CD8\(^+\) cytotoxic T lymphocytes of a cynomolgus macaque infected with simian immunodeficiency virus (SIV) mac32H-J5 recognize a nine amino acid epitope in SIV Gag p26

Anna-Maria Geretti,† Ellen G. J. Hulskotte,† Marlinda E. M. Dings,† Carel A. van Baalen,† Geert van Amerongen\(^2\) and Albert D. M. E. Osterhaus†

1 Institute of Virology, Erasmus University, 3000 DR Rotterdam, The Netherlands
2 Central Animal Laboratory, National Institute of Public Health and Environmental Protection, 3720 BA, Bilthoven, The Netherlands

A detailed analysis of simian immunodeficiency virus (SIV)-specific cytotoxic T lymphocyte (CTL) responses and the identification of the proteins and epitopes they target may improve the design of immunotherapeutic interventions and provide insights into AIDS pathogenesis. Here, we identified a new CTL epitope in the SIV Gag protein, recognized by CD8\(^+\) and MHC class I-restricted CTL clones from a long-term asymptomatic cynomolgus macaque (Macaca fascicularis) infected with SIVmac32H-J5. Using overlapping synthetic peptides, the optimal minimal epitope was characterized as a nine amino acid peptide representing amino acids 242–250 of p26 (SVDEQIQQWM). CTL recognition was shown to be abolished by amino acid substitutions observed within homologous human immunodeficiency virus (HIV)-1 and HIV-2 sequences.

Infection of macaques with several strains or clones of simian immunodeficiency virus (SIV) shows remarkable similarities with human immunodeficiency virus (HIV) infection of humans and provides a valuable model for investigating the role of cytotoxic T lymphocyte (CTL) immunity in the host defence against lentiviruses (Desrosiers, 1990; Letvin et al., 1994). A number of studies have shown that detailed analysis of SIV-specific CTL responses and the identification of the proteins and epitopes they target may help the design of immunotherapeutic interventions and provide insights into AIDS pathogenesis (Chen et al., 1992; Yasutomi et al., 1995; Hulskotte et al., 1995). Extending our previous studies of HIV-1-infected humans (Van Baalen et al., 1996), we present here a

Author for correspondence: Albert D. M. E. Osterhaus.
Fax +31 10 4365145. e-mail osterhaus@viro.fgg.eur.nl
† Department of Virology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, UK.
Table 1. CD8⁺ cytotoxic T lymphocytes expanded by stimulation with peptide p26A.5

<table>
<thead>
<tr>
<th>Effector cells</th>
<th>E:T</th>
<th>p26A.5</th>
<th>SIV Gag rVV</th>
<th>vv</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>50:1</td>
<td>72</td>
<td>63</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>25:1</td>
<td>65</td>
<td>55</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>12:1</td>
<td>55</td>
<td>34</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>CD8⁺</td>
<td>50:1</td>
<td>76</td>
<td>59</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>25:1</td>
<td>61</td>
<td>53</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>12:1</td>
<td>55</td>
<td>35</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>CD8⁻</td>
<td>50:1</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>25:1</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>12:1</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

SIVmac251. As shown in Fig. 1(a) for the CTL line designated K71/E26, of the 49 peptides tested, only peptide p26.11, covering amino acids 236–255 (IAGTSSVDEIQWMYR) of p26, sensitized targets for lysis by the CTL lines. The two contiguous peptides p26.10 and p26.12 were not recognized, indicating that the CTL epitope was contained in the central region of peptide p26.11. To define the minimal epitope within peptide p26.11, the CTL lines were tested for recognition of synthetic peptides spanning amino acids 236–255 of p26 and varying in length from 20 to 9 residues (European Veterinary Laboratory, Woerden, The Netherlands). As shown in Fig. 1(b) for CTL line K71/E26, the 9-mer peptide p26A.5, representing amino acids 242–250 (SVDEIQWM) of p26, optimally sensitized targets for lysis by the CTL lines. Two truncated peptides lacking either the N-terminal S residue (p26A.4: VDEIQWMY) or the C-terminal M residue (p26A.6: SSVDEIQW) were considerably less efficient. Further truncation at either terminus abolished recognition, probably by destroying MHC anchor residues. To study MHC class I restriction, the CTL lines were tested against MHC class I mismatched and partially matched B-LCL pulsed with peptide p26A.5. As shown in Fig. 1(c) for CTL line K71/E26, lysis was restricted to targets sharing the Mafa-A2 allele, indicating recognition in the context of this macaque MHC class I molecule. It should be noted, however, that MHC class I alleles were defined by serological techniques, and one-dimensional
isolectric focusing may be required to confirm the restriction element (Watkins, 1994).

In reciprocal experiments, PBMC (2 x 10^4 per well) were cultured for 10 days with autologous irradiated (5000 rad) B-LCL (10^4 per well) sensitized with 30 μM of peptide p26A.5, autologous irradiated feeder PBMC (10^4 per well) and rIL-2 (10 U/ml) from day 4. As a control, PBMC were stimulated with B-LCL sensitized with peptides p26.4, p26.6 or p17.6. No cytotoxic responses were mediated by these control cultures (data not shown), whereas effector cells expanded by peptide p26A.5 recognized autologous targets sensitized with the inducing peptide, as well as targets expressing endogenously processed antigen after infection with SIV Gag rVV (Table 1). The CD8+ phenotype of peptide p26A.5-specific CTL was confirmed by depletion studies (Table 1), using anti-CD8 antibody-coated magnetic beads (Dynabeads M-450, Dynal) as described (Geretti et al., 1993).

The amino acid region 242–250 of p26 partially overlaps with CD4+ T helper epitopes previously identified in immunized cynomolgus macaques (Mills & Jones, 1994). In addition, the homologous consensus sequences of the HIV-1 A and B clades fulfill the requirements for binding the human HLA-A*2.1 molecule (Brander et al., 1995). The region is conserved among several strains of the HIV-2/SIV D clade, including SIVmac251, SIVmac32H, SIVmac1A11 and SIVmac239. The consensus sequence of the HIV-2/SIV D and C clades shows one amino acid substitution at position 242 (S→T), whereas the consensus sequence of the HIV-2/SIV A and B clades shows two amino acid substitutions, at positions 242 (S→T) and 244 (D→E) (Myers et al., 1994). The homologous sequence is highly conserved among most HIV-1 clades (A–H). However, comparison of the SIVmac251 and HIV-1SF1 sequences reveals four amino acid substitutions at positions 242 (S→T), 243 (V→L), 244 (D→Q) and 248 (Q→G) (Myers et al., 1994). As shown in Fig. 2(a), targets that were either pulsed with peptide p24.11 (ADP 788/11), covering amino acids 235–254 of HIV-1SF2 p24 (GSDIAGTGSTTLQEQGWTMN), or infected with an rVV expressing HIV-1LAI Gag (TG1144; Transgene), were not recognized by CTL line K71/E26. These effector cells also failed to recognize targets infected with an rVV expressing HIV-2ROD Gag (TG1112; Transgene), suggesting either lack of generation of the epitope, or that the two amino acid substitutions at positions 242 (S→T) and 244 (D→E) of HIV-2ROD p26 were sufficient to abolish recognition.

These observations suggest that variations in the p26A.5 epitope may generate virus variants able to escape or antagonize the CTL response of monkey K71, thereby potentially affecting virus containment (Franco et al., 1995). Longitudinal studies are in progress to address this hypothesis, providing an additional basis for investigating the role of CTL immunity in the control of lentiviral infections.

We thank Professor A. McMichael (Institute of Molecular Medicine, Oxford, UK) for providing the SIVmac32H Gag p35 rVV. Dr H. C. Holmes (MRC AIDS Directed Programme, Potter Bar, South Mills, UK) for providing the ADP peptides. Dr E. W. Rud (Health Canada, Ottawa, Ontario, Canada) and Dr M. P. Cranage (Centre for Applied Microbiology and Research, Porton Down, Salisbury, UK) for providing SIVmac32H-15, and Dr R. Bonfort (Biomedical Primate Research Centre, Rijswijk, Netherlands) for MHC typing.

References


1 and EBV specific cytotoxic T lymphocyte precursors exhibit different kinetics in HIV-1 infected persons. Journal of Infectious Diseases 174, 34–45.


Received 8 November 1996; Accepted 15 December 1996