Mechanisms of decreasing HIV-1 specific CD8+ T cell activity during progression to AIDS
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Variable CD8\(^+\) T cell responsiveness to HIV Gag epitopes presented by different HLA alleles

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To study possible variations in CD8\(^+\) T cell responsiveness towards different HIV epitopes, we compared HLA-tetramer staining and peptide-specific IFN\(\gamma\) production for three Gag epitopes presented by different HLA alleles. In a cross-sectional analysis, T cells specific for an HLA-B57 epitope (KAFSPEVIPMF) appeared more responsive to antigenic stimulation than counterparts specific for HLA-A2 (SLYNTVATL) or -B8 (EIYKRWII) epitopes. This phenomenon was reproducible with T cells from individual subjects expressing HLA-B57 in combination with one or both of the other alleles. In conclusion, functional differences were be observed between T cells specific for HIV epitopes in the same protein, presented by different HLA molecules. The consistently strong KAFSPEVIPMF-specific T cell response may contribute to the protective effect of HLA-B57 in HIV infection.

Introduction

Upon infection with HIV, both cellular and humoral immunity is induced against several HIV proteins. Several lines of evidence point out that HIV-specific CD8\(^+\) T cells do contribute to the control of viraemia. Strong CTL responses are often observed in long term asymptomatic HIV infected individuals, but diminish during the progression to AIDS (30). Experimental depletion of CD8\(^+\) T cells in the SIV monkey model has been shown to lead to loss of control of acute infection and increased viral load in chronic infection (28,29). Moreover, infusion of HIV-specific CD8\(^+\) T cell clones in HIV infected individuals leads to a temporary drop in HIV load (27). Host and viral genetics also point towards an interaction between the virus and HIV-specific CTL. Immune pressure appears to select viral mutations in CD8\(^+\) T cell epitopes (19,20,22) and responses directed against structurally important regions of viral proteins may have a stronger protective effect than those directed against less restricted amino acid sequences (204). Finally, HLA alleles are genetic factors associated with differences in progression to AIDS (205). HLA heterozygosity contributes to relatively slow progression, as well as certain specific HLA alleles, including HLA-B57 (166,206,207).

HIV appears to mainly affect the CD4\(^+\) T cell compartment, resulting in strongly reduced CD4\(^+\) T cell numbers. However, the systemic immune deficiency that characterizes AIDS indicates that also other compartments of the immune system are severely compromised by the virus. In experimental animal models of virus infection, CD4\(^+\) T cells are required to sustain virus-specific CD8\(^+\) T cell responses (146,200). Therefore, defective CD8\(^+\) T cell responses in chronic HIV infection may be caused by lack of immunological help provided by the CD4\(^+\) T cell compartment (163,184). Alternatively, deterioration of the immune system may be caused by other mechanisms, such as T cell (hyper) activation and reduction of the naive T cell pool, leading to exhaustion (208). In addition, viral mechanisms like the downregulation of HLA expression by the HIV Nef protein may undermine immune control of the virus (209).

Although cytolysis of antigen pulsed artificial target cells has long been used to monitor CTL activity in vitro, new immunological techniques allow for a more detailed analysis of antigen specific CD8\(^+\) T cells, directly ex vivo. HLA tetramers stain T cell populations on the basis of their epitope specificity and independently of cellular functions (47). Intracellular cytokine staining and ELISPOT on the other hand depend on antigen induced cytokine production, most notably interferon gamma (IFN\(\gamma\)) (49). In several studies, tetramer staining and cytokine assays were combined to compare the presence and responsiveness of circulating antigen specific CD8\(^+\) T cells in chronic HIV infection. Whereas some groups detected similar numbers of cells with both assays (177,195,210), others have reported a lower number of antigen responsive, IFN\(\gamma\) producing T cells as compared to the total antigen specific, tetramer positive population (162,185,189,191,192,194). These differences in antigen induced IFN\(\gamma\) relate to differences in
disease progression (185,189). We investigated whether antigen responsiveness can vary between CD8+ T cell populations directed against distinct HIV Gag epitopes presented by different HLA alleles. T cells specific for HLA-B57, -A2 or -B8 epitopes were studied by tetramer staining, IFNγ Elispot and intracellular IFNγ staining after peptide stimulation.

Materials and Methods

Subjects and samples

All studies were performed on PBMC that were cryopreserved according to a standard computerized freezing protocol, and stored in liquid nitrogen until use. This study comprised material collected from 18 HIV-infected individuals of the Amsterdam Cohort Studies on AIDS. Subjects were selected for the expression of HLA-A2, -B8, -B57, or combinations of these alleles. In addition, 5 healthy HLA-B8 and EBV positive controls were studied for EBV-specific responses. Three HIV infected subjects were studied longitudinally over the indicated period after seroconversion or seropositive study entry. Subjects H211 and H448 entered the Cohort seropositive and had probably encountered their HIV infection within a period of 1.5 years before (211). H211 and H448 participated in a therapeutic vaccine trial (212,213), consisting of intramuscular injections with 100 μg (H211) or 1000 μg (H448) of p17/24:Ty virus-like particles (VLP), administered every 4 weeks during the marked period. Subjects H211 and H1113 were treated with highly active antiretroviral therapy (HAART), whereas H448 received lamivudine monotherapy.

Table I. CD4+ T cell numbers and viral loads of the HLA groups

<table>
<thead>
<tr>
<th>HLA group</th>
<th>CD4+</th>
<th>Viral load</th>
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<tbody>
<tr>
<td>A2 (11)</td>
<td>680</td>
<td>(400-980)</td>
</tr>
<tr>
<td>B8 (10)</td>
<td>635</td>
<td>(350-980)</td>
</tr>
<tr>
<td>B57 (8)</td>
<td>615</td>
<td>(500-680)</td>
</tr>
<tr>
<td>Total (18)</td>
<td>632</td>
<td>(350-980)</td>
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Tetrameric HLA-peptide complexes

Tetramer synthesis was performed as described previously (47,167). Peptides derived from p17 Gag (SLYTNTATI) and p24 Gag (EYKRWI, KAFSPEVIPMF) were complexed with HLA-A201, HLA-B801, and HLA-B5701 proteins respectively; EBV-specific HLA-B8 tetramers contained the lytic protein BZLF-1 derived RAKFKQQL. HLA-peptide complexes were enzymatically biotinylated, FPLC purified on a Superdex 200 HR16/60 column (Amersham Pharmacl, Little Chalfont, UK) and bound to streptavidin-phycocerythrin (PE) or streptavidin-allophycocyanin (APC) conjugates (Sigma, St Louis, MO). Subsequently, tetramers were FPLC purified using the same column.

FACS analysis

PBMC were analysed after staining for 20 min at 20 °C with tetrarmers (PE or APC), followed an additional 15 min of incubation with mAb to CD8 (PerCP; Becton Dickinson), CD27 (FITC; CLB), CD69 (APC; Becton Dickinson), Granzyme B (PE; CLB), Perforin (FITC; Pharmingen) and IFNγ (FITC or PE; Becton Dickinson) in various combinations. For antigenic stimulation, 2 x 10^6 PBMC were stimulated with 1 μg/ml of antigenic peptide at 37°C for 4 hours in medium containing 3 μM monensin as described before (189). Alternatively, a peptide concentration range (0.0001–10 μg peptide/ml) was used to determine a dose-response curve. HLA-mismatched peptide, tetramer-mismatched peptide, or medium alone were used as negative controls, and stimulation with PMA/Ionomycin was used as positive control. Stimulated cells or thawed PBMC were washed, stained for extracellular markers and tetrarmers, and for intracellular staining, cells were washed, fixed and permeabilised (Permeabilisation kit; Becton Dickinson). Stained cells were fixed (Cell Fix; Becton Dickinson) and total lymphocytes were gated and analyzed using Cellquest software (Becton Dickinson). The IFNγ gate was determined by the negative and positive controls of each individual’s CD8+ T cells. IFNγ+ fractions were calculated by dividing the percentage IFNγ+ T cells after stimulation by the percentage of tetramer positive T cells as determined in the non-stimulated control sample. Costimulation with anti-CD28 and anti-CD49 mAbs (Becton Dickinson) during stimulation with 1 μg/ml or concentration ranges of peptides moderately increased IFNγ production, but did not alter the observed differences or trends.
T cell population. After 3 h incubation at room temperature, plates were dried and spots were hand-counted after computerized visualization by a scanner (Hewlett Packard Company, Baise, Idaho, USA). Responder T cells were counted as the total number of spots after subtracting negative control values. All experiments were performed in duplo. Because the percentage of viable CD8+ T cells in PBMC was assessed in the same samples by FACS analysis, the percentage of IFNγ producing CD8+ T cells could be calculated.

**Results**

IFNγ production and CD27 expression by HLA-A2, -B8 and -B57 restricted CD8+ T cells

As observed in various previous reports (185,189,191,192,215,216), the fraction of IFNγ producing T cells can vary substantially among tetramer positive T cells. To study whether this variation may relate to T cell specificity, we compared three HIV Gag epitopes presented by different HLA alleles by tetramer staining and intracellular IFNγ staining after peptide stimulation. The KAFSPEVIPMF peptide was used for HLA-B57 (B57-KAF), SLYNTVAL for HLA-A2 (A2-SLY) and EITYKVIIL for HLA-B8 (B8-EIY). In addition, an EBV BZLF-1 HLA-B8 epitope RAKFKQLL (B8-RAK) was included. T cells were stimulated for 4 hours with 1μg peptide/ml, which results in optimally induced IFNγ production (189). As shown in figure 1A, the percentage of IFNγ producing cells in the B8-RAK tetramer positive specific CD8+ T cells varied widely (12 to 66%), both in HIV-infected and uninfected individuals. Strikingly, the IFNγ producing fraction varied distinctly for the CD8+ T cells directed against the different HIV Gag epitopes. In particular, a higher percentage of the B57-KAF specific T cells produced IFNγ upon peptide stimulation (mean 46.0 %), as compared to A2-SLY (26.0 %) or B8-EIY (25.6 %) specific T cells (p=0.007 and p=0.001 respectively, Mann Whitney). All samples were drawn during chronic (> 4 years after seroconversion), asymptomatic and untreated HIV-1 infection. The difference did not appear to relate to differences in disease progression, as the selected study subjects had similar CD4+ T cell numbers. HIV viral load was lower in the HLA-B57+ individuals than in the other HLA groups, although not statistically significant (Table I). HIV-specific T cell responsiveness to antigenic stimulation thus appeared to vary, depending on the epitope recognized and the concomitant HLA restriction element used.

Based on the expression of CD27, effector (CD27-) CD8+ T cells expressing "typical" CTL molecules like Fas ligand, granzymB and perforin can be separated from naive and memory

**Figure 1.** Functional and phenotypic analysis of CD8+ T cells directed against HLA-A2, -B8 or -B57 HIV Gag epitopes, and an HLA-B8 EBV BZLF-1 epitope. A. Antigen specific T cells were detected by tetramer staining and analysed for IFNγ production after peptide stimulation. IFNγ staining is indicated as the percentage of tetramer positive cells. Differences between the different HLA groups were computed with Mann Whitney tests, p values smaller than 0.05 are indicated. For the HIV Gag epitopes, the presenting HLA allele is indicated. Filled diamonds represent HIV-infected and open diamonds HIV-uninfected individuals. B. Percentages of CD27+ cells within the tetramer positive T cell population.

**Elispot assays**

IFNγ producing antigen specific T cells were enumerated using IFNγ specific Elispot assays as previously described (214). In short, 1 x 10^6 PBMC/well were incubated overnight at 37°C in 5% CO2 in the presence of 2μM peptide, in nylon-backed 96-well plates coated with anti-IFNγ mAb. As a positive control 2 μg/ml phytohemagglutinin (PHA) was added for antigen independent stimulation and negative controls contained PBMC in medium only. Cells were subsequently removed and after extensive washing with 0.05% (v/v) Tween 20 in PBS, biotinylated anti-IFNγ mAb, 7-B6-1 (MABTECH), was added at 1 μg/ml in PBS. After 3 h incubation at room temperature, plates were washed and developed using streptavidin-conjugated alkaline phosphatase (MABTECH) and a mixed substrate of 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Sigma, St. Louis, Missouri, USA). Reactions were stopped by extensive washing in water, the nylon membranes were dried and spots were hand-counted after computerized visualization by a scanner (Hewlett Packard Company, Baise, Idaho, USA). Responder
(CD27+) CD8+ cells (48). Low numbers of CD27-cells indicates decreased differentiation of HIV-specific CD8+ T cells, reflected in perforin deficiency (177), decreased IFNγ production and progression to AIDS3. However, no significant difference in CD27 expression between the HIV-specific T cell populations was observed (Figure 1B), and therefore the differences in IFNγ production induced by the HIV peptides did not appear to relate to their differentiation states.

Longitudinal analysis of HLA- A2, -B8 and -B57 restricted Gag specific CD8+ T cells.

To study the frequencies of CD8+ T cells directed against B57-KAF, A2-SLY, B8-EIY or B8-RAK longitudinally, three HIV-infected study subjects were selected that express HLA-B57 in combination with HLA-A2, -B8, or both. During the study period, two subjects participated in a recombinant Gag vaccination trial (212), (213) and at a later stage all three received anti-retroviral therapy. Fig. 2 shows the results obtained by tetramer staining and IFNγ Elispot. High numbers of B57-KAF-specific T cells were observed with both assays, which were consistently higher than the A2-SLY or B8-EIY responses. In subject H1113, the B57-KAF tetramer positive cells even mounted to 11% of the total CD8+ T cell population. Remarkably, both in subjects H211 and H448, considerable numbers (0.5-4% of total CD8+ T cells) of A2-SLY-specific T cells were observed with tetramers, but these cells were virtually undetectable in IFNγ Elispot. These A2-SLY tetramer positive cells, which follow similar dynamics as the B57-KAF tetramer positive cells, thus appeared to be nearly completely anergic. It is not a general phenomenon for HLA-B57 expressing individuals, as we did find A2-SLY-specific IFNγ production in PBMC from other HLA-B57+ study subjects (Fig. 3). In general, vaccination transiently increased and anti-retroviral therapy decreased the Gag-specific CD8+ T cell numbers, but did not alter the immunodominance of B57-KAF specific cells. Notably, tetramer staining and Elispot assays not always paralleled each other, indicating that cellular expansion and the capacity to produce IFNγ, at least in vitro, may be differentially regulated.

Figure 2. Longitudinal analysis of HIV- and EBV-specific CD8+ T cells in HLA-B57+ individuals. Upper panels show tetramer staining of B57-KAF (circles), A2-SLY (triangles), B8-EIY (squares) and B8-RAK (open diamonds) as the percentage of CD8+ T cells, middle panels indicate the IFNγ producing cells. The lower panels show CD4+ T cell numbers (open circles) and viral loads (filled circles). The time period in which individuals H211 and H448 were vaccinated with 0.1 or 1.0 mg of Gag VLP in 4-weekly intervals are indicated. Vertical dashed lines mark the start of anti-retroviral therapy (ART).
Increased responsiveness of B57-KAF specific T cells

Responsiveness of the different epitope-specific CD8+ T cells was further studied by varying the peptide concentration used for in vitro stimulation. Peptide concentrations ranging from 0.0001 to 10μg/ml were used to obtain dose-response relationships for IFNγ production by the tetramer positive T cell populations. Tetramer and intracellular cytokine staining were performed in the same samples and analysed simultaneously. Because peptide stimulation reduced tetramer staining, most likely due to downregulation of T cell receptor levels, IFNγ production was determined as the percentage of tetramer positive cells measured in the unstimulated sample. In figure 3A, a representative experiment is shown for B57-KAF and B8-EIY. The B57-KAF peptide shows a strong IFNγ response already at a concentration of 0.01μg/ml peptide and the maximum IFNγ response is 59% of the original amount of tetramer positive T cells. In contrast, 0.01μg/ml B8-EIY peptide elicited a relatively lower percentage of IFNγ+ T cells and the maximum IFNγ response reached only 39%. In a similar fashion, we determined the percentages of IFNγ producing T cells after stimulation with concentration ranges of the different peptides in PBMC obtained from 5 different HLA-B57 and HIV positive individuals (Fig. 3B). For subjects H211, H448, and H1113, samples from a time point before vaccination or therapy and in the same period as described in Fig. 2 were selected. Intracellular cytokine staining confirmed the absence of IFNγ responses against A2-SLY in subjects H211 and H448. We therefore identified two other study subjects, H433 and H1068, that did have measurable A2-SLY responses (> 0.5 % of total CD8+ cells) for these experiments. As documented before, 1 μg/ml of peptide was sufficient to induce optimal IFNγ production with all three peptides (189). Overall, higher percentages of IFNγ producing B57-KAF specific cells were detected over the entire peptide concentration range, as compared to A2-SLY or B8-EIY specific T cells. In addition, the peptide concentration required to reach half the maximum IFNγ response appeared to be lower for the KAF peptide than for EIY or SLY. These data firmly demonstrate that within individual subjects, the B57-KAF specific CD8+ T cells are more responsive to antigenic stimulation than the cells directed to Gag epitopes presented by other HLA alleles.

Discussion

HIV-specific CD8+ T cells are generally regarded as important suppressors of viral replication. Nonetheless, most individuals progress to AIDS despite high numbers of HIV-specific tetramer positive T cells (88,162,189). We and others have shown that in HIV infected individuals, HIV-specific or EBV-specific tetramer positive T cells vary in antigen responsiveness and that loss of function is associated with disease progression (185,189,192). The reason for the presence of antigen specific T cells that do not respond to antigenic stimulation in vitro remains unclear. Large expansions of hepatitis C virus (HCV)-specific CD8+ T cells in response to acute HCV infection have been reported to contain unresponsive or "stunned" cells. This phenomenon appeared to relate to a high exposure to antigen, resulting in expansion at the cost of functionality, and resolved after the successful control of viraemia (161). In addition to the magnitude of the antigenic stimulus, the CD4+ T cell compartment may determine the amount of antigen responsive, IFNγ producing CD8+ T cells in the total antigen specific cellular expansions (217). In healthy, CMV infected individuals, the fraction of IFNγ producing, but not the total tetramer positive CMV-specific CD8+ T cell population, correlated with the number of IFNγ producing CMV-specific CD4+ T cells (218). In a similar fashion, the variable percentages of IFNγ producing EBV-specific CD8+ T cells in HIV-infected and -uninfected individuals (Fig. 1) may depend on EBV-specific CD4+ T cells. In HIV infection, high viraemia and CD4+ T cell depletion may both contribute to the occurrence of unresponsive CD8+ T cells. To determine whether the antigen responsiveness of HIV-specific CD8+ T cells depends on the recognized epitope, we compared tetramer staining and peptide-induced IFNγ production for HLA-A2, -B8 or -B57 epitopes derived from the Gag protein. Significant differences in the capacity to produce IFNγ after in vitro stimulation were observed for the different epitope-specific T cell populations, both in PBMC from HLA-A2+, -B8+ or -B57+ individuals with similar CD4+ T cell counts and within the same PBMC sample, obtained from individuals expressing combinations of these HLA molecules. B57-KAF tetramer positive T cell populations contained the highest percentage of IFNγ producing cells after stimulation with optimal peptide concentrations (1 μg/ml). In addition, the B57-KAF specific T cells were more responsive at lower peptide
concentrations than B8-EIY or A2-SLY specific T cells from the same PBMC. These results demonstrate that T cells directed against different epitopes can have a different ability to produce IFNγ in response to antigen in vitro. Since the phenomenon was observed within the same PBMC and was independent from CD4+ T cell numbers and viral load, it appears to represent an intrinsic characteristic of the epitope-specific T cell populations. We found, however, no evidence for phenotypical differences based on CD27 expression, which distinguishes differentiated effector cells from naive and memory cells (48).

Figure 3. FACS analysis of tetramer staining and IFNγ responses after incubation with varying peptide concentrations. A. A representative experiment is shown for one sample obtained from an HLA-B57 and -B8 positive individual (1113). Responses to B57-KAF (left) and B8-EIY (right) were measured after 4 hours incubation with the indicated peptide concentrations or PMA/ionomycin. Left panels show IFNγ staining (y-axis) and CD8 staining (x-axis) on total lymphocytes. Percentages IFNγ+ within the total CD8+ T cells are indicated. Right panels show the same samples gated on CD8+ T cells, stained for tetramer (y-axis) and intracellular IFNγ (x-axis). Percentages of total tetramer positive cells are shown. B. IFNγ response curves for HIV-specific T cells in 5 individuals. The percentage of IFNγ+ T cells within the tetramer positive population are shown (y-axis), after stimulation with increasing concentrations of peptide (x-axis). B57-KAF responses are indicated by the filled circles, B8-EIY and A2-SLY responses by open circles and triangles, respectively. Peptide concentrations required for 50% maximum stimulation are indicated for B57-KAF (lowest numbers) and B8-EIY or A2-SLY.
The lower responsiveness at limiting peptide concentrations observed for B8-EiY and A2-Sly as compared to B57-Kaf may be the result of differences in peptide-MHC affinity. When the Kaf peptide has a high affinity to bind to surface MHC molecules in vivo, this could explain the low threshold of activation for IFNγ production by the B57-Kaf specific T cells. In vivo, such a high affinity peptide would result in immunodominant immune responses (219), as observed for the KAF-specific response. Alternatively, the HLA-B57 molecule carrying the KAF peptide could bind to the T cell receptor with a relatively high affinity, as compared to T cell receptor affinities for HLA-A2-Sly or HLA-B8-EiY. Either mechanism, or both, may determine the immunodominance and relatively preserved functionality of the B57-KAF response in the course of infection. It would enable CTL recognition of HIV-infected cells at low antigen dose, for example due to down-regulated HLA class I expression by HIV Nef (209), which could otherwise lead to escape from CTL recognition and cytolysis (220). In addition, it has been reported that CD8+ T cells specific for peptides with high MHC-binding affinity require less CD4+ T cell help to proliferate and differentiate (221). This may be especially relevant in HIV infection, where CD4 help is limited in the majority of patients (173,184). We have indeed recently observed that antigen unresponsiveness is particularly apparent in the presence of strongly reduced CD4+ T cell numbers (185). Especially in these situations, the presence of cells with a low activation threshold may be crucial for mounting an immune response.

HLA-B57 correlates with delayed disease progression (166,170,206,207), although the biological mechanism of this protective effect has not yet been clarified. The capacity of B57-KAF specific T cells to more readily and strongly respond to antigen may contribute to this protective effect. Indeed, among HLA-B57 individuals, immune responses of long-term asymptomatics (LTA) were considerably focussed to the KAF peptide compared to broader response to other epitopes of HLA-B57+ progressors (162). However, in the Amsterdam cohort HLA-B57+ LTA without significant levels of KAF-specific T cells could be identified (189). In these subjects, CD8+ T cell immunity against other epitopes was also low, despite stable CD4+ T cell numbers and low or undetectable viral load. They may represent a particular group of LTA with extremely good control of viral replication and therefore low CTL activity, which has also been described in another cohort (222). The protective effect of CTL may be enhanced by targeting epitopes which are unlikely to mutate because of functional constraints, as has been found for the KRWIIILGINK Gag epitope presented by HLA-B27 (204). The B57-KAF epitope may also represent an important structure in the p24 protein. No mutations of this sequence have been reported for clade B viruses in the HIV Sequence Database (Los Alamos National Laboratories, USA). The KAFSPEVIPMF sequence (aa 162-172 in the Gag sequence) forms a short loop in between the first two alpha helices in the p24 structure and is part of the second alpha helix (Molecular Modelling Database nr 5377-5379, National Center for Biotechnology Information, USA). The loop juxtapositions these two helices and places the second helix antiparallel to the sixth helix, in which the HLA-B27 epitope is contained. The B8-EiY sequence partially overlaps with the B27-KRW epitope and is also relatively conserved in clade B viruses. The B8-EiY response was however subdominant to the B57-KAF response, both in numbers and in functionality and may therefore contribute less to the control of the HIV virus. The A2-Sly peptide is part of a highly variable region of the p17 protein. Variant epitopes have been shown to act as antagonists for the CTL activity directed against the SLYNTVATL epitope (223). It is therefore conceivable that the SLYNTVATL peptide acts as an antagonist for epitope-specific CD8+ T cells from individuals originally infected with virus containing a variant peptide sequence. This would allow tetramer staining, but not peptide stimulation, as observed in two of the investigated subjects (Fig. 2).

The lack of IFNγ production of tetramer positive CD8+ T cells has been reported in several immunological settings, including anti-tumor immunity (215), acute HCV infection (161), chronic EBV (Fig.2) (191) and CMV (218) infection, AIDS related non-Hodgkin lymphoma (185), and HIV infection (189,192). T cells that are incapable of in vitro IFNγ production may reflect a subpopulation with specific functional properties or, especially when they contain the majority of antigen specific cells, a poor immune status. The latter hypothesis is supported by the observation that in chronic HIV-infection, high numbers of unresponsive T cells correlate with clinical disease progression (185,189). We have now reported that the antigen responsiveness of
T cells can depend on their epitope specificity. Finally, since the B57-KAF specific cells are present in high numbers, highly responsive to antigenic stimulation and directed against a conserved epitope, they may directly contribute to the protective effect of HLA-B57 in HIV infection.

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