

HIV p24-SPECIFIC HELPER T CELL CLONES FROM IMMUNIZED PRIMATES RECOGNIZE HIGHLY CONSERVED REGIONS OF HIV-1¹

KINGSTON H. G. MILLS,^{2*} PETER A. KITCHIN,* BERNARD P. MAHON,* AMANDA L. BARNARD,*
SALLY E. ADAMS,[†] SUSAN M. KINGSMAN,[‡] AND ALAN J. KINGSMAN^{**}

From the *National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG, UK, [†]British Bio-technology Limited, Brook House, Watlington Road, Cowley, Oxford OX4 5LY, UK, and [‡]Virus Molecular Biology Group, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

We have investigated Th cell recognition of the HIV core protein p24 by using CD4⁺ T cell clones derived from cynomolgus macaques immunized with hybrid HIV p24:Ty virus-like particles (VLP). T cell lines from two immunized animals responded to p24:Ty-VLP, control Ty-VLP, purified p24, and whole inactivated HIV, indicating the presence of T cells specific for p24 as well as the Ty carrier protein. The HIV determinants recognized by the T cell lines were identified by using a series of overlapping peptides synthesized according to the sequence of p24. Both T cell lines recognized peptide 11 (amino acids 235-249) and peptide 14 (amino acids 265-279). In addition, one T cell line also responded to peptide 9 (amino acids 215-229). Definitive identification of two T cell epitopes on p24 was confirmed at the clonal level: from a total of four T cell clones generated from one of the T cell lines, two respond specifically to peptide 11 and two to peptide 14. The T cell clones were CD4⁺ and MHC class II-restricted and secreted IL-2 in response to stimulation with purified p24, inactivated HIV or a single synthetic peptide. The specificity of the Th clones for variant peptides demonstrated cross-reactivity with two simian immunodeficiency virus isolates, but only limited responses to HIV-2 sequences. However, the Th cell epitopes identified on p24 are highly conserved between 12 HIV-1 isolates and were recognized by both of the immunized primates. These sequences may therefore be useful for priming a broadly reactive immune response to HIV-1.

The ability to induce HIV-specific Th cells is likely to be an essential requirement of any candidate vaccine designed to prevent or control HIV infection. Th cells are a vital component of the immune system and are required for both antibody and cytotoxic responses (1-3). In addition, evidence of protective immunity has been reported for other viral infections by the generation of Th cell populations directed against viral core antigens (4-7).

Previous reports have indicated that the Th cell response to HIV proteins is weak or undetectable in HIV-

infected individuals especially in the late stages of disease (8-10). In contrast, consistent proliferative responses against HIV proteins, including p24, are detected in HIV-infected non-human primates (11, 12). These animals, unlike humans, have so far failed to develop HIV-related disease symptoms. Furthermore, in HIV seropositive humans, a reduction in anti-p24 antibody levels has been shown to correlate with the onset of disease progression (13, 14). The p24 protein is therefore worthy of consideration for inclusion in a combination vaccine against HIV.

We have shown previously that a protein, p1, encoded by the yeast retrotransposon Ty can be used as a self-assembling, particulate carrier for recombinant Ag (15-17). Ty is a repetitive, mobile DNA element that moves from one genomic location to another via an RNA intermediate. The RNA is packaged into VLP³ by Ty-encoded proteins (Ty-VLP) (18, 19).

The structural components of the Ty-VLP are encoded by the Ty gene, TYA (19). If the TYA gene alone is expressed to produce protein p1, this will assemble into particles even in the absence of the other Ty-encoded proteins (19). The insertion of additional protein coding sequences at the 3' end of the TYA gene results in the production of p1-fusion proteins that retain the ability to self-assemble (15, 17). This system provides a useful means of presenting antigens in a polyvalent form as hybrid Ty-VLP. The hybrid VLPs are 50 to 80 nm in diameter, contain approximately 300 copies of the fusion protein, and have been used to elicit the production of antibodies against the non-Ty components (15, 17).

In this report we demonstrate the induction of HIV-specific CD4⁺ Th cells in cynomolgus macaques by immunization with HIV p24:Ty-VLP. MHC class II-restricted T cell clones have been derived from the immunized animals and used to delineate two Th epitopes in highly conserved regions of the HIV core protein p24.

MATERIALS AND METHODS

Ag. HIV p24:Ty VLP containing HIV *gag* codons 104-308 of isolate IIB (20) were constructed by expressing a TYA:p24 fusion gene in yeast as described previously (17). The HIV p24:Ty Ag, purified from yeast extracts on a 15 to 45% sucrose gradient, were electrophoresed on 9% SDS polyacrylamide gels and stained with Coomassie brilliant blue (19). The protein concentrations were determined by a Bradford assay obtained from Bio-Rad Laboratories. Purified p24:Ty-VLP were prepared for electron microscopy and stained with uranyl acetate as previously described (18). Nonparticulate p24 was cleaved and purified from hybrid HIV p24:Ty-VLP modified to incorporate

³ Abbreviations used in this paper: VLP, virus-like particles; SIV, simian immunodeficiency virus.

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² To whom correspondence and reprint requests should be addressed.

the recognition site for the blood coagulation factor Xa between the Ty and p24 polypeptide sequences (21). The purity of the p24 preparation was greater than 90% (21). HIV-1 (RF) was grown in H9 cells and high titer supernatants (10^6 TCID₅₀/ml) were inactivated by heating at 60°C for 1 h. Overlapping synthetic peptides corresponding to gag p17 and p24 sequence of HIV-1 (ARV-2/SF2) (22, 23) were manufactured for the MRC AIDS Directed Programme by Cambridge Research Biochemicals. Each peptide was 15 residues long with a C- or N-terminal cysteine added to facilitate chemical coupling for use in studies unrelated to those described here. Substituted analogues of two of the HIV-1 peptides (p11 and p14) were synthesized according to the sequences of the corresponding regions of HIV-2 and two SIV isolates from macaques (22). The HIV-2 ROD, SIV-M142, and SIV-M251 peptides from the corresponding region of the HIV-1 peptide 14 and the equivalent HIV-1 peptide, without the added C-terminal cysteine (amino acids 265–279), were synthesized by Jonathan Rothbard (ICRF, London). The HIV-2 and SIV peptides have a Ser substituted for the Cys in the natural sequence at position 275 to prevent cross linking of the peptides. The HIV-2/SIV-M142 and SIV-M251 peptides from the corresponding region of HIV-1 peptide 11 (amino acid 235–249) were synthesized for the MRC AIDS Directed Programme by Peptide and Programme Research Consultants, Reading, UK. The sequence of the synthetic peptides was confirmed by amino acid analysis and each peptide had a single major peak on HPLC. Peptides were dissolved in RPMI-1640 medium supplemented with 10^{-4} M 2-ME and stored at -70°C until required for use.

T cell clones. Proliferative CD4⁺ T cell lines and clones were generated from PBMC of two cynomolgus macaques (*Macaca fascicularis*) immunized with 50 μg of p24:Ty-VLP adsorbed onto alum. Animals received three 1-ml i.m. injections of the Ag preparation at 6-wk intervals. PBMC were purified by centrifugation on a percoll gradient from whole blood samples, taken either four weeks after the 1st boost (monkey H69) or two weeks after the 2nd boost (monkey H70). Cells were cultured at 1×10^6 /ml in RPMI-1640 medium supplemented with 10% FCS in the presence of antigen (p24:Ty-VLPs, 1 μg /ml) at 37°C in a CO₂ incubator. T cell lines were established by re-stimulation of the cultured T cells at 1×10^5 /ml every 14 days with antigen and autologous feeder cells (irradiated PBMC at 5×10^5 /ml). Fresh medium and IL-2 (human rIL-2, 5 U/ml) were added 5 days after Ag stimulation. T cell clones were established from T cell line 70.24 by limiting dilution at 1 cell/well in 200 μl volumes in 96 well tissue culture dishes in the presence of autologous feeder cells, p24:Ty-VLP (2 μg /ml) and IL-2 (10 U/ml). A further 25 μl of medium containing 50 U/ml IL-2 was added 5 days later. After 14 days clones were restimulated with Ag and IL-2 and expanded in 24-well plates.

Proliferation assay. Rested T cells (14 days after the last Ag stimulation) were cultured at 1×10^5 /ml in 100 or 200 μl volumes in 96 well flat-bottomed plates with antigen and irradiated autologous APC (5×10^5 /ml PBMC or 1×10^5 /ml EBV-transformed B cells). Unless otherwise indicated the concentrations of Ag used were 5 μg /ml of p24:Ty-VLP or control Ty-VLP, 1 μg /ml of purified p24 or synthetic peptide, and 1/10 to 1/100 dilution of HIV-infected H9 cell supernatant. Cultures were incubated for 2 or 3 days and pulsed for the last 4 h with 0.5 μCi of [³H]thymidine. Results are given as mean cpm of [³H]thymidine incorporation after harvesting and scintillation counting of triplicate cultures.

IL-2 assay. IL-2-release was assessed by testing the ability of supernatants, removed after 24 h of culture, to support the proliferation of the IL-2-dependent CTLL-2 cell line. Supernatants (50 μl) were added to 50 μl (1×10^4) CTLL-2 cells that were washed free of IL-2 for 2 h before the assay. CTLL proliferation was determined after 24 h by [³H]thymidine incorporation. Results are mean values for triplicate assays given as IU of IL-2, determined by comparison with a standard curve of CTLL proliferation with dilutions of human rIL-2.

MHC restriction and phenotypic analysis. The MHC restriction of the T cell lines on clones was examined by testing their proliferative response against p24:Ty VLP (1 μg /ml) and autologous APC in the presence of mAb specific for human MHC molecules. Antibodies were added to cultures at a final concentration of 1/200 and 1/1000 dilution of ascites fluid and proliferation was assayed two days later as described above. Antibody L203, specific for nonpolymorphic determinants of human MHC class II molecules (HLA-DR), was obtained from hybridoma cells purchased from the American Type Culture Collection (Rockville, MD). Antibody W6/32 is specific for a monomorphic determinant on human MHC class I molecule (HLA-A, -B, and -C). Each of the anti-human reagents cross-reacted with monkey cells: typical values for macaque PBMC were 10 and 95% positive cells with L203 and W6/32, respectively. Surface marker analysis was performed by labeling the T cells with the anti-human mAb T11, 4B4 (Coulter), OKT4 (Ortho), Leu-2a (Becton Dickinson),

specific for CD2, CDw29, CD4, and CD8 determinants, respectively. Results are expressed as the percentage of positive cells on flow microfluorimetry analysis after subtraction of background staining with a mouse IgG control.

RESULTS

Characterization of the HIV p24:Ty-VLP. The particulate nature of the HIV p24:Ty Ag was demonstrated by electron microscopy (Fig. 1). The particles have a diameter of approximately 80 nm. The purity of the Ag preparation was determined by SDS-PAGE. Examination of Coomassie-stained gels indicated that greater than 90% of the material migrates as a 70-kDa protein (data not shown). The presence of p24 Ag was confirmed by immunoblotting and immunogold electron microscopy (17).

Induction of HIV-specific T cell immunity and the generation of p24-specific T cell clones. PBMC isolated from cynomolgus macaques immunized with 50 μg of p24:Ty-VLP as an alum precipitate, were able to proliferate and incorporate [³H]thymidine in response to stimulation with purified p24:Ty-VLP. Significant T cell responses to p24 were detected 2 wk after the first immunization and for up to 8 wk after the third immunization. Furthermore, anti-p24 antibodies, as well as proliferative T cell responses, were detected in a total of eight animals after immunizations with 50 or 500 μg of p24:Ty-VLP with or without alum as adjuvant (24). T cell lines were established from the two animals that had been immunized with 50 μg p24:Ty-VLP, and maintained by repeated Ag stimulation *in vitro* for at least 6 mo. The T cell lines 69.24 and 70.24 proliferated in response to p24:Ty-VLP, control Ty-VLP, purified p24, and to whole, inactivated HIV (Table I). This specificity pattern indicated that the cell line contained T cells directed against both the Ty carrier protein and HIV p24. T cell clones were generated from line 70.24 by limiting dilution. A total of four clones were successfully maintained in culture and each of these was specific for HIV p24 (Table I).

Identification of Th cell epitopes on p24. The HIV determinants recognized by the T cell lines were identi-

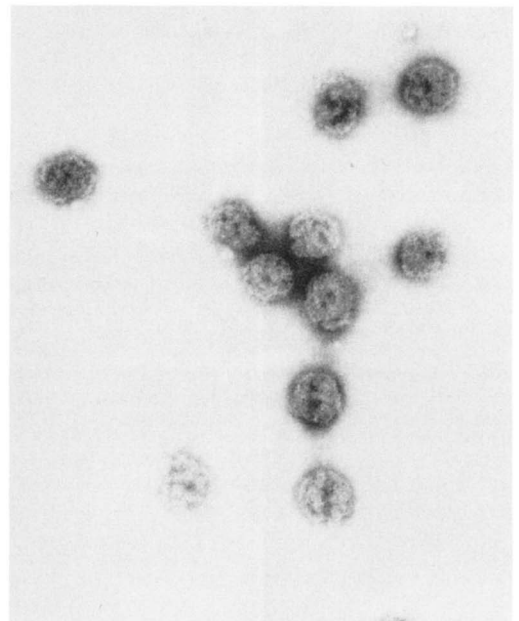


Figure 1. Electron micrograph of purified HIV p24:Ty-VLP. The particles were stained with uranyl acetate.

TABLE I
 Identification of determinants on HIV-p24 recognized by macaque CD4⁺ T cell lines and clones

T Cell	Proliferative Response (cpm) to								
	Medium	p24:Ty-VLP	p24	Ty-VLP	Whole HIV-1 RF	Synthetic peptides			
						p9 (215-229) ^a	p11 (235-249)	p14 (265-279)	p16 (285-299)
Line									
70.24									
Expt. 1 ^b	414	<u>2,246</u> ^c	ND	ND	ND	480	<u>4,900</u>	<u>2,736</u>	494
Expt. 2	4,449	<u>25,578</u>	<u>17,636</u>	<u>23,779</u>	ND	ND	<u>16,108</u>	<u>19,872</u>	ND
Expt. 3 ^a	1,360	ND	ND	ND	<u>3,580</u>	ND	ND	ND	ND
69.24									
Expt. 1 ^b	808	<u>2,459</u>	<u>2,728</u>	<u>1,626</u>	ND	<u>3,544</u>	<u>2,150</u>	<u>2,636</u>	864
Expt. 2	4,496	<u>19,405</u>	<u>18,558</u>	<u>12,224</u>	ND	<u>20,452</u>	<u>15,096</u>	<u>16,955</u>	ND
Expt. 3 ^d	4,144	ND	ND	ND	<u>12,573</u>	ND	ND	ND	ND
Clone									
70.24.29	580	<u>14,310</u>	<u>3,694</u>	788	ND	ND	<u>3,670</u>	182	ND
70.24.45	1,101	<u>14,382</u>	<u>6,938</u>	946	ND	ND	164	<u>13,456</u>	ND
70.24.51	440	<u>5,503</u>	<u>2,099</u>	392	ND	ND	252	<u>4,620</u>	ND
70.24.53									
Expt. 1	128	<u>9,843</u>	<u>8,073</u>	216	ND	ND	<u>10,101</u>	218	ND
Expt. 2 ^d	411	ND	ND	ND	<u>4,709</u>	ND	ND	ND	ND

^a Amino acids of corresponding HIV-1_{SP2} gag sequence (22).

^b Experiment was performed with EBV-B cells as APC.

^c Response values considered to be positive are underlined.

^d Experiment was performed by using different culture and harvesting facilities in a containment 3 laboratory.

fied by testing their specificity with 20 overlapping 15 amino acid-long synthetic peptides representing the entire sequence of the gag component present in the hybrid VLP (17 for p24 and 3 for p17). The proliferative T cell responses to four of the p24 peptides is shown in Table I. The remaining peptides gave responses essentially similar to that shown for peptide 16. Lines 69.24 and 70.24 both responded to peptide 11 (amino acids 235-249) and to peptide 14 (265-279). In addition, line 69.24 also recognized peptide 9 (215-229).

T cell clones generated from line 70.24 were used to definitively identify Th epitopes on HIV p24 at the clonal level. From a total of four clones examined, clones 70.24.29 and 70.24.53 responded specifically to peptide 11 and clones 70.24.45 and 70.24.51 responded specifically to peptide 14 (Table I and Fig. 2). Each of the clones proliferate and secrete IL-2 in response to Ty:p24-VLP and purified p24, and a single synthetic peptide but not on stimulation with control Ty particles. These recognition patterns indicate that the T cell clones are HIV-specific. Further confirmation was obtained by the stimulation observed of clone 70.24.53 with inactivated HIV (Table I).

Effect of sequence variation between HIV-1, HIV-2, and SIV on T-cell recognition. The regions of HIV p24 spanned by peptides 11 and 14 are highly conserved between 12 HIV-1 isolates (HXB2, BH102, BH5, PV22, BRU, MN, SF2, CDC41, WMJ21, RF, MAL, ELI) (22). However, there are a number of sequence changes in the corresponding regions of HIV-2 and SIV (Fig. 3). We therefore investigated the proliferative responses of the T cell clones to equivalent HIV-2 ROD, SIV-M142 and SIV-M251 peptides. The responses of two of the clones against these peptides are shown in Figure 3. Clone 70.24.45 (which recognizes HIV-1 gag peptide 14) did not give a significant proliferative response to the equivalent HIV-2 peptide. However, cross-reactivity was demonstrated with the two SIV peptides. It is possible that the Ile to Ser change at position 275, rather than the Ile to Cys in the natural sequences, could account for reduced activity of these peptides. However, this is unlikely since

a comparison of the HIV-1, HIV-2, and SIV sequences with reference to the proliferation observed indicates that the recognition of the epitope is most affected by amino acid substitutions at residues 270 and 271 (Fig. 3). Amino acid substitutions corresponding to natural variation between HIV-1 and HIV-2/SIV were also found to be critical for T cell recognition of peptide 11. The HIV-2 and SIV-M251 sequences in this region are identical to each other and differ from HIV-1 peptide 11 by changes at residues 245 and 246. Clone 70.24.53 gave only a weak response with its equivalent HIV-2/SIV-M142 peptide, and recognition was completely abrogated by a further substitution at residue 244 as seen in the SIV-M251 peptide (Fig. 3). The addition of C-terminal Cys residues to the HIV-1 peptides (for use in unrelated studies) may have resulted in dimer formation, however, this does not appear to have affected their activity. The response curves of clone 70.24.45 to peptide 14, synthesized with or without the additional Cys were essentially identical (in one experiment the peak responses at 1.0 µg/ml were 16,401 and 15,957 cpm, respectively). Furthermore, the synthetic peptides with the added Cys residues had similar T-cell stimulatory capacity as the full length recombinant p24 (Fig. 2).

MHC restriction and phenotype of the T cell lines and clones. An analysis of the surface markers present on the T cell lines and clones with mAb demonstrated that they were CD2⁺, CD4⁺, CDw29⁺, and CD8⁻ (Fig. 4). The effects of mAb specific for human MHC molecules on antigen-induced proliferative responses suggested that the T cell recognition was MHC class II-restricted. Antibody L203, specific for human HLA-DR inhibited the proliferative response of line 70.24 and clone 70.24.53 (Fig. 4). At a 1/100 dilution of L203 ascites, the response of line 70.24 and of clone 70.24.53 was reduced by 65 to 70% (after subtraction of backgrounds, responses were reduced from 8125 to 2500 cpm and 13,250 to 4,625 cpm, respectively). In contrast, the addition of mAb W6/32, specific for a monomorphic determinant on human MHC class I molecules (HLA-A, -B, and -C), did not significantly affect the T cell responses (Fig. 4). The net

Figure 2. Proliferation and IL-2-release by HIV p24 specific T cell clones. T cell clones 70.24.45 (a and c) and 70.24.53 (b and d) were tested for antigen specificity against a range of doses of p24:Ty-VLP, purified p24, control Ty-VLP and synthetic peptides. The response curves of clone 70.24.45 are shown with peptides p10 (amino acids 225-239), p11 (235-249) and p12 (245-269) and T-cell clone 70.24.53 with peptides p13 (255-269), p14 (265-279) and p15 (275-289). Results of proliferative responses (a and b) are shown above the corresponding curves for IL-2 release (c and d) tested in the same experiment.

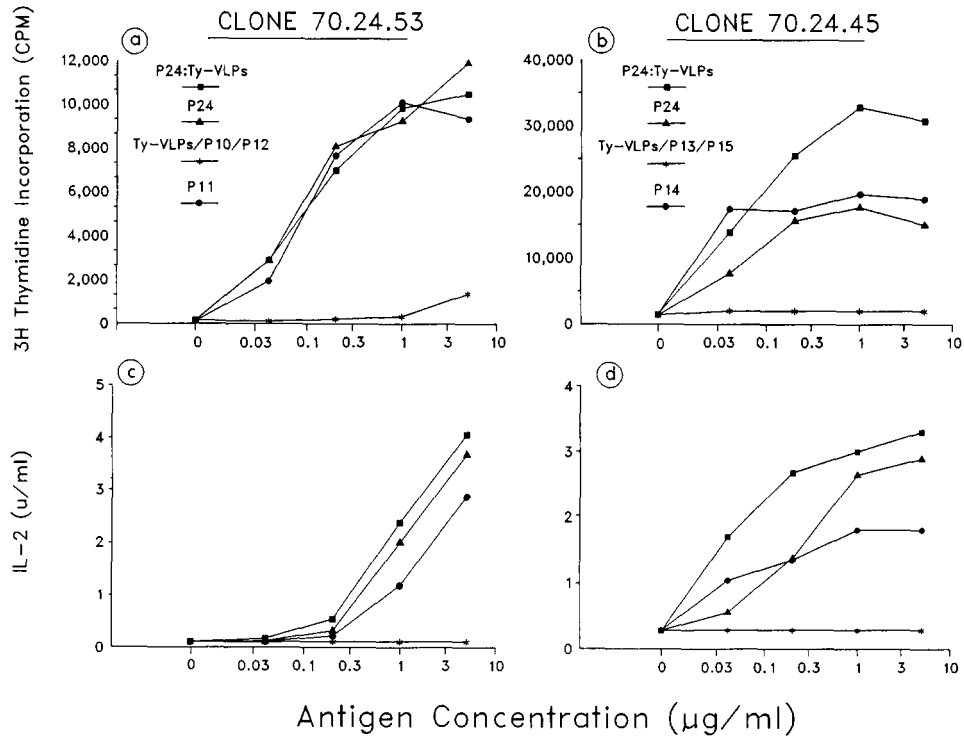
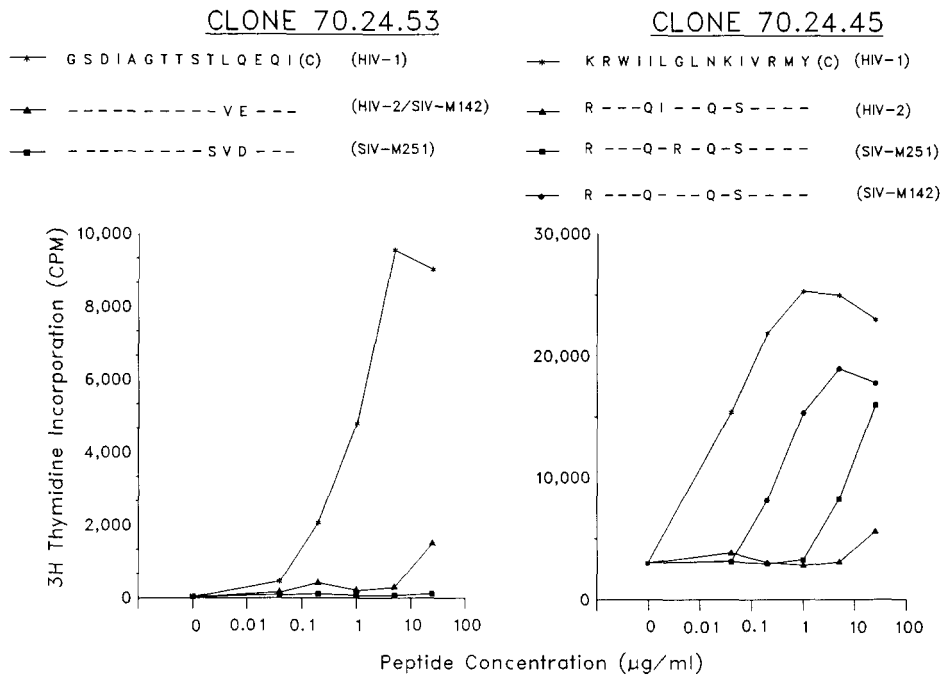


Figure 3. Specificity of HIV p24-specific T cell clones for synthetic peptides corresponding to the natural sequences of HIV-1, HIV-2 and SIV. Results are proliferative responses for clones 70.24.53 and 70.24.45 against peptides corresponding to HIV1 p24 residues 235-249 and 265-279, respectively, and against peptides synthesized according to the equivalent regions of HIV-2, SIV-M142, and SIV-M251. The HIV-2 and SIV peptides from the corresponding regions of HIV-1 residues 265-279 have a Ser substituted for a Cys in the natural sequence at position 275. Sequence of peptides is given in standard single letter code.



changes in the responses observed with W6/32 (5 to 13%) were within the experimental error of the cpm obtained in the absence of added antibody. The inability of APC from monkey H69 to present antigen to T cells from monkey H70 is also shown in Figure 4. T cell line 70.24 failed to respond to p24:Ty-VLP in the presence of irradiated PBMC from monkey H69. This confirms the MHC restriction of the response and suggests that monkeys H69 and H70 are incompatible at the DR locus.

DISCUSSION

This report describes the generation and fine specificity of HIV-specific CD4+ T cell clones generated from

primates immunized with hybrid HIV p24:Ty-VLP. The determinants recognized by the MHC class II-restricted T cell clones were mapped to conserved regions of the HIV-1 p24 protein. However, our results indicate that T cell recognition of the gag protein is affected by amino acid sequence variation between HIV-1 and HIV-2 or SIV.

Previous studies on the mapping of Th epitopes on HIV proteins have focused on envelope Ag (25-29). However, despite the presence of these epitopes, in addition to CTL epitopes (30, 31) and B cell epitopes that elicit neutralizing antibodies (32-35), attempts to protect non-human primates with envelope-based preparations has so far been unsuccessful (36, 37). There are several lines of evidence that suggest that a vaccine against HIV that

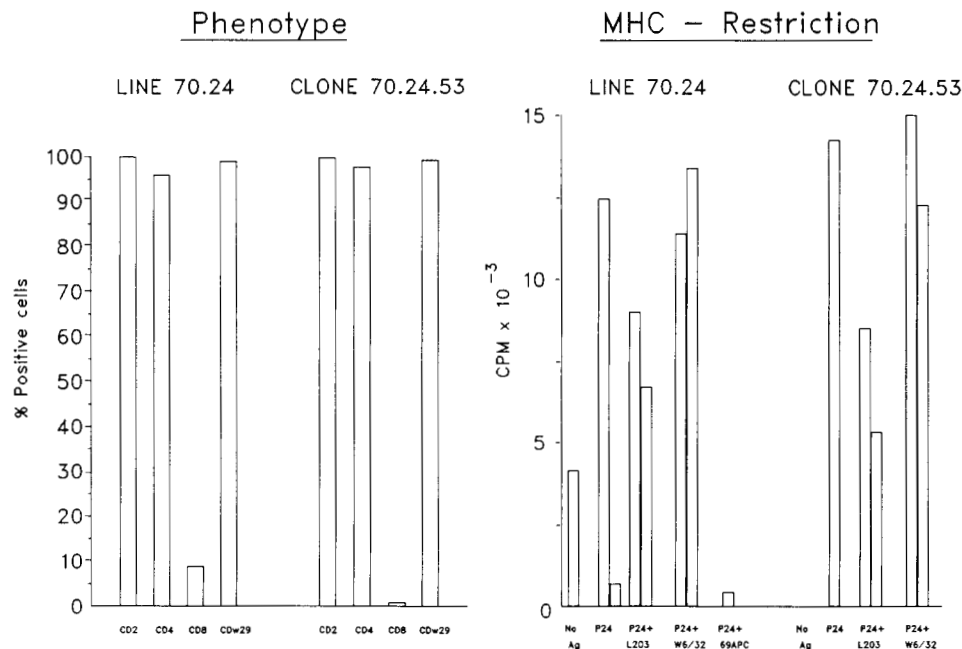


Figure 4. MHC restriction and phenotype of T cell lines and clones. Results of MHC restriction analysis are shown as cpm for proliferative responses of T cells with autologous APC alone (no Ag) or against p24:Ty-VLP (p24) in the presence 1/1000 (left-hand column) or 1/100 (right-hand column) dilution of mAb ascites specific for human MHC class II (L203) or class I (W6/32) molecules. Also shown as the response of line 70.24 to p24 in the presence of allogenic (monkey 69) APC.

includes all or part of the p24 protein in an immunogenic form, may have greater potential to confer protection than those based on envelope alone. In studies of influenza (7, 38) and hepatitis B (4-6) viruses, vaccination with core antigens has been shown to prime Th cells that cooperate with B cells in the production of neutralizing anti-envelope Ab, which are at least partially protective against live virus challenge. Furthermore, in HIV seropositive patients both T cell proliferative responses and antibody levels against p24 decline with the progression of HIV-related disease (9, 10, 13, 14). However, reported attempts to induce HIV p24 specific Th responses in immunized animals have been largely unsuccessful (9, 39). Proliferative lymphocyte responses to p24 were not detected in PBMC from chimpanzees after inoculation with vaccinia virus HIV p24 recombinants (39). Furthermore in a study of cynomolgus macaques immunized with a recombinant *gag* protein purified from *Escherichia coli*, at least six injections in IFA were required to induce a detectable proliferative response to p24 (9, 40). In contrast, significant T cell proliferation was demonstrated in PBMC from cynomolgus macaques after only one or two immunizations with hybrid HIV p24:Ty-VLP with or without alum as adjuvant (24, and this study). Specificity analysis of T cell lines and clones generated from two of the immunized animals demonstrated the induction of MHC class II-restricted T cells specific for HIV sequences. The T cells were CD4⁺ CDw29⁺ and secreted IL-2 on antigen stimulation and therefore probably belong to the helper-inducer subpopulation. These data suggest that immunization with a recombinant *gag* protein, incorporated into a hybrid polyvalent particle containing 300 copies of the Ag, is an effective method for the generating HIV p24-specific memory T cells in primates.

Fine specificity analysis of T cells from the immunized macaques against a panel of overlapping synthetic peptides, corresponding to the sequence *gag* protein, resulted in the delineation of three new Th epitopes on p24. Two of the determinants were recognized by T cell lines from two animals and definitive identification of these epi-

topes was confirmed at the clonal level; T cell clones derived from one of the T cell lines showed specific recognition of peptide 11 (235-249) or peptide 14 (265-279). Interestingly peptide 14 has also been shown to contain an epitope for CTL from HIV seropositive humans (41) and includes the sequence Lys-Ile-Val-Arg (residues 274-277) which corresponds to a consensus motif suggested by Rothbard (42) as being common to a majority of T cell determinants.

The successful generation of T cell clones specific for distinct determinants on HIV p24, has allowed us to examine the effect of sequence variation between HIV-1, HIV-2, and SIV on T cell recognition at the epitope level. The region of HIV p24 spanned by p11 is completely conserved between 12 HIV-1 variants, whereas peptide 14 corresponds to a region with a single conservative amino acid substitution (Leu to Val) in one Zairian isolate, HIV-1_{ELI} (22). However, there is a greater degree of variability seen when the sequences are compared with the corresponding regions of HIV-2 and SIV (22). The T cell clones, when tested against analogues of peptide 11 and peptide 14, with substitutions at sites corresponding to those found in the natural sequences of HIV-2 ROD, SIV-M142, and SIV-M251, showed significant cross-reactivity with SIV sequences but limited recognition of the HIV-2 peptides. An examination of the sequence changes in the HIV-2 and SIV peptides with reference to their ability to stimulate the T cell clones, reveals that Leu₂₇₀ and Gly₂₇₁ are critical for recognition of peptide 14. One or more of the amino acid substitutions at position 265, 269, 273, or 275 also appeared to affect T cell recognition, but to a lesser extent, as judged from the relatively small reduction in the response of clone 70.24.45 to the SIV-M142 peptide compared with the HIV-1 peptide. It is possible that this reduction in activity may have resulted from the substitution of Ser (rather than Cys, as in the natural sequences) for the Ile at position 275 in the HIV-2 and SIV peptides. Substitutions at Leu₂₄₅ and Gln₂₄₆, in the HIV-2 and SIV equivalents of the HIV-1 p24 peptide 11, significantly reduce the response of clone

70.24.53. A further amino acid change at residue 244 in the SIV-M251 peptide reduced the response to background levels. Therefore, natural sequence variation between HIV-1 and HIV-2 or SIV does affect T cell recognition of the *gag* protein. Substitution at critical sites may affect the capacity of the peptide or processed Ag to associate with the MHC molecule and/or its recognition by the TCR.

In a recent report on anti-*gag* mAb, Niedrig et al. (43), showed that certain antibodies were cross-reactive with SIV or HIV-2, whereas others were specific for HIV-1 sequences. In contrast, many of the B cell epitopes identified on the envelope protein, including a major neutralizing antibody site, map to hypervariable regions of the protein and consequently are often HIV-1 strain specific (33, 35). Furthermore, in a study with gp120 specific human CD4⁺ CTL clones, only limited cross-reactivity was demonstrated against synthetic peptides and gp120 molecules from different HIV-1 isolates (30). This suggests that T cell recognition, as well as antibody binding, is drastically affected by sequence variation in the envelope protein. However, the T cell epitopes on p24 defined here, map to highly conserved regions of HIV-1, and were recognized by both of the immunized primates. As these animals appear to be MHC incompatible at the class II locus, this suggests that these sequences may be effective in a broad range of haplotypes.

It remains to be defined which viral antigens or which arm of the immune response may be capable of generating protective immunity to HIV. Th cells specific for cross-reactive determinants on the HIV-1 proteins may be important for the development of anamnestic antibody or CTL responses. Therefore conserved sequences of the HIV-1 *gag* protein, that include Th cells epitopes, may be a useful component of a combination vaccine against HIV.

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