Failing immune control as a result of impaired CD8\(^+\) T-cell maturation: CD27 might provide a clue

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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. Recently developed insights into human T-cell differentiation have been used to study the phenotype of virus-specific T cells in HIV-infected individuals. Based on these results, we propose that failing immune control in human viral infection could be a result of impaired cytotoxic T-lymphocyte (CTL) maturation into fully differentiated effector T cells. Impaired maturation is not confined to HIV-specific CD8\(^+\) T cells but could also be involved in failing immunity to Epstein-Barr virus and other viral infections. We postulate that CD27\(^+\) effector CD8\(^+\) T cells might be required for adequate control of chronic viral infection and prevention of disease development.

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With the discovery of a growing number of tumor-antigens and the identification of the immunodominant T-cell epitopes, tumor-specific CD8\(^+\) T cells have also been detected using tetramers. Melan A (MART-1) [17], MAGE (melanoma-associated gene) [18] or tyrosinase-specific T cells [19] in melanoma patients were present in relative high numbers [17,20]. However, the elicited and often strongly expanded T-cell populations cannot control tumor growth and several studies indicate that this loss of control might be as a result of a loss of function of tumour-specific T cells [17,20].

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Antigen-specific T-cell differentiation

CD45RA⁺CD28⁺CD27⁺
CCR7⁺
IFN-γ⁺ Grα perforin⁻

'Naive'

CD45RO⁺CD28⁻CD27⁺
CCR7⁻
IFN-γ⁺ Grα perforin⁻

'Memory'

CD45RO⁺CD28⁻CD27⁺
CCR7⁻
IFN-γ⁺ Grα perforin⁻

'Effector'

CD45RA⁺CD28⁻CD27⁺
CCR7⁻
IFN-γ⁺ Grα perforin⁻

S.L. Rowland-Jones, that impaired maturation of antigen-specific T cells could be a key factor explaining failing CTL control.

Models of human T-cell differentiation

Upon antigen recognition, CD8⁺ T cells differentiate from naive T cells into memory T cells that provide enhanced immunity upon re-infection or reactivation of viral replication, and effect T cells that through cytosis and secretion of specific cytokines directly contribute to the control of viral replication. In humans, a differentiation model has been proposed in which naive T cells develop via the memory stage to the effector stage. This gradual differentiation process ultimately leads to fully functionally matured effecter cells [22–24].

Based on CD45RO, CD27 and CD28 expression and analysis of the replicative history and clonality of the T-cell populations [25], the phenotypically distinct and sequential stages of CD8⁺ T-cell differentiation have been proposed [22] (Fig. 1). The expression pattern of CD27, in combination with CD45 and/or CD28 expression, reflects distinct stages (snapshots) of a gradual linear differentiation from a typical memory T cell (CD27⁺), with high proliferative capacity and low cytolytic activity, to a highly differentiated T cell (CD27⁻), with strong effector functions, such as direct cytolytic capacity due to high expression of effector molecules like perforin and granzymes [26]. Thus, functional development of memory T cells is paralleled by changes in CD27 expression patterns. Memory T cells with high effector function have been designated effector memory T cells [22,26]. This might not, however, be the appropriate term because in chronic viral infection they do not necessarily have an activated phenotype, as has been shown for effector T cells during acute viral infection [27,28].

Champagne et al. [29] proposed a differentiation model using a combination of the phenotypic markers CCR7 and CD45RA, which agrees with the linear differentiation scheme based on the combined expression of the CD45RA and CD27 antigens. The composite model of human CD8⁺ T-cell differentiation depicted in Figure 1 is largely compatible with most of the results reported [22,24,27,29,30] and is appropriate for the development of our current argument, even though the exact designation of the distinct phases of T-cell differentiation is still debated. Based on the functional properties and the expression regulation of CD27 versus CCR7, the use of CD27 as a differentiation marker is more suitable (Box 1).

Phenotype of antigen-specific CD8⁺ T cells: lack of maturation of HIV-specific T cells?

In healthy individuals, the majority of CMV-specific and EBV-specific CD8⁺ T cells appear to be of the memory phenotype [31–34] with co-expression of CD45RO and CD27. Initially, it was hypothesized...
Box 1. Differential regulation of CD27 and CCR7 expression in T-cell differentiation due to functional differences

CD28 and CD27 are costimulatory molecules, which provide signals needed for the correct activation of specific T cells after T-cell receptor ligation. After the interaction of CD27 and CD28 with their ligands, T cells will expand and become effector T cells with the ability to respond directly to pathogens [a]. Because these effector T cells do not need another signal from these molecules (they are already functionally fully matured), these molecules are downregulated. Downregulation of CD27 is irreversible [b] and relates to the differentiation status of antigen-specific T cells.

CCR7 is a homing molecule, therefore, CCR7 loss is related to altered migratory capacities [c] and CCR7 expression might, therefore, provide insight into homing potential, which is probably related but not identical to the cellular differentiation status. In addition, as expected from its function, CCR7 can be re-induced on CCR7− cells upon stimulation [d] (D. van Baarle et al., unpublished), to enable migration of the antigen activated T cells to lymph nodes. Reactivation, therefore, results in T cells with a central memory CCR7+ phenotype but with functional characteristics of full-blown effector cells with the corresponding CD27− phenotype.

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that during persistent viral infection, because of chronic antigenic activation, virus-specific CTLs would be preferentially enriched in the subpopulation of highly differentiated CD45RA CD27− CTLs [7]. Indeed, significant numbers of CMV-specific [35] and EBV-specific T cells [28] are of the CD27− phenotype. In addition, CMV-specific T cells capable of rapid induction of IFN-γ reside in a subset characterized by low expression of CD27 [32].

‘HIV-specific T cells could be immature in phenotype and function.’

Although HIV-specific CD8+ T cells do not express CD28 [34], the majority of HIV-specific T cells appear to be of the CD27− memory phenotype [7,35,28]. Moreover, we do not observe an enrichment of HIV-specific T cells in the highly differentiated CD27− subset during the course of HIV infection [7]. Interestingly, the CD27− HIV-specific T cells contain less perforin and have poor ex vivo killing compared to CMV-specific T cells from the same patients, which were enriched in the CD27+ phenotype [27,35]. These data indicated that HIV-specific T cells could be immature in phenotype and function. Similar findings have been reported by Champagne et al., comparing HIV-specific with CMV-specific T cells using CD45RA and CCR7 [29], and Lieberman et al., comparing HIV-specific with EBV-specific and CMV-specific T cells based on CD45RA expression alone [36].

In a prospective longitudinal study we observed that individuals co-infected with HIV and EBV persistently had low numbers of HIV-specific CD27− T cells, despite persistent active viral replication (>100,000 viral RNA copies/ml), which one would expect to drive HIV-specific T cells to the CD27− phenotype [37]. By contrast, EBV-specific CD27− CD8+ T cells accumulated over time [34] (Fig. 2) and showed, with respect to CD27 expression, a similar phenotype as has been reported for CMV-specific T cells in HIV-infected subjects [35]. In immunosuppressed individuals who had received a renal transplant, CMV-specific CD8+ T cells were also shown to be mainly of the effector phenotype (CD27−CCR7−RO−) [31].

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Viral specificity and CD8+ T-cell differentiation
From the results on the differentiation of HIV-specific, EBV-specific and CMV-specific CD8+ T cells, an alternative hypothesis has been put forward. Different chronic viral infections could induce CD8+ memory T cells with distinct phenotypes and functional properties [27] (Fig. 3a). Virus-specific memory T-cell differentiation would be a novel concept in immunology and, thus, should be considered seriously. Below, we present an overview of recent data that refutes this hypothesis and we will argue that it is more probable that differentiation of virus-specific CD8+ T cells is related to the clinical condition, explaining the experimental results presented.

HIV-specific CD8+ T-cell maturation is related to disease progression
Although, in the majority of HIV-infected individuals, most HIV-specific T cells are of the less differentiated CD27- phenotype (90–100%), in HIV-infected long-term asymptomatics, who have been able to control virus replication for a longer period, a relatively high proportion (>20%) of HIV-specific T cells are CD45RA-CD28+CD27+ cells [34]. In addition, HIV-specific T cells in these individuals respond better to antigenic stimuli than HIV-specific T cells in individuals that progressed to AIDS. This was demonstrated by elevated IFN-γ responses to in vitro stimulation with HIV peptides [10], and an increased frequency of responders within the CD27- fraction [34]. In addition, CD27- T cells contain more granzyme B and perforin and exert stronger direct cytolytic activity compared to CD27+ cells [26,35]. Also, CMV-specific T cells in HIV infection, which have a CD27- phenotype and are high in perforin content, have higher cytolytic capacity compared to HIV-specific T cells that have higher expression of CD27 and low perforin content [27,35]. These data suggest that HIV-specific CD8+ T cells that have differentiated to the CD27- stage are associated with delayed disease progression.

Lack of maturation of T cells specific for EBV: risk for AIDS-related non-Hodgkin lymphoma
EBV-specific T cells have impaired function in individuals progressing to HIV-related disease in HIV-infection, suggesting that functional impairment and possibly also impaired maturation is not confined to HIV-specific T cells and is related to disease development [16]. HIV-infected individuals failing to control EBV infection can develop AIDS-NHL [14]. Interestingly, their EBV-specific CD8+ T cells retained CD27 expression and apparently did not differentiate to effector cells during follow up, despite high levels of EBV load in peripheral blood mononuclear cells (PBMCs) [38]. Thus, a similar lack of differentiation to the CD27+ effector phenotype for EBV-specific T cells in AIDS-NHL patients has been observed with HIV-specific T cells in patients progressing to AIDS [34] (Fig. 2). These findings argue against the idea that EBV infection might induce T cells with a different phenotype compared to HIV or CMV. Our data show that, especially in individuals with EBV-related disease, the phenotype of EBV-specific CD8+ T cells appears to be different compared to that in asymptomatic subjects (Fig. 2).

EBV-specific T cells are tumor-specific T cells in these AIDS-NHL patients, therefore, the lack of EBV-specific CD8+ T cell maturation in individuals progressing to AIDS-NHL not only provides evidence that T cells directed against other viruses can have an immature phenotype but also indicates that tumor-specific T cells can be phenotypically and thereby functionally silenced.

Factors involved in T-cell differentiation
The factors that influence differentiation of virus CD8+ T cells are only partially known. It is plausible that initial viral burden during early viral dissemination and ongoing virus replication drive T cells to a highly differentiated (effector) phenotype. Indeed, the number of expanded specific T cells correlates with low expression of CD27 and high viral load in HTLV (human T lymphotropic virus) infection [39] and the highest percentage of tumor-specific effector T cells (CD27- or CCR7-) is observed in those individuals with the highest frequencies of tumor-(melanA) specific T cells [17] (J. Haenen and T.N. Schumacher, pers. commun.). Expansion is believed to be dependent on antigenic pressure [40]. However, despite high levels of HIV RNA load and enormous T-cell expansions, HIV-specific T cells do not completely differentiate to the CD27+ effector stage, suggesting that differentiation might not be dependent on antigen loads alone. Lieberman et al. recently
T cells will be induced. Depending on the induction of virus-specific CD4+ T cells and the magnitude of CD8+ T cell expansion (determined by antigen) a certain pool of CD27+ effector T cells will be established within the CD8+ T-cell population (Fig. 4). Our data suggest that the larger this CD27+ pool is, the lower the viral setpoint, which might result in a lower risk for progression to disease (Fig. 4), which is compatible with data from Doherty et al. [50]. In the course of HIV infection, CD4+ T cells are gradually lost, leading to a progressive loss of functional HIV-specific CD4+ and CD8+ T cells. Despite continuous antigenic triggering, CD8+ T cells do not fully mature to CD27+ effector T cells and cannot sufficiently control HIV infection any longer, leading to progression to AIDS. In more advanced stages of HIV infection, CD4+ T-cell help for other antiviral responses will also be gradually lost, leading to a lack of differentiation of EBV-specific T cells and a loss of EBV-specific CTL function and subsequent development of EBV-related diseases.

Fig. 4. Possible determinants of effective cytotoxic lymphocyte (CTL) induction. A model is depicted to discuss the potential factors influencing effective CTL induction. The quantity of antigen (small light pink circles) determines the pool size of CTLs (large circles). Antigen load together with the number of CD4+ T cells leads to a certain % of effector CTLs within the total number of CTLs (large purple circles). A higher level of antigen together with a good CD4+ T helper response leads to a higher number of effector CTLs, which subsequently leads to a lower viral setpoint.

The clinical condition of the individual studied is crucial and impaired maturation of virus-specific CD8+ T cells is a common feature during disease progression.

Based on the cumulative data, we postulate that different viruses do not induce distinct virus-specific memory T cells as suggested by Appay and Rowland-Jones. Differences in CD27 phenotype observed in CMV-specific and EBV-specific versus HIV-specific T cells could be largely explained by the HIV status of the host because reportedly these virus-specific T cells display less distinct phenotypes in healthy subjects [31–33,31]. In some instances, CMV-specific T cells have less CD27 expression in healthy individuals compared to EBV-specific T cells. This does not necessarily involve the type of virus but could be associated with the particular antigens studied [52] or the HLA background. EBV-specific T cells can become more differentiated in a situation of HIV-induced chronic antigen stimulation, therefore, the clinical condition of the individual studied is crucial and impaired maturation of virus-specific CD8+ T cells is a common feature during disease progression (Fig. 3).

Thus, impaired maturation of CD8+ T cells might not be specific for HIV but might be the case in other conditions with falling antiviral or anti-tumor immune control. Because virus-specific CD27+ T cells might be crucial for the control of chronic active viral infections further research should explore whether phenotypic analyses of virus-specific or tumor-specific CD8+ T cells prove valuable as a prognostic determinant and can be of use in the evaluation of viral or tumor vaccines.

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