Clin Exp Immunol 1997; **107**:21–30

T cell receptor (TCR) BV gene repertoires and clonal expansions of CD4 cells in patients with HIV infections

M. ROGLIC, R. D. MACPHEE*, S. R. DUNCAN, F. R. SATTLER* & A. N. THEOFILOPOULOS *Department of Immunology, The Scripps Research Institute, La Jolla, and *School of Medicine, University of Southern California, Los Angeles, CA, USA*

(Accepted for publication 29 August 1996)

SUMMARY

Despite extensive investigation, the pathogenesis of T cell depletions that characterize AIDS has not been elucidated. To study this process further, we evaluated T cell antigen receptor β-chain variable gene (TCRBV) repertoires in peripheral blood lymphocytes (PBL) of 23 HIV-infected patients. Expression levels of 28 TCRBV were determined by multiprobe RNase protection assay after polymerase chain reaction (PCR) amplifications. Abnormal expansions (> 2 s.d. from mean normal values) were frequent in HIV CD4, accounting for 26% of total measured TCRBV in this population. The number and magnitude of abnormalities among individuals were inversely proportional to their CD4 counts ($P < 0{\cdot}012$ and $P < 0{\cdot}01$, respectively). While abnormalities were not randomly distributed among TCRBV subfamilies, no particular genes were expanded or contracted among all patients. Only 14% of CD8 TCRBV were proportionally expanded ($P < 0{\cdot}01$ compared with CD4), and there were limited concordances between paired CD8 and CD4 repertoires among individuals. CDR3 length analyses and TCRBV sequencing showed that most CD4 expansions comprised clonal or oligoclonal populations. Thus, T cell responses in HIV patients are characterized by severe TCRBV biases and clonal expansions among CD4 subsets, and these processes are exaggerated with disease progression. The heterogeneity and oligoclonality of the TCRBV expansions are consistent with responses to HIV-encoded or other conventional antigens rather than superantigenic effects. The presence of CD4 clonal proliferations in these patients may be important in the pathogenesis of HIV, and the absence or reduction of many T cell specificities due to oligoclonal expansions may increase susceptibility to opportunistic infections.

Keywords HIV AIDS T cell antigen receptors CD4 clonal expansions

INTRODUCTION

Elucidating the processes responsible for effects of HIV on the immune system is an important step in developing more efficacious means to ameliorate or prevent these infections. While no encompassing explanation of HIV-induced T cell alterations has yet emerged, several mechanisms have been proposed.

First, cognate recognition and subsequent responses to antigenic peptides derived from viral-genome encoded proteins could lead to expansions of selectively engaged T cells. Depending on the complexity and stability of epitopes, which may oscillate from a single immunodominant to multiple subdominant determinants [1], restricted or diverse sets of T cell clones might be engaged, respectively. Immunodominance associated with biased T cell receptor β-chain gene (TCRBV) repertoires has been identified for several conventional antigens [2,3], and more recently reported among blood and tissue lymphocytes of AIDS patients [4,5] and with *in vitro* exposure to HIV components [6].

Alternatively, or in addition, one or more viral-encoded proteins may exhibit superantigen-like properties that could, depending on the conditions, result in expansion, and subsequent anergy or deletion of T cell populations bearing appropriate TCRBV (review in [7]). Superantigen activity of HIV has previously been inferred from analyses of TCRBV expression profiles among AIDS patients [8–11], although several other studies did not observe such effects [12–16].

Finally, T cell populations could be either selectively or globally depleted by viral-induced cell death. These effects might result from either non-specific cytolysis or induction of ubiquitous, but otherwise quiescent, cellular apoptotic pathways. Lymphocytes from AIDS patients have been demonstrated to have increased apoptosis rates both spontaneously and after stimulation with mitogens and superantigens (review in [17,18]). Increased

Correspondence: A. N. Theofilopoulos MD, Department of Immunology, IMM3, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037, USA.

apoptosis has also been detected in lymph nodes of HIV^+ individuals [19]. Apoptosis in some cases has been shown to depend on binding of viral components to infected or bystander cells [20–23], although the exact mechanisms remain uncertain.

To obtain additional insights into the T cell abnormalities associated with HIV infection, we conducted a series of studies to characterize TCRBV gene expression of infected patients. These evaluations included quantitative analyses of TCRBV expression profiles and assessments of T cell clonality. We herein document severe alterations of TCRBV repertoires in AIDS patients manifested by both expansions and depletions in CD4 and CD8 subsets. Moreover, using CDR3 length assays and TCRBV sequencing, we have for the first time documented marked clonal expansions among CD4 cells of these patients.

MATERIALS AND METHODS

Patient materials

Peripheral blood samples were obtained from patients during routine clinical evaluations at the Los Angeles County Hospital AIDS Clinic. HIV infections were diagnosed by the presence of anti-HIV antibodies using Western blots. Patients were additionally stratified on the basis of clinical manifestations and CD4 counts using CDC criteria [24]. Specimens were also obtained from age-matched, healthy subjects to establish normal control values. All subjects gave informed consent under the auspices of the appropriate Institutional Review Board. Mononuclear cells were isolated by density gradient centrifugation, and T lymphocyte subpopulations were selected with anti-CD4 and anti-CD8 bound to magnetic beads (Dynal, Lake Success, NY). Flow cytometry analysis confirmed > 90% purity of the respective T cell populations. Following separation, cells were pelleted and frozen at −70°C until further use.

TCRBV template amplification

We used a modification of the semi-nested, anchored polymerase chain reaction (PCR) technique [25] to amplify TCRBV templates, thus allowing us to analyse repertoires of small cell populations. Total cellular RNA was isolated by single-step guanidinium thiocyanate isolation [26], and first-strand cDNA was synthesized from up to 1 μg of total RNA using Moloney MuLV reverse transcriptase and oligo(dT) primers (BRL, Gaithersburg, MD). dG-tails were added to cDNA by terminal deoxytransferase (Boehringer-Mannheim, Indianapolis, IN) in 0·75 mM $CoCl_2$ and 0·5 mM dGTP. A fraction of the purified product (GeneClean, La Jolla, CA) was subjected to PCR amplification using *Taq* polymerase and 0·4 μM primer concentrations. A 50 μl reaction using primers Cβ external (Cβe) (5′-CGAGGTAAGCCACAGTCTGCTCTA) and T7 poly(dC) (5′-GCCAGTAATGTATCGACACATAGGAGAT[C]$_{14}$) was performed on the dG-tailed cDNA for three cycles (94°C/20 s, 55°C/30 s, 72°C/90 s). The T7 anchor primer (5′-GCCAGTGAATTGTATCGAC) was then added for another 25 cycles (94°C/20 s, 60°C/35 s, 72°C/90 s). The PCR product was extracted with phenol/chloroform and subjected to a second PCR amplification using T7 anchor and Cβ internal (Cβi) (5′-AGAAGCCTGTGGCCAGGCCAC-CGT) primers for 15 cycles (94°C/20 s, 60°C/35 s, 72°C/90 s). The final PCR product was treated with proteinase K, phenol/chloroform-extracted and ethanol-precipitated. An aliquot of this preparation was transcribed using T7 RNA polymerase in the presence of ^{35}S-UTP to permit quantification of the synthetic RNA. All specimens were amplified and assayed in duplicate.

Multiprobe RNase protection assay

Assays were performed as previously described [27,28], using three sets of riboprobes to measure levels of 28 TCRBV genes. Briefly, radiolabelled RNA samples (1000 ct/min per μl) were hybridized with labelled probe set (2×10^6 ct/min per μl) and labelled Cβ probe (1×10^5 ct/min per μl) at 56°C overnight. Unhybridized probe and target RNA were digested with RNase A and T1. After treatment with proteinase K, phenol/chloroform extraction and ethanol precipitation, samples were dissolved in buffer and electrophoresed on 6% polyacrylamide sequencing gels. Dried gels were exposed on Kodak XRP film at −70°C for 24–72 h. A radioanalytic imaging apparatus (Ambis Systems, San Diego, CA) was used for direct β counting of gels. The net ct/min of a given band, corresponding to a specific protected TCRBV probe, was calculated by the formula ((ct/min of TCRBV-specific band)−(ct/min of background around the band))/(number of uridine residues in the specific TCRBV probe). This value was then expressed as the percentage of total analysed TCRBV transcripts.

CDR3 length analysis

Detection of clonal expansions was performed by CDR3 length analysis, as described elsewhere [29,30]. PCR reactions of 20 cycles (94°C/20 s, 60°C/35 s, 72°C/90 s) were performed using selected specimens as templates, and TCRBV-specific and ^{32}P end-labelled TCRBV-specific primers [28]. The TCRBV examined here, 1S1, 2S1, 3S1, 4S1, 10S1, 15S1 and 23S1, represent single family genes, thus simplifing analysis and interpretation. PCR products were electrophoresed on sequencing gels and analysed for evidence of clonal expansions on the basis of asymmetric or kurtotic distributions.

Cloning and sequencing

Prominent bands detected by CDR3 length analysis were cut from the polyacrylamide gels. DNA was extracted, ethanol-precipitated and reamplified by PCR using the primers and conditions detailed above. The product was cloned using the TA Cloning Kit (Invitrogen, San Diego, CA), and bacterial colonies tested for the presence of inserts by PCR. Plasmid DNA was extracted by alkaline lysis and sequenced using a BC-specific primer [29] and the *fmol* DNA Sequencing System (Promega, Madison, WI).

Statistical analysis

Two-group comparisons of continuous or ordered data were analysed by Mann–Whitney. Nominal values were compared by χ^2. Correlation coefficients (r) were established by linear regression. In some cases, quadratic regressions fit the data optimally, and are so denoted. The normal range for individual TCRBV gene expressions was defined as the mean ± 2 s.d. of values among volunteer controls. Statistical significance was defined as $P < 0{\cdot}05$.

RESULTS

Specimens

A total of 38 specimens were evaluable from 23 HIV-infected patients (mean age 41·0 ± 10·1 years). With the exception of one female, all HIV-infected subjects were homosexual males. Given the often small number of available cells, TCRBV repertoires could not always be established from both T cell subpopulations

Table 1. Clinical characteristics of HIV^+ patients

Classification	A	B	C
1	**2** (572 ± 33)	**1** (1288)	
2	**4** (350 ± 52)		**3** (307 ± 49)
3	**1** (74)		**12** (62 ± 66)

Clinical characteristics of HIV-infected subjects. Letters and italicized numbers refer to CDC classifications based on symptoms and peripheral blood CD4 counts, respectively [23]. Number of subjects per classification is in bold. Numbers in parentheses denote mean ± s.d. of aggregate CD4 counts/mm^3.

among HIV specimens. Both CD4 and CD8 repertoires were determined in eight HIV patients, CD4 were evaluable in another 12, and in three additional patients only CD8 profiles were analysed. Clinical characteristics of the subjects are outlined in Table 1. CD4 and CD8 specimens were also analysed from 12 normal volunteers (mean age 40·7 ± 13·5 years). One or more serial CD4 specimens were evaluated from six HIV-infected subjects with elapsed intervals of 112 ± 86 days (range 11–216 days) between collections. Unless otherwise noted, subsequent data analyses and displays only include results of the first specimen collection from any given individual.

TCRBV analyses

Initial experiments performed using sequential dilutions of peripheral blood lymphocytes (PBL) proved that differential amplification did not occur, and reproducible repertoires could be determined from collections of ≥ 50 000 cells. The correlation coefficients (r) between TCRBV values obtained using unamplified cellular RNA and duplicate determinations after anchor-PCR amplification were 0·91 ± 0·02. Interassay r of repeated specimen analyses were 0·92 ± 0·03.

In general, TCRBV profiles of HIV-infected patients were highly biased (Fig. 1). Of the 560 individual TCRBV gene determinations among HIV CD4 cells, 173 (31%) were abnormal, defined as a value > 2 < s.d. from determinations among normal controls (Fig. 2). Most abnormalities in the HIV population, e.g. 144 (or 26% of the total TCRBV number), represented proportional expansions of gene expression levels (> 2 s.d. of mean normal values), whereas only 29 (5%) of TCRBV values were abnormally contracted (< 2 s.d. of normal values). TCRBV expression profiles of CD8 cells were also skewed among the HIV subjects, albeit less frequently than among the CD4 (Fig. 3). Sixty-four (21%) individual CD8 TCRBV values were abnormal in these patients (P < 0·002, compared with CD4). This intergroup difference was primarily due to a lesser frequency of relative expansions among CD8. Only 42 (14%) of measured CD8 TCRBV were abnormally elevated (P < 0·001, compared with CD4), whereas the frequency of contractions (7%) in this group was similiar to that among CD4. As expected, the average number of abnormal proportional expansions per patient was greater in CD4 than CD8 profiles (7·2 ± 1·9 *versus* 3·7 ± 1·0, respectively; P < 0·001). In contrast, the mean number of abnormal TCRBV contractions per patient were similiar among CD4 (1·5 ± 1·1) and CD8 (1·9 ± 1·3) populations.

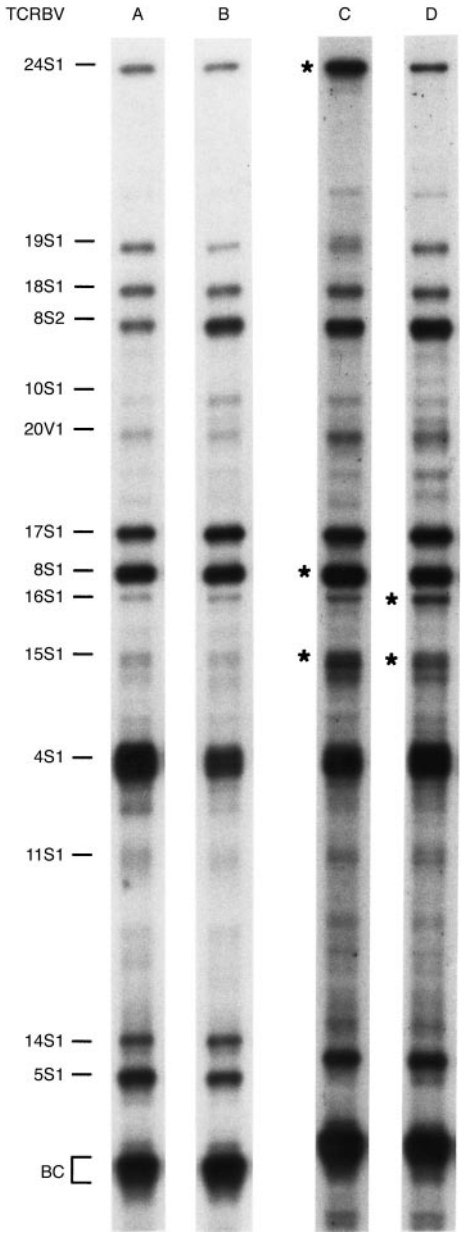

Fig. 1. Composite autoradiographs of peripheral blood CD4 lymphocytes using probe set A. Lanes A and B are normal volunteers, whereas lanes C and D show repertoires of HIV-infected subjects. *Abnormally elevated T cell antigen receptor β-chain variable gene (TCRBV) expression levels.

There were no significant intergroup differences with respect to the magnitudes of these abnormalities, since the number of s.d. these TCRBV levels ranged from mean normal values were similiar (5·5 ± 4·8 *versus* 5·6 ± 4·1, for CD4 and CD8, respectively). Similiarly, analysis limited to the number of expansions *per se* showed that magnitudes of these abnormalities were nearly identical for CD4 (5·0 ± 4·5) and CD8 (4·6 ± 3·6 s.d. from mean normal values).

There were often considerable disparities between individual patients' CD4 and CD8 profiles. Aggregate correlation coefficents between concomitant CD4 and CD8 profiles were 0·68 ± 0·31 (range 0·07–0·92). In particular, there was little concordance of abnormal TCRBV between paired CD4 and CD8 profiles (Fig. 4).

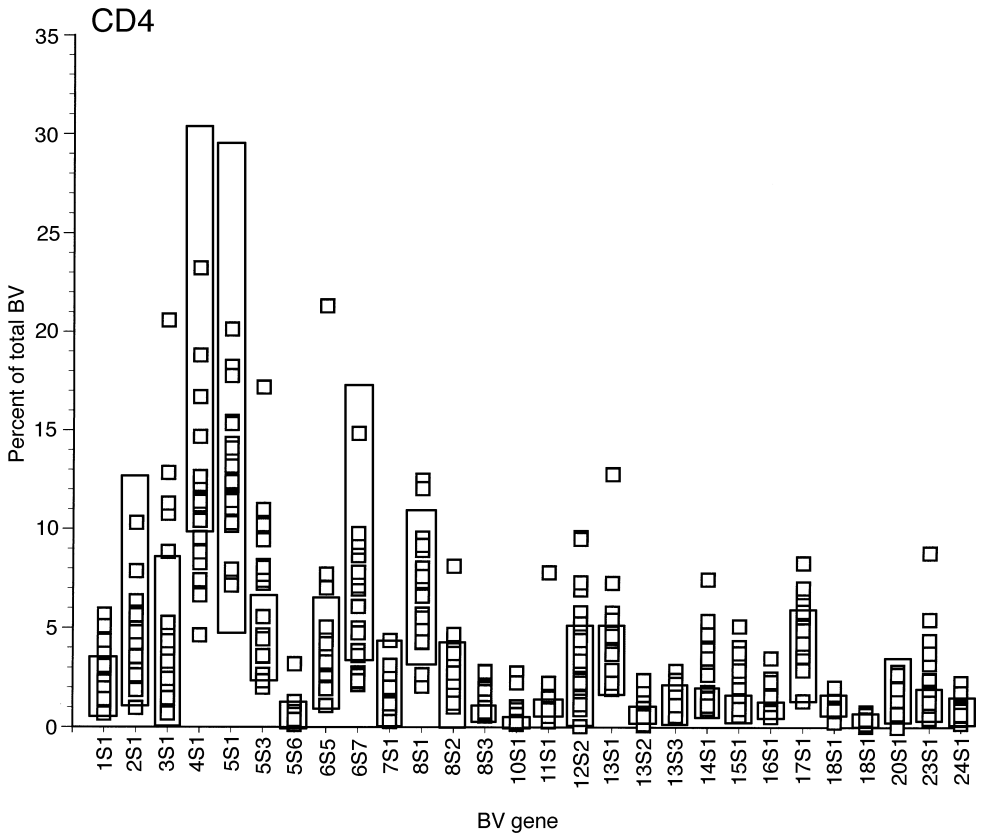

Fig. 2. Compilation of T cell antigen receptor β-chain variable gene (TCRBV) expression levels of CD4 peripheral blood lymphocytes from HIV-infected subjects. Expression levels, as a percentage of total measured TCRBV/patient, are denoted on the ordinate. Boxes represent normal ranges ($\pm$ 2 s.d. from mean normal values). Less than 5% of TCRBV gene expression levels among normal determinations fall outside these ranges.

Only 30% of TCRBV expansions among CD4 were simultaneously elevated in corresponding CD8 populations. Similarly, 23% of CD4 contractions were concomitantly decreased among respective CD8.

Abnormalities among TCRBV genes were not randomly distributed ($P < 0{\cdot}01$ by χ^2). Although no given TCRBV gene was abnormally affected in all HIV patients, most patients had CD4 expansions in one or more of five particular genes, and collectively these TCRBV accounted for 61 of 144 (42%) abnormal expansions in this population (i.e. TCRBV 5S3, 8S3, 10S1, 15S1 and 23S1). One or more of three particular TCRBV genes (TCRBV 5S6*2, 13S1 and 15S1) were found to be elevated in most CD8 repertoires and, in the aggregate, accounted for 26% of total expansions in this group. The complete absence of a measurable TCRBV gene product was only noted in one example (CD4 TCRBV 20S1) in a single patient.

Significant relationships were obvious between the number and extent of TCRBV abnormalities among individual patients and their underlying clinical status (Table 2). The number of abnormal TCRBV measurements per subject was inversely proportional to CD4 peripheral blood counts ($r = 0{\cdot}50$, $P < 0{\cdot}01$). The magnitude of TCRBV abnormalities (in s.d. from mean normal values) was also inversely correlated with CD4 counts. In general, correlations between numbers or magnitude of TCRBV abnormalities with respective clinical features were strongest in the analyses of CD4 repertoires (Table 2). Significantly, the number of abnormal TCRBV gene contractions in the CD4 subpopulations was also much greater in subjects with symptomatic HIV (Fig. 5).

Serial analyses

CD4 TCRBV profiles of individual subjects remained relatively stable over the intervals studied. Overall, r for duplicate specimens were $0{\cdot}82 \pm 0{\cdot}23$, with the strongest correlations seen among serial studies performed < 100 days apart ($r = 0{\cdot}95 \pm 0{\cdot}03$). Moreover, the number of TCRBV abnormalites among individuals tended to increase as a function of time ($r = 0{\cdot}75$, $P < 0{\cdot}054$). The relationship was particularly evident for greater numbers of TCRBV gene contractions *versus* interval duration ($r = 0{\cdot}94$, $P < 0{\cdot}002$), whereas there were no clear increases of gene expansions during the relatively short periods tested here ($r = 0{\cdot}11$, P = NS).

Assessment of clonality

Selected single-member TCRBV genes were evaluated for clonal characteristics by CDR3 length analysis of CD4 specimens from 20 patients for a total of 90 TCRBV determinations. A typical CDR3 display of polyclonal TCRBV from PBL is expected to contain a cluster of six to eight bands per lane spaced by three nucleotides, with the greatest signal intensity in the centre of the cluster of bands, diminishing to the ends in a Gaussian distribution [29,30]. There was a strong concordance for findings of non-Gaussian distribution on CDR3 gels (indicating the presence of clonal or oligoclonal expansions) and TCRBV expansions above

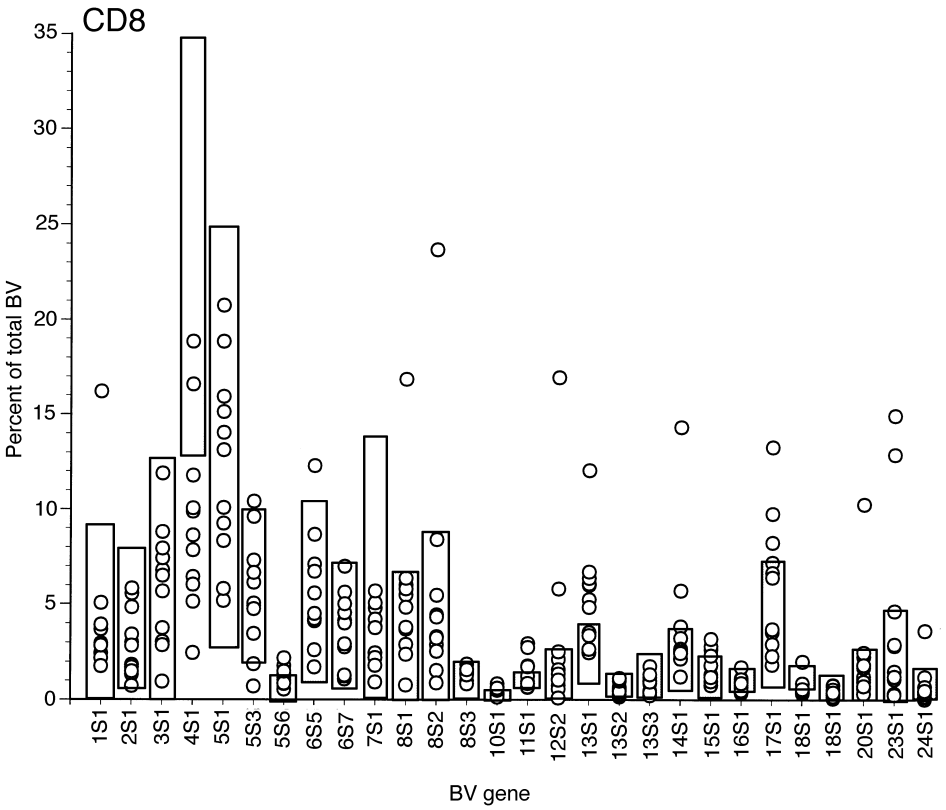

Fig. 3. T cell antigen receptor β-chain variable gene (TCRBV) expression levels of CD8 peripheral blood lymphocytes. See Fig. 2 legend for explanation.

normal ranges in HIV-infected individuals (Fig. 6). Twenty-six of 30 (87%) individual expanded TCRBV genes exhibited asymmetry and/or kurtosis on CDR3 analyses, whereas these clonal indicators were only noted in 25 of 54 (46%) specimens with normal levels and 0 of six contracted TCRBV ($P < 0{\cdot}001$). Although the reliability of CDR3 length analyses for detection of clonal proliferations has been well established [29,30], its validity was confirmed here by sequencing cDNA libraries of selected TCRBV1S1 specimens (Table 3).

DISCUSSION

By use of sensitive and quantitative multiprobe RNase protection assay and study of a relatively large number of subjects, we demonstrated that the TCRBV repertoires of HIV-infected patients were highly biased. These abnormalities, primarily consisting of relative TCRBV expansions, were most prominent in CD4 subsets, while gene expression levels of CD8 cells were also biased to a lesser degree. Both the number and magnitude of TCRBV abnormalities among individual patients correlated with the severity of their underlying disease, and became more marked as peripheral blood CD4 counts diminished. Importantly, and a novel finding of these studies, the striking relative TCRBV expansions in the CD4 subset were largely composed of clonal or oligoclonal populations, i.e. progeny of one or a very few T cells that have been intensely stimulated to undergo proliferation. These findings offer new insights into mechanisms of immune system perturbations and pathogenesis of HIV infections.

Much of the impetus for study of HIV effects on TCR repertoires derived from an initial report describing consistent reductions in T cells bearing certain TCRBV among PBL of AIDS patients [8]. As described for diverse microbial products, exogenous superantigens mediate engagements of entire TCRBV families outside ordinary binding domains, resulting in activation and expansion of the cells that bear corresponding TCRBV (review in [7]). Following this expansion, superantigen-engaged T cells subsequently decline as a result of apoptotic death and tissue sequestration [32,32]. Accordingly, the initial findings that selected TCRBV were largely deleted from the repertoire of AIDS patients were interpreted as evidence of HIV-encoded superantigens, an attractive hypothesis that seemed to explain the eventual depletion of CD4 cells with advanced disease.

Nonetheless, a large number of subsequent investigations addressing broad TCRBV repertoire changes in HIV-infected patients reported considerable discrepancies [12–16]. In some cases, TCRBV profiles seemed unperturbed, whereas others described biases that variously represented expansions or contractions. Although some of these subsequent studies also suggested that HIV may exert superantigenic effects [9–11], the findings were inconclusive and no study to date has convincingly shown the presence of such a moiety in the HIV virion [33]. Moreover, a recent demonstration that *in vitro* replication of HIV was preferentially enhanced in TCRBV12S1-expressing cells [34,35], initially hypothesized to represent a manifestation of a superantigenic effect, was subsequently shown to result from a monocyte-derived cytomegalovirus (CMV)-related superantigen [36]. The present data corroborate and extend the preponderance of evidence showing that HIV does not encode a superantigen. Findings in this and

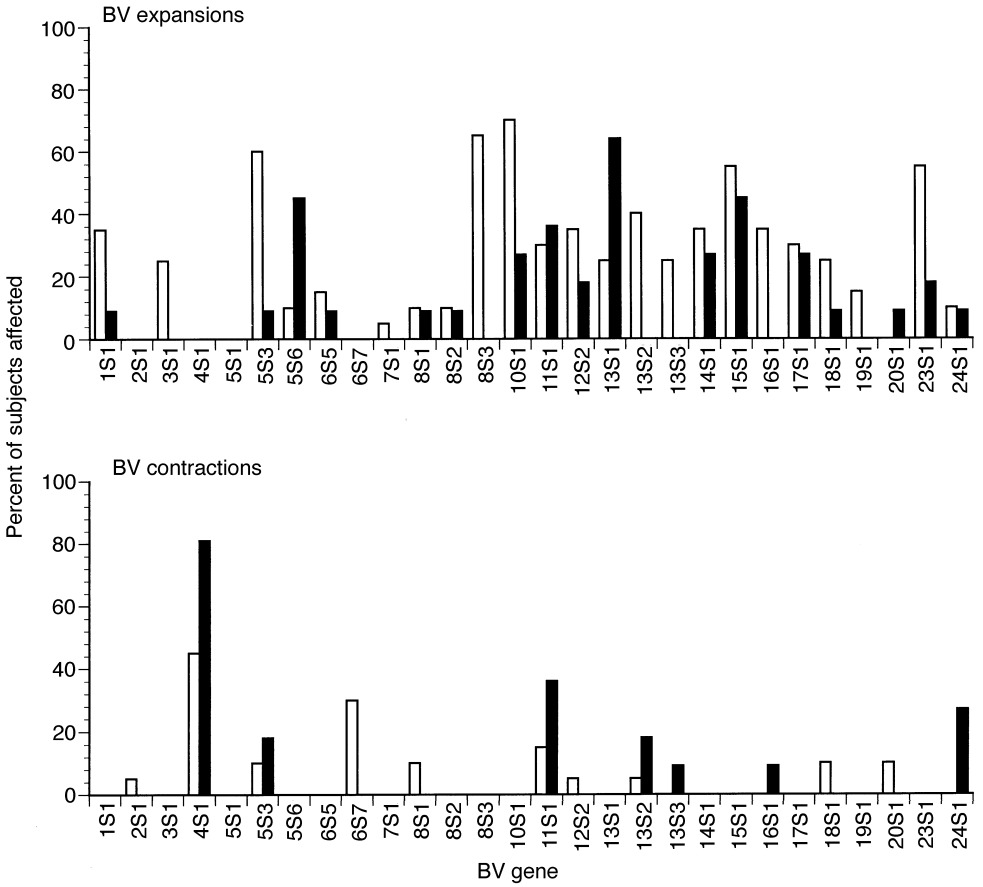

Fig. 4. Abnormal T cell antigen receptor β-chain variable gene (TCRBV) expression levels are denoted as percentages of patients affected. Top frame shows abnormal expansions, whereas lower frame depicts abnormal gene contractions. □, CD4; ■, CD8 repertoires.

other studies [11,37] show that preferential TCRBV expansions, while present, are quantitatively and qualitatively diverse. In addition, TCRBV contractions were relatively infrequent and even more diverse, unlike the consistent profile of reductions expected by a HIV superantigen. While biases in CD4 TCRBV repertoires of HIV-infected patients have been previously observed, the nature of these perturbations was not elucidated, but attributed to superantigen effects [38]. The present data show that TCRBV expansions in the CD4 subpopulation are largely clonal or oligoclonal, and thus probably represent specific proliferative responses to conventional, albeit undefined, antigen(s).

Table 2. Correlations of T cell antigen receptor β-chain variable gene (TCRBV) abnormalities with $CD4^+$ counts in HIV-infected subjects

r as a function of $CD4^+$ count (mm^3) *versus*	CD4	CD8
No. of abnormal BV	0·63 (P<0·003)	0·45*
No. of abnormal BV expansions	0·53 (P<0·012)	0·17*
No. of abnormal BV contractions	0·49 (P<0·29)	0·60 (P<0·05)
Maximal magnitude by BV abnormalities	0·65 (P<0·01)	0·27*
Mean magnitude of BV abnormalities	0·73 (P<0·002)	0·24*

Correlation coefficients (r) of BV abnormalities among HIV-infected subjects with their respective peripheral blood $CD4^+$ counts. BV abnormalities were compiled and analysed among both $CD4^+$ and $CD8^+$ repertoires. Magnitudes of BV abnormalities were analysed as s.d. from mean normal values and denoted as quadratic (second-power) regressions, which were the optimal fit to these data.

* Non-significant (P>0·05) correlations.

Although the specificity of these clonally expanded cells has not been established, inferences can be drawn from results of previous investigations using either *in vitro* expanded HIV-specific T cell clones or patient-derived polyclonal lymphoid populations. Kalams *et al.* [6] reported that single-donor cytotoxic T lymphocyte (CTL) clones recognizing a well defined epitope (pp 584–592) in the glycoprotein 41 membrane protein (gp41) exhibited restricted, and temporally stable, usage of TCR (AV14 and BV4 genes). Moreover, a limited heterogeneity among TCRBV of SIV *gag*-specific CTL has been reported [39] and expansions of specific TCRBVs (TCRBV7 and TCRBV14) were observed in acutely infected monkeys [40]. More significantly, Pantaleo *et al.* [4] characterized sequential PBL-derived T cell samples of six individuals with acute HIV and found major oligoclonal expansions in a restricted set of CD8 TCRBV families, with some degree of *in vitro* activity against transfected lymphoblastoid cell lines expressing HIV *env*. Of note, clonotypic selection and convergence became increasingly evident with disease progression, while the particular junctional region sequences of the preferentially

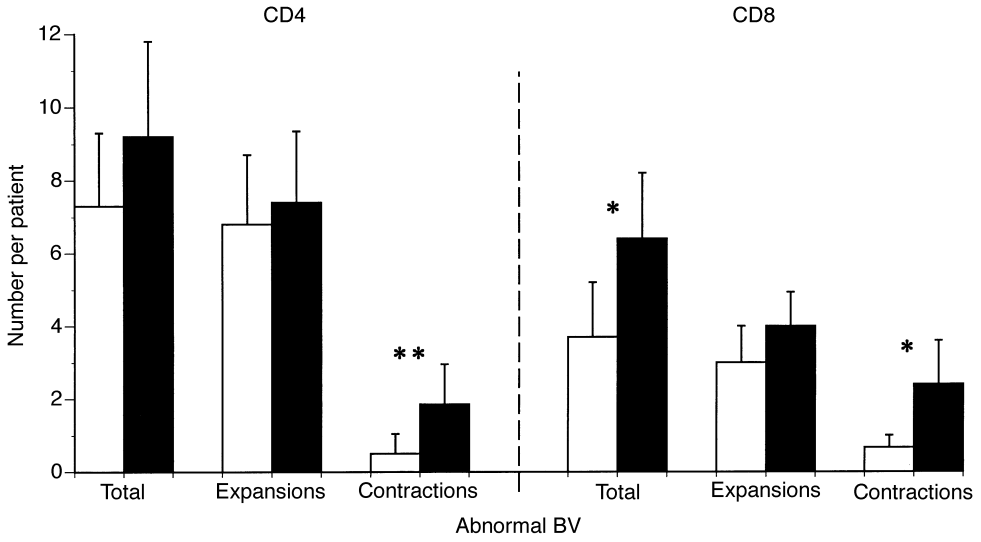

Fig. 5. Numbers and types of abnormal T cell antigen receptor β-chain variable gene (TCRBV) gene levels, as a function of clinical classification. Data are compiled separately for CD4 and CD8 subpopulations. □, Asymptomatic patients (CDC classification A); ■, those with symptomatic manifestations of their HIV infections (CDC classifications B and C). Data are denoted as means ± s.d. $*P < 0{\cdot}05$; $**P < 0{\cdot}01$ for comparisons between respective classifications.

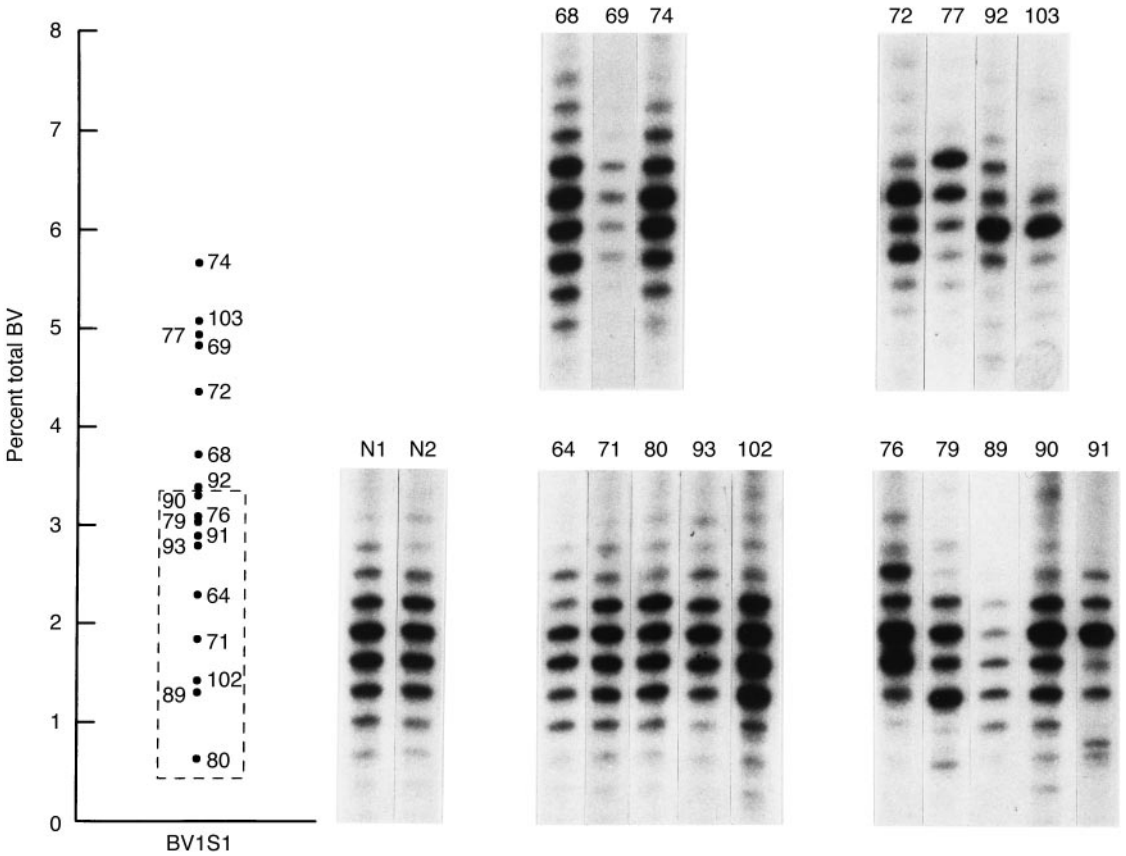

Fig. 6. Expression levels of T cell antigen receptor β-chain variable gene (TCRBV) 1S1 (as percentages of total TCRBV/respective subject) and CDR3 length analyses among CD4 specimens. Left panel displays expression levels of individual specimens denoted by numbers, with dotted-line box denoting normal range (see also Fig. 2). Corresponding CDR3 autoradiographs are depicted. The upper row depicts specimens with elevated 1S1 levels: specimens 68, 69 and 74 have normal Gaussian CDR3 distributions, whereas 72, 77, 92 and 103 exhibit asymmetry or kurtosis. The lower row of autoradiographs corresponds to specimens with normal gene expression levels. CDR3 lengths are normally distributed among uninfected, control specimens N1 and N2, as well as HIV-infected subjects 64, 71, 80, 93 and 102, whereas HIV-infected patients 76, 79, 89, 90 and 91 show abnormal distributions (asymmetry and/or kurtosis).

Table 3. CDR3 sequences of expanded T cell antigen receptor β-chain variable gene (TCRBV)1S1 clones in HIV$^+$ patients

Patient	No. identical/ no. sequenced	BV1S1	(N) D (N)	BJ
69	4/4	AGC AGC GTA G S S V	CT CCG GGG GAA A P G E 1	AAT CAG CCC N Q P 1S5
79	4/4	AGC AGC G S S	CC CAG TGG GG A Q W G 1	C ACT GAA T E 1S1
92 (lower band)	2/3	AGC AGC GTA G S S V	GC GGG GGC AT A G G I 1	C ACT GAA T E 1S1
92 (upper band)	2/4	AGC AGC GTA G S S V	GT TCC CCA GGG GGT TTC T G S P G G F 1	CT GAA GCT F G A 1S1
116	4/4	AGC AGC GTA G S S V	AG GGG AGA TCT E G R S 2	ACA GAT ACG T D T 2S3

Patient designations, numbers of sequences determined for each, and the numbers of identical (clonal) sequences found are denoted. BV-D-J nucleotide and amino acid sequences are shown. BD segments are underlined.

expanded clones varied among patients. Analysis of T cell repertoires in individual white pulps of spleens from HIV patients also documented a high compartmentalization of a limited number of VDJ specificities, probably associated with CD8 cells [40]. Although some of these CD8 clonal expansions in AIDS patients might be HIV-specific, interpretations are complicated by the fact that CD8 clonal expansions have also been documented in normal individuals [42–44]. These dominant CD8 clones in normal PBL repertoires have, in turn, been postulated to represent responses against ubiquitous and persistent pathogens.

Because monoclonal or oligoclonal expansions are only rarely, if at all, detected among CD4 PBL of normal individuals [42–44], we concentrated our efforts on identifying clonal expansions in this subset of HIV patients. We obtained direct evidence for clonal expansions in the CD4 populations of HIV-infected individuals by both non-Gaussian distribution of amplified TCRBV in the CDR3-length essay and by identification of a single or a very limited set of VDJ sequences. That the TCRBV types and corresponding junctional sequences differ among HIV-infected individuals is probably a reflection of heterogeneity among the MHC–peptide complexes being recognized. While the fine specificity of the expanded CD4 clones has not yet been defined, it seems reasonable to assume that they represent expansions driven by long-term exposure to a limited set of specific HIV-associated antigens. It is conceivable, however, that some CD4 clonal expansions in these patients represent proliferations to antigens associated with other microbial superinfections or opportunistic organisms. Nonetheless, these expansions were present in nearly all patients, including those in early stages of the disease with no evidence of other infections.

Assuming that these CD4 clones are HIV-specific raises additional questions regarding their *in vivo* function, e.g. whether they subserve helper *versus* cytotoxic activities. Vaccination with live HIV and/or recombinant proteins has been shown to elicit HIV-specific CTL responses in seronegative humans mediated by CD4 cells [45–47]. Convincing evidence also exists that identical HIV peptides can be presented by both class I and class II molecules [48]. Finally, CD4 CTL have also been observed in human infections with several other viruses, including influenza [49], herpes simplex [50], measles [51], hepatitis B [52] and Epstein–Barr virus (EBV) [53].

The unique finding that the TCR profiles of the CD4 cells in HIV-infected patients are markedly perturbed due to clonal expansions may have important implications with respect to underlying disease pathogenesis. Expanded, activated CD4 clones may be important in protective humoral and cellular immune responses against HIV (or other) infections. Alternatively, if they are of the cytotoxic type, they could also exert deleterious consequences by down-regulating specific antibody responses through lysis of B cells presenting the relevent antigen in association with class II MHC. Cytolytic CD4 may also kill non-infected CD4 cells that have taken up and processed free gp120 [45,46]. Furthermore, virus persistence may lead to the eventual exhaustion of these anti-viral CD4 cells, thereby creating an adversarial tolerance state [54]. Future studies on the activities of these cells against HIV gene-transfected cells and on their cytokine profiles may provide further insights into their functions. Finally, our demonstration that T cell populations of HIV patients largely consist of a small number of expanded clones suggests that many of the specificities of a normal TCR repertoire are present in much lower precursor frequencies. The resultant 'holes' in the repertoire may account to some degree for the increased risk of these patients to develop opportunistic infections and other complications.

ACKNOWLEDGMENTS

The authors wish to thank R. Balderas for technical assistance, and Drs D. H. Kono, R. Baccala, J. Parker and C. Taylor for advice and assistance, and M. K. Occhipinti for editorial help. This is publication number 9849-IMM from The Scripps Research Institute. The work reported herein was supported by NIH grants 1K08HL03016 and AI34215.

REFERENCES

1 Nowak MA, May RM, Phillips RE *et al.* Antigenic oscillations and shifting immundominance in HIV-1 infections. Nature 1995; **375**: 606–11.

2 McHeyzer-Williams MG, Davis MM. Antigen-specific development of primary and memory T-cells *in vivo*. Science 1995; **268**:106–11.

3 Heber-Katz E, Acha-Orbea H. The V-region disease hypothesis: evidence from autoimmune encephalomyelitis. Immunol Today 1989; **10**:164–9.

4 Pantaleo G, Demarest JF, Soudeyns H *et al.* Major expansion of $CD8^+$ T cells with a predominant Vb usage during the primary immune response to HIV. Nature 1994; **370**:463–7.

5 Dwyer M, Itescu S, Winchester R. Characterization of the primary structure of T cell receptor b chains in cells infiltrating the salivary gland in the Sicca syndrome of HIV-1 infection. J Clin Invest 1993; **92**:495–502.

6 Kalams SA, Johnson RP, Trocha AK *et al.* Longitudinal analysis of T cell receptor (TCR) gene usage by human immunodeficiency virus 1 envelope-specific cytotoxic T lymphocyte clones reveals a limited TCR repertoire. J Exp Med 1994; **179**:1261–71.

7 Kotzin BL, Leung DV, Kappler J *et al.* Superantigens and their potential role in human disease. Adv Immunol 1993; **54**:99–166.

8 Imberti L, Sottini A, Bettinardi A *et al.* Selective depletion in HIV infection of T cells that bear specific T cell receptor Vb sequences. Science 1991; **254**:860–4.

9 Soudeyns H, Routy J-P, Sekaly R-P. Comparative analysis of the T cell receptor Vb repertoire in various lymphoid tissues from HIV-infected patients: evidence for an HIV-associated superantigen. Leukemia 1991; **8**(Suppl. 1):S95–S97.

10 Soudeyns H, Rebai N, Pantaleo GP *et al.* The T cell receptor Vβ repertoire in HIV-1 infection and disease. Semin Immunol 1993; **5**:175–85.

11 Rebai N, Pantaleo G, Demares JF *et al.* Analysis of the T-cell receptor β-chain variable-region (Vβ) repertoire in monozygotic twins discordant for human immunodeficiency virus: evidence for perturbations of specific Vβ segments in $CD4^+$ T cells of the virus-positive twins. Proc Natl Acad Sci USA 1994; **91**:1529–33.

12 Boyer V, Smith LR, Ferre F *et al.* T cell receptor Vβ repertoire in HIV-infected individuals: lack of evidence for selective Vβ deletion. Clin Exp Immunol 1993; **92**:437–41.

13 Bahadoran P, Rieux-Laucat F, Le Deist F *et al.* Lack of selective Vβ deletion in peripheral $CD4^+$ T cells of human immunodeficiency virus-infected infants. Eur J Immunol 1993; **23**:2041–4.

14 Posnett DN, Kabak S, Hodtsev AS *et al.* T-cell antigen receptor Vβ subsets are not preferentially deleted in AIDS. AIDS 1993; **7**:625–31.

15 Hodara VL, Jeddi-Tehrani M, Grunewald J *et al.* HIV infection leads to differential expression of T-cell receptor Vβ genes in $CD4^+$ and $CD8^+$ T cells. AIDS 1993; **7**:633–8.

16 Bouldt-Houle DM, Rinaldo CR Jr, Ehrlich GD. Random depletion of T cells that bear specific T cell receptor Vβ sequences in AIDS patients. J Leukoc Biol 1993; **54**:486–91.

17 Ameisen JC, Estaquier J, Idziorek T. From AIDS to parasite infection: pathogen-mediated subversion of programmed cell death as a mechanism for immune dysregulation. Immunol Rev 1994; **142**:10–51.

18 Gougeon M-L, Montagnier L. Apoptosis in AIDS. Science 1993; **260**:1269–70.

19 Muro-Cacho CA, Pantaleo G, Fauci AS. Analysis of apoptosis in lymph nodes of HIV-infected persons. J Immunol 1995; **154**:5555–66.

20 Westendorp MO, Frank R, Ochsenbauer C *et al.* Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature 1995; **375**:497–500.

21 Li CJ, Friedman DJ, Wang C *et al.* Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein. Science 1995; **268**:429–31.

22 Banda NK, Bernier J, Kurahara DK *et al.* Crosslinking CD4 by human immunodeficiency virus gp120 primes T cells for activation-induced apoptosis. J Exp Med 1992; **176**:1099–106.

23 Nardelli B, Gonzalez CJ, Schechter M *et al.* $CD4^+$ blood lymphocytes are rapidly killed *in vitro* by contact with autologous human immunodeficiency virus-infected cells. Proc Natl Acad Sci USA 1995; **92**:7312–6.

24 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults. CDC MMWR 1993; **41**:1–13.

25 Davis MM, Loh EV, Elliot JF *et al.* Polymerase chain reaction with single-sided specificity: analysis of T receptor delta chain. Science 1989; **243**:217–20.

26 Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol chloroform extraction. Anal Biochem 1987; **162**:156–9.

27 Baccala R, Kono DH, Walker SM *et al.* Genomically imposed and somatically modified human thymocyte V-beta gene repertoires. Proc Natl Acad Sci USA 1991; **88**:2908–12.

28 Duncan SR, Valentine V, Roglic M *et al.* T-cell receptor biases and clonal proliferations are increased among lung transplant recipients with obliterative bronchiolitis. J Clin Invest 1996; **97**:2642–50.

29 Pannetier C, Cochet M, Darche S *et al.* The sizes of the CDR3 hypervariable regions of the murine T-cell receptor chains vary as a function of the recombined germ-line segments. Proc Natl Acad Sci USA 1993; **90**:4319–23.

30 Cochet M, Pannetier C, Regnault A *et al.* Molecular detection and *in vivo* analysis of the specific T cell response to a protein antigen. Eur J Immunol 1992; **22**:2639–47.

31 Webb S, Morris C, Sprent J. Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. Cell 1990; **63**: 1249–56.

32 Kawabe Y, Ochi S. Programmed cell death and extrathymic reduction of V$\beta 8^+$ $CD4^+$ T cells in mice tolerant to *Staphylococcus aureus* enterotoxin B. Nature 1991; **349**:245–8.

33 Westby M, Manca F, Dalgleish AG. The role of host immune responses in determining the outcome of HIV infection. Immunol Today 1996; **17**:120–6.

34 Laurence J, Hodtsev AS, Posnett DN. Superantigen implicated in dependence of HIV-1 replication in T cells on TCR Vβ expression. Nature 1992; **358**:255–9.

35 Dobrescu D, Kabak S, Mehta K *et al.* Human immunodeficiency virus 1 reservoir in $CD4^+$ T cells is restricted to certain Vβ subsets. Proc Natl Acad Sci USA 1995; **92**:5563–7.

36 Dobrescu D, Ursea B, Pope M *et al.* Enhanced HIV-1 replication in Vβ12 T cells due to human cytomegalovirus in monocytes: evidence for a putative herpesvirus superantigen. Cell 1995; **82**:753–63.

37 Dalgleish AG, Wilson S, Gompels M *et al.* T-cell receptor variable gene products and early HIV-1 infection. Lancet 1992; **339**:824–8.

38 Rebai N, Pantaleo G, Demarest JF *et al.* Analysis of the T-cell receptor β-chain variable-region (Vβ) repertoire in monozygotic twins discordant for human immunodeficiency virus: evidence for perturbations of specific Vβ segments in $CD4^+$ T cells of the virus-positive twins. Proc Natl Acad Sci USA 1994; **91**:1529–33.

39 Chen ZW, Yamamoto H, Watkins DI *et al.* Predominant use of a T-cell receptor V-beta gene family in simian immunodeficiency virus gag-specific cytotoxic T lymphocytes in a rhesus monkey. J Virol 1992; **66**:3913–6.

40 Chen ZW, Kou Z-C, Shen L *et al.* An acutely lethal simian immunodeficiency virus stimulates expansion of Vβ7- and Vβ14-expressing T lymphcytes. Proc Natl Acad Sci USA 1994; **91**:7501–5.

41 Cheynier R, Henrichwark S, Hadida F *et al.* HIV and T cell expansion in splenic white pulps is accompanied by infiltration of HIV-specific cytotoxic T lymphocytes. Cell 1994; **78**:373–88.

42 Hingorani R, Choi IH, Akolkar P *et al.* Clonal predominance of T cell receptors within the $CD8^+$ $CD45RO^+$ subset in normal human subjects. J Immunol 1993; **151**:5762–9.

43 Posnett DN, Sinha R, Kabak S *et al.* Clonal population of T cells in normal elderly humans: the T cell equivalent to 'benign monoclonal gammapathy'. J Exp Med 1994; **179**:609–18.

44 Fitzgerald JE, Ricalton NS, Meyer A-C *et al.* Analysis of clonal $CD8^+$ T cell expansions in normal individuals and patients with rheumatoid arthritis. J Immunol 1995; **154**:3538–47.
45 Hammond SA, Bollinger RC, Stanhope PE *et al.* Comparative clonal analysis of human immunodeficiency virus type 1 (HIV-1)-specific $CD4^+$ and $CD8^+$ cytolytic T lymphocytes isolated from seropositive humans immunized with candidate HIV-1 virus. J Exp Med 1992; **176**:1531–42.
46 Orentas RJ, Hildrth JEK, Obah E *et al.* An HIV envelope protein vaccine induces $CD4^+$ human cytolytic T cells active against HIV-infected cells. Science 1990; **248**:1234–7.
47 Stanhope PE, Liu AY, Pavlat W *et al.* An HIV-1 envelope protein vaccine elicits a functionally complex human $CD4^+$ T cell response that includes cytolytic T lymphocytes. J Immunol 1993; **150**:4672–86.
48 Takeshita T, Takahashi H, Kozlowski S *et al.* Molecular analysis of the same HIV peptide functionally binding to both a class I and a class II molecule. J Immunol 1995; **154**:1973–86.
49 Kaplan DR, Griffin R, Braciale VL *et al.* Influenza virus-specific human cytotoxic T cell clones: heterogeneity in antigen specificity and restriction by class II MHC products. Cell Immunol 1984; **88**:193–8.
50 Yasukawa M, Zarling JM. Human cytotoxic T cell clones directed against herpes simplex virus-infected cells. I. Lysis restricted by HLA class II MB and DR antigens. J Immunol 1984; **133**:422–7.
51 Jacobson S, Sekaly RP, Jacobson CL *et al.* HLA class II-restricted presentation of cytoplasmic measles virus antigens to cytotoxic T cells. J Virol 1989; **63**:1756–62.
52 Celis E, Ou D, Otvos L. Recognition of hepatitis B surface antigen by human T lymphocytes. Proliferation and cytotoxic responses to a major antigenic determinant defined by synthetic peptides. J Immunol 1988; **140**:1808–15.
53 Moss DJ, Misko IS, Burrows SR *et al.* Cytotoxic T-cell clones discriminate between A- and B-type Epstein–Barr virus transformants. Nature 1988; **331**:719–21.
54 Moskophidis D, Lechner F, Pircher H *et al.* Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. Nature 1993; **362**:758–61.