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Synthetic Peptides Coupled to the Lipotriptide P₃CSS Induce *in vivo* B and T_{helper} Cell Responses to HIV-1 Reverse Transcriptase

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Abstract

To evaluate the ability of the lipotriptide P₃CSS to increase peptide-specific immune responses *in vivo*, we immunized mice from different inbred strains (BALB/c, C3H/HeJ, C57BL/6) with the 22-mer lipopeptide conjugates P₃CSS-[RT-(522-543)] and P₃CSS-[RT-(528-549)] of HIV-1 reverse transcriptase (RT) which included an immunodominant T_h epitope [*i.e.* RT-(528-543)] characterized previously. Analysis of T and B cell responses to these lipopeptide conjugates indicated that specific T_h responses could be readily induced *in vivo*. The peptide segments could also efficiently prime mice for secondary recognition of native RT. The use of shorter peptides permitted a delineation of the minimal T cell recognition site of this RT C-terminal region [*i.e.* RT-(528-540)]. Close to this T cell epitope we identified a B cell determinant containing the motif EQVD [RT-(546-549)] which was recognized in three different strains of mice (H-2^b, H-2^d and H-2^k). A comparison with X-ray analysis of the C-terminal region of HIV-1 reverse transcriptase indicated exposed positions of these T_h and B cell epitopes. Both the presence of T and B cell sites and its limited polymorphism make the region RT-(528-549) a promising candidate for vaccine design. The use of the P₃CSS adjuvant/carrier principle as a nontoxic adjuvant may be of major importance in the development of vaccines applicable to humans.

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Abbreviations: LP = lipopeptide; P₃CSS = N-palmitoyl-S-(2,3-dipalmitoyloxy-[2RS]-propyl)-[R]-cysteinyl-seryl-serine; RT = reverse transcriptase; T_h = T helper cell

Introduction

Synthetic peptide vaccines have been successfully used to protect against viral and bacterial infections (1). We have shown that the C-terminal segment of the HIV-1 reverse transcriptase (RT) contains a highly immunogenic determinant that induces proliferative T cell responses in different strains of mice (2) and overlaps with a B cell epitope (3, 4). This region of the RT is highly conserved among various strains of HIV-1, HIV-2 and SIV (5) and might be a candidate for subunit vaccines, provided it could elicit both cellular and humoral antiviral responses. As free peptides are often poorly immunogenic, iscoms, liposomes, proteoliposomes or lipopeptides have been used as adjuvants (6–9). We selected the lipopeptide adjuvant N-Palmitoyl-S-(2,3-dipalmitoyloxy-[2RS]-propyl)-[R]-cysteinyl-seryl-serine (P₃CSS), derived from the cell wall of Gram-negative bacteria, to enhance the immunogenicity of the free peptides (10). P₃CSS lacks the toxicity of the conventional complete Freund's adjuvant, and B and T cell epitopes coupled to it are known to induce both B and cytotoxic T cell responses (11, 12). We describe the induction of B and T cell responses following three consecutive immunizations with 22-mer peptide-lipopeptide conjugates representing a part of the highly immunogenic C-terminal region of RT. In addition, we used shorter overlapping peptides to define the structural features of these B and T cell epitopes.

Materials and Methods

Mice

BALB/c (H-2^d), C3H/HeJ (H-2^k), and C57BL/6 (H-2^b) mice (age 6–8 weeks) were obtained from our animal facilities.

Peptides

The overlapping peptide segments corresponding to the C-terminal region of the HIV-1 BRU RT or the N-terminal with P₃CSS conjugated peptide-lipopeptides (Table 1) were synthesized as previously described (13). Sample purity was analyzed by RP-HPLC (Beckman System Gold, San Ramon, USA). Lipopeptides were analyzed by electrospray-mass spectrometry.

Immunization

Mice were immunized i. p. three times with 100 µl of a 100 µM solution (10 nmol) of the lipopeptide conjugates P₃CSS-[RT-(522–543)], or P₃CSS-[RT-(528–549)], or the corresponding free peptides dissolved in PBS. Boost injections were carried out under identical conditions on days 14 and 28.

T cell separation

Four days after the last boost immunization, spleens were removed and homogenized. After erythrocyte lysis, splenocytes were cultured for 1 h at 37°C in Petri dishes (10 ml; 1 × 10⁷ cells/ml) to remove adherent cells. B cells were depleted using goat anti-mouse

Table 1. Synthesized

Peptide sequences

IEQLIKKEK\

KEK\

IEQLIKKEK\

QLIKKEK\

IKKEK\

KEK\

IEQLIKKEK\

QLIKKEK\

bIKKEK\

KEK\

KEK\

KEK\

IgG- and IgM-coated
per 2 × 10⁷ cells/ml
contained less than
analysis on a FACSc
in complete RPMI (c
µg/ml streptomycin,

T cell proliferation

For the restimulation
from non-immunize
incubated for 1 h in

Table 1. Synthesized peptides of HIV-1 RT.

Peptide sequences	Abbreviations
IEQLIKKEKVYLAWVPAHKGIG	RT-(522-543)
KEKVYLAWVPAHKGIGGNEQVD	RT-(528-549)
IEQLIKKEKVYLAWVP	RT-(522-537)
QLIKKEKVYLAWVPAH	RT-(524-539)
IKKEKVYLAWVPAHKG	RT-(526-541)
KEKVYLAWVPAHKGIG	RT-(528-543)
AWVPAHKGIGGNEQVD	RT-(534-549)
IEQLIKKEKVYL	RT-(522-533)
QLIKKEKVYLAW	RT-(524-535)
IKKEKVYLAWVP	RT-(526-537)
KEKVYLAWVPAHK	RT-(528-540)
KEKVYLAWVPAH	RT-(528-539)
LAWVPAHKGIGG	RT-(533-544)
AHKGIGGNEQVD	RT-(538-549)
KEKVYLAW	RT-(528-535)
YLAWVPAHK	RT-(532-540)
VPAHKGIG	RT-(536-543)
HKGIGGNE	RT-(539-546)
IGGNEQVD	RT-(542-549)
VYLAWVPA	RT-(531-538)
YLAWVPA	RT-(532-538)
LAWVPAH	RT-(533-539)
AWVPAHK	RT-(534-540)
WVPAHKG	RT-(535-541)
VPAHKG	RT-(536-542)
PAHKGIG	RT-(537-543)
EQVD	RT-(546-549)
NEQVD	RT-(545-549)
GNEQVD	RT-(544-549)
GGNEQVD	RT-(543-549)

IgG- and IgM-coated magnetic beads (Paesel-Lorei, Frankfurt, Germany, 400 µl of beads per 2×10^7 cells/ml for 3 h by gently shaking at 4 °C). The remaining unlabeled cells contained less than 1% surface Ig-positive cells, as determined by cytofluorometric analysis on a FACScan (Becton Dickinson, Heidelberg, Germany). Cells were cultured in complete RPMI (containing 10% FCS, 4 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 mM Na-pyruvate, and 60 µM 2-mercaptoethanol).

T cell proliferation assay

For the restimulation of T cell enriched spleen cells, bone marrow-derived macrophages from non-immunized mice were cultured for 10 days as described (14) and then incubated for 1 h in 96-well microtiter plates (5×10^3 macrophages/well, triplicate

cultures) with different concentrations of free peptides or lipopeptide conjugates (*i.e.* final concentration: 25 μ M, 5 μ M, or 0.5 μ M solutions in complete RPMI) before adding T cells (2×10^5). After 4 days of culture, the cells were pulsed for 24 h with 2.3×10^4 Bq of [3 H]dThd (Amersham, Braunschweig, Germany) per well. After freezing and thawing, cells were collected on glass fiber filters with an automatic cell harvester, and the [3 H]dThd incorporation was measured in a liquid scintillation counter (BetaPlate, Pharmacia, Freiburg, Germany).

Antibody production and ELISA

Sera from immunized mice were collected on day 32 (*i.e.* when spleen cells were assayed for specific T cell proliferation). The detection of the specific antibodies was performed by a standard ELISA as described recently (11).

Results

P₃CSS-coupled HIV-1 RT peptides elicit proliferative T cell responses to both lipopeptide-peptide conjugates and native RT peptides

Two 22-mer peptides, RT-(522-543) and RT-(528-549) (Table 1), from the C-terminal part of HIV-1 RT were chemically prepared. These RT-segments contain both T and B cell sites (2-4), the minimal structures of which have not yet been investigated in detail. The peptides RT-(522-543) and RT-(528-549) were also covalently coupled to P₃CSS. P₃CSS is a lipotriptide derived from the lipoprotein of Gram-negative bacteria, which is known to be a very efficient immunoadjuvant (9, 11). After three immunizations of BALB/c mice on day 0, 14, 28 with the lipopeptide-peptide conjugate P₃CSS-[RT-(528-549)], T cell-enriched splenocytes from day 32 were assayed *in vitro* for specific proliferation against the lipopeptide conjugates and the free peptides. We found specific dose-dependent secondary responses to the lipopeptide conjugate P₃CSS-[RT-(528-549)] as well as to the peptide RT-(528-549) (Fig. 1). In contrast, immunization with either the free peptide RT-(528-549) (Fig. 1) or PBS (data not shown) did not elicit specific T cell proliferation at any concentration of free peptides tested. However, *in vitro* restimulation of these immunization groups with the conjugated P₃CSS-[RT-(528-549)] induced a moderate dose-dependent response.

Structural features of the C-terminal T cell epitopes as defined by overlapping peptides and lipopeptide conjugates

With the goal of defining the minimal RT fragment necessary for T cell recognition, BALB/c mice were immunized with the lipopeptide conjugates P₃CSS-[RT-(528-549)] or P₃CSS-[RT-(522-543)], and the resulting proliferative peptide-specific T cell responses were measured. Both the free 16-mer RT-(528-543) and the 13-mer RT-(528-540) peptides could stimulate T cell proliferation after priming with P₃CSS-[RT-(528-549)] (Fig. 1).

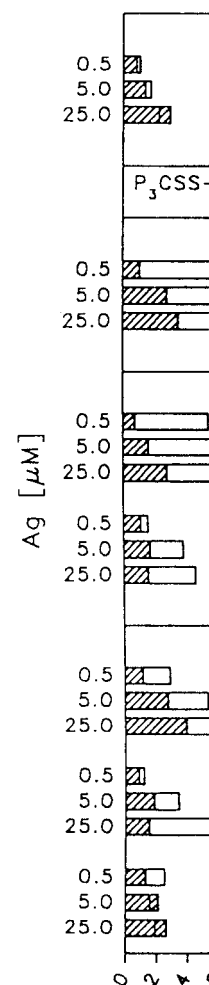


Figure 1. Proliferative responses of T cells from mice immunized with the peptide RT-(528-549) or lipopeptide-peptide conjugate P₃CSS-[RT-(528-549)] and restimulated with the same or different concentrations of free peptide RT-(528-549) or lipopeptide conjugates. The [3H]dThd uptake ratio between experimental and control cells from triplicate cultures was determined. Restimulation was of HIV-1 BH10.

Immunization with the lipopeptide conjugate P₃CSS-[RT-(528-549)] induced a moderate dose-dependent response. In contrast, immunization with either the free peptide RT-(528-549) (Fig. 1) or PBS (data not shown) did not elicit specific T cell proliferation at any concentration of free peptides tested.

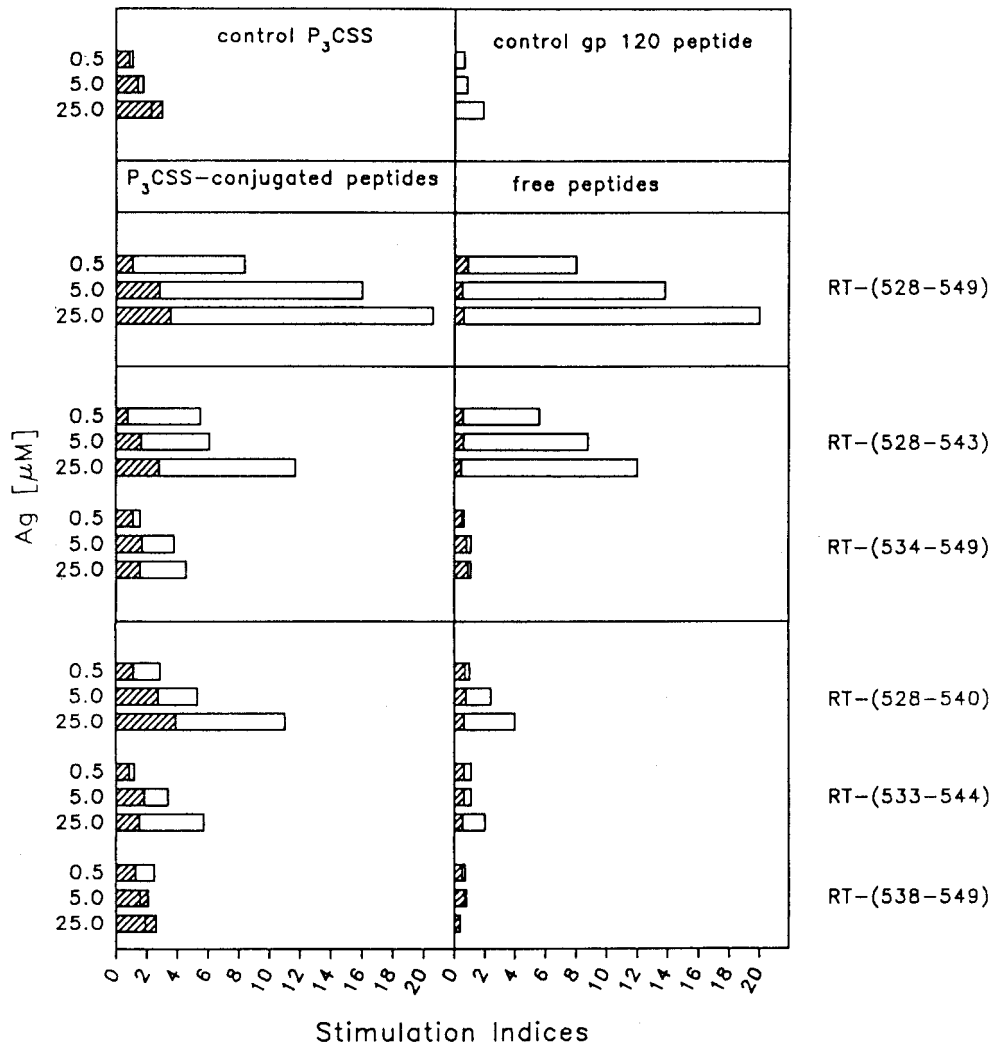


Figure 1. Proliferative responses of spleen T cells from BALB/c mice (H-2^d). Following immunizations on days 0, 14, and 28 with P₃CSS-[RT-(528-549)] (empty bars) or with the peptide RT-(528-549) (hatched bars), splenocytes were restimulated *in vitro* with lipopeptide-peptide-conjugates or unconjugated peptides on day 32 and assayed for [³H]dThd uptake (CPM) after 5 days of culture. Stimulation indices were determined as ratio between experimental cpm and background cpm of T cells and antigen-presenting-cells from triplicate cultures. The SD was below 10%. Background cpm were between 500 and 2000 cpm. Peptides were used at concentrations of 0.5, 5, and 25 μM. Control restimulation was performed with lipotriptide P₃CSS and a 27-mer peptide from gp120 of HIV-1 BH10 (³⁰³TRKSIRIQRGPGRAFVTIGKIGNMRQA³²⁹).

Immunization with P₃CSS-[RT-(522-543)] induced similar but lower proliferative responses to those peptides (data not shown), while immunization with the unconjugated peptide RT-(528-549) or PBS resulted only in a marginal T cell proliferation following restimulation with lipopeptides comparable to that induced by P₃CSS. No T cell responses were detectable after restimulation with unconjugated peptides. To further characterize the

peptide recognition site also in different mouse strains we used a panel of overlapping peptides (see Table 2). The shortest peptide recognized by BALB/c lymphocytes was RT-(528-540) (Fig. 1). The peptides RT-(524-535), RT-(526-537), RT-(528-539), RT-(533-544) and RT-(538-549) were not recognized (data not shown), indicating that KEKV [RT-(528-531)] at the N-terminus as well as PAHK [RT-(537-540)] at the C-terminus seem to be necessary for specific T cell recognition. Overlapping 9-mer peptides covering sequence positions 528-549 (Table 1) did not trigger T cell proliferation (data not shown). Some lipopeptides (e.g. P₃CSS-[RT-(528-543)]) also induced proliferative responses in mice that had not been primed with RT lipopeptides. This could be due to a primary cytokine releasing effect of these lipopeptides (9). Similar immunization of a different mouse strain [C3H/HeJ] with P₃CSS-[RT-(522-543)] or P₃CSS-[RT-(528-549)] also revealed proliferative responses to the peptides recognized by BALB/c mice (Table 2). Peptide RT-(526-541) that overlaps RT-(528-543) was also recognized in this mouse strain. In contrast, C57BL/6 mice only showed marginal secondary T cell responses after immunization with P₃CSS-[RT-(522-543)], and after immunization with P₃CSS-[RT-(528-549)] no response against RT-(526-541) was found (Table 2).

The lipopeptide-conjugate P₃CSS-[RT-(528-549)] elicited RT-specific antibody production

Sera from BALB/c mice primed with the lipopeptide conjugate P₃CSS-[RT-(528-549)] exhibiting secondary T cell responses, were tested for their ability to recognize a series of RT peptides. Ab titers to the lipopeptide P₃CSS-[RT-(528-549)], but not to the control lipopeptide P₃CSS, could be detected (Fig. 2). Since peptides like P₃CSS-[RT-(528-543)], P₃CSS-[RT-(534-549)] and P₃CSS-[RT-(528-540)] were also recognized (data not shown), shorter overlapping peptides, covering RT residues 528-549 (Table 1) were screened with sera from mice immunized with P₃CSS-[RT-(528-549)] (Fig. 2). P₃CSS-[RT-(528-549)] elicited the highest Ab titers; a very strong B cell response could be mapped to the C-terminal tetrapeptide EQVD P₃CSS-[RT-(546-549)]. In contrast, mice primed with the unconjugated peptide RT-(528-549) and control mice injected with PBS (data not shown) did not show specific Ab responses.

To test whether this recognition pattern was restricted to the H-2^d haplotype, we also immunized mice from the strains C3H/HeJ (H-2^k) and C57BL/6 (H-2^b) with either P₃CSS-[RT-(528-549)], P₃CSS-[RT-(522-543)] or PBS. Results shown in Figure 3 indicate that C3H/HeJ mice immunized with P₃CSS-[RT-(528-549)] also recognized the peptides containing the EQVD-motif (Fig. 3A). C57BL/6 mice that exhibited only marginal proliferative T cell responses were found to develop high Ab titers to the peptides containing the tetrapeptide motif EQVD [RT-(546-549)] (Fig. 3A) as well as to P₃CSS-[RT-(536-543)], while P₃CSS-[RT-(532-540)] was not recognized. This might be due to partial T cell activation resulting in

Table 2. T cell proliferate responses to HIV-1 synthetic RT peptides.

T cell stimulation indices ^a	
	C57BL/6
	C3H/HeJ

Table 2. T cell proliferate responses to HIV-1 synthetic RT peptides.

	T cell stimulation indices ^a					
	C3H/HeJ			C57BL/6		
	P ₃ CSS-[RT-(528-549)]	P ₃ CSS-[RT-(522-543)]	PBS	P ₃ CSS-[RT-(528-549)]	P ₃ CSS-[RT-(522-543)]	PBS
Immunization ^b :	P ₃ CSS- free	P ₃ CSS- free	P ₃ CSS- free	P ₃ CSS- free	P ₃ CSS- free	P ₃ CSS- free
Challenge ^c :	P ₃ CSS- free	P ₃ CSS- free	P ₃ CSS- free	P ₃ CSS- free	P ₃ CSS- free	P ₃ CSS- free
Peptide sequences						
RT-(522-537)	3.5	1.7	1.6	1.7	4.4	0.9
RT-(524-539)	1.1	1.6	0.7	0.9	2.8	0.8
RT-(526-541)	8.1	1.9	4.1	2.7	3.5	0.8
RT-(528-543)	9.6	1.7	4.9	4.0	4.7	1.1
RT-(534-549)	5.8	1.2	3.1	1.1	2.3	1.3
RT-(522-533)	2.4	1.9	0.4	0.9	1.8	1.0
RT-(524-535)	1.7	2.8	0.5	1.0	1.6	0.8
RT-(526-537)	3.2	0.7	1.1	0.9	3.8	1.2
RT-(528-540)	10.1	2.4	5.3	3.2	4.8	1.0
RT-(528-539)	1.2	1.1	1.4	1.1	3.5	0.7
RT-(533-544)	7.0	0.6	3.4	1.6	2.1	1.3
RT-(538-549)	4.0	0.8	2.3	1.1	2.7	1.2

^a [³H]dThd uptake measured by standard proliferation indices of exp cpm/background cpm of triplicate cultures.^b Mice were immunized either with P₃CSS-[RT-(528-549)], or P₃CSS-[RT-(522-543)] or PBS.^c Cultures were challenged with the different P₃CSS-coupled or uncoupled (free) peptides.

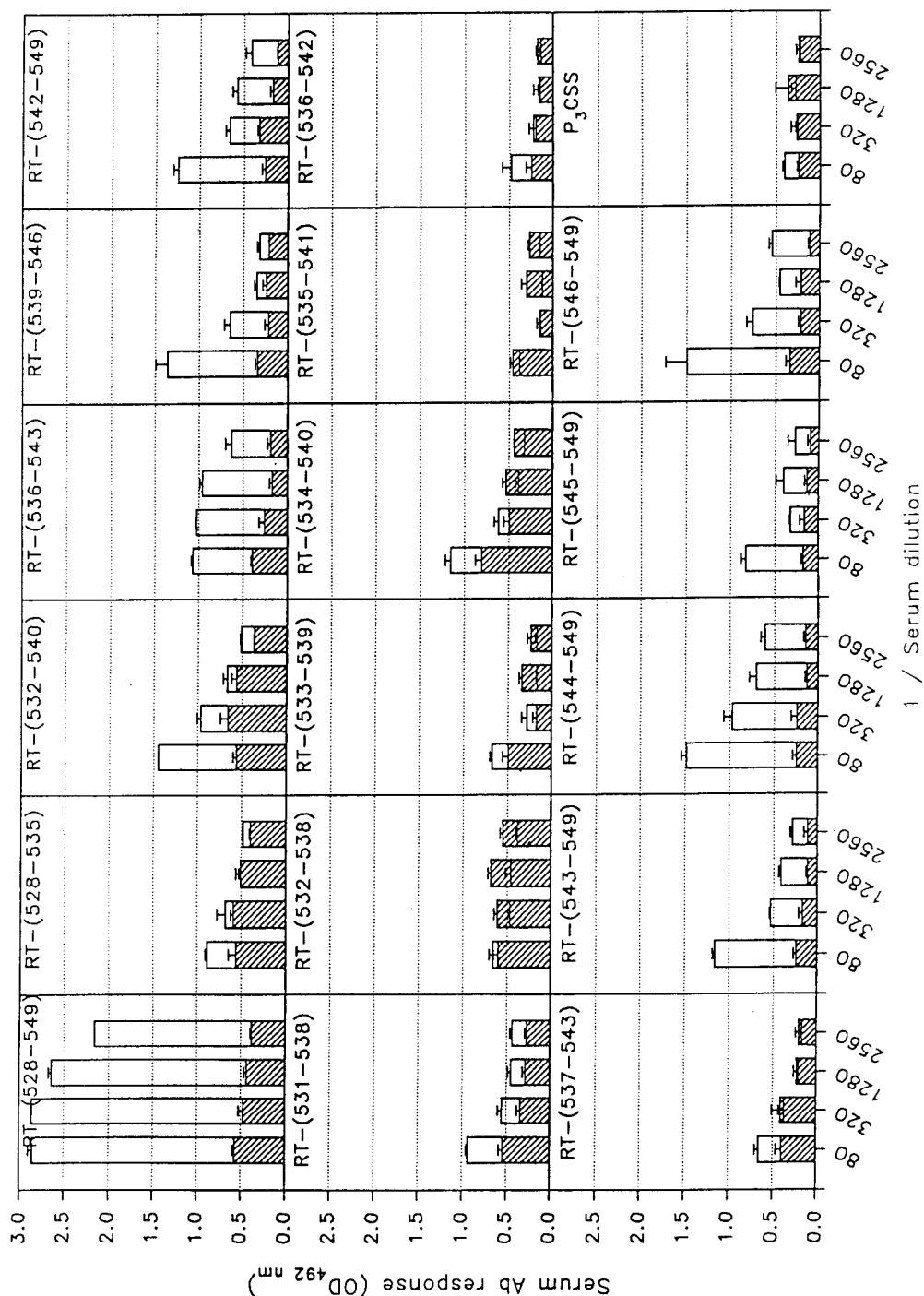


Figure 2. Specific antibody production after immunization of BALB/c mice (H-2^d) with P₃CSS-[RT-(528-549)] (empty bars) or the free peptide RT-(528-549) (hatched bars). Immunization was performed as described in Figure 1. Antibody-production was measured by a standard ELISA. Values are given as the means of triplicate assays \pm SD. Microtiter plates were coated with 0.5 μ g/well of the indicated P₃CSS conjugated peptides.

cytokine production immunization with 3B) induced a moderate [RT-(543)] in the mouse EQVD, which is r

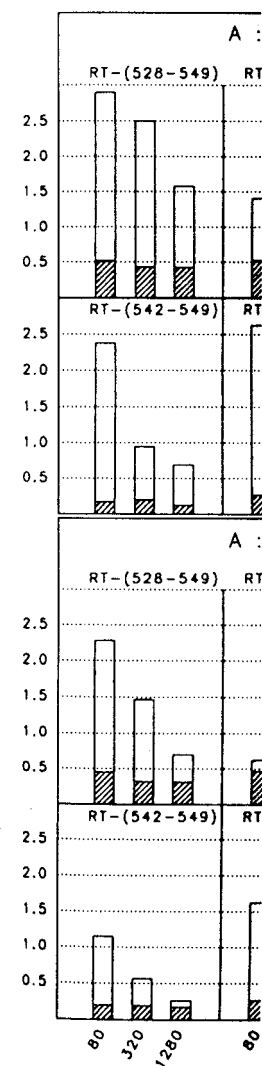


Figure 3. Specific antitoxin (A) performed as described in Figure 1. Antitoxin were coated with 0.5 μ g C3H/HeJ- (H-2^k) or C57BL/6-mice (H-2^b) injection of PBS (hatched bars).

cytokine production without significant proliferation (15–17). In contrast, immunization with the lipopeptide conjugate P₃CSS-[RT-(522-543)] (Fig. 3B) induced a moderate specific Ab response to peptide P₃CSS-[RT-(536-543)] in the mouse strains C3H/HeJ and C57BL/6, but not to the motif EQVD, which is not present in P₃CSS-[RT-(522-543)].

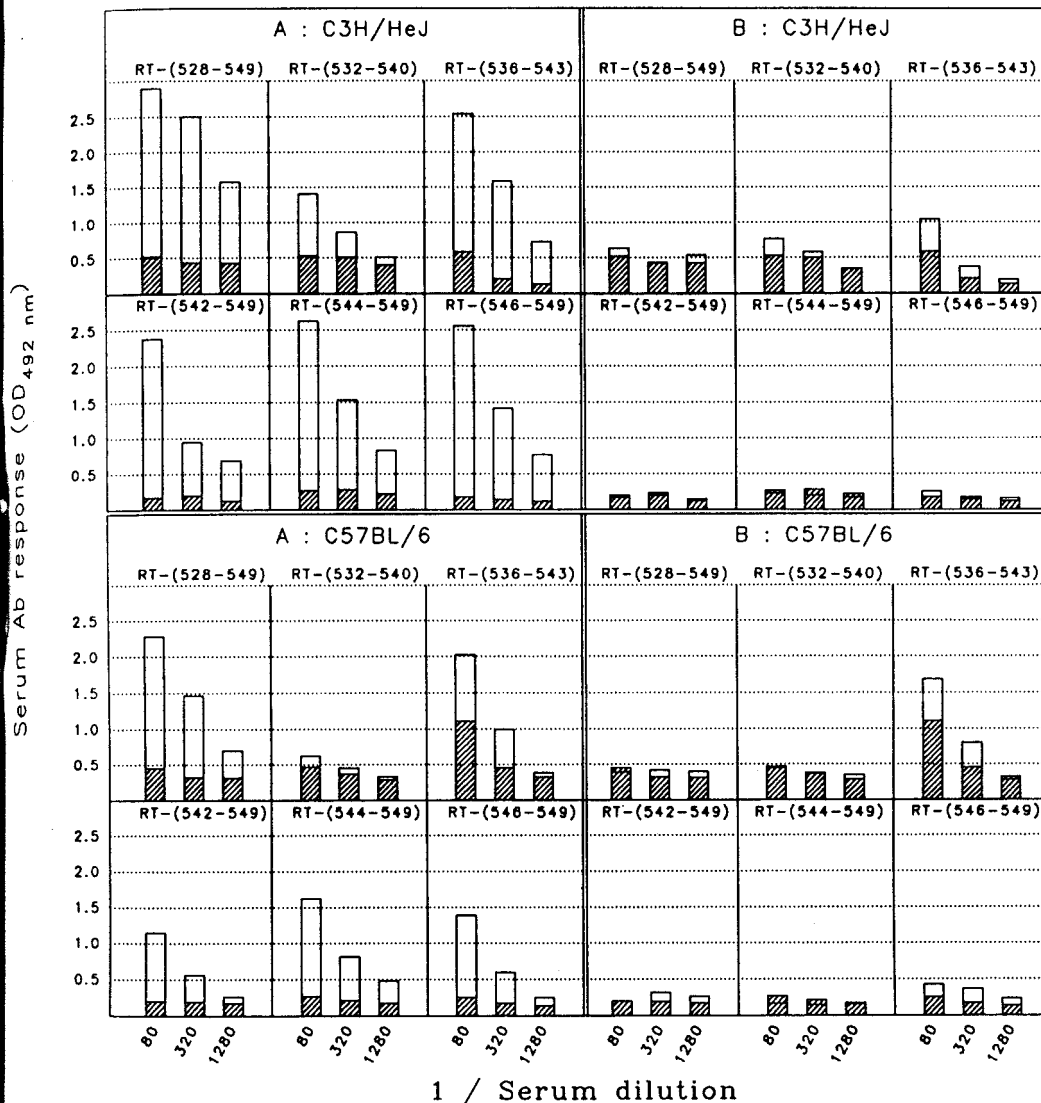


Figure 3. Specific antibody production by C3H/HeJ- or C57BL/6-mice after immunization with A: P₃CSS-[RT-(528-549)] or B: P₃CSS-[RT-(522-543)]. Immunization was performed as described in Figure 1. Antibody-production was measured by a standard ELISA and expressed as the means of triplicate assays, SD was < 10 %. Microtiter plates were coated with 0.5 µg/well of the indicated P₃CSS conjugated peptides. – A: Sera from C3H/HeJ- (H-2^k) or C57BL/6-mice (H-2^b) after immunization with P₃CSS-[RT-(528-549)] (empty bars) or injection of PBS (hatched bars). B: Sera from C3H/HeJ (H-2^k) or C57BL/6-mice (H-2^b) after immunization with P₃CSS-[RT-(522-543)] (empty bars) or injection of PBS (hatched bars).

/c mice (H-2^d) with
549) (hatched bars).
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plicate assays ± SD.
P₃CSS conjugated

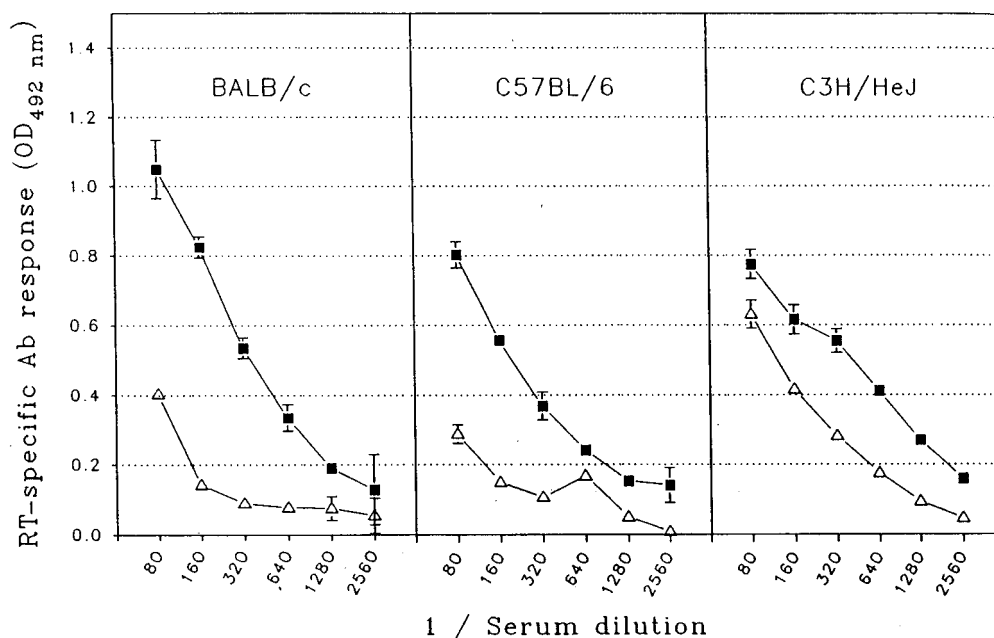


Figure 4. Recognition of native HIV-1 RT by sera from mice immunized with P₃CSS-[RT-(528-549)] (filled squares) or with PBS (empty triangles) under the conditions described in Figure 1. Sera were tested for their binding capacity to RT-coated wells (0.25 µg/well) in a standard ELISA (means of triplicate determinations ± SD).

When the same sera from these mouse strains were assayed for Ab responses to the whole RT molecule, specific binding was detectable in the sera of BALB/c and C57BL/6 mice (Fig. 4). However, we also observed recognition of RT by serum Ab from unprimed C3H/HeJ mice. To investigate a possible crossreactivity of RT with endogenous peptides, a search for local homologies was performed using the FASTA (18) program for sequence comparison (with parameters appropriate for small peptides) and position-dependent scoring matrices (19) on recent releases of protein sequence databases (EMBL-SWISS-PROT, Heidelberg, Germany, Rel. 24, NB-RF/PIR R. 36 and MIPS R. 36.01). Some local similarities in the N-terminal and central region of HIV RT to RTs of mouse-related viruses (e.g. mouse mammary tumor viruses strain C3H and mouse intracisterna A-particle, a defective retrovirus) could be detected (data not shown). This suggests, that possible latent infections with murine retroviruses could account for the observed crossreactivity.

Discussion

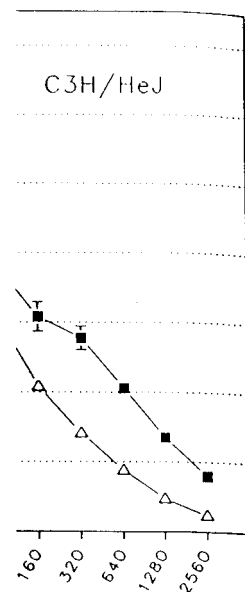
The results of this study show that P₃CSS constitutes an effective adjuvant capable of inducing T_h and B cell responses to peptide sequences from the C-terminal region of HIV-1 RT. The C-terminal region was chosen because it contained a T_h site (2) as well as B cell sites (3, 4) that have not yet been

characterized in immunodominant immunogenic regions. B cell sites in the C-terminal region were included in the vaccine design (2) with CFA as an adjuvant. Therefore, we could not test the response to peptides from the C-terminal region *in vivo* B and T_h cell responses.

Lipopeptide-adjuvanted B cell responses (9, 10) provide additional evidence for the use of various animal models at the N- or C-terminal coupling of an antigen. K.-H. WIESMÜLLER and his group developed lipopeptides to study B cell responses: MARTINEC by Boc chemistry terminal parts of the same model, DELOP by our group with a lysine spacer called MAPS (mimetic adjuvant peptide) able to induce Ab responses. The efficiency of the preparation.

FERNES et al. (3) identified B cell epitopes on the RT molecule, and located deletion mutants of human B cell sites in infected individuals. P₃CSS-[RT-(532-540)] further supports the use of RT-(542-540) to minimize the recognition of RT. To our knowledge, Tetrapeptides have not been identified. Nef protein (25) is a target for antibodies to native RT (26, 27) but the function is not yet clear, since the role of the RT in the progression of disease (28) is still unknown.

The RT T_h epitope as RT-(528-540).



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SD).

assayed for Ab
detectable in the
we also observed
H/HeJ mice. To
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or small peptides)
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Germany, Rel. 24,
larities in the N-
lated viruses (e.g.
intracisterna A-
not shown). This
etroviruses could

effective adjuvant
quences from the
as chosen because
ave not yet been

characterized in detail. In previous investigations aimed at defining immunodominant epitopes of HIV-1 RT, we have identified 4 highly immunogenic regions that contain T_h sites which partially overlap with B cell sites in the context of H-2^d and thus might be important for subunit vaccine design (20). However, the immunogens applied had been prepared with CFA as adjuvant which cannot be used for human vaccination. Therefore, we covalently coupled the nontoxic lipopeptide adjuvant P₃CSS to peptides from the C-terminal region of HIV-1 RT, and investigated the *in vivo* B and T_h cell responses.

Lipopeptide-antigen conjugates have been shown to induce both T and B cell responses (9, 12); furthermore, it has already been shown that lipopeptides provide adjuvant function to otherwise poorly immunogenic peptides in various animal models (11, 14, 21–23). The lipo-amino acid P₃C located at the N- or C-terminal part of a given peptide is more effective than the coupling of an analogue of P₃C at an intermediate position (reviewed in 23, K.-H. WIESMÜLLER, personal communication). Approaches to couple lipids or lipopeptides to HIV-derived peptides have also been described by other groups: MARTINON et al. (21) used a C₁₄-fatty acid peptide (HDA) prepared by Boc chemistry coupled to N-terminal, and in some experiments, also C-terminal parts of peptides from the V3 region of HIV-1 Env. Using the same model, DEFOORT et al. (22) synthesized the lipopeptide P₃C developed by our group (10) using Fmoc chemistry, and modified it by linking with a lysine spacer to 4 molecules of the same HIV Env peptide to a so-called MAPS (multiple antigen peptide system). Both preparations were able to induce Ab production, CTL and T cell help to HIV-1 gp120, but the efficiency of the HDA preparation did not reach the same level as the P₃C preparation.

FERNS et al. (3) and TISDALE et al. (4) describe linear and conformational B cell epitopes of RT in BALB/c mice after immunization with the native molecule, and located a B cell site at RT-(532-539) using truncated expression mutants of the RT molecule. Part of this region overlaps with the human B cell site RT-(511-536) that is recognized by sera from HIV-infected individuals (24). The B cell responses to the overlapping peptides P₃CSS-[RT-(532-540)] and P₃CSS-[RT-(536-543)] as well as to native RT further supports these observations. In addition, we found a strong recognition of RT-(542-549) in all three mouse strains analyzed, and were able to minimize the recognition site to the tetrapeptide EQVD [RT-(546-549)]. To our knowledge, this is the first description of this B cell epitope. Tetrapeptides have already been reported as B cell epitopes in the HIV-1 Nef protein (25). Other epitopes that were recognized using monoclonal antibodies to native RT were localized in the N-terminal and central region of the RT (26, 27). The biological relevance of Ab responses to HIV RT is not yet clear, since some groups reported declining Ab titers with progression of disease (28) while others did not (29, 30).

The RT T_h epitope previously described was here more precisely defined as RT-(528-540). The observation that this sequence includes the B cell site

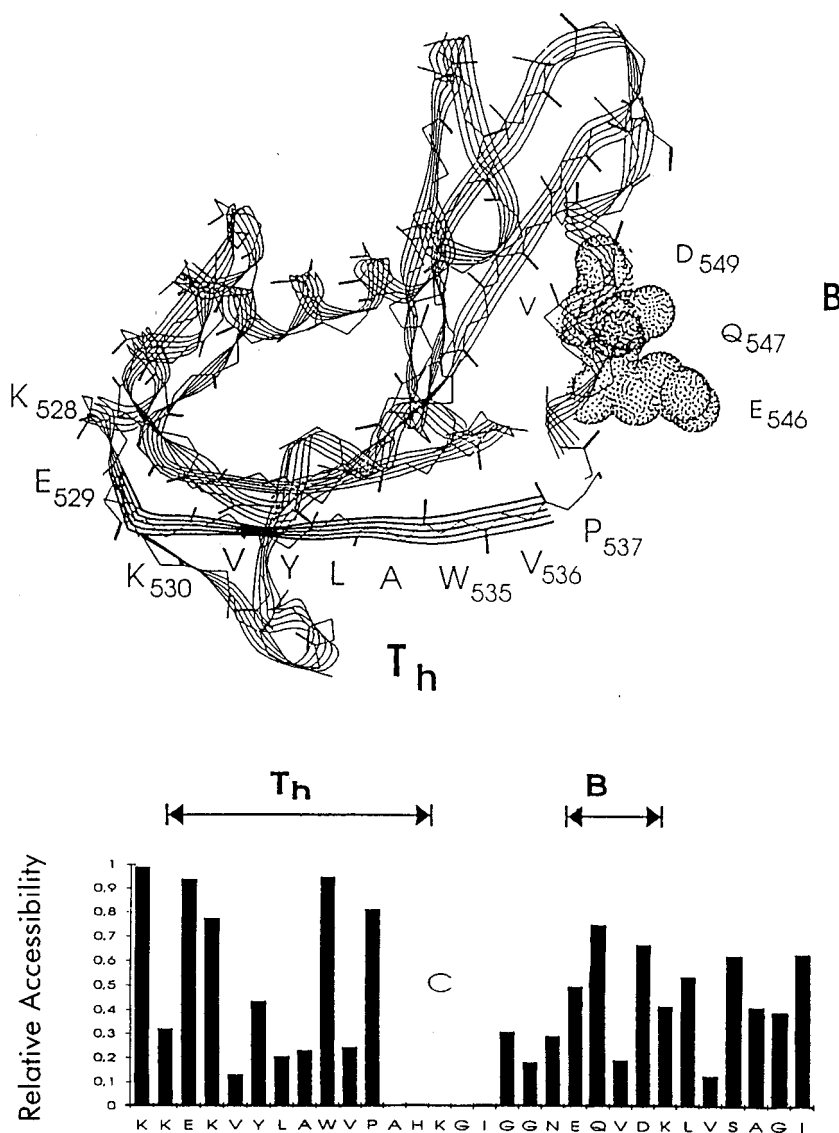


Figure 5. X-ray structure of the RNase H domain of HIV-1 reverse transcriptase (1HRH). The relative accessibility of the amino acids of the T cell site RT-(528-540) (T_h) and the B cell site RT-(546-549) (B) is calculated using a grid based method and a water probe sphere of 0.14 nm. The reference state is defined by residues, which are inserted in a polyglycine helix. For amino acids RT-(538-542) no structural data are available.

RT-(532-539) may explain the strong immune response that could be elicited in different detection systems. This is in accordance with the data showing that RT-(528-542) binds very efficiently to the MHC class II molecule A^d and induces T_h responses *in vivo* as well as *in vitro* after priming with native RT (20). Recently a murine CTL epitope has been located to RT-(514-528) (31) that is closely related to the T_h epitope RT-(528-539) described in this paper, indicating that in the C-terminal region of HIV-1 RT MHC class I- and class II-restricted presentation might occur.

There are recent data as well as to MHC and humans (34-36) lymphocytes in the responses of HIV CTL responses to individuals indicating lines derived from CD4 counts < 200 with the HLA-A2 MHC K^k and K^d .

The high immune response (550) correlates with the data obtained by X-ray B cell epitope RT-(528-539) located on the protein seeking helical secondary strandlike segment (3D-structure) and of the EQVD epitope continuous B cell epitope recognition of the EQVD epitope antibody binding; residue might explain the size.

In summary we show that the immune responses by lipopeptides findings indicate that the C-terminal region is a candidate for vaccine development.

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There are recent reports of some peptides that can bind to MHC class II as well as to MHC class I (32, 33) or that can be recognized both in mice and humans (34–36). To test whether RT-(528–549) is immunogenic for T lymphocytes in humans as well, we are now investigating the T cell responses of HIV seropositive individuals. Our first results investigating CTL responses to the C-terminal region of HIV 1 RT in HIV-infected individuals indicate that peptide RT-(528–549) can be recognized by CTL lines derived from PBMC of patients at different stages of disease even at CD4 counts $< 200/\text{mm}^3$. In 2 patients this recognition occurred in context with the HLA-A2 molecule, which shares C-terminal anchor motifs with MHC K^k and K^d (37).

The high immunogenicity of the C-terminal region of HIV 1 RT-(510–550) correlates with structural and topological features of the protein (Fig. 5) obtained by X-ray analysis of the HIV RT/RNase H domain. Both, the B cell epitope RT-(546–549), and the T cell epitope RT-(528–540) are located on the protein surface. The B cell epitope can be assigned to surface seeking helical segments, and the T_h cell epitope spans the complete strandlike segment (including a N-terminal region which is buried in the 3D-structure) and 1 or 2 neighbouring loop residues. The glutamine residue of the EQVD epitope protrudes from the surface. The accessibility of the continuous B cell epitope EQVD in the 3-D structure is consistent with the recognition of the native structure of the RT/RNase domain. The structure of the EQVD epitope suggests a critical role of the glutamine residue for antibody binding; a complementary pocket for binding of this exposed residue might explain why this epitope can be minimized to a very small size.

In summary we could demonstrate an effective induction of B and T_h cell responses by lipopeptide antigen conjugates in a viral model. Moreover, our findings indicate that the C-terminal part of the HIV-1 RT is a promising candidate for vaccines.

Acknowledgements

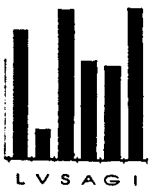
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Received March

Abstract

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