% nonfat powdered milk in PBS-T for 30 min at 37 °C. Biotinylated sCD4 (1 μg/ml) or biotinylated CDR3-like peptide (100 μg/ml) were incubated with 100 μl of each washes in PBS-T, and plates were incubated at 37 °C for 2 h. Following four washes in PBS-T, 100 μl of an alkaline phosphate-streptavidin conjugate was added to each well. The conjugate was used at a 1:3000 dilution in PBS-T. The plates were incubated at 37 °C for 30 min and then washed three times in PBS-T. Finally, a 1 mg/ml 4-nitrophenyl phosphate disodium (Sigma) solution in 1 M diethanolamine, pH 9.8, was added for 20 min at 37 °C, and the absorbance was measured at 405 nm.

**Real-time Analysis by BIACore™**—The kinetic parameters (association rate constant (k_a) and dissociation rate constant (k_d)) were determined by surface plasmon resonance analysis using a BIACore instrument (BIACore AB, Uppsala). Using BIACore 3.0.2 software, k_a and k_d were determined by the so-called global method (48). The apparent equilibrium constant K_eq is the ratio k_a/k_d. All experiments were carried out at 25 °C. Free NH_2 from the extrusion lineage residue in CM1, CM2, CM6, CM7, and CM9–CM11 and from the intrasequence lineage residue in CM4 and free COOH from the glutamic acid residue in CM5 were used to chemically immobilize molecules on the sensor chip. Peptides CM3 and CM6 were chemically immobilized by the hydroxyl group of tyrosine and CM7, CM10, CM11, and CM1 by the N1′-carboxyldimiazole (Sigma-Aldrich). The surface plasmon resonance signal for immobilized peptides was found to be ~30–50 resonance units after completion of the chip regeneration cycle, corresponding to 30–50 μg of peptide/mm². The binding kinetics for immobilized peptides were determined by injecting sCD4 (20 μg/ml) in Heps-buffered saline buffer (running buffer) at a flow rate of 30 μl/min. For the inhibition
FIG. 1. Reactivity of overlapping dodecapeptides derived from the sequence of anti-CD4 mAb ST40 with biotinylated sCD4 (A) and quantitative analysis of the binding (B). The membrane on which the peptides were synthesized was incubated with 1 μg/ml biotinylated sCD4 or with 1 μg/ml biotinylated sCD4 preincubated with 10 μg/ml mAb ST40. In A, CDRs are indicated (H1, H2, and H3 and L1, L2, and L3 correspond to CDR1, CDR2, and CDR3 of the heavy and light chains, respectively), and peptide spots are numbered from 1 to 220. In B, shaded areas indicate the cellulose-bound peptides that reacted with biotinylated sCD4 (cutoff taken at 80 arbitrary units). Boldface amino acids belong to the CDRs. Results correspond to the mean ± S.D. of values obtained from three independent experiments.
study, mAb ST40 (20 µg/ml) and PDP (20 or 200 µg/ml) were co-injected onto the sensor chip-bound CD4 (30–50 pg/mm²). The kₐ increase was calculated as the ratio of kₐ determined with inhibitor to that obtained without inhibitor.

**HIV-1 Promoter Activation Assay**—The HeLa P4 HIV-1 LTR β-galactosidase indicator cell line (49) was provided by O. Schwartz (Institut Pasteur, Paris). HeLa P4 cells, which stably express the β-galactosidase reporter gene cloned downstream of the HIV-1 LTR promoter, were plated in six-well plates at 5 × 10⁵ cells/ml in Dulbecco’s modified Eagle’s medium containing 1% penicillin/streptomycin mixture (Gibco), 1% Glutamax, 1 mg/ml Geneticin (G418), and 10% fetal calf serum. The cells were exposed to 1 × 10⁶ infectious HIV-1_LAI at 1000 × 50% tissue culture infective dose/ml prepared from the supernatant of chronically infected CEM T-cells, as described previously (50). After incubation for 1 h at 37 °C, the cyclic PDPs CM1, CM2, CM6, CM7, and CM9–CM11, at concentrations ranging between 12.5 and 200 µg/ml, were added individually to the cell culture medium. Next, cell cultures were transferred at 37 °C in a 5% CO₂ atmosphere to allow infection (note that the HIV-1 infection provides the viral transactivator Tat protein necessary for the HIV promoter in the target cells). After 3 days in culture, cells were lysed, and β-galactosidase activity was determined by incubating 200 µl of total cellular extracts for 1 h at 37 °C in 1.5 ml of buffer containing 80 mM Na₂HPO₄, 10 mM MgCl₂, 1 mM 2-mercaptoethanol, and 6 mM o-nitrophenyl β-D-galactopyranoside. β-Galactosidase activity was evaluated by measuring absorbance at 410 nm. Incubation of infected HeLa P4 cells with anti-CD4 mAb ST40 at 20 µg/ml or anti-HLA class II mAb B8-12 (kindly provided by M. H., Immunotechn-Coulter, Marseille, France) at 20 µg/ml served as positive and negative controls, respectively. Additional controls consisted of linear Lyso-3 peptide (biotinylated YKKSSTGSPPKRWYDIT), derived from the light chain CDR2 of anti-lysozyme mAb HyHEL-5 (40), and the cyclized 97026c peptide described above.

### RESULTS

**Sequence of Anti-CD4 mAb ST40**—The nucleotide sequences of the VH and VL regions from anti-CD4 mAb ST40 were established as described under “Experimental Procedures.” Nucleotide sequences of three individual clones were determined for each chain type and shown to be similar. Comparison of this sequence with other known antibody sequences showed that the VH region of mAb ST40 belongs to subgroup IIA according to the classification of Kabat et al. (44) and displays 95.5% homology to the VH region of the V-Gam 3.8 family. mAb ST40 used a member of the JH2 germ line gene (52) from the JH-Gam 3.8 family. mAb ST40 used a member of the JH2 germ line gene (52) from the JH-Gam 3.8 family.

**Table I**

| PDP      | Peptide sequence | kₐ (10⁻⁴ s⁻¹) | kₜ (10⁻⁴ s⁻¹) | K₀ (nM) |
|----------|------------------|---------------|---------------|---------|
| CM1      | KCRISCKASSGYSFTCK| 9.4           | 3.0           | 3.2     |
| CM2      | KCSGYFTNAGQGKC   | 13.7          | 2.2           | 1.6     |
| CM3      | WYCTHYDGVFFAFCY  | 7.7           | 16.9          | 22.0    |
| CM4      | YCGGGTYLEIKCK    | 3.0           | 8.2           | 27.0    |
| CM5      | YCQDTAYFTCARGGRC| 10.3          | 3.5           | 3.4     |
| CM6      | KGCGVLWSRGRDFDCK| 21.9          | 3.6           | 1.6     |
| CM7      | KCRRDFDYWGGTCK   | 22.4          | 7.2           | 3.2     |
| CM8      | YCPASLVLSQGRC    | 2.4           | 20.4          | 86.4    |
| CM9      | KDSNMYQPGQPKCK   | 8.5           | 9.1           | 10.6    |
| CM10     | KLLPEDIFAPPSGCK  | 8.2           | 4.5           | 5.5     |
| CM11     | KWCFAFGTYLEIKCK  | 8.1           | 3.7           | 4.6     |
| 97026c   | CKSSQSSLDSDKHTYKWC| Not measurable | Not measurable |

All peptides were cyclized through N- to C-terminal disulfide bridging.

**CD4 and CDR3-like Loop Specificity of Soluble Cyclic Peptides Derived from the ST40 Antibody Sequence**—The 11 peptides (CM1–CM11), selected from the initial 220 overlapping peptides on the basis of their reactivity with sCD4 in the form of membrane-bound peptides, were synthesized by conventional solid-phase synthesis and N to C terminus-cyclized through cysteine oxidation (Table I). Their binding to whole CD4 and to a CDR3-like loop peptide (corresponding to residues 81–92 in D1 of the CD4 molecule) was assessed by enzyme-linked immunosorbent assay (Fig. 2). Soluble cyclic peptides reacted specifically with sCD4 in a dose-dependent manner, which was not the case for the three irrelevant cyclic peptides 97026c, Dig23c, and Dig97c, the latter two including an extra lysine residue like the CM peptides. Peptides selected from either the V₃ region (Fig. 2A) or the V₃ region (Fig. 2B) displayed CD4 binding activity in a 1–100 µg/ml concentration range. Peptides CM2, CM6, and CM7 (Fig. 2C), derived from the ST40 V₃ region, and peptides CM9 and CM11 (Fig. 2D), derived from the ST40 V₃ region, strongly recognized CDR3-like peptide 81–92, whereas other synthetic peptides did not significantly bind this antigen. The linear forms of peptide CM9 and several other PDPs were markedly less reactive than the cyclic form (data not shown), indicating a beneficial effect of N- to C-terminal cyclization on binding properties. Furthermore, the absence of reactivity of the 12-mer Lys-Cys-cyclized
peptides Dig23c and Dig97c showed that the additional cysteine and lysine residues used for cyclization/solubilization are not implicated in the CD4 and CDR3-like binding. Taken together, these results indicate that the selected soluble cyclic peptides derived from mAb ST40 have the capacity to specifically bind the CD4 molecule, but only some of them also demonstrated a specificity for the CDR3-like loop.

The results of the BIAcore study, in which the kinetic parameters $k_d$ and $k_a$ of the interaction between immobilized peptides and soluble CD4 were measured, are summarized in Table I. All 11 peptides exhibited measurable binding to sCD4. No measurable binding was obtained with the irrelevant cyclic peptide. The calculated $K_d$ values ranged from 1.6 to 86.4 nM. Peptides CM2 and CM5–CM7, derived from the CDR1 and CDR3 VH regions of mAb ST40, showed the highest affinity. The $K_d$ values obtained with the peptides showed a 4–8-fold increase in value as compared with the value obtained with the parental ST40 mAb (0.37 nM). This increase is mainly due to a lower dissociation rate of the mAb ($3.3 \times 10^{-4}$ s$^{-1}$) in comparison with that obtained with the PDPs.

Fig. 2. Enzyme-linked immunosorbent assay binding curves of biotinylated sCD4 or CDR3-like peptide 81–92 on adsorbed cyclic peptides derived from the sequence of mAb ST40. Plates were coated with various concentrations of the cyclic peptides synthesized from the $\text{V}_\text{H}$ sequence (A and C) and from the $\text{V}_\text{L}$ sequence (B and D). Probing was performed either with biotinylated sCD4 (1 μg/ml) (A and B) or biotinylated CDR3-like peptide (100 μg/ml) (C and D). Irrelevant peptides (Dig23c, Dig97c, and 97026c) were used as negative controls. Each value represents the mean ± S.D. of triplicate determinations.

Inhibition of HIV-1 Promoter Activation in Virus-infected Cells by PDPs—The ability of the PDPs to inhibit HIV-1 promoter activity was measured in HeLa P4 cells stably transfected with the $\beta$-galactosidase reporter gene under the control of the HIV-1 LTR promoter. Infection of the indicator cell line with HIV-1 Lai strongly stimulated the HIV-1 promoter activity (mean $A_{410\text{ nm}}$ increased from 0.014 to 0.548). As shown in Fig. 3A, no inhibition of the HIV-1 LTR-driven $\beta$-galactosidase gene expression was observed when HIV-1 Lai-infected indicator cells were cultured with anti-HLA class II mAb B8-12, whereas 65% inhibition was found following incubation with mAb ST40. Irrelevant linear and cyclic peptides did not affect the $\beta$-galactosidase gene expression. In contrast, treatment with the cyclic PDPs CM2, CM9, and CM11 significantly inhibited the HIV-1 LTR-driven $\beta$-galactosidase gene expression induced by HIV-1 Lai. Several other cyclic PDPs (CM1, CM6, CM7, and CM10) showed no effect. Peptide CM9, corresponding to the sequence $\text{DSYMNWYQQKPG}$ of the CDR1 framework-2 light chain region, was the strongest inhibitor. As shown in Fig. 3B, peptide CM9 inhibited, in a dose-dependent manner, the HIV-1 LTR-driven $\beta$-galactosidase gene expression induced by HIV-1 Lai. At a concentration of 63 μg/ml, peptide CM9 showed −50% of the effect of the parental antibody used at 20 μg/ml. Taken together, these results indicate that the PDPs CM2, CM9, and CM11, initially selected among all the overlapping dodecapeptides of the VH and VL domains of anti-CD4 mAb ST40, are able to inhibit the HIV-1 promoter, a property previously ascribed to mAb ST40 (24).

Inhibition of ST40 Binding to CD4 by Three Paratope-derived Peptides—Competitive binding assays were performed to examine the ability of peptides CM2, CM9, and CM11 to block the binding of the parental ST40 mAb to sensor chip-bound CD4 (Table II). The three PDPs competed with the anti-CD4 antibody for binding to sensor chip-bound CD4, as determined by BIAcore analysis. This competition led to the enhancement of the dissociation rate of the antibody to the CD4 molecule. A 1000–2000-fold $k_d$ increase was obtained when peptides were used at a concentration of 200 μg/ml. This inhibitory effect was
dose-dependent since a peptide concentration of 20 μg/ml caused only a 30–50-fold increase in the dissociation rate.

**DISCUSSION**

The identification, by using multiple peptide synthesis, of PDPs able to bind antigen was recently described by our group; several of these peptides display a significant fraction of the affinity of the whole antibody (40). Therefore, this approach could conceivably be used to screen peptide ligands mimicking the biological effect of a given antibody. With this perspective in mind, we have studied an anti-CD4 mAb (ST40) that shows interesting pharmacological activities. The ST40 antibody binds to the CDR3-like loop in D1 of CD4 and has been described as a strong inhibitor of HIV promoter activity and provirus transcription (24). We have established the V₉ and V₆ amino acid sequences of this antibody and assessed the reactivity of sCD4 with overlapping 12-mer peptides derived from these sequences by the Spot method (40, 41). Eleven peptides were found to react strongly and specifically with the CD4 antigen. We demonstrated that soluble cyclic peptides derived from peptides reactive in the Spot assay were able to recognize the CD4 molecule and a cyclic CDR3-like loop peptide corresponding to region 81–92 of CD4. Among the CDR3-like loop-specific PDPs, three (CM2, CM9, and CM11) were found to block HIV promoter activity and to compete efficiently with the parental mAb for binding to CD4.

An interesting feature was that PDPs showing the strongest reactivity with CD4 in the Spot assay included both residues from the CDRs and residues from the framework flanking the hypervariable regions, extending our previous observations (40). Antibody variable domains comprise a framework of β-sheets surmounted by antigen-binding loops. We can postulate that critical residues, identified in the Spot assay and confirmed by preliminary Alascan analysis (data not shown), located in the β-sheet framework closely underlying the CDRs, probably do not participate in direct interaction with CD4, but could induce a binding conformational state mimicking some of
that Glu87, Asp88, Glu91, and Glu92 in the CDR3-like loop are

negative electrostatic potentials of the CDR3-like region were

putative CD4 dimerization occurring in D1 predicted that the

D1. Moreover, electrostatic potential contours calculated for a

putative dimerization of CD4 involves the CDR3-like loop in

formation. From these observations and from the results ob-

tained in our laboratory on the interactions of mAb HyHEL-

5/lysozyme (40) and mAb Tg10/thryoglobulin and mAb 4D8/

angiotensin II.2

Based on the observations that CDR3-like synthetic peptides
can bind CD4, Langedijk et al. (30) have proposed that the

putative dimerization of CD4 involves the CDR3-like loop in

D1. Moreover, electrostatic potential contours calculated for a

putative CD4 dimerization occurring in D1 predicted that the

negative electrostatic potentials of the CDR3-like region were

completely compensated for by positive charges on the opposite

CD4 molecule in the dimer (30). Recent results (29) suggest

that cyclization helps peptides to mimic the CDR con-

formation between soluble linear peptides from the HyHEL-

5/lysozyme (40) and that cyclization/solubilization are not implicated in this binding. Positively charged residues, like Arg100H and Lys39, have been found to be critical amino acids by the Spot method in the peptide/CD4 interactions. Moreover, preliminary results obtained by Alascan analysis of PDPs confirm the contribution of these positively charged residues in CD4 binding (data not shown). However, positively charged amino acids probably reflect only a part of the interaction between ST40 and CD4 since other contributor residues in the CDRs were found by using Alascan analysis.

With regard to the measured binding kinetics of the interaction

between soluble linear peptides from the HyHEL-5 para-

tope and lysozyme (40), a 1-log decrease in the $k_d$ was observed in the peptide/CD4 binding, whereas association rates were in

the same order of magnitude in the two models. In the case of anti-reovirus mAb 87.92.6 (34), it has been reported that the increased conformational stability of cyclic CDR peptides could increase the binding affinity. In addition, other reports (26, 57) suggest that cyclization helps peptides to mimic the CDR con-

formation. From these observations and from the results ob-

tained with the CM peptide series, it seems that constraining

the structural features of the ST40 paratope. Three points

are worth of favor in this hypothesis. First, some framework amino

acids that modulate the peptide/CD4 interaction (i.e. Tyr27, Trp47, Gly49, and Arg64 in the ST40 VH sequence and Tyr36 in the ST40 VH segment) belong to the vernier zone, which contains residues that adjust the CDR structure and fine-tune the fitting to the antigen (54). Second, some residues possess an aromatic structure (i.e. Tyr27 and Trp47 in the heavy chain and Tyr36 in the light chain) characterized as protruding into the antigen-binding site surface to stabilize the antigen/antibody interaction (55, 56). Third, framework arginine residues (i.e. Arg64 in VH and Arg18 in VL) modulate the peptide/CD4 bind-
ing, in keeping with previous work demonstrating the critical role of Arg64 in the interaction of a CDR3 VH Peptide with phosphatidyserine (36). These critical residues from the framework regions of the ST40 antibody possess one or several of these characteristics, in agreement with previous results obtained in our laboratory on the interactions of mAb HyHEL-5/lysozyme (40) and mAb Tg10/thryoglobulin and mAb 4D8/angiotensin II.2

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