Antibody Responses Raised against a Conformational V3 Loop Peptide of HIV-1

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Received February 15, 1995. Accepted April 20, 1995

Abstract: The amino acid sequence of the principal neutralizing determinant (PND) of 224 cases of human immunodeficiency virus type 1 (HIV-1) was determined and the most frequently occurring sequence was used as a peptide antigen for studying virus-specific antibody responses. In our present study, a linear peptide of the most frequent PND was first synthesized and then oxidized to create a disulfide-bridged loop conformation. Then, in order to construct a macromolecular structure for the purpose of increasing antigenicity, the synthetic peptide was conjugated to a core peptide. We compared the immunogenicity of the disulfide-bridged loop PND peptide antigen (AG4) and the linear PND peptide antigen (AG5). After immunizing rabbits 5 and 6 times with both peptides, the results obtained using ELISA revealed that AG4 (conformational-loop type) was more capable of inducing a high titer of antigen-specific antibodies than was AG5 (linear type). Despite an amino acid sequence homology of 72%, a 1:8 dilution of serum raised against AG4 inhibited 81.9% of HIV-1mediated cell fusion, suggesting that conformational V3 loop peptide is able to elicit an antibody response which is strongly HIV-1-specific.

Key words: HIV-1, Antibody response, Conformational V3 loop peptide

Sera from many HIV-1-infected individuals have been shown to be capable of neutralizing a broad spectrum of virus isolates (3, 25, 35, 36). The induction of high titer, broadly cross-reactive, HIV-1-neutralizing antibodies by immunization is considered to be vital for the development of an effective HIV vaccine. Among the amino acid sequences encoding the viral envelope, the principal neutralizing determinant (PND) has been shown to be one of the strongest in terms of neutralization by antibody generated as a result of HIV-1 infection (5, 14, 21, 26).

We have been developing a multicomponent synthetic peptide vaccine composed of three types of V3 regions (Thai-A, Thai-B, and IIIa), one CD4 binding site and one gag region, p17 (HGP-30), in order to elicit the production of broadly neutralizing antibodies (7, 17–19). Immunization with this multicomponent vaccine yielded an antiserum which exhibited strong synergistic effects. In order to elicit even more broadly cross-reactive neutralizing antibodies, it was necessary to determine the most effective amino acid sequence of the V3 region. We therefore determined the most frequent amino acid sequence of the PND by analyzing the PND of 224 cases of HIV-1. There has been some controversy as to which type of structure is more effective, a conformational-loop type or a linear type antigen. McKeating et al suggested that rat monoclonal antibodies directed to a conformational-loop epitope had stronger neutralizing activity than antibodies to a linear epitope (11). However, a macromolecular synthetic vaccine composed of disulfide-bridged loop PND peptides could not be constructed using the multiple antigen peptide (MAP) system (15, 31) or the m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) method (19). Vogel et al indicated that such findings, that the significance of antibodies specific for linear V3 sequences had been questioned as a fact that the majority of neutralizing antibodies in an

Abbreviations: BSA, bovine serum albumin; CFA, complete Freund’s adjuvant; CPE, cytopathic effect; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); ELISA, enzyme-linked immunosorbent assay; GA, glutaraldehyde; HIV-1, human immunodeficiency virus type 1; HPLC, high-performance liquid chromatography; IFA, incomplete Freund’s adjuvant; IR, inhibition ratio; MAP, multiple antigen peptide; MBS, m-maleimidobenzoyl-N-hydroxysuccinimide ester; 2-ME, 2-mercaptoethanol; MW, molecular weight; PBS, phosphate-buffered saline; PKA, polylysine antigen; PND, principal neutralizing determinant; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SH, sulphydryl; V3, third hypervariable domain.
HIV-infected patient recognized conformation-dependent or discontinuous epitopes, would limit the potential of synthetic peptide vaccines (33). In this study, we developed new methods for constructing a macromolecular peptide antigen which included a conformational V3 loop region capable of inducing a strong virus-specific antibody response.

Materials and Methods

Viruses. HIV-1<sub>IIIB</sub> used in this study were provided by the NIH AIDS Research and Reference Reagent Program. HIV-1<sub>GUN-1</sub> was isolated from a Japanese hemophilia B patient with AIDS and was donated by Dr. Hoshino, Department of Hygiene, Gunma University School of Medicine, Japan (28).

Experimental animals. Six JW/CSK rabbits, 12 weeks of age, were obtained from the Shizuoka Laboratory Animal Center Co., Ltd. These were maintained in horizontal laminar flow cabinets and provided with sterile food and water.

Synthetic peptides. All peptides were synthesized using an automated model 430A peptide-synthesizer (Applied Biosystems, Foster City, Calif., U.S.A.). Since synthesized peptides were up to approximately 40 amino acid residues in length, we designed a synthetic peptide with 37 amino acid residues of which 13 were from the CD4 binding site and 22 were from the V3 region (Fig. 1). After synthesizing the V3 region peptide, the peptide in the CD4 binding site was also synthesized from the amino-terminal region of PND since simultaneous immunization with the V3 region and the CD4 binding site has been reported to synergistically induce neutralization (11, 19). The synthesized peptides were purified using reverse-phase high-performance liquid chromatography (HPLC) as described previously (20).

Modification of the synthetic peptide. All the synthetic PND peptides were designed to contain a cysteine at both ends. Immediately after synthesis, peptides were coupled to each other by disulfide-bridges since the concentration of peptide during synthesis was high. Reduction of the synthetic peptide was necessary for construction of intramolecular disulfide-bridges and was carried out as follows. One hundred milligrams of the disulfide-bridged peptides formed just after synthesis were added to 0.36 M 2-mercaptoethanol (2-ME), and 0.05 M Tris (pH 8.2) at a total volume of 100 ml, and the solution was incubated overnight at room temperature. During this step, the intermolecular disulfide-bridged peptides were reduced. Instead of the highly concentrated peptides obtained during synthesis, these peptides were diluted to 1:50 and then were dialyzed overnight at 4°C using a Spectra/Por 7 (MW < 1,000) tubing. The peptides were re-oxidized during dialysis and had formed an intramolecular disulfide-bridged loop. These peptides were then lyophilized (Fig. 1).

Quantitative analysis of free SH-groups by 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). DTNB was shown by Ellman (1959) to be useful for determination of sulfhydryl groups and the method has gained broad acceptance (1, 4, 34, 38). Using this procedure, 3.96 mg of DTNB (Ellman's reagent) were dissolved in 1 ml of 0.1 M sodium phosphate buffer (pH 7.0–7.5). If the pH of the buffer is lower than pH 7.0, DTNB is very insoluble. As positive controls, 1 μM, 10 μM and 100 μM L-cysteine were prepared in 500 μl 0.1 M Tris (pH 8.0) containing 8 M urea. The molecular mass of the synthetic peptide was around 4,000 Daltons. Then, 1 μM and 10 μM of the peptide that had just been synthesized and 1 μM and 10 μM of the re-oxidized peptide were prepared in 500 μl 0.1 M Tris (pH 8.0) containing 8 M urea. To each sample, 100 μl of DTNB solution was added and the absorbance was measured at 412 nm.

Conjugation. We used poly-lysine antigen (PKA) as
a core structure, followed by conjugation with glutaraldehyde (GA), as described in detail in previous reports (9, 24). The molecular mass of the synthetic peptide was around 4,000 Daltons and that of PKA was around 8,000 Daltons. Their ratio in the mixture was determined to be 20:1 (peptide:PKA). Forty milligrams of re-oxidized, intramolecular disulfide-bridged loop peptide and 1 mg of PKA were added to a solution containing 4 ml of 1/15 M phosphate-buffered saline (PBS) and 4 ml of 0.2% glutaraldehyde (pH 7.5) and the peptides were then conjugated overnight at 4°C. Since the NH₂ group and not the NH₃ group is the target for GA, lowering the pH below the pK of each amino group during conjugation allows for reactions mainly involving the α-amino terminus. The Schiff base is unstable but quickly changes to a more stable structure by means of Amadori rearrangement.

**Immunization.** Three rabbits were immunized by subcutaneous injection with 100 μg/kg of AG4 or AG5 in complete Freund’s adjuvant (CFA) on day 0. On days 28, 56, 84, 112 and 140, they received booster injections of 100 μg/kg of AG4 or AG5 in incomplete Freund’s adjuvant (IFA). Antisera were collected on days 42, 70, 98, 126 and 156, respectively.

**Enzyme-linked immunosorbent assay (ELISA).** Antibody responses were determined by ELISA as previously described (8). Briefly, two types of antigens were used for ELISA. One antigen was the common consensus PND (NTRKSIHGPGRAFYTTGEIIG) that was used for construction of AG4 and AG5, and the other was the V3 region peptide of HIV-1IIIB (NTRKSIRIQRGPRAFVTIGKI). First, 96-well microplates (Nunc, Roskilde, Denmark) were coated with 5 μg/100 μl/well of each antigen in PBS. Blocking was performed with 200 μl of 1% bovine serum albumin (BSA) in PBS for 4 hr. Incubation with antisera serially diluted in the same buffer was carried out for 2 hr at 37°C. All wells were treated with peroxidase-labeled, affinity purified, antirabbit IgG (Organon Teknika N.V., West Chester, Pa., U.S.A.) for 1.5 hr. α-Phenylenediamine dihydrochloride (Sigma Chemical Co., St. Louis, Mo., U.S.A.) in 0.1 M citrate-phosphate buffer (pH 5.0) containing 0.01% H₂O₂ was added. Antibody titers are expressed as the reciprocals of the final detectable dilution that gave an optical density at 490 nm (OD₄₉₀) of ≥0.2 OD units.

**Inhibition assay of HIV-1-mediated cell fusion.** Neutralizing activity against HIV-1 infection was evaluated as the fusion-inhibiting activity of antisera, which has previously been described (22, 27). Briefly, infected cell partners were produced in CEM, a CD4+ cell line. Cells were infected with HIV-1_{IIIB} and HIV-1_{GUN-1} isolates. They were then cultured for 10–14 days during which time the culture reached maximum CPE (cytopathic effect) and the majority of the cells had been killed. The surviving fractions of the cells that were stably infected were identified as “grow throughs.” The uninfected partners were MOLT-4. Infected CEM cells were prepared at a concentration of 0.25 × 10⁶/ml and 50 μl of the cells were added to each well of a 96-well microplate (Nunclon, Nippon Inter Med, Tokyo), following which 10 μl of immune or preimmune sera were added. Uninfected MOLT-4 cells were prepared at a concentration of 1.75 × 10⁶/ml and 50 μl of the cells were also added to each well (infected cells:uninfected cells=1:10). The plates were then incubated for 24 hr at 37°C. Giant cells (cells with a diameter greater than 5 times that of the input cells) were counted using an inverted microscope.

The inhibition ratio (IR) at each dilution was obtained as follows:

\[
IR = \left(1 - \frac{S_i}{S_p}\right) \times 100\%.
\]

Where \(S_i\) is the mean number of fusion cells in the presence of immune sera and \(S_p\) is the mean number of fusion cells in the presence of preimmune sera. IR was defined as the percentage of syncytium formation reduced by the immune serum compared with preimmune sera.

**Results**

**Analysis of Amino Acid Sequences of the V3 Region**

We analyzed the amino acid sequence of the PND of 224 cases of HIV-1-seropositive individuals. These included 34 recently diagnosed Japanese cases (unpublished data), 26 reported Japanese cases (7), and 21 reported Thai cases (16, 32). The remainder were pre-
previously reported cases from the United States (2, 29). We then determined the most frequently occurring amino acid sequence (Fig. 3) and it proved to be almost the same as that reported by LaRosa et al (10). This sequence was designated the common consensus PND (10). The PND amino acid sequences used in this study were as follows.

**Common consensus PND:**

CTRPNNTRKSIHIGPRAFYTGEIIGDIRQAHC

**IIIa PND:**

CTRPNNTRKSIHIGPRAFYTGEIIGDIRQAHC

**GUN-1 PND:**

CTRPNNTRKSIHIGPRAFYTGEIIGDIRQAHC

**Demonstration of Intramolecular Disulfide-Bridged Loop Peptide**

We compared the molecular weight of the synthetic peptide before and after modification using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (9% acrylamide) (Fig. 4). Lane 1 consisted of sample markers (Bio-Rad, low molecular weight), lane 2 was the synthetic peptide before modification without 2-ME, lane 3 was the synthetic peptide before modification with 2-ME, lane 4 was the modified peptide without 2-ME, and lane 5 was the modified peptide with 2-ME. Lane 2 revealed bands of high molecular weight whereas lane 3 did not even though the amino acid sequences were the same, suggesting that the synthetic peptides were connected by disulfide-bridges before modification. In contrast, the modified peptides in lanes 4 and 5 failed to yield bands of high molecular weight.

The presence of free SH-groups in each synthetic peptide was determined using DTNB and their concentration was measured by absorbency using Lambert and Beer's law (4).

\[ A = \log_{10} \frac{I_0}{I} = \varepsilon_{412} \cdot C \cdot D. \]

In this equation, \( A \) is absorbance, \( \varepsilon_{412} \) is the molar absorbency, \( C \) is the concentration, and \( D \) is path length of the cell. Using DTNB in 8 M urea, the \( \varepsilon_{412} \) is \( 14,290 \text{ M}^{-1} \text{ cm}^{-1} \). The absorbance and concentration

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**Table 1. Concentration of the free SH-groups of L-cysteine and the synthetic peptide before and after modification by DTNB**

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>Absorbance at 412 nm</th>
<th>Concentration of free SH-groups (µM)</th>
</tr>
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<tbody>
<tr>
<td>L-cysteine</td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>0.013</td>
<td>0.91</td>
</tr>
<tr>
<td>10</td>
<td>0.137</td>
<td>9.59</td>
</tr>
<tr>
<td>100</td>
<td>1.541</td>
<td>107.8</td>
</tr>
<tr>
<td>Synthetic peptide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before modification</td>
<td>-0.002</td>
<td>NA</td>
</tr>
<tr>
<td>100</td>
<td>0.004</td>
<td>0.28</td>
</tr>
<tr>
<td>After modification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.001</td>
<td>0.07</td>
</tr>
<tr>
<td>100</td>
<td>0.004</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Each concentration of L-cysteine is approximately equal to the concentration of free SH-groups. However, the synthetic peptide before and after modification shows hardly any free SH-groups. NA: not applicable.
of free SH-groups are shown in Table 1. The absorbance of L-cysteine was almost equivalent to that of a free SH-group on a molecular basis. One hundred micromoles of synthetic peptide before and after modification contained only 0.28 µM of free SH-groups. This indicated that the synthetic peptide contained no free SH-groups compared with L-cysteine.

These data demonstrated that the modified peptide formed a conformational-loop configuration.

Antibody Titers by ELISA

After conjugation with GA, we compared the immunogenicity of the intramolecular disulfide-bridged loop antigen (AG4) with that of the linear antigen (AG5). Antibody titers of individual rabbits were determined after the 2nd, 3rd, 4th, 5th and 6th injection and are presented in Table 2. Both AG4 and AG5 were constructed from the common consensus PND, therefore, the antibody raised against this peptide had a higher titer than that against the IIIb PND peptide. The titer of the antibody obtained after immunization with AG4 against the common consensus PND peptide was higher than 12,800 after the 2nd injection, which was approximately the same as the titer obtained after the 6th injection of AG5. A high titer was obtained after the 4th or 5th injection of AG4. However, in the case of AG5, similar titers were never obtained, even after repeated immunization. The antibody titer obtained by immunizing with AG4 against the common consensus PND peptide was higher than 51,200. In contrast, the titer obtained using AG5 was low. Immunization with AG4 antigen yielded an antibody titer about 10-fold higher than did immunization with AG5 antigen. Furthermore, the period required for AG4 to induce a high titer antibody was shorter than for AG5 (Fig. 5, A and B). The titer of antibody obtained with AG4 against the CD4 binding site peptide was 51,200 when the antibody titer against the common consensus PND peptide was 204,800. However, with AG5, the antibody titer against the CD4 binding site peptide was 6,400 when the titer against the common consensus PND peptide was 12,800. This indicates that AG4 was also more effective in inducing antibodies directed to the CD4 binding site peptide than was AG5.

HIV-1-Mediated Cell Fusion Inhibition Assay

To investigate the function of the antigen-specific antibodies raised against AG4 or AG5, we next examined

<table>
<thead>
<tr>
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<th>Against common consensus PND peptide</th>
<th>Against IIIb PND peptide</th>
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<tbody>
<tr>
<td></td>
<td>AG4</td>
<td>AG5</td>
</tr>
<tr>
<td>Following</td>
<td>12,800</td>
<td>1,600</td>
</tr>
<tr>
<td>2nd injection</td>
<td>25,600</td>
<td>3,200</td>
</tr>
<tr>
<td></td>
<td>12,800</td>
<td>3,200</td>
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<tr>
<td>Following</td>
<td>25,600</td>
<td>6,400</td>
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<tr>
<td>3rd injection</td>
<td>51,200</td>
<td>12,800</td>
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<td>25,600</td>
<td>12,800</td>
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<tr>
<td>Following</td>
<td>25,600</td>
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<tr>
<td>4th injection</td>
<td>51,200</td>
<td>12,800</td>
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<td></td>
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<tr>
<td>Following</td>
<td>51,200</td>
<td>6,400</td>
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<tr>
<td>5th injection</td>
<td>102,400</td>
<td>12,800</td>
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<tr>
<td></td>
<td>51,200</td>
<td>12,800</td>
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<tr>
<td>Following</td>
<td>102,400</td>
<td>12,800</td>
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<tr>
<td>6th injection</td>
<td>204,800</td>
<td>12,800</td>
</tr>
<tr>
<td></td>
<td>102,400</td>
<td>12,800</td>
</tr>
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</table>

Two groups of three rabbits each were immunized with 100 µg/kg of AG4 or AG5, respectively. The antibody titers are expressed as the reciprocal number of the endpoint dilution.

a) Common consensus PND peptide was used as the antigen for ELISA.
b) IIIb PND peptide was used as the antigen for ELISA.

Fig. 5. Time course of HIV-1 antibody responses. (A) Common consensus PND peptide was used as the antigen for ELISA. (B) IIIb PND peptide was used as the antigen for ELISA. Closed circles represent means of antigen-specific antibody titers of sera of three rabbits immunized with AG4. Open triangles represent means of antigen-specific antibody titers of sera of three rabbits immunized with AG5. Error bars represent means ± S.E.M. of triplicate experiments.
IR against both HIV-1\textsubscript{IIIB}- and HIV-1\textsubscript{GUN-1}-mediated syncytium formation. The amino acid sequence homology between the IIIB PND sequence and the common consensus PND sequence used for AG4 and AG5 was approximately 72%. The IR against HIV-1\textsubscript{IIIB} is plotted in Fig. 6A. The 1:8 dilution of serum resulting from immunization with AG4 inhibited HIV-1\textsubscript{IIIB} at an IR of 81.9%, and the 1:32 dilution, at an IR of 65.6%. In contrast, the IR of the 1:8 dilution of serum resulting from AG5 was no more than 35.2%. The IR against HIV-1\textsubscript{GUN-1} is plotted in Fig. 6B. The amino acid sequence homology between the GUN-1 PND sequence and the common consensus PND sequence was around 80%. Fifty percent inhibition of HIV-1\textsubscript{GUN-1} was achieved with a 1:8 dilution of antiserum obtained using AG4 and 30% inhibition of HIV-1\textsubscript{GUN-1} was obtained with a 1:32 dilution. AG5 antiserum, however, showed a lower level of inhibition. These results demonstrated that the AG4 configuration was more capable of inducing a high titer of cross-reactive, inhibiting antibodies against HIV-1-specific syncytium formation than was that of AG5.

**Discussion**

The synthetic peptide before modification (Fig. 4, lane 2) shows bands of high molecular weight without 2-ME, however, in the presence of 2-ME (lane 3), it does not. This indicated that, before modification, the synthetic peptides are interlinked with each other by disulfide-bridges and that 2-ME reduced this linkage. In contrast, the modified peptide has no high molecular weight bands, without or with 2-ME (lanes 4 and 5). This suggested that the modified peptide did not contain inter-disulfide connections but rather possessed either a free SH-group or an intramolecular disulfide-bridged loop. The concentrations of free SH-groups of each peptide are shown in Table 1 and indicate that 100 \mu M of synthetic peptide before and after modification contain only 0.28 \mu M of free SH-groups. These results demonstrated that the modified peptide was a single substance and lacked free SH-groups, possessing instead the intramolecular disulfide-bridged loop configuration.

In general, the major disadvantage of synthetic peptide vaccines is low antigenicity due to their low molecular weights. Although there have been many reports on the use of a single peptide conjugated with an adjuvant (12, 15, 23, 30, 31, 37), controversy remains as to whether these vaccines provide sufficient immunogenicity for protecting against HIV challenge. We previously conjugated a peptide using \textit{m}-maleimidobenzoyl-N-hydroxysuccimide ester (MBS) (19), a heterobifunctional coupling reagent with which peptides can be linked to PKA through cysteines and free amino groups (6). However, after constructing an intramolecular disulfide-bridged loop, the peptide was found to lack the free SH-group needed for conjugation with MBS. GA was then suggested as a promising alternative. It is a bifunctional coupling reagent capable of linking two compounds, primarily through their amino groups. Furthermore, GA cross-linking provides one of the most stable types of linkage available (9, 24). After construction of an intramolecular disulfide-bridged loop, GA is able to conjugate the peptide to a core peptide. In the process of conjugation, however, the pH of the reaction solution is critical. Lowering the pH well below the pK of the amino group slows down the coupling rate since the target is the NH\textsubscript{3} group and not the NH\textsubscript{2} group (6). In order to develop an efficacious peptide antigen, it is...
necessary to conjugate to an α-amino rather than to an ε-
amino terminal site since the former maintains the natural
structure. The pK of the α-amino terminal site is 9.8 ±
1.0 and the pK of the ε-amino terminal site is 10.5 (13).
The α-amino terminal site conjugates more readily than
the ε-amino terminal site at pH 7.5. Therefore, in the
present study, GA conjugation was carried out at this pH
in order to ensure a stable macromolecular antigen. One
disadvantage of this method is that the macromolecular
antigen often becomes insoluble due to the absence of
free amino groups, making it difficult to determine its
size. The molecular mass of AG4 or AG5 used in this
study was presumed to be around 4–8 × 10⁴ Daltons.

In spite of using the same dose for immunization,
the antibody titer of AG4 was 10-fold higher than AG5
(Table 2). In addition, the time required to elicit a high
antibody titer with AG4 was brief compared with that
required for AG5 (Fig. 5, A and B). It appears that the
V3 intramolecular disulfide-bridged loop configuration is
more easily recognized than the linear V3 structure.
Our present study clearly demonstrates that the anti-
genericity of the macromolecular synthetic peptide with the
V3 loop configuration is about 10-fold stronger than
that of the linear V3 peptides.

As for the effectiveness of the antibody produced by
immunizing with synthetic peptide vaccines, the IR of the
1:8 dilution of the AG4 antiserum was 81.9% against
HIV-1MN and that of AG5 was 35.2%. The IR of the
AG4 antiserum was more than 65%, up to a 1:32 dilu-
tion. A 50% inhibition by AG4 antiserum was reached
at approximately a 1:48 dilution, whereas inhibition by
AG5 failed to reach this level (Fig. 6A). Even with an
amino acid sequence homology of 72%, the IR of AG4
was appreciably stronger. The IR of HIV-1G11-mediated
cell fusion was lower than that of HIV-1MN, since
HIV-1G11, which was isolated from a Japanese HIV-1-
infected patient is presumed to possess high levels of via-
bility and infectivity. In spite of this, when the AG4 anti-
serum was used against HIV-1G11, the IR of the 1:8
dilution was 50% and that of the 1:32 dilution was
around 30%. However, the IR obtained with the AG5
antiserum was considerably lower (Fig. 6B).

Vaccines which include the common consensus PND
peptide have greater potential for inducing more effective
and broadly cross-reactive antibodies directed to the
PND of the various quasispecies of HIV-1 compared
with vaccines containing the IIIb PND or MN PND
alone since former consists of the most frequently occur-
arino acid sequence. Furthermore, the confor-
amational V3 loop peptide was demonstrated to be more
efficacious than the linear V3 peptide in terms of neu-
tralizing activity. Based on the observations above, the
development of a multicomponent vaccine containing the
conformational V3 loop peptide may provide us with an
ideal means of dealing with variation and quasispecies of
HIV-1.

We are grateful to Ms. T. Kanako for her excellent technical
support and Dr. H. Hoshino for donating the HIV-1G11 isolate.
This work was supported in part by a Grant-in-Aid for Scientific
Research from the Ministry of Education, Science and Culture of
Japan (No. 01570242 and No. 04269209), and by a grant
from the Waksman Foundation.

References

1) Acharya, A.S., and Taniuchi, H. 1976. A study of renatura-
tion of reduced hen egg white lysozyme. J. Biol. Chem.
251: 6934–6946.
2) Balfe, P., Simmonds, P., Ludlam, C.A., Bishop, J.O., and
Brown, A.J.L. 1990. Concurrent evolution of human immuno-
deficiency virus type 1 in patients infected from the same
source: rate of sequence change and low frequency of inac-
Human immunodeficiency virus 1: pseudovirion of a
group specific neutralizing epitope that persists despite
Biophys. 82: 70–77.
5) Goudsmit, J., Debouck, C., Meloen, R.H., Smit, L., Bakker,
M.A., Asher, D.A., Wolff, A.V., Gibbs, C.J., Jr. and Gaj-
duske, D.C. 1988. Human immunodeficiency virus type 1 neutral-
eization epitope with conserved architecture elicits ear-
y type-specific antibodies in experimentally infected
atory manual, p. 78–85, Cold Spring Harbor Laboratory,
New York.
7) Inami, S., Taniguchi, N., Ishibashi, K., Nagao, T., Aoki, I.,
Ishii, N., and Okuda, K. 1991. Serum antibody directed
against synthetic peptides derived from HIV-1 protein
sequence obtained from 26 Japanese HIV-1-infected indi-
viduals. AIDS 5: 1140–1141.
8) Jackson, R.J., Fujihashi, K., Xu-Amano, J., Kiyono, H.,
Elsom, C.O., and McGhee, J.R. 1993. Optimizing oral vac-
cines: induction of systemic and mucosal B-cell and antibody
responses to tetanus toxoid by use of cholera toxin as an adju-
Gluatraldehyde: nature of the reagent. J. Mol. Biol. 65:
525–529.
10) LaRos, G.J., Davide, J.P., Weinhold, K., Waterbury, J.A.,
Profy, A.T., Lewis, J.A., Langlois, A.J., Dressman, G.R.,
Boswell, R.N., Shadduck, P., Holly, L.H., Kapplus, M.,
Bolognesi, D.P., Matthews, T.J., Emini, E.A., and Putney,
S.D. 1990. Conserved sequence and structural elements in the
HIV-1 principal neutralizing determinant. Science 249: 932–
935.
Synergistic interaction between ligands binding to the CD4


