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Definition of an immunodominant T cell epitope contained in the envelope gp41 sequence of HIV-1

S. J. D. BELL*, D. A. COOPER*†, B. E. KEMP‡, R. R. DOHERTY§ & R. PENNY* *Centre for Immunology, St. Vincent's Hospital, Sydney, †National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, ‡St. Vincent's Institute for Medical Research, Melbourne, Victoria, §National Centre in HIV Virology and Macfarlane Burnet Centre for Medical Research, Fairfield Hospital, Melbourne, Australia

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SUMMARY

The majority of the immunodominant amino acid sequences of HIV-1 that have been characterized to date are coded for by hypervariable gene sequences. These variable sequences are however interspersed with sequences that are highly conserved between HIV strains. Immunogenic viral products with amino acid sequences that vary minimally between strains, and that consistently elicit both humoral and cellular immune responses, may be ideal for inclusion in a subunit vaccine. We studied HIV-seronegative and HIV-infected persons, classified as asymptomatic (AS), ARC or AIDS. Initially, we assessed the cellular immune status of each subject from results of T cell phenotype analyses, assays for serum levels of surrogate markers of disease progression, and responses to mitogens and recall antigen. In addition, we tested whether three short synthetic peptides derived from the conserved sequences of the envelope gp120 (aa 262–284) and gp41 (aa 579–601), and core p17 (aa 106–125) regions of the HTLV-IIIB isolate, could elicit B cell as well as T cell responses in HIV-infected subjects. Only the gp41-derived sequence was immunogenic at both B and T cell levels. To further characterize the gp41 epitope, we used a series of overlapping synthetic peptides derived from a conserved region of the envelope gp41 (aa 572–613). We thus identified an immunodominant 12-mer peptide sequence, gp41{8}(aa 593–604), which consistently elicited both T cell blastogenic and B cell (antibody) responses in AS HIV-seropositive individuals but not in ARC and AIDS patients. Linear regression analysis showed that in AS persons there was a strong positive correlation ($P < 0{\cdot}0005$) between the absolute $CD8^+$ T cell numbers and the magnitude of blastogenic responses to the gp41{8}(aa 593–604). Furthermore, those AS subjects with T cells that proliferated in response to this gp41 analogue also had significantly greater serum levels of antibody to the same short peptide sequence than symptomatic ARC and AIDS patients. These results suggest that cellular responses to the immunodominant and highly conserved envelope sequences of HIV-1, associated with increased $CD8^+$ T cells, may be important in the pathogenesis of HIV disease.

Keywords HIV conserved sequence T cell epitopes B cell epitopes synthetic peptide

INTRODUCTION

Early after the molecular cloning of HIV, it was discovered that the sequence of the virus genome varied between HIV isolates [1]. The nucleotide sequences of at least 19 distinct HIV-1 isolates have been determined [2], indicating that there is considerable variation in both structural (e.g. envelope) and functional gene sequences (e.g. *tat* and *nef*) [3,4]. Sequence variability is pronounced in the envelope, yet the extent of variation is not constant over the whole envelope region, and the variable sequences are interspersed with sequences that are highly conserved between different HIV strains [5–8]. If an amino acid sequence that elicited neutralizing antibody or cytotoxic T cell responses was found to have a variable sequence, it may not be ideal for inclusion in a subunit vaccine, as the variability may prevent broad spectrum protection against infection by a large variety of HIV isolates to which an at risk individual might be exposed.

With due consideration of the sequence variability between HIV-1 strains, we tested conserved-sequence HIV-derived synthetic peptides with sequences matching those of the native HTLV-IIIB isolate of HIV-1 [2] for their potential to elicit both humoral and cellular immune responses in HIV-infected individuals and normal volunteers. Initially we tested three conserved-sequence 20- to 23-mer synthetic peptides, namely gp120 (aa 262–284), gp41{5}(aa 579–601) and a core p17 analogue (aa 106–125). We and other groups have previously demonstrated

Correspondence: Stephen J. D. Bell, Centre for Immunology, St. Vincent's Hospital, Sydney, N.S.W. 2010, Australia.

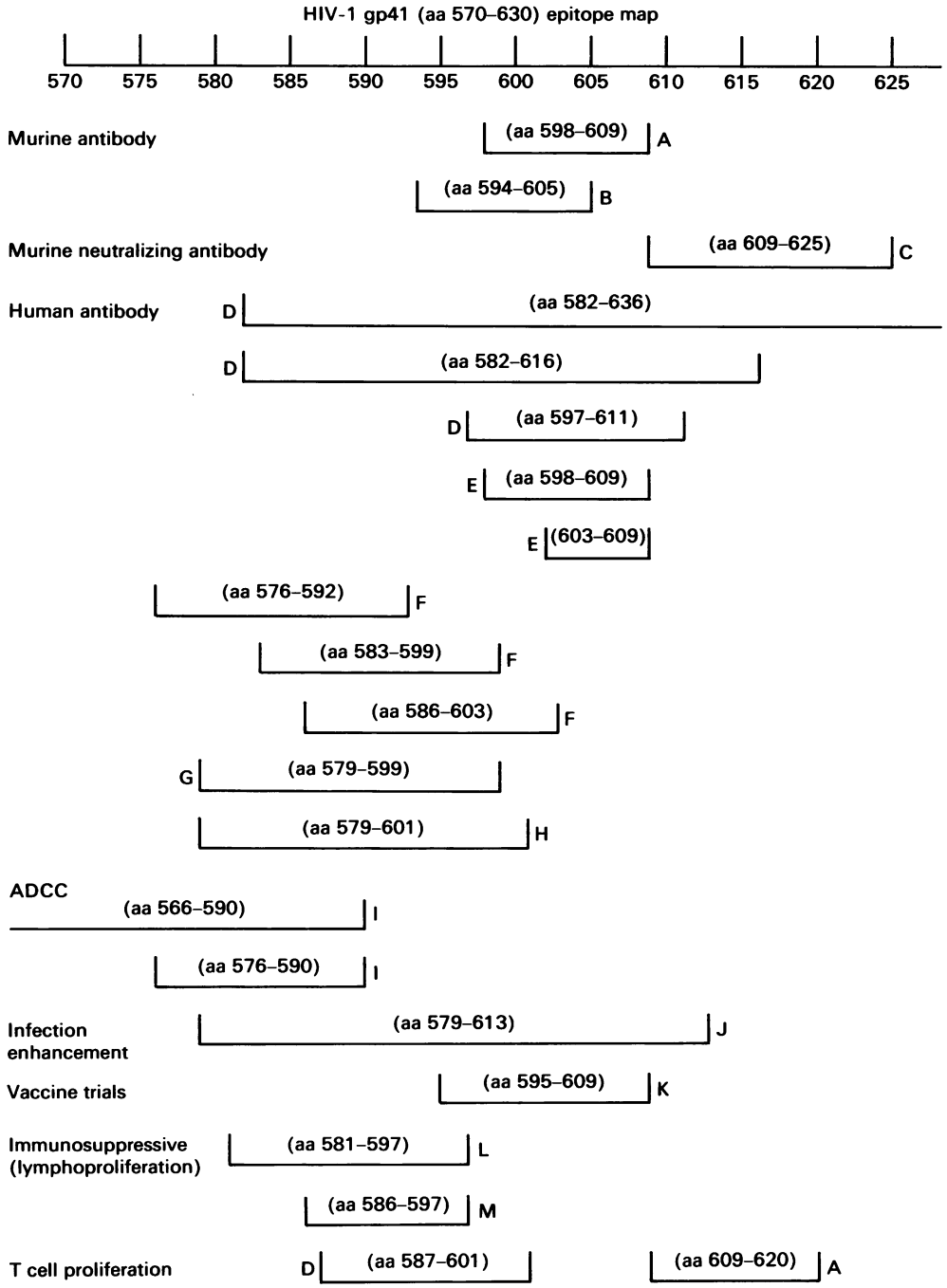

Fig. 1. Epitope map of immunodominant HIV-envelope gp41 (aa 570–630).A, [20]; B, [33]; C, [34]; D, [19]; E, [17]; F, [35]; G, [11]; H, [10]; I, [36]; J, [37]; K, [38]; L, [14]; M, [39]. ADCC, Antibody-dependent cell-mediated cytotoxicity.

that synthetic peptide sequences derived from the envelope gp41, showing sequence homology to our gp41{5}(aa 579–601), elicited predominantly humoral responses in animals and man (for references, see legend to Fig. 1). In this study, the gp41{5}(aa 579–601) sequence was found to be more immunogenic at both T and B cell levels than the core p17 and gp120-derived peptides. To characterize definitively the T cell epitope in this conserved sequence, we then tested for T cell proliferative responses and serum antibodies to a series of 10 overlapping gp41-derived synthetic peptides, that ranged in length from 12- to 35-mer, and that spanned the gp41 (aa 572–613), of the HTLV-IIIB isolate of HIV-1 [2].

We thus identify a short synthetic peptide sequence, gp41{8}(aa 593–604), that consistently elicits humoral and

cellular immune responses during the asymptomatic disease period only.

MATERIALS AND METHODS

Study group

Healthy HIV-seronegative ($n=20$) and HIV-infected persons were recruited for this study. The cohort of HIV-infected persons included asymptomatics (AS) ($n=14$) and symptomatic patients with ARC ($n=20$) or AIDS ($n=11$).

HIV-derived synthetic peptides

Synthetic peptides were synthesized by the Merrifield procedure [9], with the aid of an Applied Biosystems Model 430 synthesizer using double coupling cycles supplied by the manufacturer. Two conserved-sequence synthetic peptide analogues of HIV-1, namely envelope gp120 (amino acids 262–284, GSLAEEEV-VIRSANFTDNAKTI), and *gag* p17 (amino acids 106–125, EEQNKSKKKAQQAAADTGHS), as well as a series 10 short overlapping synthetic peptides, with sequences collectively representing a 40-residue section of the immunodominant envelope gp41 sequence of the HTLV-IIIB isolate of HIV-1 [2], were tested for the presence of T and B cell epitopes. We have previously tested these same peptide sequences for the development of an HIV-specific antibody assay [10]. The amino acid sequences of the overlapping gp41-derived synthetic peptides were as follows:

{1}(aa 572–591)	(GIKQLQARILAVERYLKDQQ)
{2}(aa 574–591)	(KQLQARILAVERYLKDQQ)
{3}(aa 576–591)	(LQARILAVERYLKDQQ)
{4}(aa 578–591)	(ARILAVERYLKDQQ)
{5}(aa 579–601)	(RILAVERYLKDQQLLGIWGCSGK)
{6}(aa 579–599)	(RILAVERYLKDQQLLGIWGCS)
{7}(aa 582–596)	(AVERYLKDQQLLGIW)
{8}(aa 593–604)	(LGIWGCSGKLIC)
{A}(aa 572–582)	(GIKQLQARILA)
{β}(aa 579–613)	(RILAVERYLKDQQLLGIWGCSGKLICTTAVPNWAS)

Only six overlapping peptides, ranging in length from 12- to 22-mer, and spanning a 33-residue sequence (gp41 aa 572–604), were available for testing throughout the whole study period (Table 1).

HIV immunoblot assay

Western blot assays were performed using serum samples from all subjects, to test for antibody responses to native HIV-1 peptides (Bio-Rad Novopath Immunoblot Assay, Richmond, CA).

T cell subset phenotype analysis

The numbers of circulating T cells and their subsets were determined using fluorescein-conjugated monoclonal antibodies (Coulter Electronics, Hialeah, FL). Briefly, 80 μl aliquots of anti-coagulated donor blood were mixed with subset-specific MoAbs. After incubation at room temperature, erythrocytes were lysed and lymphocytes were fixed, using Q-Prep (Coulter). Bound fluorescence was then measured on the EPICS Profile Flow Cytometer (Coulter). A complete blood count and differential was performed simultaneously. Absolute $CD4^+$ and $CD8^+$ lymphocyte counts were derived using the total leucocyte count, the lymphocyte fraction from the differential leucocyte count, and the proportion of antibody-positive cells detected with the flow cytometer.

Disease progression markers

Quantitative enzyme immunoassays were performed for the detection of HIV p24 antigen (Abbott Laboratories, North Chicago, IL) and β_2-microglobulin measurements (IMx β_2-microglobulin assay, Abbott Laboratories) on all sera. Quantitative radioimmunoassays were also performed to measure the serum neopterin concentrations (IMMUtest Neopterin, Henning Berlin GMBH, Berlin, Germany).

Anti-HIV synthetic peptide enzyme immunoassays

Enzyme immunoassays (EIA) for the detection of *in vivo*-synthesized serum antibodies to the envelope gp120 (aa 262–284), gp41-derived synthetic peptides, and the *gag* p17 (aa 106–125) were performed, and the cut-off point of the peptide EIAs was calculated as described earlier [11]. The optical density (OD) was measured at 495 nm wavelength.

T cell proliferation analyses

Peripheral blood mononuclear cells (PBMC) were tested for their ability to respond to the soluble antigens, tetanus toxoid (TT) and purified protein derivative (PPD) of tuberculin (Commonwealth Serum Laboratories (CSL), Melbourne, Australia), as well as the mitogens pokeweed (PWM) and phytohaemagglutinin (PHA) (Sigma Chemical Company, St Louis, MO). All recall antigens and PHA were used at a concentration of 5 μg/ml, and PWM at 2 μg/ml. PBMC were also cultured in the presence of the HIV-derived synthetic peptides described above. All peptides were used at a concentration of 2 μM per culture. Briefly, 200 000 PBMC were cultured in 0·2 ml of RPMI 1640 medium, which contained pooled 10% human AB serum (HIV-seronegative) (Red Cross Blood Transfusion Service, Sydney, Australia), penicillin and streptomycin antibiotic (50 nM each) (CSL), 2 mM HEPES buffer solution (pH 7·4), and 2·5 mM glutamine. Antigens and PWM cultures were maintained for 6 days, while PHA cultures were only left for the last 3 days, as maximal responses occurred on day 3. After 6 days at 37°C in 5% CO_2, each culture was pulsed overnight with 50 μl of a 20 μCi/ml of [^{3}H]-thymidine (Amersham, Amersham, UK), harvested on glass-fibre filter papers and counted in a liquid scintillation spectrometer (Beckman, Fullerton, CA). The mean background ct/min incorporated in the absence of antigen in HIV-seronegative controls ranged from 926 to 2811 ct/min, and from 366 to 4960 dpm in HIV-seropositive patients. Standard errors of the mean of triplicate cultures were less than 20%. Stimulation indices (SI) were calculated as follows:

$$\text{SI} = \frac{\text{mean ct/min of cells with antigen} - \text{mean ct/min without antigen}}{\text{mean ct/min without antigen}}$$

Mean SI $\pm$ 2 s.d. of blastogenic responses to the gp120, gp41-, and p17-derived synthetic peptides in 20 HIV^- normals were $1{\cdot}38 \pm 0{\cdot}33$, $1{\cdot}62 \pm 0{\cdot}36$ and $1{\cdot}00 \pm 0{\cdot}35$ respectively. That is, mean SI + 2 s.d. were all $< 2{\cdot}5$. On the basis of these observations, we regarded SI $\geqslant 2{\cdot}5$ as positive responses.

Statistical analysis

The correlation coefficients and significance of differences between experimental groups were determined. Correlation was calculated using X-Y pairs for linear regression analysis, and significance of differences was calculated using two-tailed

Table 1. Humoral and cellular responses to gp41-derived overlapping peptides

	gp41 (572–591) {1}		gp41 (574–591) {2}		gp41 (578–591) {4}		gp41 (579–601) {5}		gp41 (582–596) {7}		gp41 (573–604) {8}	
Asymptomatics												
	T cell	Ab	T cell	Ab	T cell	Ab	Tcell	Ab	Tcell	Ab	Tcell	Ab
Case 22	−	−	+	−	−	−	−	+	−	+	+	+
Case 30	−	+	+	−	−	−	−	+	−	−	−	+
Case 36	−	−	+	−	−	−	+	+	+	−	+	+
Case 37	−	+	−	+	−	+	−	+	−	+	+	+
Case 38	+	+	−	−	−	−	−	+	−	+	−	+
Case 43	−	+	−	−	NT	−	−	+	−	+	+	+
	1/6	4/6	3/6	1/6	0/5	1/6	1/6	6/6	1/6	4/6	4/6	6/6
ARC												
Case 21	−	+	−	−	+	+	−	+	+	+	−	−
Case 27	+	−	−	−	+	−	+	+	−	−	−	−
Case 28	−	+	−	+	−	+	+	+	−	+	−	+
Case 32	−	+	+	+	−	+	−	+	−	+	−	+
Case 33	−	+	−	−	−	−	−	+	−	−	−	+
Case 34	+	+	−	+	−	+	+	+	−	+	−	+
Case 35	−	+	+	−	−	+	−	+	−	−	−	+
Case 39	−	+	−	−	−	−	−	+	−	+	−	−
Case 40	+	+	−	−	−	+	−	+	+	+	−	+
Case 41	−	−	+	−	−	−	+	+	−	−	+	+
Case 42	−	+	+	−	−	−	−	+	−	+	−	+
	3/11	9/11	4/11	3/11	2/11	6/11	4/11	11/11	2/11	7/11	1/11	8/11
AIDS												
Case 23	−	+	−	−	−	+	−	+	−	+	−	+
Case 24	−	+	−	−	−	+	−	+	−	+	−	+
Case 25	−	+	−	−	−	+	−	+	−	−	+	+
Case 26	−	+	−	+	−	+	+	+	−	+	−	+
Case 29	−	+	+	−	−	−	−	+	−	−	−	+
Case 31	+	+	+	−	−	−	+	+	−	+	−	+
	1/6	6/6	2/6	1/6	0/6	4/6	2/6	6/6	0/6	4/6	1/6	6/6

Humoral (Ab) compared with cellular (T cell) *in vitro* reactions to a series of overlapping gp41-derived synthetic peptides. +, either a positive blastogenic response (SI $\geqslant$ 2·5) or a positive serum antibody response (OD > cut-off) [11]. Ten gp41 analogues were tested at different times, but only six peptides, namely peptides {1}(aa 572–591); {2}(aa 574–591); {4}(aa 578–591); {5}(aa 579–601); {7}(aa 582–596); and {8}(aa 593–604) were available throughout the study period. NT, Not tested.

Student's *t*-test. χ^2 analysis was used to test for differences in observed frequencies of blastogenic responses between different patient groups.

RESULTS

T cell phenotype analysis

Results showed the characteristic decline in $CD3^+$ and $CD4^+$ T cell numbers that became more marked as disease progressed [12–14], and a concomitant increase in $CD8^+$ cell numbers in AS and ARC individuals was also noted. In AIDS, however, mean $CD8^+$ cell numbers decreased further and patients were severely lymphopenic (results not shown).

Disease progression markers

In accordance with earlier documented data [12,14–16], mean serum p24 antigen and serum β_2-microglobulin levels were elevated in the symptomatic patients. Elevated serum neopterin levels (> 15 nmol/*l*) were found in 84% of HIV-infected individuals, and the highest mean neopterin levels in this cohort were seen in the AS group. However, the range of serum neopterin levels in ARC patients was broad, so mean serum levels did not differ significantly between AS, ARC and AIDS groups (results not shown). Serum p24 antigen levels, which are regarded as a measure of viral activity [12], were detected in 50% of HIV-infected individuals, with the lowest frequency of p24 antigenaemia seen in the AS group (results not shown). In this cohort, serum β_2-microglobulin levels in AS and ARC individuals were not different from those seen in seronegatives ($P \geqslant 0{\cdot}1$), but AIDS patients had higher serum levels than

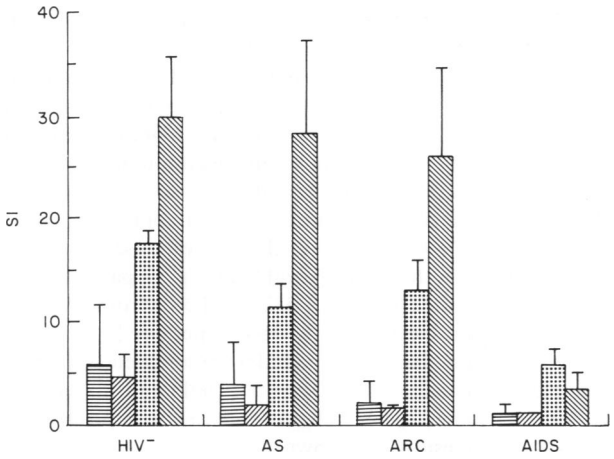

Fig. 2. Blastogenic responses to recall antigens and mitogens. Comparison of mean lymphocyte stimulation indices (±s.e.) of seronegatives, with asymptomatic (AS), ARC and AIDS patients. Tetanus toxoid (TT), purified protein derivative of tuberculin (PPD), and phytohaemagglutinin (PHA), were used at a concentration of 5 μg/ml, and pokeweed mitogen (PWM) at a concentration of 2 μg/ml. ■, Tetanus toxoid; ■, PPD; ▨, PWM; ▧, PHA.

seronegatives ($P \leqslant 0{\cdot}05$), suggesting a high cell membrane turnover in AIDS [16].

T cell responses to recall antigens and mitogens

Comparison of the mean ct/min of [^{3}H]-thymidine incorporated by *in vitro*-unstimulated PBMC from seronegatives and HIV-infected individuals, indicated that lymphoid cells from AIDS patients, without *in vitro* stimulation, had significantly higher basal levels of activation than HIV-seronegatives (2966 ct/min and 1600 ct/min [^{3}H]-thymidine incorporation, respectively; $P \leqslant 0{\cdot}05$).

Interestingly, the levels of p24 antigenaemia in ARC, but not AIDS patients, correlated strongly with the basal activation levels (ct/min) of *in vitro*-unstimulated PBMC ($P \leqslant 0{\cdot}005$). Mean blastogenic responses to TT and PPD, as well as responses to PWM and PHA, are shown in Fig. 2. As expected, PBMC from AIDS patients did not respond to recall antigens TT and PPD. Mean blastogenic responses in AIDS patients, induced by mitogens (PWM and PHA), were lower than in seronegatives and HIV-infected (non-AIDS) patients (all $P \leqslant 0{\cdot}05$). Decreased or lost recall antigen and mitogen responses in HIV-infected subjects did not correlate with decreased CD4$^+$ cell numbers ($P > 0{\cdot}1$).

T cell responses to conserved HIV-derived core and envelope synthetic peptides

Figure 3 shows the mean SI of all members of the different diagnostic groups (including non-responders, i.e. SI < 2·5), that were recorded after *in vitro* culture of cells with the three conserved sequence synthetic peptides used in initial experiments, namely the gp120 (aa 262–284), gp41{5}(aa 579–601), and the *gag* p17 (aa 106–125). Sixty-four per cent of all HIV-infected patients showed T cell responses to at least one or more of these three soluble HIV-derived synthetic peptides. T cells from 50% of HIV-seropositives responded to the core p17-derived peptide, 48% responded to the gp41{5}(aa 579–601), and 41% had T cells that proliferated in culture with the gp120-

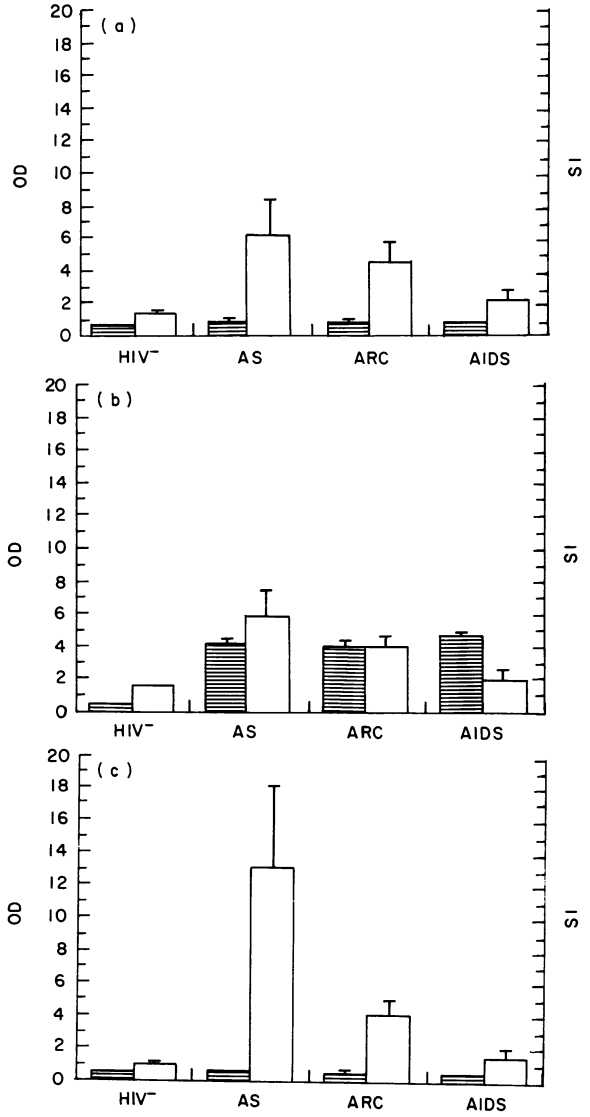

Fig. 3. T cell blastogenic (SI) compared with serum antibody (ODI) responses to three envelope- and core-derived synthetic peptides. Comparison of mean stimulation indices (SI ± s.e.m.), with serum antibody levels (OD (optical density) ± s.e.m.) of HIV-seronegatives, and HIV-seropositive asymptomatic (AS), ARC and AIDS patients. (a) gp120 (aa 262–284); (b) gp41{5}(aa 579–601); and (c) p17 (aa 106–125) were added to cell cultures (triplicate) for 6 days, each at a concentration of 2 μM per culture, and serum levels of antibodies with specificity for the same short peptides were determined using a solid-phase synthetic peptide EIA. (a) ■, gp120 (aa 262–284 OD); □, gp 120 (aa 262–284) SI; (b) ■, gp41{5}(aa 579–601) OD; □, gp41{5}(aa 579–601) SI; (c) ■, p17(aa 106–125) OD; □, p17 (aa 106–125) SI.

derived peptide. The magnitude of the mean proliferative responses to all three HIV-derived peptides, in AS and ARC individuals, was higher than in seronegatives ($P \leqslant 0{\cdot}05$). However, mean SI of cells from patients with AIDS were no different from seronegatives ($P > 0{\cdot}1$), indicating that the majority of patients with AIDS did not have the ability to respond to the HIV peptides we tested. Interestingly, in AS the highest mean responses (SI) were those induced by the core p17 peptide ($P \leqslant 0{\cdot}005$) (Fig. 3c). Most of the responses directed towards the gp120 (aa 262–284), and the *gag* p17 (aa 106–125), were either

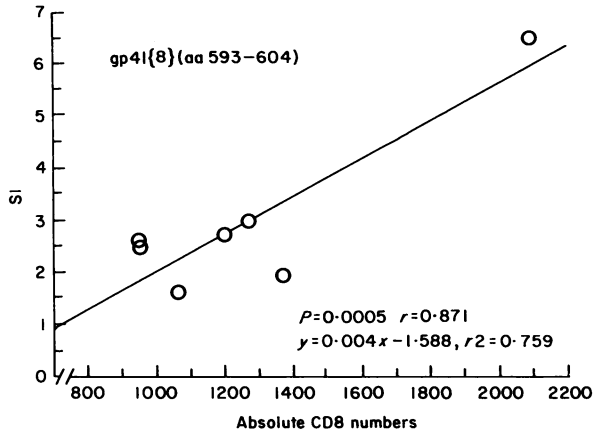

Fig. 4. Relationship between absolute CD8 T cell numbers and SI of blastogenic responses to the immunogenic gp41{8}(aa 593–604). Absolute $CD8^+$ T cell numbers in peripheral blood of asymptomatic (AS) HIV-infected individuals correlated positively with the magnitudes of blastogenic responses to the immunogenic gp41{8}(aa 593–604) ($P=0·0005$).

T cell or B cell responses. Simultaneous T and B cell responses to the gp120 and *gag* p17 peptides were seen to occur in less than 10% of individuals from any disease group (results not shown). The 23-mer gp41{5}(aa 579–601) however elicited both T cell and B cell responses in the same individuals.

Blastogenic responses induced by conserved-sequence overlapping gp41-derived peptides

As the gp41{5}(aa 579–601) was found to be the most immunogenic of the three conserved-sequence envelope and core synthetic peptides we initially tested, we proceeded to use a series of 10 overlapping gp41-derived synthetic peptides to define the gp41 epitope.

PBMC from HIV-seronegatives did not respond to the HIV-derived synthetic peptides we tested. HIV-seropositive patients, at all disease stages, responded at both humoral and cellular levels to one or more of these synthetic peptides. Eighty-three per cent of patients from this heterogeneous population had PBMC that showed $SI \geqslant 2·5$, after culture with one or more of these six HIV-derived, overlapping synthetic peptides. All AS patients had T cells that responded to at least one peptide. In all HIV-infected persons, $CD4^+$ T cell numbers did not correlate with the magnitude of blastogenic responses (i.e. SI values). Interestingly, in the AS group only, T cell responses (SI) to three overlapping and immunodominant synthetic peptides, namely peptides {2}, {5} and {8} (Table 1), correlated positively with the absolute $CD8^+$ T cell numbers (all $P \leqslant 0·05$) (Fig. 4) and with the CD8% (results not shown). The mean proliferative responses (of the whole group) to the overlapping gp41-derived peptides are shown in Fig. 5. Gp41{8}(aa 593–604) elicited significant blastogenic responses in four of six AS individuals in this cohort, and more recent studies indicate that about 75% of AS subjects consistently respond to this sequence (data not shown). Only one of 11 symptomatic ARC patients, however, had T cells that responded to this gp41-derived synthetic peptide. χ^2 analyses of the test data obtained using peptide {8} to induce blastogenesis indicated that the difference in frequencies of responses between AS, and those seen in ARC and AIDS groups, was significant ($P=0·0003$ and $P=0·0001$, respectively).

Interestingly, those AS individuals whose T cells responded more strongly (i.e. higher mean SI) to the immunodominant peptide sequences {2}, {5} and {8} also had the highest serum p24 concentrations (all $P \leqslant 0·05$) (Fig. 6). In the symptomatic patient group there was no correlation ($P > 0·1$).

Peptide {8}(aa 593–604) and the overlapping sequence peptide {5}(aa 579–601) induced T cell responses with the highest frequency in the total cohort (Table 1). No patients (0/7) responded to peptide {A}(aa 572–582), and cells from only one of seven patients responded to the 35-mer peptide {β} (aa 579–613), which has a sequence that includes the complete sequences of the immunogenic peptides {5}(aa 579–601) and {8}(aa 593–604). All seven patients were however seen to be seropositive for {β}(aa 579–613) (results not shown).

Antibody responses to the same HIV-derived synthetic peptides

Uninfected individuals were seronegative for the envelope- and core-derived synthetic peptides we tested. Visual inspection of the Western blot results indicated that patients from all disease stages had serum antibodies to the native gp120, gp41 and *gag* p17 virus components, and that antibody responses to these bands became weaker as disease progressed (results not shown). In accordance with earlier reports [10,11,17–19], we found that the HIV-derived gp41{5}(aa 579–601) peptide contained a highly immunogenic B cell epitope, and infected persons from all stages of disease progression were seropositive for this sequence. Less than 30% of all patients were however found to be seropositive for gp120 (aa 262–284), and the *gag* p17 (aa 106–125). By testing for serum antibodies to the other conserved-sequence overlapping gp41 analogues, we found that 87% of the total cohort of HIV-infected individuals was seropositive for gp41{8}(aa 593–604), and all asymptomatic patients were seropositive for peptides {5}(aa 579–601), and peptide {8}(aa 593–604) (Table 1).

These results also indicated that AS, ARC, and AIDS individuals produce antibodies to native HIV (*in vivo*), that could also recognize the immunodominant HIV-derived synthetic peptides we tested *in vitro*.

Gp41{5}(aa 579–601)-specific antibody levels (OD) did not vary significantly between patient groups (mean ± s.e.m.: AS = 4·21 ± 0·26; ARC = 4·03 ± 0·23; AIDS = 4·58 ± 0·29; all $P > 0·1$) (mean OD of HIV^- normals; AS, ARC and AIDS groups are shown in Fig. 3b). Antibody levels to only one of the overlapping gp41-derived peptides we tested, namely gp41{8}(aa 593–604), differed significantly between patient groups, with serum levels in the symptomatic ARC and AIDS patients (OD) found to be significantly lower than in AS (mean ± s.e.m.: ARC = 2·03 ± 0·28; AIDS = 2·03 ± 0·16 *versus* AS = 3·03 ± 0·11; both $P \leqslant 0·005$).

T and B cell responses to the same short HIV-derived synthetic peptides

Initial studies with the three conserved-sequence, core- and envelope-derived synthetic peptides revealed that significant B and T cell responses seen in the same individuals, and directed towards the same short peptide, only occurred in response to the gp41{5}(aa 579–601) peptide. One hundred per cent seropositivity in infected individuals, irrespective of their clinical condition, was observed to the gp41{5}(aa 579–601), and 48% of all

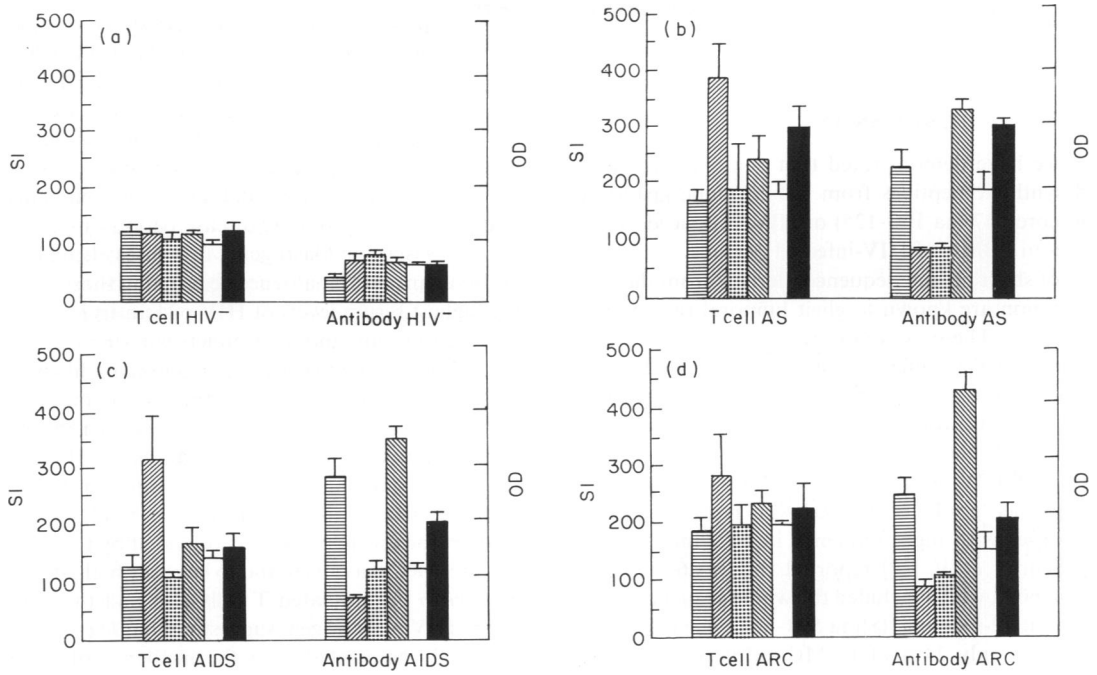

Fig. 5. Comparison of the blastogenic responses (SI) with serum antibody responses (OD) to overlapping gp41-derived synthetic peptides. Mean SI (SI = SI × 100) (±s.e.m.) and OD ((optical density) = OD × 100) (±s.e.m.) of both responder and non-responder members of asymptomatic (AS), ARC and AIDS patient groups in the cohort, are compared with mean SI and OD of HIV-seronegatives. HIV-seronegatives did not respond at either T or B cell levels. (a) Controls. (b) AS. (c) AIDS. (d) ARC. ■, gp41{1}(aa 572–591); ■, gp41{2}(aa 574–591); ▩, gp41{4}(aa 578–591); ▧, gp41{5}(aa 579–601); □, gp41{7}(aa 582–596); ■, gp41{8}(aa 593–604).

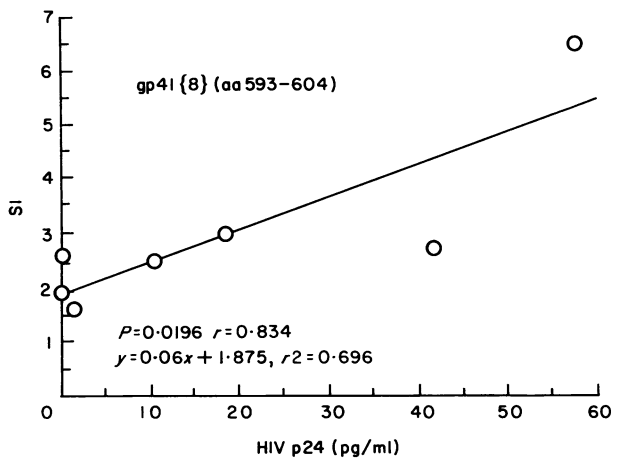

Fig. 6. Relationship between HIV p24 antigenaemia and SI to the immunogenic gp41{8}(aa 593–604). Mean serum levels of HIV-1 p24 antigen in peripheral blood of asymptomatic (AS) HIV-infected individuals correlated positively with the magnitudes of blastogenic responses to the immunogenic gp41{8}(aa 593–604) ($P = 0{\cdot}0196$).

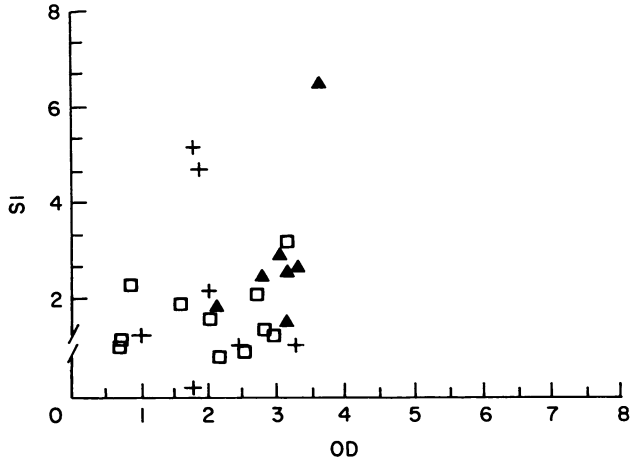

Fig. 7. Relative magnitudes of antibody and blastogenic responses to gp41{8}(aa 593–604). Serum antibody levels (OD) seen in any one individual in the asymptomatic (AS), ARC and AIDS patient groups were compared with the magnitudes of blastogenic responses (SI) to the immunodominant gp41{8}(aa 593–604). Linear regression analysis of these paired data indicated a direct correlation between OD and SI in AS only ($P \leqslant 0{\cdot}05$). ○, HIV⁻; ▲, AS; □, ARC; +, AIDS.

seropositives also had T cells that proliferated during culture with this peptide.

To define this gp41 epitope, we then used the series of six overlapping gp41-derived synthetic peptides that spanned aa 572 to aa 613. The profiles of the T and B cell responses (of the individual patients) to these overlapping gp41-derived peptides are shown in Table 1. One hundred per cent of these patients had serum antibody specific for the gp41 peptides, and 83% of these same patients had PBMC that responded by proliferation to one or more of these overlapping gp41-derived peptides. Comparative magnitudes of B and T cell responses to the most immunodominant peptide in AS, namely gp41{8}(aa 593–604), are shown in Fig. 7. Linear regression analyses of paired samples indicated that there was a direct correlation between the relative magnitudes of the T cell and antibody responses to immunodo-

minant gp41-derived synthetic peptides {5} and {8} ($P \leqslant 0{\cdot}05$) (results not shown).

DISCUSSION

In this study we have demonstrated that conserved-sequence HIV-derived synthetic peptides from the envelope gp120 (aa 262–284) and core p17 (aa 106–125) of HIV-1 induced blastogenic responses in PBMC of HIV-infected persons.

A number of short peptide sequences derived from the gp41 (aa 570–630) region are known to elicit humoral responses in animals and man. There was however a paucity of data describing human T cell proliferative responses to peptides from this immunodominant and conserved region. We have now identified a 12-mer envelope-derived peptide, gp41{8}(aa 593–604), that consistently elicits both antibody and T cell blastogenic responses in AS HIV-infected persons. Previously, a 12-mer peptide that spanned gp41 (aa 598–609) was reported to elicit antibody responses with high frequency in HIV-infected persons [17,18]. Schrier *et al.* [20] reported that a 26-mer gp41 peptide (aa 584–609), which included the whole sequence of our immunodominant 12-mer gp41{8}(aa 593–604), elicited blastogenic responses in only 24% of PBMC cultures from HIV-infected persons of undefined disease status, although patients tended to be in earlier stages of HIV infection. We found that a 35-mer gp41(β)(aa 579–613) analogue also only elicited blastogenic responses with low frequency (i.e. only one of seven patients, four AS and three ARC). Together, these data suggest that a shorter peptide sequences may be more immunogenic *in vitro* than longer ones in HIV-infected persons.

By virtue of the small size of our immunogenic 12-mer gp41{8}(aa 593–604), it is possible that the requirement for processing by antigen-presenting cells (APC) is bypassed, and that such a short peptide sequence may associate directly with complementary HLA determinants at the cell surface, thus giving rise to a 'complete' antigen that has the potential to elicit T cell blastogenic responses. A recent report by Choppin *et al.* [21] provides support for this hypothesis. This group has demonstrated *in vitro* that the short and immunogenic gp41 peptide (aa 584–604) that spanned the entire length of our shorter gp41{8}(aa 593–604) had the potential to associate spontaneously *in vitro* with diverse class 1 and class II HLA molecules.

These observations suggest that our short gp41{8}(aa 593–604) may also associate spontaneously with HLA molecules of diverse haplotype, thus acquiring the potential to elicit blastogenic responses in a large proportion of an MHC diverse population of humans.

To date there have been no reports indicating a direct relationship between the decline in blastogenic responses to mitogens or soluble antigens that occurs following HIV-infection, and the associated decline in absolute $CD4^+$ T cell numbers. HIV-infected persons have been reported to have HIV-specific $CD8^+$ CTL [22–24]. $CD8^+$ T lymphocytes have also been shown to have the ability to suppress HIV replication [25]. Our finding that the magnitudes of blastogenic responses to immunodominant HIV analogues correlated with absolute $CD8^+$ T cell numbers in asymptomatic, but not symptomatic, patients adds support for the suggested role of HIV-specific $CD8^+$ T cells in the control of HIV infection. Furthermore, if the $CD8^+$ T cells from the AS patients do themselves proliferate to any significant degree during culture with gp41 analogues, then a proportion of them may be $CD8^+$ CTL, whose function is also to suppress HIV replication in AS. If HIV-specific T cells function in this way during asymptomatic disease, the absence or disappearance of such $CD8^+$ suppression may also be associated with progression to symptomatic disease.

Our other interesting finding that warrants discussion was that in the asymptomatic disease period only, the strongest *in vitro* HIV-specific blastogenic responses elicited by our immunodominant HIV analogues occurred in those persons that had the highest serum levels of HIV p24 antigen.

Native virion and its products entering into the circulation of the host during productive infection are likely to elicit T cell blastogenic responses to the immunodominant HIV-envelope sequences [26]. Therefore the *in vitro* microblastogenesis responses elicited by our immunodominant HIV analogues may reflect the ongoing clonal proliferation responses that were initiated *in vivo* during productive viral infection. Indirect support for this hypothesis is provided by the observation that AS and ARC patients found to have the highest basal activation of *in vitro* unstimulated T cells also had the highest levels of serum HIV p24 antigen, suggesting that *in vivo* T cell activation is directly associated with the incidence of productive virus replication. However, these relationships with p24 antigenaemia were not observed in patients with AIDS (all $P > 0{\cdot}1$).

Specific anti-viral antibodies, in the presence of complement, have been shown to enhance simian immunodeficiency virus (SIV) infection of rhesus macaques [27]. A potential contraindication for the inclusion of gp41 amino acid sequences in a subunit vaccine arises from reports indicating that a gp41 peptide, homologous to our gp41{8}(aa 593–604), may enhance HIV infection *in vitro* [28,29].

Clinicians treating HIV-infected persons have found it difficult to reach consensus on which combination of an array of frequently used surrogate markers will provide the most reliable data to predict a good or poor prognosis [12,16,30–32]. Having identified this short and conserved-sequence HIV-derived synthetic peptide that consistently elicits cell-mediated immunity in asymptomatic patients only, we may now have at our disposal a tool with which to predict the risk of disease progression in HIV-infected persons.

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