Journal of Peptide Science, Vol. 2, 165–175 (1996)

# A Combinatorial Peptide Library Around Variation of the Human Immunodeficiency Virus (HIV-1) V3 Domain Leads to Distinct T Helper Cell Responses

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*Received 2 October 1995*
*Accepted 30 November 1995*

Abstract: The hypervariable domain of the HIV gp120, the V3 loop domain, represents a target for neutralizing antibodies and for HIV vaccine strategies. In this study, we have investigated in murine species the potential cross-reactivity of immune responses elicited by immunization either with individual V3 peptides, derived from distinct HIV sequences (BRU, RF, SF2, MN and ELI sequences), or with a V3 combinatorial peptide library.

We observed that individual V3 peptides are immunogenic but elicit a specific B- and T-cell immune response that is mainly restricted to the sequence of the immunizing peptide. In particular, T-cell responses that depend on T-cell receptor recognition of peptides bound to the molecules encoded by the major histocompatibility complex were significantly influenced by small differences in the peptide amino acid sequence. The combinatorial V3 peptide library, previously described as B- and T-cell immunogens, induced a more broadly reactive immune response, specially when T-cell cytokine secretion was used as a readout for restimulation of T-cells with individual V3 peptides.

These data suggest that amino acid variations in the sequence of an antigenic peptide could lead to the induction of different transducing signals in the primed T-cell population and to the activation of T-cells with distinct cytokine secretion properties. These observations may have implications in the understanding of antigenic variability and in the design of vaccine strategies.

Keywords: HIV; peptide library; immune response; cytokines

## INTRODUCTION

Variations in the amino acid sequence of antigenic peptides affect B-cell and T-cell response to antigens [1–3]. In contrast to B cells, which recognize soluble antigens, T lymphocytes recognize antigens as peptide fragments in association with major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells [4,5]. The generation of an effective immune response to foreign antigens depends on the initiation of a T helper (TH) cell response and is therefore regulated by molecules encoded by MHC [6].

Several approaches have been used to determine amino acids involved in MHC (agretope) or T-cell receptor (TCR) (epitope) binding sites. Critical residues on peptide antigens have been mapped by using amino acid substitutions [7–12]. The importance of MHC polymorphisms on the specificity of the interactions between peptide and MHC has been assessed using site-directed mutagenesis [13–19]. Recently, crystallography of the MHC molecules [20,21] and peptide elution studies have provided additional information [22–25]. MHC molecules can select

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Abbreviations: CFA, complete Freund's adjuvant; HIV, Human immunodeficiency virus; IFA, incomplete Freund's adjuvant; MA, mixotope; PD-MS, plasma desorption mass spectrometry; TH, T helper cell.


CCC 1075-2617/96/030165-11

peptides according to the complementarity between their allele-specific pockets in the MHC groove and the side chains of the amino acid residues in antigenic peptides that represent allele motifs; such allele motifs include amino acids referred to as anchor residues, separated by a variable anchor spacer sequence that extrudes into the groove [26–29].

B- and T-cell responses to the envelope glycoprotein (gp120) are an important component of the specific immune response to HIV infection [30–32]. These responses include the recognition of the hypervariable V3 loop of HIV-1 gp120 that plays an important role in the generation of neutralizing antibodies and in the tropism of HIV. Peptides derived from this domain have been shown to be immunogenic and to elicit HIV-neutralizing antibodies [33–38].

In the present study, we have analysed the immune response against individual V3 peptides from distinct HIV sequence isolates (BRU, MN, RF, SF2 and ELI) and against a combinatorial peptide library, composed of $7.5 \times 10^5$ related V3 peptides, called mixotope (MA) [39]. This library was previously described by ourselves as potent B- and T-cell immunogens. Variation in the HIV proteins is a way by which the virus evades neutralizing antibodies [40,41] and also escapes possible control by cytotoxic T cells [42–45]. An additional feature of HIV infection is the simultaneous presence of a high number of viral mutants at any given time [46]. We analysed in different strains of rodents the fine specificity and the cross-reactivity of the B- and T-cell response to individual V3 peptide sequences and to a combinatorial V3 peptide library, specially with regards to the profile of cytokine secretions. This approach represents a new and interesting model to explore the immune response to antigenic variability.

## MATERIALS AND METHODS

### Media and Reagents

For all cultures, RPMI 1640 (Gibco, Courbevoie, France), containing 20 mM HEPES (Sigma, St Louis, MO), 100 U penicillin/ml, 100 $\mu$g streptomycin/ml (Specia, Paris, France) and 10% heat-inactivated FCS (Gibco) was usually supplemented with 2 mM L-glutamine, $5 \times 10^{-5}$ M $\beta$-mercaptoethanol (Merck, Darmstadt, G) and 1 mM sodium pyruvate (Gibco).

### Animals

Fischer (RT-$1^{lv1}$), and Lou M (RT-$1^{u}$) female syngeneic rat strains were bred and maintained in the animal unit of Institut Pasteur, Balb/c (H-$2^{d}$), C57bl/6 (H-$2^{b}$) and CBA (H-$2^{k}$) female mice were obtained from IFFA CREDO.

### Peptide Synthesis

The mixotope antigen was synthesized as previously described [39,46], using the BOC–TFA scheme and an Applied Biosystems Model 430A peptide synthesizer (Foster City, CA), on a *t*-BOC-*p*MeBzl-Cys PAM resin (1 mmol; loading of the starting resin: 0.72 mmol/g). Side-chain protecting groups were: Glu (Obzl), Ser (Bzl), Thr (Bzl), Arg (Tos), Tyr (BrZ), Cys (4-MeBzl), Met (O) and His (Bom). Each peptide was treated twice with 20% mercaptoethanol/2% DIEA in DMF for 120 min in order to remove the DNP group protecting the histidine residue. Peptidyl–resin cleavage and side-chain deprotection were performed by low and high hydrogen fluoride (HF) procedure when side-chain sulphoxide protection was used on Met, or high HF for all other peptidyl–resins. Crude peptides were purified by preparative reverse-phase HPLC and analysed for identity by PD-MS (plasma desorption mass spectrometry).

The individual V3 sequences used were the RF, BRU, MN, SF2 and ELI peptides, and divergences between the amino acid sequences of these peptides are indicated in Table 1.

Table 1 Divergence Scores[a]

| | BRU | RF | SF2 | MN | ELI |
|---|---|---|---|---|---|
| BRU | 0 | 26.9 | 26.8 | 33 | 52.6 |
| RF | | 0 | 8.0 | 15.1 | 38.4 |
| SF2 | | | 0 | 7.1 | 41.3 |
| MN | | | | 0 | 38.2 |

[a]The divergence between sequences of peptides listed below:

BRU: TRPNNNTRKSIRIQRGPGRAFVTIGKIGNMR
QAHC,
MN: TRPNYNKRKRIHIGPGRAFYTTKNIIGTIRQAHC,
SF2: TRPNNNTRKSIYIGPGRAFHTTGRIIGDIRKAHC,
RF: TRPNNNTRKSITKGPGRVIYATGQIIGDIQKAHC,
ELI: ARPYQNTRQRTPIGLGQSLYTTRSRSIIGQAHC;

was determined by calculating divergence scores by summing scores of each amino acid replacement within the sequence using a scoring matrix [47] and by adding 2.2 for each amino acid residue represented in one but not in the paired sequence and 6.0 for each gap in the sequence.

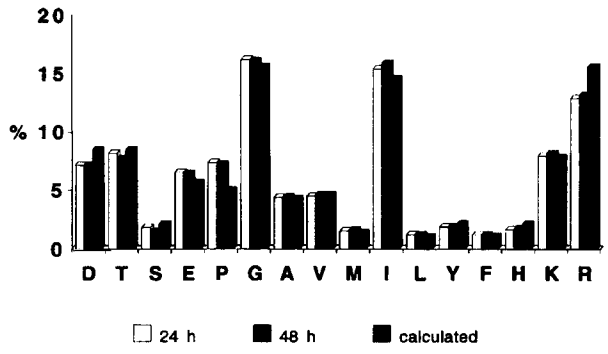


Figure 1 Amino acid composition determined after 24 and 48 h of acid hydrolysis, compared with the theoretical composition, calculated on the basis of an equimolecular amount of each amino acid introduced in the degenerated positions.

The mixotope antigen was composed of the following peptide sequence in which degenerated amino acids are indicated in brackets:
NNT(KR)(KR)(GRS)I(HPRPSTY)(IMR)( – Q)( – R)GP
GR(AV)(FILV)(HVY)(AT)(IRT)(EGK)(KQ)( – 1)IGC

The most probable amino acid residues in each degenerate position of the central region of the V3 loop from 138 randomly selected isolates of HIV-1 were coupled using the dicyclohexyl carbodiimide/hydroxybenzotriazole method. In each degenerate position, all amino acids have a percentage occurrence higher than 7% were arbitrarily selected. After HF cleavage and deprotection (respectively in low and high concentrations of HF), the crude peptide was purified by three consecutive TFA/diethylether precipitation steps. The mixotope antigen was checked for identity by amino acid analysis after total acid hydrolysis, the only possible analytical control of the final product (Figure 1). In each of these positions, the different amino acids or the gap were represented in an equivalent fraction, thus generating $7.5 \times 10^5$ distinct sequences. Other analytical methods were also performed, including microsequencing, that did not provide additional information, and plasma desorption mass spectrometry, that confirmed that the mixotope antigen is a random peptide mixture.

### Immunization Procedure

Rodents were immunized at the base of the tail with either 100 $\mu$g (rats) or 50 $\mu$g (mice) of peptides in complete Freund's adjuvant (CFA) (Difco, Detroit, MI). Twenty days later, animals were boosted in the same way with half the amount of antigen emulsified in incomplete Freund's adjuvant (IFA).

### Proliferation Assay

Five days after the last immunization the draining lymph nodes and the spleen were removed. T lymph node cells from rats were purified by passage through a nylon wool column. A total of $5 \times 10^5$ T-cells were cultured with $5 \times 10^5$ syngeneic irradiated antigen-presenting cells (30 Grey, Philips RT) from thymic rat and antigen in flat-bottomed 96-well plates (Falcom, Becton Dickinson, France) in a humidified $CO_2$ incubator at 37°C.

Five days later, a pulse with 18.5 kBq of 3H-TdR to each well (CEA, Gif-sur-Yvette, France) was made in order to measure T-cell proliferation: 12–16 h later the wells were harvested onto glass fibre filter strips using a multiharvester (Skatron, Liergyen, Norway) and the radioactivity incorporated into DNA was determined by liquid scintillation counting (LKB, Wallac, Turku-Finland).

### Cytokine Secretion Assays

Supernatants from spleen and lymph node T-cells ($10^6$ cells/ml) of immunized rodents were analysed for lymphokine secretion after antigen restimulation *in vitro* (20 $\mu$g/ml).

Interleukin (IL)-2 production was tested using an IL-2-dependent T-cell line (CTLL-2, ATCC). CTLL-2 ($10^4$ cells) were added to the appropriate antigen supernatants and incubated for 24 h including a 10 h pulse with tritiated thymidine (0.5 $\mu$Ci/well) before harvesting. Units of IL-2 were determined by comparison with standard curves using recombinant IL-2 (Genzyme).

IL-3 production was measured using an IL-3-dependent cell line (32D CL, ATCC). 32D CL ($10^4$ cells) were added to the supernatants and incubated for 48 h before 3H-TdR labelling.

IL-5 production from rats was measured using an IL-5-dependent cell line (Ly H7 B13, ATCC). Samples were incubated with $10^4$ cells for 48 h and pulsed with 3H-TdR to measure cell proliferations.

IFN-$\gamma$ was measured using a B-cell lymphoma-sensitive cell lines (WEHI-279, ATCC). Inhibition of cell proliferations ($10^4$ cells) was determined by 18 h of 3H-TdR incorporation after three days of culture.

IL-4 and IL-5 production from mice were determined by ELISA. Briefly, samples were analysed with pairs of rat anti-IL-5 and anti-IL-4 mAb in a two-site sandwich ELISA. Microtitre plate wells were first coated with the monoclonal antibody (1 $\mu$g/ml) at 4°C overnight. Plates were then washed (PBS, tween 1‰) and blocked by the addition of 1% BSA in PBS.

Samples and standard dilutions (recombinant (r) IL-4 and rIL-5 lymphokines) were added and incubated for 2 h. Plates were washed and the antibody–biotin conjugate (1 $\mu$g/ml) was added for 1 h at room temperature. Streptavidin–horseradish peroxidase conjugate was incubated for 1 h, washed extensively and revealed with substrate (OPD, orthophenylenediamine, Sigma), in solution phosphate buffer 0.1 M, pH 5.5, containing $H_2O_2$. The optical density was measured through a multichannel spectrophotometer at 492 nm.

### Cytokine mRNA

Total cellular RNA were extracted from $20 \times 10^6$ spleen cells 18 h after *in vitro* restimulation with the antigen using the RNAzol procedure (Bioprobe, France) and precipitated. Reverse transcription was performed using 2 $\mu$g of total RNA. Aliquots of the RT-RNA were amplified in a 50 $\mu$l reaction volume containing oligonucleotide primers (0.5 $\mu$M) to the $\beta$-actin and the IL-4, 200 $\mu$M of deoxynucleotide triphosphate (dNTP) (Pharmacia), 60 mM KCl, 50 mM *tris*-HCl, ph 8.3, 5 mM $MgCl_2$. PCR products, amplified using (Perkin Elmer/Cetus Thermocycler) for 25, 35 and 45 cycles, respectively, for $\beta$-actin, IFN-$\gamma$ and IL-4, were subjected to electrophoresis on 2% agarose gels, visualized by staining with ethidium bromide or hybridized with $^{32}$P-labelled IL-4 probes (kindly provided by J. McKnight).

### Antibody ELISA Assay

Microtitre plate wells (Nunc, Denmark) were coated for 24 h at 4°C with 0.5 $\mu$g/0.1 ml of synthetic peptides. The plates were saturated before the addition of the appropriate dilutions of sera ($1/50^e$). IgG was detected by a rabbit anti-rat peroxidase (ICN biomedicals, Lisle, Il) and revealed with OPD substrate. The optical density was determined at 492 nm.

## RESULTS

### B- and T-cell Immunogenicity of V3 Peptides

Humoral responses from two rat strains (Lou M and Fischer) immunized with individual V3 peptides (BRU, MN, RF, SF2 and ELI) were analysed. Divergence between the amino acid sequence of these peptides ranged from 7% to 52% (Table 1). Results are summarized in Table 2. All peptides induced an antibody response against themselves, except the RF peptide that was immunogenic in the Lou M rat strain only. IgG antibodies elicited by RF and SF2 peptide immunizations were the main antigenic cross-reactive antibodies. Divergence between the sequence of both peptides was 8% only. However, divergence of 7% only between SF2 and MN did not result in a similar cross-reactivity, indicating that the degree of homology shared by two peptides was not sufficient to determine the induction of a cross-reactive immune response.

We investigated the ability of V3 peptides to generate specific lymph node T-cells (Figure 2). In both rat strains, all peptides stimulated specific T-cell responses as assessed by T-cell proliferation after *in vitro* restimulation with the same V3 peptides, except the RF peptide that generated specific T-cells only in Lou M rat strain. These results suggested that the inability of the RF peptide to elicit T-cell and B-cell responses in the Fischer rat strain could be related to the absence of a T-cell determinant in the RF peptide sequence. Proliferative responses of T-cells from individual peptide-immunized rats were restricted to the immunizing peptides. Moreover, we observed that from RF-immunized Lou M rats, T-cells can be restimulated with the combinatorial V3 peptide library, which represents 750,000 related V3 peptides (Figure 2(a)). These results suggest that the T-cell response elicited by immunization with individual V3 peptides was thus restricted to the individual peptide sequence.

### Analysis of the Immune Response Elicited by a Combinatorial Peptide Library

We then investigated the immune repertoire elicited by the combinatorial V3 peptide library, called mixotope (MA), especially the nature of the cytokines secreted. Thus, T-cell activation was assayed by measuring both proliferation and cytokine secretions. In all rat strains immunized with MA in Freund's adjuvant, T-cells from both the spleen and the lymph node organs proliferated (Figure 2(f) and (k)) and secreted IL-2, IFN-$\gamma$ and IL-5 cytokines (Figure 3) in response to MA. No T-cell response to MA was observed in rats immunized with Freund's adjuvant alone. Since in the rat no specific reagents exist for the detection of the IL-4 protein, we used PCR in order to explore IL-4 mRNA expression.

In contrast to IFN-$\gamma$, which was also detected at the mRNA level using PCR (Figure 4), no IL-4 message could be observed in either Lou M and Fischer rat strains from PCR products. Using a specific probe, the MA IL-4 only message was

Table 2 Rat Humoral Responses[a]

| | Antibody recognition | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | BRU | | MN | | RF | | SF2 | | ELI | |
| | Lou | Fischer | Lou | Fischer | Lou | Fischer | Lou | Fischer | Lou | Fischer |
| BRU | + + | + + | − | +/− | − | − | − | +/− | − | − |
| | + + | + + | − | − | − | − | − | − | − | − |
| MN | − | − | +/− | + + | − | − | − | − | − | − |
| | − | − | + | + + | − | − | − | +/− | − | − |
| | − | − | + + | + + + | − | − | − | − | − | − |
| | − | − | + + | + + + | − | − | − | − | − | − |
| | | − | | + + + | | − | | − | | − |
| RF | + + + | − | + + | − | + + + | − | + + + | − | − | − |
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| | − | | − | | + + + | | + + | | − | |
| SF2 | − | − | +/− | − | + | + + + | + + + | + + + | − | − |
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| | − | | + | | +/− | | + + + | | − | |
| | − | | − | | +/− | | + + | | − | |
| ELI | − | − | − | − | − | − | − | − | + | + + + |
| | − | − | − | − | − | − | − | − | + + + | + + + |
| | − | | − | | − | | − | | − | |
| | − | | − | | − | | − | | + + + | |
| | − | | − | | − | | − | | + + | |

[a]Fischer, Lou M rat strains were immunized with the different peptides BRU, MN, ELI, $SF_2$ and RF. Antibodies from individual animal were analysed against the different peptides. Optical density (OD) was determined at 492 nm ( − < 0.150; 0.150 < ± < 0.500; 0.500 < + < 1.000; 1.000 < + + < 1.500; 1.500 < + + + ).

hybridized. We observed that the IL-4 message could be detected, but was not significantly increased following restimulation with MA from rats immunized with MA (Figure 4).

The cytokine secretion profile initially explored in the mouse models [48] was in accordance with the cytokine profile obtained in these rat strains. Thus, Balb/c (H-$2^d$), CBA (H-$2^k$) and C57Bl/6 (H-$2^b$) have shown that in all mouse strains immunized with MA, T lymphocytes restimulated with MA secreted IL-2, IL-3, IFN-$\gamma$ and IL-5 but not IL-4 cytokines.

The identification of functional TH-cell subsets secreting distinct patterns of cytokines have increased the understanding of the immune regulation and the immune effector function of TH cells [49,50]. TH1 cells secrete IL-2, and IFN-$\gamma$ cytokines, which mediate delayed type hypersensitivity responses, activate macrophages and play a key role in defence against intracellular pathogens, whereas TH2 cells secrete IL-4, IL-5 and IL-10 cytokines, which help B-lymphocytes and promote the synthesis of IgG1, IgE and IgA antibody isotypes. Other cytokines such as Il-3 are secreted by both TH1 and TH2 T-cells. TH0 cells have been described, which secrete IL-2, IFN-$\gamma$, IL-4 and IL-5.

Since no IL-4 was detectable under our experimental conditions, the T-cell population generated by MA immunization seems to include the TH1 cell subset and a particular subset of TH2 cells that secreted only IL-5 whatever the haplotypes with MA.

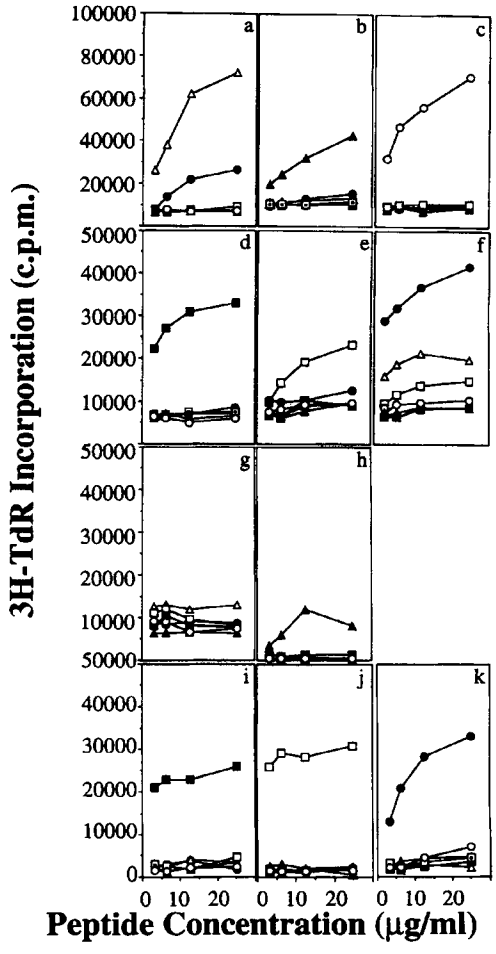


Figure 2 Rat T-cell proliferative responses. Lou M (a,b,c,d,e and f) and Fischer (g,h,i,j and k) rat strains were immunized with the different synthetic peptides RF (a,g); $SF_2$ (b,h); ELI (c); BRU (d,i); MN (e,j); MA (f,k). Lymph node T-cells from three immunized rats were restimulated using the different peptides (RF △; $SF_2$ ▲ ELI ○; BRU ■; MN □; MA ●; and medium ⊡). Proliferation was determined after five days of culture by 3H-Tdr incorporation. Experiments were carried out at least three times. Results represent mean of triplicates with SD less than 10%.

### Qualitative Changes in Cytokine Secretion may be Related to the Peptide Amino Acid Composition

Next, we investigated the cytokines secreted by MA-specific T-cells following restimulation with individual V3 peptides.

Immunization with the combinatorial V3 peptide antigen induced T-cell proliferation in response to restimulation with RF and MN peptides in the Lou M strain, whereas none of the peptides was able to restimulate T-cells from Fischer rats (Figure 2(f) and (k)).

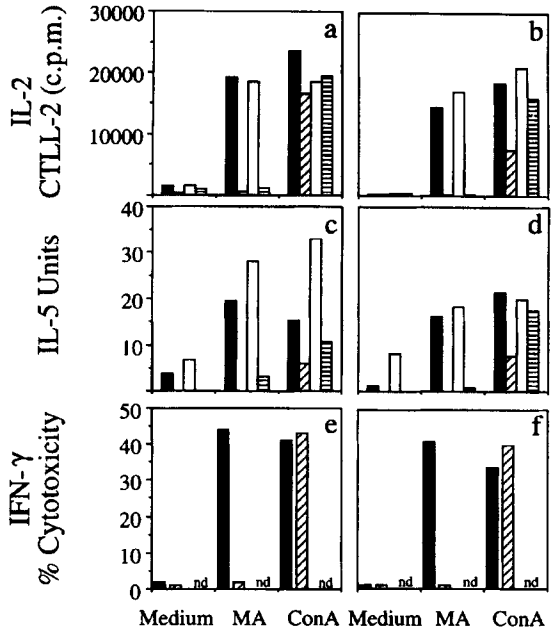


Figure 3 Cytokine secretions from rat. Fischer (a,c,e) and Lou M (b,d,f) rat strains were immunized with the combinatorial peptide library (■, □) or with Freund's adjuvant alone (▨, ▤). Cytokines IL-2 (a,b); IL-5 (c,d); and IFN-γ (e,f) were analysed from T cells derived from lymph nodes (■, ▨) or spleen (□, ▤) and restimulated either with medium alone, MA or Con A. The experiment was carried out twice.

Depending on the MHC haplotype we observed that the minimal response of T-cells restimulated with individual V3 peptides involved the secretion of IL-5 and/or IFN-γ in the absence of IL-2 secretion and in the absence of T-cell proliferation (Figure 3). No cytokine secretion was observed in T-cells from rats immunized with Freund's adjuvant alone (not shown). ELI induced none of the cytokines tested; being the most divergent sequence, ELI may be considered as a negative control V3 peptide.

As in the rat model, the extensive study of the cytokines secreted by T lymphocytes from the three different mouse haplotypes varied depending on the individual V3 peptide used for *in vitro* restimulation (Figure 6). In contrast to T-cell proliferation and Il-2 secretion, cytokine secretions revealed a more broad cross-reactivity between the different V3 individual peptides.

This suggests either that specific T-lymphocytes with different functional properties were elicited during immunization with MA or that peptide amino acid variations associated with the V3 domain significantly influenced the restimulation of T-cells, which require the recognition of peptides bound to

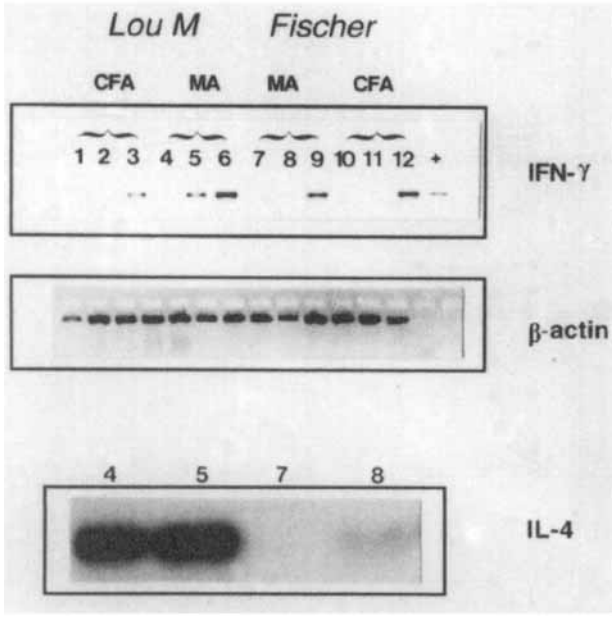


Figure 4 Cytokine RNA message from rat. IFN-$\gamma$ and IL-4 compared to $\beta$-actin were carried out by PCR approach from Lou M [1–6] and Fischer [7–12] rat spleen cells immunized with either MA [4–6, 7–9] or Freund's adjuvant alone [1–3, 10–12] and restimulated *in vitro* for 20 h with medium alone [1,4,7,10], MA [2,5,8,11] and Con A [3,6,9,12]. For IL-4 message, hybridization was carried out with IL-4 specific probe.

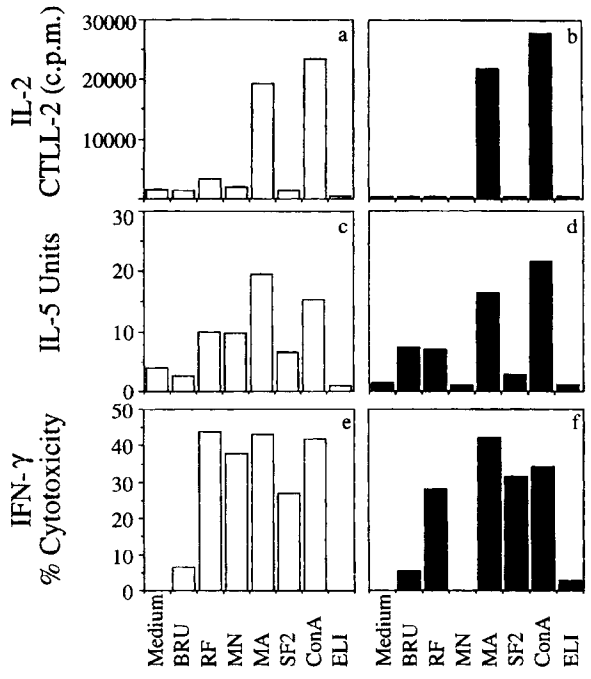


Figure 5 Cytokine secretions related to $V_3$ sequences in rat. Lou M (□) and Fischer (■) rat strains were immunized with the combinatorial peptide library and T-cells from the spleen were restimulated with the different synthetic peptides (20 $\mu$g/ml). Specific supernatants were analysed for IL-2 (a,b), IL-5 (c,d) and IFN-$\gamma$ (e,f) cytokine secretions.

MHC molecules. Differences in amino acid peptide sequences may thus generate distinct T-cell signals leading to particular cytokine secretion patterns.

## DISCUSSION

The observation that protective immune responses include B-cell and T-cell responses directed towards highly variable regions of virus surface proteins is not unique to HIV-1 infection. Other virus, such as influenza virus undergo antigenic shifts and drifts in response to immune pressure, which lead to the generation of new subtypes and possible escape mutants [42–45, 51]. A critical question regarding amino acid variations within immunogenic domains is to what extent they affect the immune response, and specially the T-cell response. Indeed, sequence changes could prevent the interaction of the processed antigenic fragment with the relevant MHC molecule; also, even if the variant peptide retains the ability to associate with MHC molecules, the sequence changes may affect interactions of the peptide–MHC complex with the TCR 52–55].

Our studies show that immunization with individual V3 peptides results in a specific B- and T-cell immune response polarized on the sequence of the immunizing peptide, suggesting that small amino acid differences in peptide sequences are sufficient to limit immune recognition. Antibody cross-reactivity between RF and SF2 peptides could be related to the very limited nature of their primary sequence divergence [56], but this difference in amino acid sequence was nevertheless insufficient to generate a cross-reactive T-cell response. The observation that amino acid variations in V3 peptide affect T-cell recognition was initially made by Callahan *et al.* [52] in T-cell clones. Our results using primary T-cells confirm this initial finding and underline the important role of the MHC molecule restriction in the context of peptide presentation.

In mice and rats immunized with MA, a combinatorial peptide library (MA) that contains 750,000 related V3 peptides, including both B- and T-cell epitopes, had very interesting features. Indeed, the T-cell responded to MA restimulation by secreting the TH1 cytokines IL-2 and IFN-$\gamma$ and the TH2 cytokine IL-5. However, when restimulation was performed

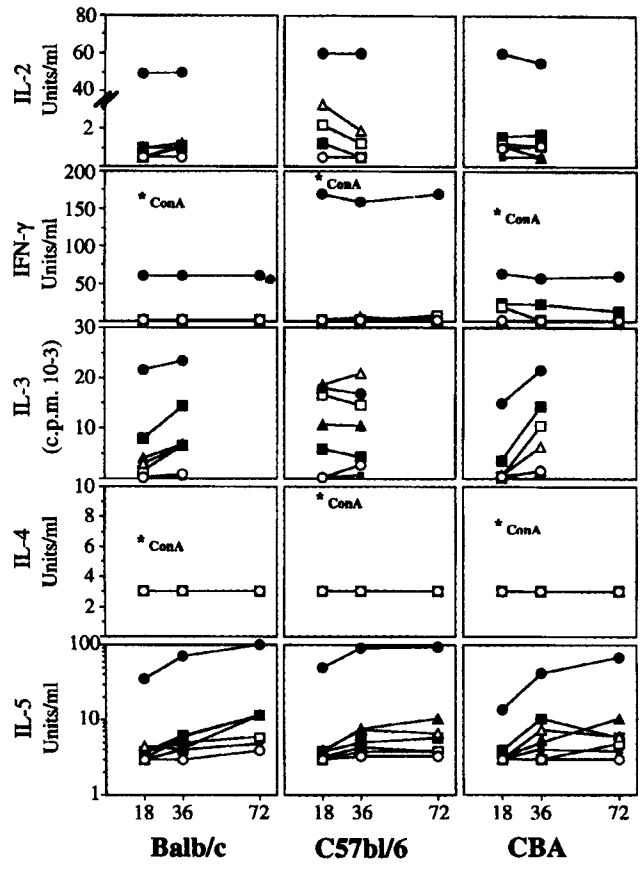


Figure 6 $V_3$ specific lymphokine secretions in mouse. Spleen cells from CBA (H-$2^k$), C57bl/6 (H-$2^b$), Balb/c (H-$2^d$) mice immunized with the combinatorial peptide library were carried out for lymphokine secretions. T-cells were restimulated with the different synthetic peptides (20 μg/ml) (RF △; $SF_2$ ▲; ELI ○; BRU ■; MN □; MA ●; and medium ⊡) and supernatants were analysed for IL-2, IL-3, IL-4, IL-5 and IFN-γ cytokines at 18, 36 and 72 h of culture. The experiment was carried out twice.

with different individual V3 peptide sequences, dissociated profiles of cytokine secretion were observed, depending both on the sequence of the individual V3 peptide and on the MHC haplotype of the immunized rodent. These findings suggest that the V3 combinatorial peptide library opens the V3 T-cell repertoire by allowing the stimulation of various T-cell clones with distinct functional properties. Antigenic variants could lead to distinct T-cell signals and therefore to distinct T-cell function through changes in the interaction between peptide–MHC complexes and TCR. Accordingly, variation in amino acid composition could induce qualitative changes in the cytokine secretion pattern from a given T-cell. These observations are consistent with findings from other laboratories that have described closely related agonist and antagonist peptides able to give distinct signals to T-cells, resulting either in T-cell proliferation, anergy or apoptosis [58–61].

The possible correlation between the emergence of new viral subtypes and the evolution of the immune repertoire in the course of a viral infection needs to be re-evaluated, specifically with regards to the functional properties of the stimulated T-cells, including their differentiation towards TH1 and/or TH2 subsets. These T helper cell subsets could play an important role in the control of a viral infection and therefore represent a major parameter in the design of vaccine strategy.

## Acknowledgement

We thank H. Gras-Masse and S. Giannini for discussing and reviewing the manuscript.

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