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Immunological reactivity of a human immunodeficiency virus type I derived peptide representing a consensus sequence of the GP 120 major neutralizing region V3

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Summary. To reduce the opportunities for human immunodeficiency virus type 1 (HIV-1) to evade vaccine induced immunity, the development of subunit vaccines must focus on the characterization of immunogenic epitopes, which are major targets for the immune system. The most dominant site for elicitation of neutralising immune response is located on the external envelope glycoprotein gp 120 within the third variable domain (V3). To overcome virus type specificity of antibodies directed to the V3-domain we designed a 36 amino acids long gp 120/V3-consensus peptide (V3-C_{36}) based on published biological data and sequence comparisons of various HIV-1 virus isolates. This peptide contains a conserved core sequence which is suggested to form a surface-exposed β-turn. This peptide also includes T-cell epitopes defined in mice and humans, an ADCC-epitope and two highly conserved cysteine residues which were oxidized to form a cystine derivate, thus allowing correct peptide folding. In ELISA-tests, this peptide reacts with at least 90% of randomly selected sera of European and African patients infected with HIV-1 and is recognized by three different HIV-1/V3 "type-specific" antisera (MN, RF, IIIB-strain). Using this peptide as immunogen in rabbits, antisera could be raised with highly cross-reactive and HIV-1/IIIB strain neutralizing properties. Moreover, HTLV/HIV-1/IIIB specific cytotoxic T-lymphocytes (CTLs) of BALB/c mice infected with a gp 120 recombinant vaccinia virus recognized the central 16- and 12-mer peptides of the V3-C_{36} consensus peptide in cytolytic assays, indicating perfect compatibility of the consensus peptide with the IIIB-primed CTLs. The DNA-sequence encoding the V3-consensus loop region might be an important component in newly designed recombinant subunit vaccines. In addition, due to its broad

serological reactivity, the V3-consensus peptide might play an important role in special diagnostic purposes.

Introduction

Several domains of the HIV-1 glycoprotein complex have been shown to induce virus neutralizing antibodies in experimental animals when applicated in the appropriate form. Amongst those, the most dominant neutralization site is located within the third variable domain (V3) of the HIV-1 outer envelope protein gp 120. The V3-loop of the gp 120 was described as the only hypervariable surface exposed antigenic region without consensus sequence for N-glycosylation and was early discussed in its immunological importance [20]. Deletion of the flanking cysteine residues 296 and 331 results in non infectious mutants. Actually, this region was verified by several laboratories to be the dominant neutralizing epitope on the external glycoprotein of HIV [8, 11, 21, 24, 25]. Small peptides are sufficient to induce neutralizing antibodies [7, 13, 14, 23]. This sequence contains a potential, in various virus isolates highly conserved β-turn motif (GPGR) [19, 15]. Flanking residues seem to be critical for antibody binding, virus type specific neutralization, inhibition of syncytia formation and stabilization of the structure of the V3-loop. Recent publications indicate that disease progression in AIDS patients and vaccine induced immunity in experimental animals might be directly correlated with V3-specific antibody titers [1, 6]. This hypothesis is supported by the observation that virus is transmitted from HIV positive mothers to their children during pregnancy in the absence of high-affinity maternal antibodies to gp 120/V3. In contrast, mothers with high titers of V3-specific antibodies could be shown only in few cases to transmit their HIV to their children during pregnancy [6].

How anti-V3-neutralizing antibodies actually inhibit infection and syncytia formation is not clear to date. One possibility is sterical hindrance by bound antibodies inhibiting direct interaction of the gp 120/C4 region with the CD4 receptor molecule. Recent results [3, 12] suggest the involvement of cellular, cell surface exposed proteases as potential secondary adsorption site for gp 120/V3 and propose cleavage of gp 120 in the GPGR motif of the V3-loop. This process possibly induces structural rearrangements thus stabilizing the interaction of the gp 120/C4 region with the CD4 receptor [12, 16] or enabling gp 41 mediated membrane fusion events [3].

The characterization of the V3-loop as an ADCC-target region (aa 315–329) is of special interest with respect to the induction of immune response capable to eliminate HIV-infected cells [17, 18]. Further, a HIV-1/IIIB derived minimal peptide of gp 120/V3 was shown to be recognized as target for cellular immune response in BALB/c mice immunized with IIIB-*env* recombinant vaccinia virus [27–29]. CTLs raised against the IIIB- and against the MN-specific sequences by an *env*-recombinant vaccinia virus were completely not cross-reactive. Reciprocal exchange of a single amino acid between the two peptides led to a complete reversal recognition by primed CTLs. In addition, CTL

responses to this determinant were detected iwth the H2-D^d haplotype only. V3-IIIB derived synthetic peptides were shown to stimulate T-helper cell function by PBL from HIV infected, asymptomatic patients [5]. Recently also CTL activity could be demonstrated and characterized in a similar patient population [4].

Sequence analysis of HIV-1 virus isolates in Europe and U.S.A. demonstrated that V3 heterogeneity is limited [15, 26]. The majority of the sequenced virus isolates could be classified as variants similar to MN- or SC-like strains. Sequence alignment revealed five arbitrary virus families: the first one consisting of MN, SF, SC, second of CDC4 and NY5, third of RF, fourth of ELI, Z6 and MAL and the last of Z3 [22]. Based on serum cross reactivities Goudsmit et al. [9] discriminated three virus families including following prototype members: SF and RF; ELI, Z6 and MAL; Z3, CDC4 and NY5.

The knowledge about V3-heterogeneity and its involvement in ADCC- and CTL-reaction in virus neutralization in combination with geographic distribution of individual V3-family clusters stimulated us to design a V3-consensus domain. This approach aims to investigate whether the antigenicity and immunogenicity of the epitope may be extended by using a rationally designed variant of the HIV-1 dominant neutralizing epitope V3 (V3-C_{36}). The corresponding DNA sequence could be included as an important component in future subunit vaccines. Another approach aims to develop a group specific diagnostic, possibly prognostic antigen for detection of V3 specific antibodies in patients.

Materials and methods

Sequence comparison

The design of the V3 consensus sequence was based on the comparison of a considerable number of different, independent HIV-1 isolates from Europe, U.S.A. and Africa. Optimal sequence alignments of the various V3 domains were achieved according to chemical and functional similarities of the individual residues. The number of theoretically necessary mutation events in DNA sequence was not included. The constructed sequence includes the neutralization-active highly conserved GPGR motif, which is predicted to form a β-turn (unpubl. data), the H2-D^d restricted mouse CTL epitope, the ADCC-target domain and the flanking cysteine residues thus allowing the formation of a disulfide bridge and stabilization of the peptide structure. The sequence of this V3-peptide 36 amino acids in length (V3-C_{36}) is shown in Fig. 1 B.

Peptide synthesis

Peptide synthesis was done in a 9050 peptide synthesizer (Milligen) using Fmoc (9-fluorenylmethyloxycarbonyl)-protected amino acids. The first residue was coupled to the resin by an acid-stable amide anchor group (Tentagel AM; purchased from Rapp polymere, Tübingen, Federal Republic of Germany). t-Butyl (Ser, Thr, Tyr, Cys, Asp, Glu, His), t-butoxycarbonyl (Lys), and 4-methoxy-2,3,6-trimethylbencenesulfonyl (Arg) groups were used for side chain protection. Fmoc protected amino acids were converted to hydroxybenzotriazol-activated esters by treatment with 0.75 mmol of hydroxybenzotriazol and 0.6 mmol diisopropylcarbodiimide per mmol of amino acid for 10 min in

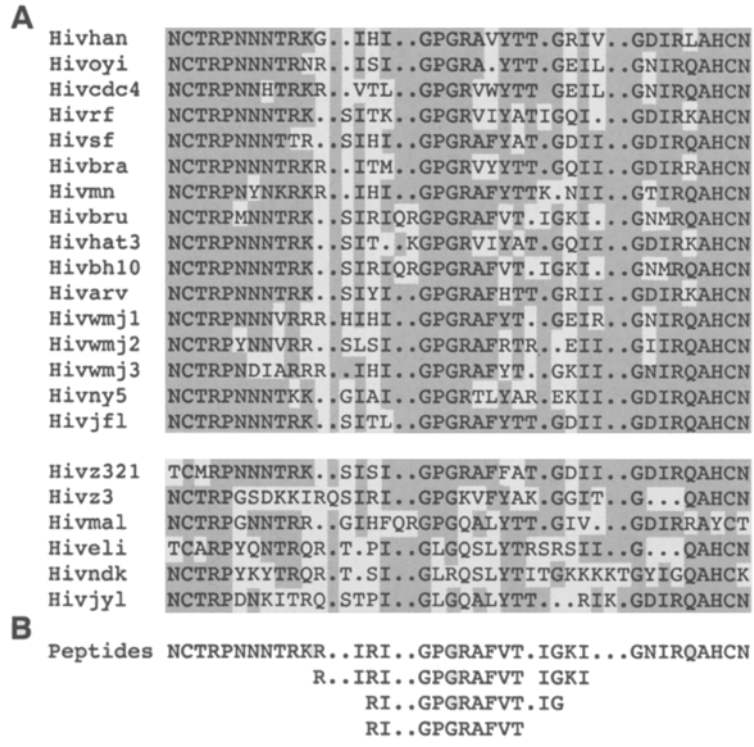

Fig. 1. A Sequence comparison of the gp120 major neutralizing epitope V3 of various European, American and African HIV-1 virus strains. Shaded sections within the group of the depicted isolates are highly conserved (> 50%). **B** V3-consensus peptides: 36-mer (V3-C_{36}), 16-mer (V3-C_{16}), 12-mer (V3-C_{12}), and 10-mer (V3-C_{10})

N,N′dimethylformamide. The subsequent coupling reaction was performed in dimethylformamide. After the coupling reactions, Fmoc-groups were removed with 20% piperidine in dimethylformamide followed by a series of washed with dimethylformamide (all solvents were purchased from Merck AG, Darmstadt, Federal Republic of Germany; Fmoc-protected amino acids were from Bachem AG, Bubendorf, Switzerland; all chemicals from Aldrich, Steinheim, Federal Republic of Germany). After synthesis, side chain protecting groups were removed by a 12 h treatment in trifluoracetic acid containing 5% mercaptoethanol, 3% thioanisol, 3% phenol at room temperature. The peptide was precipitated in an excess of ice-cold t-butyl-ethyl ether, washed several times, suspended in high volume of 1.5% ammoniumcarbonate in order to allow the preferential formation of intramolecular cysteine derivates and stirred over night. The peptide was lyophilized and purified by reversed phase high performance liquid chromatography (HPLC) using a gradient of 0–70% acetonitrile in 0.1% trifluoracetic acid. The sequences of the peptides were checked by amino acid analysis and the peptides were stored as lyophilized material at 4 °C. In addition to V3-C_{36} several subfragments were synthesized.

ELISA test

500 ng of purified V3-C_{36} peptide per well were coupled over night in 0.2 M sodium carbonate buffer, pH 9.5 to 96 well microtiter plates (Dynotech). Coupling of shorter peptides representing central sequences of various V3 domains were adjusted to the molar ratio of the

described 36 amino acid V3-consensus peptide. Free protein binding sites were saturated by a 2 h incubation with gelatin solution (5 mg/ml; Sigma Chemicals, Munich, Federal Republic of Germany). Before and after addition of the respective serum dilutions in PBS (2 h, 37 °C) the plates were washed several times with PBS containing 0.5% Tween 20. The peroxidase labeled second antibody (rabbit anti humans, swine anti rabbit IgG, rabbit anti mouse IgG or rabbit anti goat IgG; Dako, Hamburg, Federal Republic of Germany) was added in a dilution of 1/1000 in PBS/0.5% Tween 20. Staining was done in 0.1 M phosphate buffer, pH 6.0 containing 0.5 mg/ml o-phenylenediamin and 0.1% H_2O_2 for 10 min, the reaction was stopped with 1 M H_2SO_4, and optical density was determined at 486 nm.

Production of anti peptide sera

For the production of anti peptide specific antibodies in rabbits, 200 µg of the dissolved V3-C_{36} were used in an emulsion with complete Freund's adjuvant (CFA). Booster injections were performed four times with 200 µg of the V3-C_{36} in an emulsion of incomplete Freund's adjuvant in four week intervals (serum A).

In a second approach, V3-C_{36} was coupled to ovalbumin via carboxyterminal COOH-group of the peptide using water soluble 1-ethyl-3,3-dimethyl-aminopropyl-carbodiimide (EDC; Sigma Chemical Company, St. Louis, MO, U.S.A.). For the first immunisation 500 µg of the coupled peptide (OvaV3-C_{36}) were emulgated in complete Freund's adjuvant. Booster-injections were performed in 4 week intervals with 500 µg of the coupled peptide in incomplete Freund's Adjuvant (serum B). The V3-C_{36} anti peptide sera were analysed by ELISA using various V3-derived peptides and by conventional Western blot analysis using p55/V3 fusion proteins as antigen [31].

HIV neutralisation assay

100 tissue culture infectious doses ($TCID_{50}$) of HIV-1/HTLV-IIIB were incubated for 1 h at 37 °C with different dilutions of heat inactivated rabbit antisera. About 10^6/ml Jurkat $CD4^+$ cells suspended in RPMI 1640 culture medium with 10% inactivated fetal calf serum and 2 µg/ml polybrene were infected with the preincubated mixtures and seeded into 4 individual microtiter wells (100 µl/well). After three days 50% of culture medium was exchanged by fresh medium without polybrene; 20% fresh medium was added in 3–4 days intervals. After 2 and 3 weeks, 50% of the medium was replaced. The removed medium was concentrated with PEG (polyethylenglycol) and tested for reverse transcriptase activity with poly rA : dT and Mg^{++} as described previously [10]. Cell fusion was evaluated by microscope controls. Cultures with either reverse transcriptase activity or giant cell formation were considered as positive, cultures without parameters were regarded negative.

Mouse cytotoxic T-cell recognition

Spleen cells from BALB/c mice, harvested 2 months after immunization with an gp 120-IIIB recombinant vaccinia virus VSC25 (1×8^8 PFU/mouse) were used as effector cells. The isolated cells were restimulated for 7 days with the gp 120-IIIB recombinant vaccinia virus (2×10^7 PFU/cell). Target cells (p 815 mastozytoma cells) were incubated for 1 h with different concentrations of the peptides (10 µM, 0.1 µM, 1 nM) and tested in a 3 h cytolytic assay for ^{51}Cr-release using different target/effector-cell ratios [27].

Results

Reactivity of the V3-C_{36} peptide with AIDS patient sera

As a consequence of the isolate-specificity of neutralizing antibodies directed against the V3-loop, we designed a 36 amino acid consensus peptide (V3-C_{36})

including the highly conserved GPGR-motif, a mouse-specific T-cell epitope and the highly conserved flanking cysteine residues essential for the stabilisation of the secondary and tertiary structure of the peptide. The design was based on published biological data and sequence comparisons of a number of different and independent HIV-1 isolates derived from Europe, U.S.A. and Africa (Fig. 1).

The cysteine residues were oxidized following peptide synthesis by air oxygen and the cyclized peptide was seperated from linear and oligomerized material by reversed phase HPLC. The purified peptide was tested in ELISA with 97 sera from HIV-1 infected persons in various stages of the disease. To exclude regional strain specific effects, sera from European (n = 58) and African (n = 39) seropositive donors were used. Almost 90% of all randomly selected sera of HIV-1 infected persons reacted positively with the V3-C_{36} independent of the clinical stage of the disease and the origin of the tested sera. This is in clear contrast to recent investigations reporting the reactivity of 86 random AIDS patient sera with the commonly used HIV-1/IIIB or LAV (BRU) V3 sequences: only 14% of the tested sera showed positive reaction [15]. At least 83% (45/54) of sera derived from asymptomatic HIV-1 patients recognized the V3-C_{36} peptide in ELISA. Differentiation between sera from European, mostly infected with HIV-1 types of US-origin, and African asymptomatic patients revealed that the positive reaction with V3-C_{36} of African sera (13/18; 73,2%) is only slightly reduced if compared to the reactivity of the European sera (30/36; 83%). Part of the sera from early infection status, which did not recognize the consensus peptide were also negative for gp 120 detection in Western blot analysis. Sera taken two months later from the same patients were positive to V3-C_{36}. All sera from LAV-, ARC- and AIDS (symptomatic) patients tested showed positive reaction in ELISA, independent from their origin of Europe and Africa. The serological data are summarized in Fig. 2.

Reactivity of V3-C_{36} with HIV-1 virus typespecific sera

To analyse the isolate- or group-specificity of V3-C_{36} we tested the reactivity of HIV-1 typespecific sera [24] with the peptide. These sera were raised in goats with peptides representing the central region of the V3 domain (aa 301–321) of strains IIIB, MN, and RF coupled to tetanus toxoid: V3-IIIB, V3-RF and V3-MN specific peptide antisera recognized V3-C_{36} in ELISA. The reactivity of the 1×10^{-2} diluted sera is shown in Table 1, indicating that the peptide is recognized by all HIV-1 type specific sera tested so far. End point titers were determined using serial dilutions of V3-specific antisera. V3-C_{36} was recognized best by the V3-IIIB specific antiserum with titers of 2.4×10^4, the corresponding antisera to V3-MN and V3-RF showed titers of 3.2×10^3 and 1.6×10^3, respectively. Another anti V3-peptide serum, raised in rabbits against a short V3-consensus peptide developed in our laboratory (V3-S_{12}; SIHIGPGRAFVT; [21]) reacted with titers of 3.2×10^3 (Table 1). Moreover, abundant reaction with V3-C_{36} could be shown in ELISA using a murine monoclonal antibody

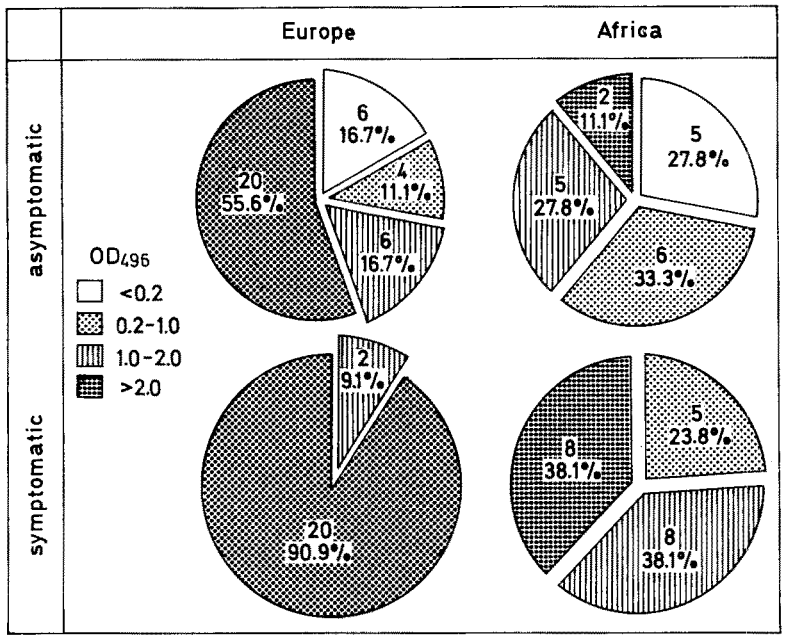

Fig. 2. Reactivity of AIDS patient sera in ELISA using the cyclized and purified V3-C_{36} peptide as antigen. 1/100 serum dilutions of asymptomatic and symptomatic European and African donors were tested

directed to the central V3-IIIB sequence. A 4.8×10^{-4} dilution of the monoclonal antibody was sufficient to specifically recognize the V3-C_{36} peptide.

Reactivity of antipeptide-specific antibodies directed to the V3-C_{36} peptide

Using the V3-C_{36} peptide as immunogen we were able to induce polyclonal V3-specific antibodies in rabbits (Table 1). Resulting anti-V3 sera recognized the peptide with titers ranging from 1×10^4 to 5×10^4, depending on the V3-C_{36} formulation. When applied as a free peptide, maximum titers of 10^4 were reached in rabbits (serum A), when V3-C_{36} was conjugated to ovalbumin with carbodiimide before inoculation maximum titers of 5×10^4 (serum B) were obtained (Table 1). Serum B was further used to test the cross reactivity of V3-C_{36} specific antibodies in Western blot analysis using chimeric *gag*/V3IIIB and *gag*/V3-consensus hybrid proteins, produced by recombinant vaccinia viruses as target antigens [31]. Serum B recognized both chimeric proteins with the same sensitivity independently from the V3-strain specificity. Serum dilution of 1×10^{-6} easily detected the V3 epitope of the tested fusion proteins in immunoblots.

Further, we tested the specificity of V3-C_{36} specific serum B using shortened derivates of the V3-C_{36} peptides as targets in ELISA experiments (Table 2). A 16-mer (V3-C_{16}) and a 12-mer (V3-C_{12}) peptide representing the central region of the V3-C_{36} peptide were sufficient to be recognized by serum dilutions of 3.2×10^{-3}. Further shortening of the peptide to 10 (V3-C_{10}) or 8 amino acids (V3-C_8) drastically reduced the recognition by serum B. To confirm the cross

Table 1. Reactivity of type-specific antisera, a murine monoclonal antibody and of two V3-C_{36} specific antisera in a peptide ELISA

Antiserum to	Titer
V3-IIIB[a]	2.4×10^4
V3-MN[a]	3.2×10^3
V3-RF[a]	1.6×10^3
V3-S_{12}[b]	3.2×10^3
mab-V3IIIb[c]	4.8×10^4
V3-C_{36} (serum A)[d]	1.0×10^4
OVA-V3-C_{36} (serum B)[d]	4.8×10^4

[a] End point titers of V3-type-specific antisera [24]

[b] End point titer of an antiserum against a 12-mer consensus peptide [21]

[c] End point titer of a murine V3-IIIB monoclonal antibody (Du Pont Nea 9301)

[d] End point titers of antisera raised in rabbits against the V3-C_{36} peptide

reactivity of serum B with V3-IIIB derived peptides in ELISA, three different peptides shortened asymmetrically from the COOH-terminus were used (Table 2). A 16-mer V3-IIIB peptide (V3-$IIIB_{16}$) was recognized from serum dilutions up to 3.2×10^{-3}, thus showing a comparable reactivity with serum B as the corresponding V3-C_{16} peptide. V3-IIIB derived shortened peptides (V3-$IIIB_{11}$; V3-$IIIB_{10}$) required significantly lower serum dilutions for specific recognition (4×10^{-2}; 1×10^{-2}). A similar reactivity was already mentioned for the V3-C_{36} subfragments. A single amino acid exchange (P to L) in the β-turn motif of the V3-C_{36} peptide reduced the reaction of the peptide with serum B almost by a factor of 10. Further analysis used peptides that represent the V3-corresponding region (16 aa) of unrelated HIV-1 isolates (V3-MN_{16}, -RF_{16}, -$SF2_{16}$, and -$WMJ2_{16}$), also including African virus strains (V3-$Z6_{16}$, -$Iy1_{16}$, and -Mal_{16}) as antigens. The interpretation of the ELISA experiments revealed a broad cross reactivity of serum B also with peptides derived from unrelated HIV-1 V3-sequences. However, the quantity of cross reaction is dependent on the individual isolate and is slightly reduced as compared to the recognition of the original 16-mer V3-C_{16} consensus peptide (Table 2). In competition ELISA experiments, none of the shortened peptides, depicted in Table 2 was capable to block or even significantly reduce the reactivity of patient sera or serum B to V3-C_{36}. Using the complete peptide in the competition experiment, reactivity was reduced almost to background level (data not shown).

Table 2. Crossreactive properties of an antiserum raised in rabbits against the 36-mer V3-consensus peptide in ELISA experiments

Peptide[a]	aa-Sequence	Titer serum B
V3-C_{16}	RIRIGPGRAFVTIGKI	3.2×10^3
V3-C_{12}	RIGPGRAFVTIG	3.2×10^3
V3-C_{10}	RIGPGRAFVT	1.0×10^2
V3-C_8	GPGRAFVT	1.0×10^2
V3-L_{16}	RIRIGLGRAFVTIGKI	4.0×10^2
V3-$IIIB_{16}$	RIQRGPGRAFVTIGKI	3.2×10^3
V3-$IIIB_{11}$	RIQRGPGRAFV	4.0×10^2
V3-$IIIB_{10}$	RIQRGPGRAF	1.0×10^2
V3-MN_{16}	RIHIGPGRAFYTTKNI	1.6×10^3
V3-RF_{16}	SITKGPGRVIYATGQI	8.0×10^2
V3-$SF2_{16}$	SIYIGPGRAFHTTGRI	8.0×10^2
V3-$WMJ2_{16}$	SLSIGPGRAFRTREII	4.0×10^2
V3-$Z6_{16}$	STPIGLGQALYTTRGR	8.0×10^2
V3-$IY1_{16}$	STPIGLGQALYTTRIK	4.0×10^2
V3-MAL_{16}	GIHFGPQALYTTGIV	2.0×10^2

[a] Equimolar ratios of the peptides indicated were used for immobilization on ELISA plates; the numbers following the designation of the HIV-strain correspond to the length of the tested peptides; *C* and *L* correspond to the used type of the tested consensus peptide

Table 3. HIV neutralization assay. Neutralizing activity of the V3-C_{36} derived antiserum (serum B)

Serum	Serum dilution	RT activity (cpm) $\times 10^3$	Syncytia formation
Mock	–	2.0	–
B+	1:4	3.2	–
B–	1:4	48.2	+

B+ Preimmune serum; *B–* immune serum

Reactivity of V3-C_{36} peptide specific antibodies in HIV-IIIB specific neutralization tests

To prove the functionality of V3-C_{36} induced antibodies in vitro, we performed a virus neutralization test (NT) using Jurkat cells as targets for infection with HIV-IIIB. This virus strain was selected with respect to the good recognition of V3-IIIB derived peptides in the ELISA experiments reported. As shown in Table 3, 1:4 dilutions of the antipeptide serum B inhibited virus replication and syncytia formation. As shown by RT assays done with the supernatant of

the infected cells and by microscopic control of the cell culture, neither RT activity in the cell culture supernatant, nor formation of syncytia could be detected up to 14 days p.i., indicating a complete neutralization of HIV-IIIB virus by antibodies of the V3-C_{36} peptide. These results indicate that neutralizing antibodies can be induced by synthetic peptides which are not identical with any of the reported V3 sequences. Thus we suggest that induction of a group-, not only type-specific humoral immune response on the basis of synthetic antigens should be possible.

Recognition of V3-C_{36} peptides by cellular immunity

A murine H2-D^d restricted CTL-cell epitope is located in the V3 loop sequence of HIV-IIIB and has been characterized in its immunological properties (27–29). To confirm the functional conservation of this epitope in our V3-C_{36} peptide, recognition to V3-C_{36} derived peptides was tested in a 3 h cytolytic assay. Spleen cells were isolated from BALB/c mice two months after immunization with an gp 120-IIIB recombinant vaccinia virus VSC25. After a 7 days in vitro restimulation with the gp 120-IIIB recombinant vaccinia virus, spleen cells were used as effector cells. The p818 mastozytoma target cells were incubated with different concentrations of the indicated peptides (Fig. 3; 10 μm, 0.1 μm, 0.001 μm). Incubation of the target cells with the consensus 16-mer peptide (V3-16) resulted in specific lysis comparable to that of the HIV-IIIB/BH10 specific peptide present in the gp 120 protein sequence of VSC25 used for priming (Fig. 3, BH10 (VSC25) and V3-16). Shortening of the consensus peptides by two residues from the NH_2- and COOH-terminal end of the peptide (V3-12) decreased in

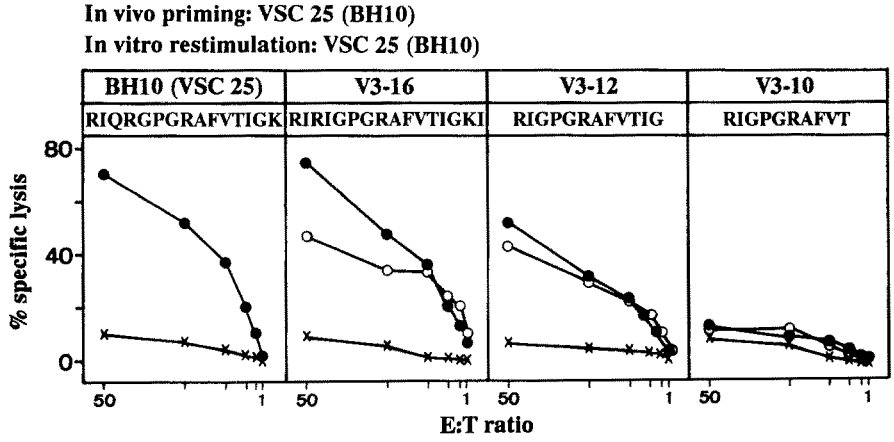

Fig. 3. Cross reactivity of H2-D^d restricted murine CTLs after vaccinia-env-IIIB mediated in vivo priming and in vitro restimulation with a IIIB/BH10-derived 16-mer V3 peptide *BH10 (VSC25)* and different truncated derivatives of the V3-C_{36}-consensus peptide (*V3-16; V3-12; V3-10*). p815 target cells were preincubated with different concentrations of the indicated peptides [10 μM (●), 0.1 μM (○) and 1 nM (×)]. % specific lysis achieved by the use of different E/T ratios (1–50) are indicated

H2-D^d restricted recognition by the effector cells (Fig. 3). Further shortening by 2 amino acids from the carboxy terminus (Fig. 3, V3-10) completely abolished the killing effect. Reduction of the peptide concentration also decreases the H2-D^d restricted recognition of the peptide-treated targets by the effector cells.

Discussion

The envelope proteins of HIV-1 show a high extent of overall variability in the amino acid sequences of the individual isolates. Variability, however, is concentrated in defined regions, which show all parameters for a high probability of surface exposition of the protein in combination with consensus-sites for carbohydrate modification. Variable domains are stabilized by flanking conserved cysteine residues to form highly exposed loop structures representing antigenic domains. Although the variable region 3 contains no consensus sites for N-glycosylation, but a fairly conserved central sequence motif (GPGR), the immunological recognition of this epitope is, however, highly isolate specific and thus may allow the virus to evade the humoral immune response by continuously forming mutant virus type in individual patients.

Isolate specificity of antibodies to the V3-region of gp 120 was overcome by designing a consensus sequence, which was tested in its properties in form of a cyclized synthetic oligopeptide. In ELISA-tests using sera from HIV-1 positive individuals from Europe and Africa, the overall reactivity of the compound could be shown to be 90%. A negative reaction was only observed in the group of asymptomatic patients. Those sera were also not reactive with gp 120 on Western blots, but showed conversion to positive reaction in a serially taken serum sample. Therefore, it may be concluded that non-reactivity of these sera is due to a very early phase of HIV infection, where envelope specific immunglobulines are not found. All sera with a gp 120 positive reaction on Western blots irrespective of their origin in Europe and Africa tested so far showed positive reaction with the V3-C_{36} peptide. The reactivity of the peptide is thus not isolate- but group specific. Due to its broad serological reactivity, the V3-C_{36} peptide might have special value for diagnostic purposes. Since vertical transmission of the virus during pregnancy can be correlated to the absence of maternal V3-specific antibodies [6], determination of V3-antibody titers might be a further criterion for the decision whether to continue pregnancy or not. The high degree of cross reactivity was confirmed by the recognition of the V3-C_{36} peptide with a strain specific monoclonal antibody (V3-IIIB) and polyclonal antisera directed to the V3-regions of HIV-IIIB, RF and MN. Sera obtained after inoculation of the V3-consensus peptide in rabbits recognized peptides spanning the core-sequences of the V3-domain of various HIV-isolates equally well, indicating that antibodies directed to the V3-consensus sequence are not virus isolate specific.

The HIV-IIIB neutralizing capacity of the V3-C_{36} anti-peptide serum could be shown in vitro, thus underlining the functional significance of the antibodies raised in rabbits against the V3-C_{36} peptide. In addition, mouse cytotoxic T-

cells specific for HIV-IIIB recognized target cells presenting the V3-consensus peptides in context to H2-D^d. Those reactivities show that the functional properties of the V3-region to induce humoral and cellular immune response were not destroyed by the construction of the consensus sequence. It can be concluded that by comparison of amino acid sequences and functional parameters of the V3-loop, a domain with a new, naturally not existing sequence can be proposed, which exhibits additional new properties. The functional importance of the V3-region to induce HIV-neutralizing immune reaction as well as V3 specific CTLs in the course of virus infection makes the V3 consensus sequence with its group-, not isolate-specific properties a candidate-epitope to be included in a potential vaccine inducing cross protection to a variety of HIV-isolates.

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