Characterization of HLA-B57-restricted human immunodeficiency virus type 1 Gag- and RT-specific cytotoxic T lymphocyte responses

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HLA-B57 has been shown to be strongly associated with slow disease progression in human immunodeficiency virus type 1 (HIV-1)-infected patients from the Amsterdam Cohort. Since HIV-1-specific CTL can control and eliminate virus-infected cells, we sought to characterize the dominant HLA-B57-restricted CTL responses at the epitope level. It was found that HLA-B57-restricted CTL responses were targeted at multiple proteins of HIV-1, with CTL specific for Gag and RT being the most pronounced. Gag-specific CTL recognized peptides ISPRTLNAW (aa 147–155) and STLQEQIGW (aa 241–249), which had previously been reported as HLA-B57-restricted. The RT-specific CTL response in one long-term survivor studied in great detail persisted for 10 years and was dominated by HLA-B57-restricted CTL that recognized the newly defined epitope IVLPEKDSW (RTLAI, aa 244–252). This epitope could be recognized in the context of both HLA-B*5701 and HLA-B*5801. Interestingly, three epitope variants of IVLPEKDSW were observed, which coincided with the strongest detectable CTL response to RT. One variant (T2E7) was not recognized by IVLPEKDSW-specific CTL despite the fact that this variant bound to HLA-B*5701 with a similar affinity as the index peptide. Finally, only viruses which contained the epitope index sequence were obtained suggesting efficient virus control by CTL. In conclusion, we report the characterization of dominant HIV-1 Gag- and RT-derived, HLA-B57-restricted CTL epitopes which are associated with longer time to AIDS. Further characterization of CTL responses restricted by HLA-B57 and other protective HLA alleles may contribute to the development of effective AIDS vaccines.

Introduction

The clinical course and outcome of human immunodeficiency virus type 1 (HIV-1) infection are highly variable. The time to AIDS appears to follow a quasi-Gaussian distribution as a consequence of multiphasic and multifactorial virus–host interactions (Klein & Miedema, 1995; Haynes et al., 1996). In a small proportion of HIV-1-infected individuals, who seem to represent the extreme of the right-hand tail of the distribution, an extraordinarily benign disease course beyond the median time to AIDS is observed. This group of so-called long-term survivors appears heterogeneous and consists largely of long-term asymptomatic patients; however, in most cases minor signs of disease progression can be observed. These individuals may harbour peculiar combinations of virus characteristics, host genetic determinants, antiviral immune responses and environmental co-factors that could favour slow disease progression. Among the group of long-term survivors, there may also be some individuals who are protected from progression to AIDS for life. It seems likely that such a rare phenomenon can only result from unique features not observed in other patients progressing to AIDS (Klein & Miedema, 1995; Haynes et al., 1996).

It is widely held that HIV-1-specific CTL are among the favourable determinants for delaying disease progression, since in vitro experiments have shown that HIV-1-specific CTL
kill infected target cells via MHC class I-restricted recognition (Plata et al., 1987; Walker et al., 1987) and can suppress virus replication via secretion of various antiviral cytokines (Walker et al., 1986; Tsubota et al., 1989; Cocchi et al., 1995; Buseyne et al., 1996). This view is further underscored by in vivo associations of emerging HIV-1-specific CTL responses with the profound reduction of viremia during the acute phase of infection (Borrow et al., 1994, 1997; Koup et al., 1994; Price et al., 1997) and with the relatively sustained control of viremia during the asymptomatic phase (Klein et al., 1995). Interestingly, several HLA alleles are consistently associated with either rapid disease progression (Kaslow et al., 1990; Itescu et al., 1992; Sahmoud et al., 1993; Klein et al., 1994) or longer time to AIDS (Klein & Miedema, 1995; Goulder et al., 1996; Haynes et al., 1996; Kaslow et al., 1996). This suggests that there may be qualitative differences in MHC class I-restricted HIV-1-specific CTL responses that could variably impact on the rate of virus replication and hence on time to AIDS. Characterization of HIV-1 CTL responses restricted by these so-called risk and protective HLA alleles should be one of the first steps taken to reveal the molecular basis of the correlates of immune protection (Harrer et al., 1996; Van der Burg et al., 1997).

Since the association between HLA-B57 and long-term survival appeared to be the strongest correlate of slow disease progression among patients from the Amsterdam Cohort and independently confirmed observations in other cohort studies (Haynes et al., 1996; Kaslow et al., 1996), we sought to characterize the dominant HLA-B57-restricted CTL responses to HIV-1 Gag and RT proteins at the epitope level.

Methods

Study population

Nested case-control study on HLA and long-term survival of HIV-1 infection. Within the Amsterdam Cohort Studies on HIV-1 infection and AIDS, a nested case-control study was designed to examine the relationship between host genetics and duration of HIV-1 infection. Long-term survivors (cases) were HIV-1-seropositive homosexual men ascertained during the Cohort Study in November 1984 and were found seropositive for HIV-1 at the next visit in February 1985. He subsequently attended the Municipal Health Service in Amsterdam at three-monthly intervals (at least 50 visits) until October 1997 and so far he has remained asymptomatic. Standard laboratory measurements were routinely performed and were found to be in the normal range: CD4+ T cell counts (mean ± SD), 860 ± 190 cells/µl; CD8+ T cell counts, 520 ± 170 cells/µl; and CD4+/CD8+ T cell ratio, 1.7 ± 0.37 (Klein et al., 1995). HIV-1 RNA was always undetectable in serum samples (< 10³ RNA copies/ml). HIV-1 could only be isolated from 3 out of 16 different blood samples tested with a frequency of < 1 tissue culture infectious dose per 10⁶ CD4+ T cells.

Patient H433 was a Caucasian homosexual man with HLA type: A2, B7, B57, Cw6, DR1, DQ1, HLA-DR3, DQ1, DQ2. He was found to be HIV-1-seropositive when he entered the Cohort Study in January 1985. After 7 years, he developed a generalized cytomegalovirus infection of which he died. Since six months before AIDS diagnosis his CD4+ T cell counts were not severely decreased (310 cells/µl), anti-retroviral therapy was considered at that time. This patient is one of three HLA-B57-positive participants from the Cohort who have developed AIDS so far.

Both long-term survivor H090 and progresor H433 did not carry the 32 bp deletion in the C-C chemokine receptor-5 (CCR5) gene (De Roda Husman et al., 1997), and both individuals were infected with non-syncytium-inducing HIV-1 variants only as determined in the MT2 assay (Koot et al., 1993).

In vitro restimulation and expansion of HIV-1-specific CTL. HIV-1-specific CTL in PBMC samples of HIV-1-seropositive individuals were expanded in vitro using an antigen-specific stimulation protocol as previously described (Van Baalen et al., 1993; Klein et al., 1995). This method results in the generation and expansion of CTL which are biased towards recognition of HIV-1a1-derived sequences, sequences highly similar to clade B or highly conserved epitopes among all clades of HIV-1.

T cells were stimulated with autologous Epstein–Barr virus-transformed B-lymphoblastoid cell lines (B-LCL) infected at an m.o.i. of 5 with recombinant vaccinia viruses (rVV) expressing single genes of HIV-1a1 (Myers et al., 1995). Cells were harvested after 20–22 h infection at 37°C and 5% CO₂ and fixed with 1% (w/v) parafomaldehyde for 15 min at room temperature. Subsequently, fixed cells were incubated with 0.2% glycine in PBS for 15 min and washed once with complete medium (RPMI 1640; Life Technologies) containing antibiotics and heat-inactivated, pooled human serum (10%) from 8–10 healthy HIV-1-seronegative blood donors. Fixed stimulator cells were stored at 4°C and kept for a maximum period of 4 weeks.

HIV-1-specific CTL responses were quantified using standard methods for limiting dilution analysis (Lefkovits & Waldmann, 1984). Briefly, 24 replicate wells of eight serial dilutions of PBMC ranging from 10⁷ with complete medium supplemented with rIL-2 (10 U/ml) and 10 U/ml of recombinant IL-2 (rIL-2; Proleukin; kindly provided by R. Rombouts, Chiron Benelux BV, Amsterdam, The Netherlands). The cultures were restimulated at day 7 with complete medium supplemented with rIL-2 (10 U/ml) and 10³ irradiated (50 Gy) B-LCL (mixture of
equal amounts of allogeneic B-LCL, APD, BSM and JY) in complete medium supplemented with rIL-2 (10 U/ml) and leucogagglutinin (Leuco A, 1 μg/ml; Sigma). When sufficient amounts of cells were obtained, effector cells were tested for cytotoxicity at various effector:target ratios (see below).

**Recombinant vaccinia viruses (rVV).** Recombinant vaccinia viruses (rVV) have been constructed from the Copenhagen strain of vaccinia virus. The following rVV expressing single genes of HIV-1 (Myers et al., 1995) were used for this study: TG.4163 (RT); TG.1144 (Gag) (Rautmann et al., 1989); TG.3183 (Env) (McChesney et al., 1990); TG.1147 (Nef) (Guy et al., 1987); TG.3196 (Tat); TG.4113 (Rev); TG.1160 (Vif); and control rVV 186poly, containing no specific insert. M. P. Kiény (Transgène SA, Strasbourg, France) and Y. Rivière (Institut Pasteur, Paris, France) kindly provided all the rVV.

**Peptides.** Peptides were synthesized by solid-phase strategies on an automated peptide synthesizer (Abimed AMS 422) using Fmoc chemistry. Peptides were analysed by reverse-phase HPLC; they were dissolved in DMSO at 10–50 mg/ml, aliquotted and stored at −70 °C until use.

**51Cr-release assays.** Standard 51Cr-release assays were performed as previously described (Van Baalen et al., 1993; Klein et al., 1995; Van der Burg et al., 1995a). Briefly, autologous or (partially) HLA-matched B-LCL were infected at an m.o.i. of 5 with rVV expressing single genes of HIV-1, control rVV 186poly and labelled with 100 μCi Na51CrO4 (Amersham) for 16 h at 37 °C and 5% CO2. After three wash steps, 4,000 target cells were added to each well. Alternatively, 51Cr-labelled and uninfected B-LCL were pre-incubated for 30 min at room temperature with synthetic peptides at a final concentration of 5–10 μg/ml or in 10-fold serial dilutions in titration experiments as indicated. After 4 h, supernatants were harvested and radioactivity was measured with a γ-counter (Cobra-II; Canberra Packard Benelux). 51Cr release was expressed as specific lysis (%) using the following formula: ([experimental release − spontaneous release]/[maximum release − spontaneous release]) × 100. Spontaneous 51Cr release was always <15% of the maximum release. Wells were considered positive when 51Cr release exceeded 10% specific lysis. This arbitrary threshold was always greater than the mean +3 × SD. CTL precursor (CTLp) frequencies were calculated using methods previously described (Strijbosch et al., 1987). CTLp frequencies for specific antigens were computed as differences between CTLp frequencies determined on specific versus control targets.

**Results**

**Association of HLA-B57 with long-term AIDS-free survival**

To address which HLA alleles contribute to delayed disease progression in HIV-1 infection, HLA phenotype frequencies were analysed in a nested case-control study among homosexual men from the Amsterdam Cohort. Long-term survivors (cases) were HIV-1-seropositive participants who remained AIDS-free for over 9 years (median 10–8 years, range 9.2–11.1) with normal CD4+ T cell counts (mean 538 cells/μl, range 408–953 in the ninth year of follow-up). Control subjects were HIV-1-seropositive participants who developed clinical AIDS within 8 years after seroconversion or seroprevalent entry in the study (median 3–7 years, range 0–8–7.9). None of the HLA alleles which are frequently present in north European Caucasian populations differed significantly between the total HIV-1-infected population studied here and the local healthy control group (data not shown) (Klein et al., 1994). The Bw4 group of HLA-B alleles was observed more frequently among long-term survivors compared to subjects who developed AIDS. This association was mainly due to a significantly increased frequency of HLA-B57 (P = 0.006) and, to a lesser extent, a slightly increased frequency of HLA-B27 (P = 0.06) (Table 1). In addition, HLA-CW6 was also over-represented in the group of long-term survivors as a result of linkage disequilibrium with HLA-B57. Furthermore, similar associations were observed in a prospective study of 148 cohort participants with a known date of HIV-1 seroconversion. Kaplan–Meier survival analysis showed in this case that individuals with HLA-B57 had a relative risk of 0.163 (P = 0.07) for developing AIDS compared to individuals without HLA-B57 (data not shown). Since the association between HLA-B57 and long-term survival appeared to be the strongest correlate in the Amsterdam Cohort and independently confirmed observations made in other cohort studies (Kaslow et al., 1996; Goulder et al., 1996), we sought to further characterize the HLA-B57-restricted HIV-1-specific CTL responses.

**Table 1. Associations between HLA and long-term survival of HIV-1 infection**

<table>
<thead>
<tr>
<th>HLA</th>
<th>Long-term survivors (n = 23)</th>
<th>AIDS cases (n = 86)</th>
<th>RR*</th>
<th>P</th>
<th>Local controls (n = 804)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cw6</td>
<td>50.0</td>
<td>12.5</td>
<td>6.5</td>
<td>0.007</td>
<td>20.0</td>
</tr>
<tr>
<td>Bw4</td>
<td>78.3</td>
<td>43.8</td>
<td>4.3</td>
<td>0.005</td>
<td>52.0</td>
</tr>
<tr>
<td>B57</td>
<td>26.1</td>
<td>2.3</td>
<td>12.6</td>
<td>0.0006</td>
<td>5.2</td>
</tr>
<tr>
<td>B27</td>
<td>13.0</td>
<td>3.5</td>
<td>4.1</td>
<td>NS†</td>
<td>8.4</td>
</tr>
<tr>
<td>B51</td>
<td>21.7</td>
<td>11.8</td>
<td>2.1</td>
<td>NS</td>
<td>10.3</td>
</tr>
<tr>
<td>B18</td>
<td>8.7</td>
<td>9.3</td>
<td>1.1</td>
<td>NS†</td>
<td>7.2</td>
</tr>
</tbody>
</table>

* Relative risks (RR) were odds ratios according to Haldane (1955). RR and corresponding probability (P) indicate the likelihood of long-term survival relative to progression to AIDS in the presence of particular HLA alleles. The Chi-square (χ2) test was used to evaluate whether the calculated RR differed significantly from unity.

ns, Not significant; indicates that the RR did not differ from unity taken at the 5% level except where marked (†, P = 0.06).
HIV-1-seropositive when he entered the Cohort Study in January 1985 and died of AIDS 7 years later.

**HLA-B57-restricted CTL responses against multiple HIV-1 proteins**

HIV-1-specific CTL responses against Gag, RT, Env, Tat, Rev, Vif and Nef were quantified using limiting dilution analysis of a blood sample from long-term survivor H090 obtained in February 1990. In addition, the contribution of HLA-B57-restricted CTL responses was determined in parallel with HLA-B*5701-matched B-LCL as targets in split-well $^{51}$Cr-release assay (Fig. 1a). In agreement with our previous observations, dominant CTL responses were targeted at Gag, RT and Tat (Klein *et al.*, 1995; Van der Burg *et al.*, 1997; Van Baalen *et al.*, 1997). Minor responses were detected against...
Env, Nef, Rev and Vif. The contribution of HLA-B*5701-restricted CTL was 50% in the case of Gag-specific CTL and 85% in the case of RT-specific CTL. HLA-B*5701-restricted CTL responses against the other proteins tested represented only minor components of the total HIV-1-specific CTL response (Fig. 1a).

Similar experiments were carried out for patient H433 who developed AIDS in about 7 years. For this purpose, a blood sample from April 1988 was analysed which was obtained about 3 years after he entered the Cohort Study. At that time, he was still asymptomatic and both CD4$^+$ T cell numbers and the CD4$^+$/CD8$^+$ T cell ratio were only slightly decreased (450 cells/$\mu$l and 0.31, respectively). Dominant CTL responses of progressor H433 comprised CTL against Gag (2165 per $10^6$ PBMC) followed by the responses to Env and Nef (both 1180 per $10^6$ PBMC) and a minor response to RT (157 per $10^6$ PBMC) (Fig. 1b). Using HLA-matched targets, it was determined that 84% of the CTL response against Gag was HLA-B*5701-restricted. CTL responses against Env and Nef consisted of about 20% of HLA-B*5701-restricted CTL. The frequency of HLA-B*5701-restricted RT-specific CTL was 104 per $10^6$ PBMC and no CTL responses were detected against Tat, Rev and Vif (Fig. 1b). Since HLA-B*5701-restricted HIV-1 Gag- and RT-specific CTL constituted dominant responses in the
long-term survivor with the most stable disease course, we decided to characterize these further at the epitope level.

**Identification of HLA-B57-restricted Gag-specific CTL epitopes**

The Gag-specific CTL epitopes were identified with a series of peptides containing the HLA-B57 peptide-binding motif (Falk et al., 1995; Barber et al., 1997). Despite the fact that a phenylalanine has been reported as the C-terminal anchor residue (Goulder et al., 1996) and that this residue has been found in some endogenous peptide sequences eluted from HLA-B17 alleles (Falk et al., 1995; Barber et al., 1997), we decided to focus on a motif with tryptophan at position 9. The rationale behind this was based on previous observations in our laboratory using functional binding assays (Van der Burg et al., 1997). We subsequently tested against the panel of Gag-derived HLA-B*5701 than tryptophan (not shown).

Gag-specific CTL bulk cultures were expanded from a blood sample of patient H090 from August 1988, and were subsequently tested against the panel of Gag-derived HLA-B57 motif-bearing peptides. We found that peptide GagLAI STLQEQIGW (aa 241-249) was recognized by CTL from this patient (Fig. 2a). Positive wells from the limiting dilution experiments of patient H433 that showed consistent cytolytic activity for GagLAI were also non-specifically expanded in vitro. Bulk cultures were subsequently tested on the panel of 9-mer peptides and strongly recognized peptide STLQEQIGW (aa 241-249) and, to lesser extent, peptide ISPRTLNAW (aa 147-155) (Fig. 2b). Both peptides are highly conserved among the published HIV-1 sequences (Myers et al., 1995) and have also previously been observed as being part of dominant HLA-B57-restricted CTL responses to HIV-1 (Johnson et al., 1991; Goulder et al., 1996).

**Characterization of a novel HLA-B57-restricted epitope in HIV-1 RT**

From a blood sample of subject H090 obtained in October 1986, five oligoclonal cytotoxic T cell lines were obtained which showed consistent specificity for HIV-1LAI RT. These CTL were CD8+ and granzyme B-positive (data not shown). Using B-LCL that were partially matched for the HLA type of patient H090, it was shown that HLA-B*5701 was the restriction element for all of these RT-specific CTL (Fig. 3a). The CTL epitope specificity was determined using a set of 536 consecutive 10-mer peptides each with a nine residue overlap and spanning the entire sequence of HIV-1 RTLAI (Van der Burg et al., 1997) (Fig. 3b). It was shown that peptides 243-245 were recognized by all RT-specific CTL from patient H090, with RT peptide p243 (aa 243-252; IVLPEKDSW) being the optimal. Note, RT-specific CTL from another HLA-B57-positive long-term survivor previously studied in our laboratory also recognized the 10-mer peptides p243 and p244 (Van der Burg et al., 1997).

Both the 10-mer sequence (p243) and the peptide sequence lacking the N-terminal proline (p244/9) were titrated in order to assess the optimal epitope length (Fig. 3c). The 9-mer sequence IVLPEKDSW was recognized at much lower concentrations than the 10-mer peptide p243 (median value half-maximal killing 4.7 nM vs 251 nM, respectively). Therefore, it was concluded that HIV-1 RTLAI peptide p244/9 (aa 244-252; IVLPEKDSW) constituted the optimal epitope sequence.

Positive wells from the limiting dilution experiments of patient H433 which showed consistent cytolytic activity for HIV-1 RTLAI were non-specifically expanded in vitro. Bulk cultures were subsequently tested on the panel of RT-derived HLA-B57 motif-bearing 9-mer peptides. Bulk cultures of RT-specific CTL showed consistent reactivity toward peptides IVLPEKDSW and RTLAI (aa 375-383; ITTESIVW) (Fig. 4). Of note, two 10-mer peptides containing the latter sequence were also recognized by another HLA-B57 patient previously studied in our laboratory (Van der Burg et al., 1997).

According to reported similarities in peptide-binding motifs for HLA-B17 alleles (Falk et al., 1995; Barber et al., 1997), it is to be expected that a number of HLA-B57-restricted CTL epitopes will also be recognized in the context of HLA-B58 alleles (Goulder et al., 1996). Similarly to the situation demonstrated for peptide HIV-1 Gag TSTLQEQIGW (Goulder et al., 1996; Bertoletti et al., 1998), we found here that HLA-B*5701-restricted RT-specific CTL from patient H090 could also recognize peptide RTLAI IVLPEKDSW in the context of HLA-B*5801 (Fig. 3d).

**Longitudinal analysis of RT-specific CTL and HIV-1 variants**

The kinetics of the RT-specific CTL responses of long-term survivors H090 were studied at the epitope level (Fig. 5). The RT-specific CTL response reached peak levels 44 months after
Serial dilutions of variant peptides were tested for their ability to inhibit binding of the fluorescein-labelled index peptide p244/9 to HLA-B*5701 (Van der Burg et al., 1995b). To determine whether these peptides could still be recognized by IVLPEKDSW-specific CTL, they were also tested in standard ³¹Cr-release assays.

Due to the low numbers of infected CD4⁺ T cells in patient H090, biological variants of HIV-1 could only be obtained from three out of 16 different blood samples tested. Eight clones were obtained from PBMC donated in February 1989, a single clone was from October 1990, and eight clones were from August 1994. With respect to the epitope sequence, the isolates from February 1989 consisted of three variants: four clones contained the index sequence of the epitope, three clones harboured amino acid substitutions at positions 2 and 7 (V2 → T2/D7 → E7), and one clone was observed with a single mutation at position 2 (V2 → M2) (Fig. 5). Interestingly, we did not observe any sequence variation in the stretch of 30 nucleotides flanking either side of the epitope sequence (not shown).

The clone from October 1990, and the eight remaining isolates from August 1994 all contained the index sequence IVLPEKDSW (Fig. 5). The M2 variant of p244/9 bound to HLA-B*5701 with somewhat lower affinity than the index sequence, but was very well recognized by IVLPEKDSW-specific CTL of patient H090 (not shown). The T2E7 variant was not recognized by IVLPEKDSW-specific CTL of patient H090 (half-max. killing > 10 µM), despite the fact that this variant bound to HLA-B*5701 with similar affinity (IC₅₀ = 6 µM) as the index peptide.

In total, seven virus isolates were obtained from patient H433, four from April 1988 and three from October 1989. Sequencing revealed that all biological isolates contained a glutamic acid (E) at position 2 (V2 → E2). The latter variant was clearly recognized by IVLPEKDSW-specific CTL of patient H090, albeit at a lower level than the index peptide p244/9 (not shown). In addition, previous binding experiments also indicated that the E2 variant bound significantly less strongly to HLA-B*5701 compared to the index peptide (not shown).

Discussion

To start unravelling the correlates of immune protection to AIDS, we have analysed the distribution of HLA class I alleles in a nested case-control study of long-term survivors and progressors from the Amsterdam Cohort. HLA-B57 was strongly associated with longer time to AIDS and independently confirmed observations made in other study populations (Klein et al., 1995; Goulder et al., 1996; Haynes et al., 1995; Kaslow et al., 1996). Since MHC class I-restricted CTL responses to HIV-1 can interfere with virus replication via secretion of antiviral cytokines (Walker et al., 1986; Tsubota et al., 1989; Cocchi et al., 1995; Buseyne et al., 1996) or via cell-mediated cytotoxicity (Plata et al., 1987; Walker et al., 1987),
we aimed at the identification of HLA-B57-restricted CTL responses to HIV-1.

HLA-B57-restricted CTL responses were vigorous and involved broad recognition of epitopes from multiple proteins of HIV-1. Dominant responses appeared to be directed against HIV-1 Gag and RT, but minor responses to Env, Nef, Tat, Rev, and Vif were also observed. The major CTL responses directed at Gag and RT were subsequently characterized at the epitope level. The Gag-derived epitopes that were recognized by CTL from the patients we studied involved two highly conserved sequences, namely ISPRTLNAW (aa 147–155) and STLQEQIQGW (aa 241–249). Previously, these epitopes have been described by others as being part of dominant HLA-B57-restricted CTL responses to HIV-1 (Johnson et al., 1991; Goulder et al., 1996).

The major component of the RT-specific CTL response in long-term survivor H090 was targeted at a newly defined HLA-B57-restricted epitope IVLPEKDSW (aa 244–252) and persisted for more than 10 years. These observations add to the findings of Kalams et al. (1994) who studied clonotypes of TcR-Vβ CDR3 sequences of a dominant CTL response to an HLA-B14-restricted epitope in gp41 that persisted for more than 5 years. However, it remains unclear whether our findings here were due to persistent clones or persistent immunodominance.

The epitope data obtained here using functional assays clearly show that peptides with a valine at position 2, maybe in conjunction with other auxiliary residues such as a proline at position 4 (Falk et al., 1995; Barber et al., 1997), can clearly bind to HLA-B*5701. So far, this has not been observed in the sequences of endogenous peptides eluted from HLA-B17 alleles (Falk et al., 1995; Barber et al., 1997). From previous in vitro binding assays in our laboratory, we also concluded that a phenylalanine as C-terminal anchor residue was less efficient than tryptophan (not shown) (Van der Burg et al., 1995; Borrow et al., 1997). For example, in progressor H433, we did not observe CTL responses to Tat, Rev and Vif and only a weak response against RT was seen. It could well be that CTL observe CTL responses to Tat, Rev and Vif and only a weak response against RT was seen. It could well be that CTL recognize four HLA-B57-restricted epitopes. Moreover, these epitopes are shown here, as well as in other studies, to be part of dominant CTL responses of long-term survivors (Goulder et al., 1996; Van der Burg et al., 1997). The obvious difference in disease course may be explained by taking into account the longevity of these responses as well as the entire clonal composition of dominant and sub-dominant HIV-1-specific CTL.

For future AIDS vaccines, it may be worthwhile to include the type of immune responses that mimic the dominant and persistent responses observed in long-term survivors. With respect to the HLA restriction elements of HIV-1-specific CTL epitopes, these would ideally have to be alleles common in ethnic populations with a high prevalence of HIV-1 infection. Coincidentally, HLA-B17 alleles are quite common in particular
sub-Saharan populations living in areas with a high prevalence of HIV-1 infection (Imanishi et al., 1992). As with the findings for peptide Gag TSTLSEQIGW (Goulder et al., 1996; Bertolotti et al., 1998), we observed here that the HLA-B*5701-restricted epitope RTLAI IVLPEKDSW could also be recognized by CTL in the context of HLA-B*5801. This finding again underscores the striking similarity of the peptide-binding motifs of the structurally related HLA-B17 alleles (Falk et al., 1995; Barber et al., 1997). Unravelling of the molecular basis of the correlates of immune protection, in particular the role of HIV-1-specific CTL responses restricted by HLA-B57 as well as other ‘protective’ HLA class I alleles, will hopefully contribute to the further development of effective AIDS vaccines.

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References


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