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# Recognition of envelope and tat protein synthetic peptide analogs by HIV positive sera or plasma

Dale A. McPhee, Bruce E. Kemp*, Simon Cumming, David Stapleton*, Ian D. Gust and Richard R. Doherty

*NHMRC Special Unit for AIDS Virology, Macfarlane Burnet Centre for Medical Research, Fairfield Hospital, Fairfield* and **Department of Medicine, University of Melbourne, Repatriation General Hospital, Heidelberg, Victoria, Australia*

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A series of synthetic peptides corresponding to segments of HIV encoded proteins were selected using criteria described by Welling et al. [(1985) FEBS Lett. 188, 215]. Synthetic peptide analogs to gp120 (2–13), (55–65), gp41 (582–596) (659–670) and $tat_{III}$ (71–83) were recognized by 41–67% of sera or plasma from individuals known to be infected with HIV on the basis of virus isolation or Western blot screening. The peptide which reacted with most sera or plasma was gp41 (582–596), a conserved region in the transmembrane glycoprotein. An extended peptide analog, gp41 (579–599), tested against the same samples showed almost 100% reactivity, confirming independent studies identifying a highly immunodominant region of gp41. There was an unexpected high prevalence of antibodies (52%) to the $tat_{III}$ peptide.

HIV; $Tat_{III}$; Predicted antigenicity; Antibody response; Synthetic peptide

## 1. INTRODUCTION

Infection with human immunodeficiency virus (HIV) has been traditionally confirmed by detecting antibodies against proteins derived from partially purified virus or antigens synthesized in virus-infected cells [1]. As these preparations contain some non-viral antigen, problems of specificity have been encountered. To overcome these difficulties considerable interest has arisen in the use of synthetic peptides and recombinant proteins [2–8] which might only detect antibodies directed against antigenic sites on HIV. A variety of algorithms have been proposed for predicting antigenic epitopes [9–11]. Recently Welling et al. [12] suggested an empirical method based on the frequency of occurrence of amino acids in known antigenic regions of some well characterized proteins. Using this procedure, several potential antigenic epitopes have been identified in the envelope glycoproteins gp120 and gp41 as well as the $tat_{III}$ gene product of HIV-1. Synthetic peptides of 11–21 residues corresponding to these regions were recognized by antibodies in sera or plasma from viraemic patients.

*Correspondence address:* B.E. Kemp, Dept of Medicine, University of Melbourne, Repatriation General Hospital, Heidelberg, Victoria 3081, Australia

## 2. MATERIALS AND METHODS

### 2.1. *Synthesis*

Peptides were synthesized by the Merrifield procedure [13] with the aid of an automated Applied Biosystems synthesizer using double coupling cycles supplied by the manufacturer. The *N-t*-butyloxcarbonyl amino acid derivatives were obtained from the Protein Research Foundation (Osaka, Japan). Side chain protection was the same as those supplied by Applied Biosystems with the exception of arginine for which the w-$NO_2$ derivative was used. Chain assembly was monitored using ninhydrin [14]. Peptides were synthesized with a carboxyl or amino terminal cysteine to facilitate conjugation to proteins, often important for raising antisera to the peptide. The assembled peptides were simultaneously cleaved and deprotected using anhydrous HF containing 10% anisol (v/v) [15]. The peptide was precipitated with diethylether and washed with ethylacetate before extracting with 60% acetonitrile and 0.1% trifluoroacetic acid (v/v). Previously we have used acetic acid to extract peptides; however, extraction with the commonly used HPLC solvents results in improved purity. Synthetic

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peptides were purified by preparative reversed phase HPLC (25 mm × 400 mm, C18, 250 A Amicon resin with a gradient of 1000 ml, 0–60% acetonitrile in 0.1% trifluoroacetic acid over 15 h). The synthetic peptides were approx. 95% pure as judged by analytical reversed phase HPLC (Brownlee RP 300 column 4.6 × 50 mm, 0–60% acetonitrile in 0.1% trifluoroacetic acid at 1 ml/min over 30 min) and by quantitative amino acid analysis following acid hydrolysis.

Peptide analogs synthesized were gp120 (2–13) RVKEKYQHLWRC, gp120 (55–65) ASDAKAYDTEV, gp41 (582–596) AVERYLKDQQLLGIC, gp41 (659–670) ELLELDKWASLC, and $tat_{III}$ (71–83) SQTHQVSLSKQPC. The extended gp41 peptide synthesized for comparisons to published data was gp41 (579–599) RILAVERYLKDQQLLGIWGCS.

### 2.2. *Plasma and serum specimens*

The panels of plasma and sera used were selected from samples submitted to the State and National HIV Reference Laboratories, Fairfield Hospital, Melbourne. Samples were selected so that patients in various stages of disease were represented. Infection with HIV-1 had been confirmed by virus isolation from associated peripheral blood lymphocytes [16] or serology using a Western blotting procedure [17]. There were 44 culture positive plasma and 42 Western blot positive sera. Sequential plasma specimens were available for 2 patients both in advanced stages of disease.

### 2.3. *Enzyme immunoassay (EIA)*

Wells of polyvinyl plates (96 well Nunc Immunoplates) were coated with 100 μl peptide solution (10 μg/ml in carbonate buffer, pH 9.6) and held overnight at 4°C. The plates were washed 10 times in phosphate-buffered saline (PBS) containing 0.05% Tween 20 and 0.01% $NaN_3$. Non-specific adsorption was blocked with PBS/1% bovine serum albumin (BSA) for 1 h at 37°C (200 μl/well) and the plates were rewashed 10 times. A 1:50 dilution of serum or plasma was added (100 μl/well) and the plates incubated at 37°C for 1 h. The plates were again washed and 100 μl/well of a 1:80 dilution of alkaline phosphatase conjugated rabbit anti-human IgG (Behring Enzygnost) was added and plates incubated for 45 min at 37°C. After washing the plates in washing buffer, distilled water, alkaline phosphatase substrate (2 mg/ml) in diethanolamine buffer was added and incubated for 30 min at room temperature. The reaction was stopped with 1.5 M NaOH and the wells read using a microtiter plate reader.

## 3. RESULTS

### 3.1. *Predicted antigenic epitopes*

The sequence data for the HIV-1 envelope protein, gp160, was examined [18] using the Welling antigenicity program [12] and a number of potential antigenic epitopes were identified (fig.1A). Epitopes present in regions considered as variable according to published comparisons of nucleotide sequences [19], or containing glycosylation sites were excluded. The following peptides corresponding to the envelope protein sequence were synthesized, gp120 (2–13), (55–65), gp41 (582–596) and (659–670). The $tat_{III}$ protein sequence was similarly examined (fig.1B) and the peptide $tat_{III}$ (71–83) synthesized.

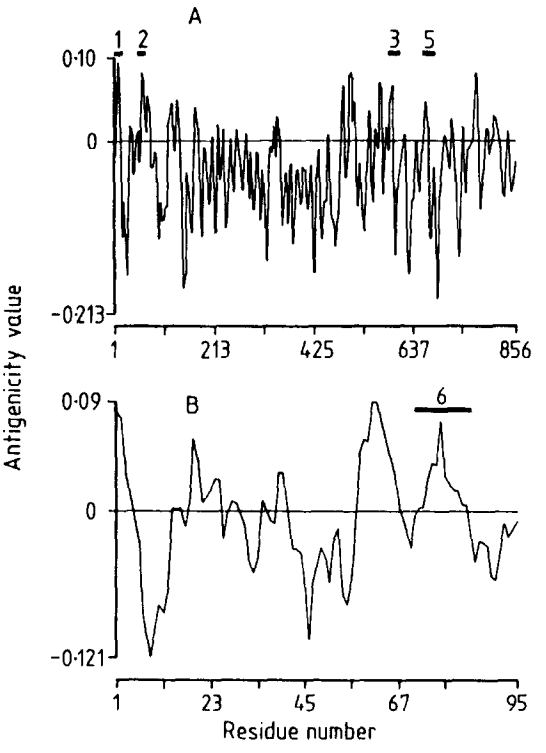


Fig.1. Profiles of HTLV IIIb env (a) and $tat_{III}$ (b) gene predicted amino acid sequences using the Welling program. Positions of the five synthetic peptide analogs tested are marked and labelled 1, gp120 (2–13); 2, gp120 (55–65); 3, gp41 (582–596); 5, gp41 (659–670); and 6, $tat_{III}$ (71–83).

### 3.2. *Serological response to the synthetic peptide analogs*

Each of the five synthetic peptides were recognized by some of the plasma or sera tested from the three categories (healthy seropositive, ARC and full blown AIDS) (fig.2). Reaction to the peptides varied from patient to patient with some sera or plasma recognizing all five and others recognizing only one. There was no clear correlation to disease status with the exception of gp41 (582–596). Antibodies against gp41 (582–596) were most frequently detected in the sera of healthy seropositive patients (15/16, 94%) but less

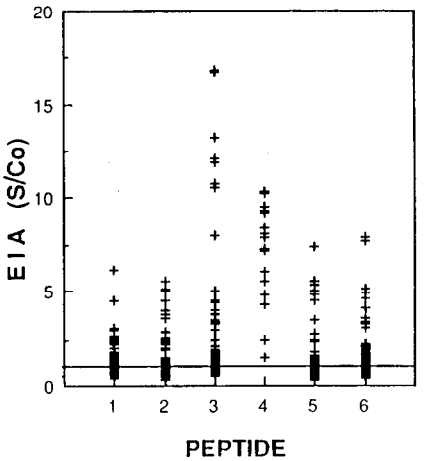


Fig.2. Combined values of 86 samples (42 sera and 44 plasma) tested by reactivity to five synthetic peptide analogs [1, gp120 (2–13); 2, gp120 (55–65); 3, gp41 (582–596); 5, gp41 (659–670); and 6, $tat_{III}$ (71–83)], plus samples that were also tested against the extended peptide labelled 4, gp41 (579–599). The cut off value was determined as the mean of seronegative control sera (5) plus 2 standard deviations. The sample/cutoff ratio for the EIA test is that value plotted for each sample. The number of positive samples/total number tested were 44/86, 40/86, 58/86, 19/19, 35/82 for peptides 1, 2, 3, 4, 5 and 6, respectively.

commonly in patients with ARC/LAS (11/16, 69%) and AIDS (29/45, 64%). Ten samples (12%) did not have detectable antibodies to any of the five synthetic peptides and of these 8 were from AIDS patients and 2 were from patients with ARC or LAS. Only 10 patients had antibodies to all five peptides and of these 8 were patients with AIDS.

### 3.3. *Serological response to an extended gp41 peptide*

Wang and others [8] have reported a synthetic peptide encompassing gp41 (582–596), which reacted specifically with antibodies in serum from AIDS, ARC and asymptomatic carriers. We therefore synthesized an extended peptide, gp41 (579–599), and directly compared it and gp41 (582–596) using a second panel of 60 samples (comprising sera collected from 36 patients with AIDS, 17 with ARC/LAS and 3 healthy seropositive individuals). Whereas gp41 (582–596) was recognized by only 40 of the sera (67%) the extended peptide gp41 (579–599) was recognized by all but 1 sample, which was from a patient with terminal AIDS.

## 4. DISCUSSION

Several approaches have been used to identify immunodominant epitopes for HIV-1 envelope gene products [3–6,8]. These have used a process of elimination using recombinant peptide antigens [3–5] or involved the synthesis of a large number of synthetic peptide analogs over a large region of the genome (3 of 10 [8]; 3 of 17 [6]). We identified five synthetic peptide analogs that were recognized by antibodies in 41–67% of sera or plasma from patients positive for HIV culture or serology. Thus by using the Welling program to identify potential antigenic epitopes and additional criteria of conservation among isolates, and excluding glycosylation sites we were able to identify five, out of five tested, antigenic epitopes. Additionally gp41 (582–596) was found to be part of a region recognized by almost 100% of patients sera, regardless of disease status.

The extended peptide gp41 (579–599) was recognized by antibodies in 59 out of 60 samples from patients varying in disease status. One patient with AIDS did not recognize this peptide. We concur with the conclusions of Wang et al. [8] who found 93.6 of ARC and 98.3% of AIDS patients sera recognized gp41 (579–599). The shorter peptide, gp41 (582–596) was recognized more by antibodies from healthy seropositives (94%) than by those with ARC or AIDS (59% and 67% respectively).

When peptide analog gp41 (582–596) [AVERYLKDQQLLGIC] is compared to peptide analogs synthesized by Wang et al. [8], gp41 (579–599) [RILAVERYLKDQQLLGIWGCS], and Gnann et al. [6], gp41 (593–604) [LGLWGCSGKLIC], there would appear to be at least two antigenic sites, one within gp41 (582–596) which may be focussed around the lysine at position 588 (see Wang et al. [8]), and a second within gp41 (593–604) possibly focussed around the tryptophen at position 596 (see [7]).

Interestingly the recognition of a $tat_{III}$ peptide (71–83) by 52% of patients sera is much greater than recognition of the $tat_{III}$ protein in AIDS patients reported elsewhere [21] (Sodroski, J., personal communication). This peptide spans amino acids coded by the region immediately 5′ to the putative splice site between the second and the third exons of $tat_{III}$ mRNA [22] and covers some

15% of the length of this critical regulatory protein. No clinical correlates were apparent for patients whose sera recognized this analog, but the central role of this protein in regulation of viral replication warrants further mapping of immunodominant regions.

Although this approach was useful in predicting native antigenic regions in HIV envelope and $tat_{III}$ proteins, more detailed studies of these regions are needed to fully define the antigenic epitope(s), as shown here for gp41 (582–596) which comprised part of a highly immunodominant region of gp41 [8].

Development of a sensitive and specific synthetic peptide diagnostic assay for HIV antibodies requires that conserved epitopes are universally recognized. We have shown declining reactivity to immunodominant epitopes in some patients with advanced disease (McPhee, D.A., unpublished results), and conclude that a reliable assay will require more than a single synthetic peptide to reach necessary sensitivity standards.

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