Mechanisms of decreasing HIV-1 specific CD8+ T cell activity during progression to AIDS
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Virus-specific CD27− effector CD8+ T cells are associated with protection from disease in HIV-infection

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Despite readily detectable virus specific CD8+ T cells in most HIV-infected patients, immune surveillance is eventually lost, leading to progression to AIDS. In three clinically distinct groups, long-term asymptomatics, progressors to opportunistic infections and progressors to EBV-associated non-Hodgkin lymphoma's (NHL), CD45RO and CD27 expression of HIV- and EBV-specific CD8+ T cells were studied longitudinally. Lack of differentiation of HIV- and EBV-specific CD8+ T cells to the CD27− cell stage was found to be associated with rapid progression to AIDS or EBV-related NHL, respectively. EBV-specific CD27− T cells showed elevated IFNγ production in response to viral peptides in vitro compared to CD27+ T cells, indicative for strong effector function. Therefore, we conclude that maturation of virus-specific T cells into high IFNγ producing CD27-negative effector cells is required for adequate immune control of viral infection in humans.

Introduction

In human viral infections such as Human Immunodeficiency Virus (HIV), Epstein-Barr virus (EBV), and Cytomegalovirus (CMV), high levels of antigen specific CD8+ T cells are found (23,26,125,175). Most chronic infections are adequately controlled by virus-specific T cells. Although apparently HIV-specific T cells initially control viral replication, HIV-specific T cell responses are lost in individuals progressing to AIDS (30). In addition, HIV-infected individuals are at high risk for EBV-positive AIDS-related Non-Hodgkin’s lymphomas (AIDS-NHL), which are thought to develop because of progressive loss of EBV-specific T cell immunity (159).

Recently primed virus-specific T cells are mostly of the CD45RO+CD27+ phenotype with cytolytic activity only after restimulation (48). These CD27+ T cells irreversibly switch off CD27 expression when stimulated for prolonged periods (53) and eventually revert to CD45RA+ T cells. (66,95,96,180) The readily detectable effector T cells, like high Granzyme B expression and direct cytolytic activity, would predict these cells to be critical in control of virus replication (181,182).

Significant numbers of CMV- (177,182) EBV (126) and Hepatitis C Virus- specific T cells (183) were reported to be of the CD27− phenotype. In contrast, in HIV infected individuals the majority of HIV-specific T cells have a CD27+ memory phenotype, while only minor populations of CD27− effector cells are found (26,177). To define the role of CD8+ CD27−, so-called effector T cells in human viral infections in relation to clinical outcome, we investigated the differentiation status of HIV- and EBV-specific T cells in the course of HIV-1 infection. We studied kinetics of virus-specific CD8+ T lymphocytes using MHC class I-peptide tetrameric complexes Altman, and assessed their CD45RO and CD27 surface expression in different groups of HIV-infected individuals. In addition, we studied the functional consequences of differentiation towards the CD27− phenotype in these individuals by measuring IFNγ production.

Methods

Study population

This study was performed on participants of the Amsterdam Cohort studies on AIDS and HIV-1 infection. Peripheral blood mononuclear cells (PBMC) were cryopreserved according to a standard computerised freezing protocol. HIV-1-seropositive male individuals were selected according to duration of follow-up, availability of samples and HLA-type (HLA-A2 and/or B8). In a cross-sectional analysis, 33 HIV-1-infected individuals were studied for the presence and phenotype of HIV-specific CD8+ T cells. Furthermore, we analysed longitudinal PBMC samples from 9 HIV-1 infected individuals for HIV-specific CD8+ T cells and 11 HIV-1 infected individuals for EBV-specific CD8+ T cells, including several individuals who were studied for both antigen-specific T cells. Characteristics of these groups are in part published elsewhere (26) and summarised in Table 1.
**Table 1: Characteristics HIV-1 infected individuals**

<table>
<thead>
<tr>
<th>group</th>
<th>n</th>
<th>median age</th>
<th>median follow-up</th>
<th>median CD4 count</th>
<th>viral load</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV+ cross-section</td>
<td>33</td>
<td>36 (24-46)</td>
<td>96 (12-173)</td>
<td>350 (100-1060)</td>
<td>36 (1-1300)</td>
</tr>
<tr>
<td>HIV+ longitudinal</td>
<td>9</td>
<td>35 (24-40)</td>
<td>119 (92-141)</td>
<td>280 (100-790)</td>
<td>25 (1-800)</td>
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<tr>
<td>EBV+ longitudinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIDS-NHL</td>
<td>5</td>
<td>36 (25-46)</td>
<td>87 (25-124)</td>
<td>100 (60-190)</td>
<td>6574 (74-879,630)</td>
</tr>
<tr>
<td>AIDS-OI</td>
<td>3</td>
<td>35 (33-43)</td>
<td>128 (39-134)</td>
<td>90 (40-230)</td>
<td>66667 (481-444,444)</td>
</tr>
<tr>
<td>LTA</td>
<td>3</td>
<td>39 (35-44)</td>
<td>128 (95-162)</td>
<td>340 (240-360)</td>
<td>1023 (130-64,815)</td>
</tr>
</tbody>
</table>

A number of patients; B median age at AIDS-diagnosis or for LTA last time point studied; C median seropositive follow-up in months, from HIV-1 seroconversion or first seropositive visit until AIDS-diagnosis or last time point studied; D median CD4+ T cell numbers at AIDS-diagnosis or for LTA last time point studied; E Median and range of viral load: HIV load (copies/μl serum) and EBV load (copies per 10⁶ PBMC) for all time points studied as measured by real-time quantitative taqman PCR assay as described in detail elsewhere; F patient group studied consisted of HIV-1 infected individuals who progressed to AIDS-related non-Hodgkin's lymphoma (AIDS-NHL), AIDS with opportunistic infections (AIDS-OI) and Long-term asymptomatic individuals (LTA).

Flow cytometry and tetramer staining

MHC class I tetramers complexed with EBV and HIV-peptides were produced as previously described (47). The peptides used were two immunodominant epitopes from EBV lytic cycle proteins, the HLA A2-restricted epitope GLCTLVAML (A2-GLC) from BMLF-1 and the HLA B8-restricted epitope RAKFKQQL (B8-RAK) from BZLF-1, and one immunodominant epitope from the EBV latent antigen EBNA-3A, the HLA B8-restricted epitope FLRGRAYGL (B8-FLR). From HIV p17Gag and Pol proteins, SLVYATL and ILKEPVHGQ were complexed with HLA-A2; p24Gag and Nef peptides EIYKRWII and FLKEKGGGL were refolded in HLA-B8 proteins.

PBMC were stained with appropriate MHC class I tetramers and anti-CD8, anti-CD45RO, anti-CD27 antibodies. Cells were analysed using a FACSCalibur flow-cytometer (Becton Dickinson).

Intracellular IFNγ staining after antigen specific stimulation

Two million PBMC/ml were stimulated with 1μg EBV (RAK), used in the tetrameric complexes, or PMA/ionomycin (positive control) or not stimulated (medium alone as negative control) at 37°C for 4 hours in the presence of 3μM monensin. After incubation, cells were washed and stained for extracellular markers as described above. For intracellular staining cells were fixed with 4% paraformaldehyde, permeabilised (Permeabilisation kit, BD) and stained with IFNγ-PE (BD) for 30 minutes at 4°C. At least 200,000 events in the lymphogate were acquired using a FACSCalibur flow cytometer (Becton Dickinson).

ELispot assay for single cell IFNγ-release

IFNγ producing antigen-specific T cells were enumerated after stimulation with peptide using IFNγ specific ELispot assays as previously described (144). PBMC were stimulated overnight at 37°C in 5% CO2 in triplicate wells at 1 x 10⁵ cells/well in the absence or presence of 2 μM peptide or, as a positive control to test the capacity of PBMC to produce IFNγ in general, Phytohaemagglutinin (PHA) (Murex Diagnostics, Dartford, UK). The number of specific T cell responders per 10⁶ PBMC was calculated after subtracting negative control values.

Statistical analysis

For group comparisons Mann-Whitney tests were performed. Cross-sectional data were analysed by Spearman correlation tests and ANOVA stepwise regression analysis. Repeated measurements analyses were performed after cube root transformation of all variables. In analyses, we corrected for dependency between observations within a person assuming a compound symmetry (CS) structure using the software program SAS/STAT (SAS Institute Inc. Cary, USA).
In the course of HIV infection, we applied the CD27 and CD45RO T cell antigen expression patterns, revealing distinct subsets of T cells (figure 1, right panels). As shown in figure 3, early in HIV infection (within 3 years after study entry or HIV-seroconversion) the majority of the HIV- (left panel) and EBV-specific CD8+ T cells (middle panel) had a CD45RO+CD27+ phenotype, as also illustrated by the low ratio of CD27-/CD27+ T cells for HIV- (n=9) and EBV-specific T cells (n=11), respectively (figure 4A). In contrast to the HIV-specific T cells, a substantial proportion (30-50%) of the EBV-specific T cells was of the CD27+ phenotype already early in HIV- infection. (fig. 1a) In the course of HIV-1 infection, the CD27-/CD27+ ratio of HIV-specific CD8+ T cells remained stable, whereas in most individuals, the CD27-/CD27+ ratio of EBV-specific T cells increased, indicating that EBV-specific CD8+ T cells had differentiated and increasingly accumulated at the CD27+ stage (figure 4A). Late in HIV-1 infection (between 6 and 12 years after study entry or HIV-seroconversion), the ratio CD27-CD27+ was significantly higher (median=1.1) for EBV-specific CD8+ T cells than for HIV-specific CD8+ T cells (median=0.3) (p<0.014, Mann-Whitney test).

Results

Stable numbers of HIV- and EBV-specific CD8+ T cells in the course of HIV-1 infection
To study HIV- and EBV-specific T cells in the course of HIV-infection, PBMC from HIV-infected individuals at several timepoints in the course of HIV-infection were stained using HLA-HIV-peptide and HLA-EBV-peptide tetrameric complexes (figure 1, left panel). As shown in figure 2, high numbers of HIV- (range 0.1-2.7% of CD8+ T cells) and EBV-specific T cells (range 0.1-3.7% of CD8+ T cells) were present. Overall, no change in the number of both HIV- (p=0.97, Wilcoxon test) and EBV-specific T cells (p=0.97, Wilcoxon test) was observed in the course of HIV-infection (figure 2), suggesting that the HIV- and EBV-peptides used in the tetrameric complexes are still recognized by specific CD8+ T cells after several years of HIV-positive follow-up.

Lack of differentiation of HIV-specific T cells to CD27 T cells in the course of HIV-infection
To investigate the differentiation stage of HIV-versus EBV-specific T cells in individuals who experienced relatively long periods of high HIV-viraemia, we applied the CD27 and CD45RO T cell antigen expression patterns, revealing distinct subsets of T cells (figure 1, right panels). As shown in figure 3, early in HIV infection (within 3 years after study entry or HIV-seroconversion) the majority of the HIV- (left panel) and EBV-specific CD8+ T cells (middle panel) had a CD45RO+CD27+ phenotype, as also illustrated by the low ratio of CD27-/CD27+ T cells for HIV- (n=9) and EBV-specific T cells (n=11), respectively (figure 4A). In contrast to the HIV-specific T cells, a substantial proportion (30-50%) of the EBV-specific T cells was of the CD27+ phenotype already early in HIV- infection. (fig. 1a) In the course of HIV-1 infection, the CD27-/CD27+ ratio of HIV-specific CD8+ T cells remained stable, whereas in most individuals, the CD27-/CD27+ ratio of EBV-specific T cells increased, indicating that EBV-specific CD8+ T cells had differentiated and increasingly accumulated at the CD27+ stage (figure 4A). Late in HIV-1 infection (between 6 and 12 years after study entry or HIV-seroconversion), the ratio CD27-/CD27+ was significantly higher (median=1.1) for EBV-specific CD8+ T cells than for HIV-specific CD8+ T cells (median=0.3) (p<0.014, Mann-Whitney test).

Figure 2. HIV and EBV-specific T cells in the course of HIV-infection
Percentage of HIV- (left panel) and EBV-specific (right panel) CD8+ T cells as determined by tetramer-staining and subsequent FACS analysis early (within 2 years after study entry or seroconversion) and late (between 6 to 8 years after entry or seroconversion) in HIV-infection
Lack of differentiation of EBV-specific T cells into CD27+ T cells in AIDS-NHL patients
Althoug hh  i n  HIV-infecte d  individual s  th e  media n  throughou tt  follo w  up . T o  stud y  whethe r  thi s  coul d  test) ..  Progressor s  t o  opportunisti c  infection s  (AIDS -
4b ,,  i n  AIDS-NH L  patient s  EBV-specifi c  T  cell s  increased ,,  i n  a  smal l grou p  o f individual s  EBV -
epitop ee  specifi c  T  cell s  i n  th e  LT A  group . Late r  i n  specific specific  CD8  fo rr  eac h  patien t 2  epitope s  wer e  tested )  o r  specific specific  CD8+  T  cells  in one  AIDS-NHL  patient
++
CD27~/CD27  wit hh  opportunisti c  infection s  (PROG/AIDS-OI , n=3 , fo rr  3  individual s  2  epitope s  wer e  teste d  an d  fo r  2
++
CD27~/CD27  in infecte dd  individual s  wh o  eithe r  progresse d  t o  AID S  individual ss  1  epitop e  wa s  tested) , wh o  likel y  hav e  patien tt  2  epitope s  wer e  tested) . A s  show n  i n  figur e
maintenanc ee  o f th e  CD27-expressio n  i n  AIDS-NH L
remaine dd  clinicall y  stabl e  (LTA , n=3 , fo r  eac h
infection). infection).

Abundance of HIV-specific CD27~ T cells is associated with slower progression to AIDS
Although in the majority of HIV-infected persons most HIV-specific T cells expressed CD27, in some HIV-infected individuals a higher percentage of HIV-specific CD27~ cells was observed. To investigate the effect of CD27~ HIV-specific T cells on disease progression we correlated the percentage of HIV-tetramer+ T cells lacking CD27 expression with viral load and months of AIDS free follow-up in HLA-A2 or HLA-B8 positive participants of the Amsterdam Cohort. In samples drawn between 1 and 3 years after seroconversion (n=11) an inverse correlation between HIV viral load and months AIDS free survival was found (Spearman, R=0.78, p=0.005) (figure 5a) as described before (9). For the same samples an inverse correlation was observed between the percentage of CD27~ HIV-specific T cells and viral load (R=0.79, p=0.004). (fig 5b) Interestingly, high percentages of HIV-specific CD27~ cells detected between 1 and 3 years after seroconversion (n=11) and in samples randomly drawn during HIV-Infection (n=33) correlated with delayed disease progression (Spearman: R=0.77, p=0.005 and R=0.46, p=0.006, respectively (figure 5c and d, respectively), whereas the total number of tetramer+ T cells did not correlate with protection from disease (R=0.22, p=0.49)(data not shown).

In a multivariate stepwise analysis, the percentage of CD27~ HIV-specific T cells was predictive for progression rate independent of viral load (CD27~: β=0.82, p=0.002; viral load: β=0.112, p=0.75).

Virus-specific CD8+ CD27~ T cells are high IFNγ producers
To investigate the effect of CD27~ differentiation on anti-viral CD8+ T cell function, we tested the relation between the number of virus-specific CD27~ T cells and—as a read-out for effector function—the number of IFNγ producing T cells. We selected those HIV-1 infected individuals in whom a significant accumulation of CD27~ EBV-specific T cells was observed (3 progressors to OI and 3 LTA). Using regression analyses (mixed linear model) the number of EBV-specific CD27~ T cells measured at all time points (n=34) was positively

### Figure 3. Longitudinal changes in CD45RO and CD27 expression on virus specific T cells.
Percentage CD45RO CD27+, CD45RO+CD27+, CD45RO+CD27 and CD45RO-CD27 HIV- and EBV-specific T cells as determined by FACS analysis in the course of HIV-1 infection in a. one long-term asymptomatic HIV-carrier and EBV-specific CD8+ T cells in one AIDS-NHL patient and b. one slow progressor to AIDS (opportunistic infection).

Although in HIV-infected individuals the median ratio of EBV-specific CD27+/CD27+ was increased, in a small group of individuals EBV-specific CD8+ T cells maintained CD27-expression throughout follow up. To study whether this could be related to clinical outcome, we compared the ratio CD27+/CD27+ for AIDS-NHL patients (n=5, for 3 individuals 2 epitopes were tested and for 2 individuals 1 epitope was tested), who likely have defective EBV-specific immunity (159), with HIV-1 infected individuals who either progressed to AIDS with opportunistic infections (PROG/AIDS-OI, n=3, for each patient 2 epitopes were tested) or remained clinically stable (LTA, n=3, for each patient 2 epitopes were tested). As shown in figure 4b, in AIDS-NHL patients EBV-specific T cells predominantly were of the CD27+ phenotype (low CD27+/CD27+ ratio), whereas an increase in the CD27+/CD27+ ratio was observed for all EBV-epitope specific T cells in the LTA group. Later in HIV-infection, the CD27+/CD27+ ratio in LTA was significantly higher (median 1.48) than in AIDS-NHL patients (median 0.43) (p<0.034, Mann-Whitney test). Progressors to opportunistic infections (AIDS-OI) also showed a trend towards higher percentages of EBV-specific CD27~ T cells (n=6) (median ratio CD27+/CD27+ = 0.80). The maintenance of the CD27-expression in AIDS-NHL patients occurred despite high levels of EBV load in PBMC, which was comparable to EBV load in LTA and other progressors to AIDS (table 1).
correlated with the number of EBV-specific CD8+ T cells producing IFNγ (β = 1.2), which was highly significant in multivariate (p<0.001) analyses controlling for EBV load and the number of CD4+ T cells. (fig. 6).

To investigate in detail whether the overall increase in IFNγ- producing antigen-specific T cells was indeed caused by an increase in the percentage of virus specific CD27+ T cells, we compared the distribution over the different T cell subsets of EBV-specific T cells defined by tetramer-staining and by IFNγ production, using intracellular IFNγ staining after stimulation with the specific EBV peptide. In a long-term asymptomatic HIV-infected individual early in HIV-infection (left panels), the tetramer+ T cells resided for 33% in the CD27− subset (fig 7b), whereas 60% of the IFNγ+ EBV-specific T cells resided in this subset (fig. 7c). Moreover, at the later time point the percentage tetramer+ T cells had not increased, but EBV-specific T cells were two-fold more enriched in the CD27+ fraction (right panels). Concomitantly, the percentage of IFNγ producing T cells had doubled (fig. 7b) and these cells were also further enriched in the CD27+ T cell fraction (61%). Thus, the overall increase in IFNγ-producing antigen-specific T cells was indeed related to an increase in the percentage of virus specific CD27+ T cells. (fig 7)

**Discussion**

In this study, we investigated the differentiation status of HIV- and EBV-specific CD8+ T cells in the course of HIV-1 infection to evaluate the role of virus-specific CD27+ CD8+ T cells in human viral infections in relation to clinical outcome. Differentiation of HIV- or EBV-specific T cells into CD27+ T cells was associated with slower progression to AIDS or protection against AIDS-NHL, respectively. In AIDS-NHL patients, EBV-specific CD8+ T cells remained of the CD27+ phenotype in the course of HIV-1 infection, despite high levels of EBV load in PBMC. For HIV-specific T cells the percentage of CD27+ T cells appeared to be predictive of disease progression, independent of HIV serum load. Since percentages of CD27+ HIV-specific T cells did not substantially change over time, assessment of CD27 expression on HIV-specific T cells at any time during the natural course of infection appeared to have a prognostic value for progression to AIDS.
Figure 5. Impact of CD27+ T cells on disease progression. a. Correlation between percentage HIV-RNA load, between 1 and 3 years after seroconversion, and AIDS free survival (n=11). Correlations were calculated using Spearman non-parametric regression analysis. b. Correlation between percentage HIV-specific CD27+ T cells between 1 and 3 years after seroconversion, and viral RNA load (n=11). c. Correlation between percentage HIV-specific CD27+ T cells, as determined by FACS-analysis between 1 and 3 years after seroconversion, and AIDS free survival (n=11). d. Correlation between percentage HIV-specific CD27+ T cells at random timepoints during the natural course of HIV-infection, and AIDS free survival (n=33).

Defective maturation of HIV-specific T cells is compatible with the observed lower perforin levels in CD27+ T cells. (177) We provide evidence that fully differentiated CD8+ T cells specific for HIV or EBV are required to prevent progression to AIDS or AIDS-NHL, respectively. The accumulation of virus specific CTL in the CD27- CD8+ population in these clinical conditions provides evidence that these T cells may be critical for controlling viral infections that are chronically active as reflected by active viral replication. This is compatible with the finding that these T cells have augmented antiviral effector function compared to CD27+ T cells, as reflected by the correlation between the percentage CD27- T cells and high IFNγ production by virus specific CD8+ T cells. In addition, CD27- T cells have also been shown to contain more granzyme B and perforin and exert stronger direct cytolytic activity compared to CD27+ cells (181,182).

Since development of CTL activity is known to be dependent on CD4+ T cell help (163,184) and HIV-specific CD4+ T cells are believed to be irreversibly lost during acute infection (184), HIV-specific CD8+ T cells may not be able to efficiently differentiate to CD27+ T cells despite high HIV viraemia. Indeed, we observed significant numbers of CD27- cells only in LTA individuals that experience a relatively stable clinical course, have modest viral loads and are known to still have measurable HIV-specific CD4+ T helper activity (173). In contrast, most likely EBV-specific CD4+ T cells were present before HIV seroconversion, and these cells may decrease more gradually. When EBV-specific CD4+ T cells are eventually lost, due to immune activation-induced increased EBV-reactivation, also functional differentiation to EBV-specific CD27+ T cells will be hampered and the individuals become at high risk of developing NHL.

Figure 6. Impact of CD27- T cells on IFNγ production. Correlation between numbers of EBV-specific CD27- T cells (cube root transformed), as determined by FACS-analysis, and numbers of EBV-specific IFNγ producing T cells (cube root transformed), as determined by elispot assay after in vitro stimulation with peptide, in 6 HIV-infected individuals.
Figure 7. Distribution of tetramer* and IFNγ producing virus specific T cells over the CD27+ memory and CD27- effector populations in a long-term asymptomatic HIV-infected individual. The distribution of a. the total number of EBV-specific (left panels), as assessed by tetramer staining and b. the number of IFNγ-producing EBV-specific CD8+ T cells (left panels), as assessed by intracellular IFNγ staining after in vitro stimulation with peptide, over the CD27+ and CD27- T cells (right panels) in an HIV-positive long-term asymptomatic individual at an early and late time point in HIV-infection. FACS staining for CD8, CD45RO, CD27 and tetramer or IFNγ after peptide stimulation was performed as described in Methods.

In addition, as shown previously, CD4+ T cell numbers indeed correlated with the number of EBV-specific IFNγ producing T cells, suggesting a role for CD4+ T cells in maintaining functional capacity of CD8+ T cells (185). Furthermore, the expression of CD27 has been shown to decrease after interaction with its specific ligand, CD70 (186) and CD70 expression is upregulated by T helper 1 cytokines (187). Therefore, it could be that helper T cells, directly or indirectly, contribute to this differentiation process of CD8+ T cells.

Lack of CD27- CD8+ virus-specific T cells may be explained by high rate of activation and rapid death of these cells induced by high and continuous antigen exposure. However, given the functional defects in CD4+ and CD8+ T cells (42), the apparent selective depletion of HIV-specific helper T cells (184) and the evidence for APC defects in HIV-infection (188), we favor the hypothesis that in HIV-infection maturation of virus-specific CD8+ T cells to the CD27- stage may be impaired.

Yet, another alternative explanation for the lack of CD27 T cells may be that virus-specific CD8+ T cells do not differentiate due to escape mutations which abolish CD8+ T cell activation. Although escape mutations have been described to occur in HIV-epitopes (19-22), we did not find escape mutations in HIV-Gag epitopes studied in several individuals included in this study population (189). In addition, if escape mutations would have occurred, this would have led to lack of antigen-recognition and rapid decay kinetics of the virus-specific T cells as has been reported to occur for HIV-specific responses after occurrence of viral mutations (19,22) and HIV load-reduction in HAART treated individuals (23,89,90). However, we observe no substantial decrease in the number of virus-specific T cells in the course of HIV-infection suggesting that the corresponding T cell epitopes are still recognized by the T cells. Furthermore, we also show lack of CD27- EBV-specific T cells in individuals progressing to AIDS-NHL. Since EBV does not mutate frequently nor rapidly, it is likely that another mechanism is at play.

In conclusion, we provide evidence that fully differentiated CD8+ T cells specific for HIV but also EBV are required to prevent progression to AIDS or AIDS-NHL, respectively. Thus, our data indicate that
CD27- effector CD8+ T cells important for disease protection

impaired maturation of CD8+ T cells is not specific for HIV, but may well be a general phenomenon in other conditions with failing anti-viral or anti-tumor immune control. Our data support the notion that phenotypic analyses of virus or tumour specific CD8+ T cells based on CD27 expression may be informative for protective immunity to virus-induced disease and tumour development. Altogether our results warrant further prospective studies into this issue.

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