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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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CD8⁺ MHC class I-restricted cytotoxic T-lymphocyte (CTL) responses are essential in controlling most non-lytic virus infections (reviewed in [1]). On recognition of viral antigens, CD8⁺ T cells are activated to kill virus-infected cells by excretion of perforin and granzymes or by Fas receptor triggering [2,3], or they inhibit viral replication by releasing cytokines, most notably interferon- γ (IFN- γ) [4].

Our knowledge of virus-specific T-cell populations has increased considerably with the introduction of MHC class I tetrameric complexes to specifically stain these MHC class I-restricted antigen-specific T cells [5]. In human chronic viral infections, such as HIV, Epstein-Barr virus [(EBV) a widespread, persistent γ -herpesvirus] and cytomegalovirus [(CMV) a widespread β -herpesvirus], high levels of antigen-specific CD8⁺ T cells are found [6–9] with frequencies of $\leq 10\%$ in chronic infection [10] and of $\leq 40\%$ in acute infection [8].

In healthy individuals, most chronic viral infections are adequately controlled by virus-specific T cells. By contrast, in HIV infection, despite the initial control of viral replication by HIV-specific T cells and a slow rate of clinical progression in some

individuals [11,12], eventually HIV-specific T cells become incapable of suppressing virus replication and preventing progression to AIDS [13]. In addition, HIV-infected individuals and other immunosuppressed individuals are at high risk for clinical complications involving other persistent viruses, such as EBV, the aetiological agent causing EBV⁺ AIDS-related non-Hodgkin's lymphoma (AIDS-NHL) and post-transplant lymphoproliferative disorders, and CMV, which causes CMV-disease and retinitis in immunocompromised subjects. Compelling evidence is now available that clinical complications might develop because of the progressive loss of virus-specific T-cell immunity [13,14]. For HIV-specific [15], as well as for EBV-specific T cells [16], functional loss of these CD8⁺ T cells is associated with progression to AIDS and AIDS-NHL, respectively.

'in different clinical settings, including antiviral immunity in HIV infection, antiviral immunity against persistent viruses in immunosuppressed situations and immunity to tumors, CTLs apparently fail to maintain control.'

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 tumor-specific T cells [17,20].

Thus, in different clinical settings, including antiviral immunity in HIV infection, antiviral immunity against persistent viruses in immunosuppressed situations and immunity to tumors, CTLs apparently fail to maintain control. This has been ascribed to viral escape mutations in HIV infection, which frustrate CD8⁺ T-cell activity (reviewed in [21]), however, most studies observed persistent high numbers of virus-specific T cells. This suggests that the corresponding T-cell epitopes remain to be recognized and do maintain the corresponding T-cell populations. Furthermore, for other viruses, like EBV or CMV, this explanation is less probable because these viruses do not have a high mutation frequency.

Therefore, we propose, based on cumulative findings from several laboratories, and in contrast to the accompanying Opinion by V. Appay and

Fig. 1. Differentiation scheme of CD8⁺ T cells using CD27, CD28, CCR7 and CD45RO/RA. Based on CD45RO, CD27, CD28 and CCR7 expression the following maturation pathway in CD8⁺ T-cell differentiation has been proposed [22]. Naïve CD8⁺ T cells express CD45RA. In addition, costimulatory receptors, like CD27 and CD28, are expressed and chemokine receptors, like CCR7, which potentiate migration to peripheral lymph nodes, are also expressed. Activation of unprimed CD8⁺ T cells results in the loss of CD45RA expression and the addition of CD45RO expression. The CD45RO⁺CD27⁺ memory T cells have high proliferative capacity but only gain cytolytic activity after restimulation *in vitro* [26] and still express CD28 and CCR7. After homing to the lymph nodes CCR7 will be downregulated. Chronic stimulation subsequently downregulates CD28 expression [53]. Furthermore, the CD27⁺ T cells irreversibly switch off CD27 expression when stimulated for prolonged periods either *in vitro* or *in vivo* [37]. Finally, these cells re-express CD45RA, through an as yet unknown mechanism, and acquire the CD45RA⁺RO⁻CD27⁻CD28⁻CCR7⁻ phenotype [22,25]. Recent data indicate that these cells can be induced to divide and re-express CD45RO again [24,30] (R.A.W. van Lier, pers. commun.). The readily detectable effector functions of CD27⁻ T cells, like high granzyme B expression and direct cytolytic activity are compatible with an important role for these cells in controlling virus replication. Also, populations of CD45RO⁺CD27⁻CD28⁺ and CD45RA⁺CD27⁻CD28⁻ T cells have been described [23,24]. Because it is unclear how these T-cell populations develop and what their functional properties are, future research should reveal the relevance of these T cells in the differentiation pathway. Abbreviations: Gr, granzyme; IFN, interferon.

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 naïve T cells into memory T cells that provide enhanced immunity upon re-infection or reactivation of viral replication, and effector T cells that through cytotoxicity and secretion of specific cytokines directly contribute to the control of virus replication. In humans, a differentiation model has been proposed in which naïve T cells develop via the memory stage to the effector stage. This gradual differentiation process ultimately leads to fully functionally matured effector 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 cytotoxic 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 although 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' 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 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.

References

- a Hamann, D. *et al.* (1997) Phenotypic and functional separation of memory and effector human CD8⁺ T cells. *J. Exp. Med.* 186, 1407–1418
- b Hintzen, R. *et al.* (1993) Regulation of CD27 expression on subsets of mature T lymphocytes. *J. Immunol.* 151, 2426–2435
- c Sallusto, F. *et al.* (1999) Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401, 708–712
- d Champagne, P. *et al.* (2001) Skewed maturation of memory HIV-specific CD8 T lymphocytes. *Nature* 410, 106–111

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].

Fig. 2. Lack of HIV-specific and Epstein-Barr virus (EBV)-specific CD27⁻ CD8⁺ T cells in HIV-infected individuals progressing to AIDS or AIDS-related non-Hodgkin lymphoma (NHL), respectively. Ratio CD27⁻:CD27⁺ CD8⁺ T cells in HIV-infected individuals early (within two years after study entry or seroconversion) and late (between six and eight years after entry or seroconversion) in HIV-1 infection for (a) HIV-specific (n=9) and EBV-specific (n=11) T cells and (b) EBV-specific T cells in two different groups of HIV-infected individuals: Long-term asymptomatic individuals and progressors to AIDS with opportunistic infections (n=6, for each patient two epitopes were tested) and progressors to AIDS-NHL (NHL, n=5, for three individuals two epitopes were tested and for two individuals one epitope was tested). A low CD27⁻:CD27⁺ ratio is observed for both HIV-specific T cells as well as EBV-specific T cells in AIDS-NHL patients, suggesting that impaired differentiation of virus-specific T cells is associated with disease progression. Abbreviations: LTA, long-term asymptomatic individual; PROG, progressor to AIDS. Adapted from [34] © (2002) Lippincott Williams and Wilkins.

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].

Fig. 3. Phenotypic enrichment in memory CD8⁺ T cells according to viral specificity or clinical course. Four main phenotypic subsets as well as their respective enrichment in memory virus-specific T cells are depicted according to (a) viral specificity, as proposed by Appay and Rowland-Jones and (b) according to HIV-induced clinical course, as proposed here. Phenotypes in brackets represent intermediate or minor populations. Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; HCV, hepatitis C virus.

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 seriously considered. 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 CD45RO⁺ 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 EBV-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 as 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 specific CD8⁺ T cells are only partially known. It is plausible that initial viral burden during early virus 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. Haanen 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 levels alone. Lieberman *et al.* recently

Fig. 4. Possible determinants of effective cytotoxic T-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 poolsize 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.

proposed that persistent high antigenemia might result in impairment of CD8⁺ T-cell function [41].

In addition to antigen, antigen-specific helper T cells and functional antigen-presenting cells (APCs) might have a role because CD4⁺ T cells have a major role in the maintenance of functional CD8⁺ T cells [42,43]. A crucial role for CD4⁺ T cells in the cellular immune response to immunization has been shown [44]. Progressive loss of CTLs in the absence of adequate helper-cell function has been demonstrated in several murine viral infections [45,46] and CD4⁺ T cells are needed for long-term persistence of adoptively transferred virus-specific CTLs in humans [47,48].

Thus, the failure of CD8⁺ T cells to function and mature properly in various clinical conditions related to HIV infection could be a consequence of impaired T-cell help or perturbed APC function. Most recently, interleukin-15 (IL-15), and to some extent IL-7, have been shown to be crucial for the expansion of CD8⁺ CTLs during primary infection and for the maintenance of CD8⁺ CTLs [49]. Lack of IL-15 production and restoration of T-cell responses by exogenous IL-15 in HIV infection has been reported and calls for further studies on the role of cytokine dysregulation in antigen-specific CD8 differentiation in human viral disease in general.

Conclusions

From the collective data reported, the following sequence of events related to the differentiation of virus-specific T cells in viral infection emerges. After encountering the virus, virus-specific CD4⁺ and CD8⁺

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.

'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 [31–33,51]. 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 failing 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.

References

- 1 Wodarz, D. (2002) The importance of lytic and nonlytic immune responses in viral infections. *Trends Immunol.* 23, 194–200
- 2 Riera, L. *et al.* (2000) Murine cytomegalovirus replication in salivary glands is controlled by both perforin and granzymes during acute infection. *Eur. J. Immunol.* 30, 1350–1355
- 3 Topham, D.J. *et al.* (1997) CD8⁺ T cells clear influenza virus by perforin or Fas-dependent processes. *J. Immunol.* 159, 5197–5200
- 4 Guidotti, L.G. *et al.* (1996) Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunity* 4, 25–36
- 5 Altman, J.D. *et al.* (1996) Phenotypic analysis of antigen-specific T lymphocytes. *Science* 274, 94–96
- 6 Ogg, G.S. *et al.* (1998) Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* 279, 2103–2106
- 7 Ogg, G. *et al.* (1999) Longitudinal phenotypic analysis of human immunodeficiency virus type 1-specific T lymphocytes: correlation with disease progression. *J. Virol.* 73, 9153–9160
- 8 Callan, M.F.C. *et al.* (1998) Direct visualization of antigen-specific CD8⁺ T cells during the primary immune response to Epstein-Barr virus *in vivo*. *J. Exp. Med.* 187, 1395–1402
- 9 Jin, X. *et al.* (2000) High frequency of cytomegalovirus-specific cytotoxic T-effector cells in HLA-A*0201-positive subjects during multiple viral coinfections. *J. Infect. Dis.* 181, 165–175
- 10 Kostense, S. *et al.* (2001) High viral burden in the presence of major HIV-specific CD8⁺ T-cell expansions: evidence for impaired T-cell effector function. *Eur. J. Immunol.* 31, 677–686
- 11 Rinaldo, C.R. *et al.* (1995) High levels of anti-human immunodeficiency virus type 1 (HIV-1) memory cytotoxic T-lymphocyte activity and low viral load are associated with lack of disease in HIV-1 infected long-term nonprogressors. *J. Virol.* 69, 5838–5842
- 12 Pontesilli, O. *et al.* (1998) Longitudinal analysis of human immunodeficiency virus type-1 specific cytotoxic T-lymphocyte responses: a predominant gag-specific response is associated with nonprogressive infection. *J. Infect. Dis.* 178, 1008–1018
- 13 Klein, M.R. *et al.* (1995) Kinetics of Gag-specific CTL responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long-term asymptomatics. *J. Exp. Med.* 181, 1365–1372
- 14 Kersten, M.J. *et al.* (1997) EBV-specific cytotoxic T-cell responses in HIV-1 infection: different kinetics in patients progressing to opportunistic infection or non-Hodgkin's lymphoma. *J. Clin. Invest.* 99, 1525–1533
- 15 Kostense, S. *et al.* (2002) Persistent numbers of tetramer⁺ CD8⁺ T cells but loss of interferon- γ HIV-specific T cells during progression to AIDS. *Blood* 99, 2505–2511
- 16 van Baarle, D. *et al.* (2001) Dysfunctional Epstein-Barr virus (EBV)-specific CD8⁺ T lymphocytes and increased EBV load in HIV-1 infected individuals progressing to AIDS-related non-Hodgkin's lymphoma. *Blood* 98, 146–155
- 17 Dunbar, P.R. *et al.* (2000) A shift in the phenotype of melan-A-specific CTL identifies melanoma patients with an active tumor-specific immune response. *J. Immunol.* 165, 6644–6652
- 18 Valmori, D. *et al.* (2001) Frequent cytolytic T-cell responses to peptide MAGE-A10(254–262) in melanoma. *Cancer Res.* 61, 509–512
- 19 Valmori, D. *et al.* (1999) Analysis of the cytolytic T-lymphocyte response of melanoma patients to the naturally HLA-A*0201-associated tyrosinase peptide 368–376. *Cancer Res.* 59, 4050–4055
- 20 Lee, P.P. *et al.* (1999) Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. *Nat. Med.* 5, 677–685
- 21 Klennerman, P. *et al.* (2002) HIV: current opinion in escapology. *Curr. Opin. Microbiol.* 5, 408–415
- 22 Hamann, D. *et al.* (1999) Faces and phases of human CD8⁺ T-cell development. *Immunol. Today* 20, 177–180
- 23 Tomiyama, H. *et al.* (2002) Differentiation of human CD8⁺ T cells from a memory to memory/effector phenotype. *J. Immunol.* 168, 5538–5550
- 24 Wills, M.R. *et al.* (2002) Identification of naïve or antigen-experienced human CD8⁺ T cells by expression of costimulation and chemokine receptors: analysis of the human cytomegalovirus-specific CD8⁺ T-cell response. *J. Immunol.* 168, 5455–5464
- 25 Hamann, D. *et al.* (1999) Evidence that human CD8⁺ CD45RA⁺ CD27⁺ cells are induced by antigen and evolve through extensive rounds of division. *Int. Immunol.* 11, 1027–1033
- 26 Hamann, D. *et al.* (1997) Phenotypic and functional separation of memory and effector human CD8⁺ T cells. *J. Exp. Med.* 186, 1407–1418
- 27 Appay, V. *et al.* (2002) Memory CD8⁺ T cells vary in differentiation phenotype in different persistent virus infections. *Nat. Med.* 8, 379–385
- 28 Roos, M.T. *et al.* (2000) Changes in the composition of circulating CD8⁺ T-cell subsets during acute Epstein-Barr virus and human immunodeficiency virus infection in man. *J. Infect. Dis.* 182, 451–458
- 29 Champagne, P. *et al.* (2001) Skewed maturation of memory HIV-specific CD8 T lymphocytes. *Nature* 410, 106–111
- 30 Dunne, P.J. *et al.* (2002) Epstein-Barr virus-specific CD8(+) T cells that re-express CD45RA are apoptosis-resistant memory cells that retain replicative potential. *Blood* 100, 933–940
- 31 Gamadia, L. *et al.* (2001) Differentiation of cytomegalovirus-specific CD8⁺ T cells in healthy an immunosuppressed virus carriers. *Blood* 98, 754–761
- 32 Gillespie, G.M. *et al.* (2000) Functional heterogeneity and high frequencies of cytomegalovirus-specific CD8(+) T lymphocytes in healthy seropositive donors. *J. Virol.* 74, 8140–8150
- 33 Hislop, A.D. *et al.* (2001) EBV-specific CD8⁺ T-cell memory: relationships between epitope specificity, cell phenotype and immediate effector function. *J. Immunol.* 167, 2019–2029
- 34 van Baarle, D. *et al.* (2002) Lack of Epstein-Barr virus (EBV)- and HIV-specific CD27⁺ CD8⁺ T cells is associated with progression to viral disease in HIV-infection. *AIDS* 16, 2001–2011
- 35 Appay, V. *et al.* (2000) HIV-specific CD8(+) T cells produce antiviral cytokines but are impaired in cytolytic function. *J. Exp. Med.* 192, 63–76
- 36 Chen, G. *et al.* (2001) CD8 T cells specific for human immunodeficiency virus, Epstein-Barr virus and cytomegalovirus lack molecules for homing to lymphoid sites of infection. *Blood* 98, 156–164
- 37 Hintzen, R. *et al.* (1993) Regulation of CD27 expression on subsets of mature T lymphocytes. *J. Immunol.* 151, 2426–2435
- 38 van Baarle, D. *et al.* (2002) Absolute level of Epstein-Barr virus DNA in human immunodeficiency virus Type 1 infection is not predictive of AIDS-related non-Hodgkin lymphoma. *J. Infect. Dis.* 186, 405–409
- 39 Nagai, M. *et al.* (2001) Increased activated human T-cell lymphotropic virus type I (HTLV-I) Tax11–19-specific memory and effector CD8⁺ cells in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis: correlation with HTLV-I provirus load. *J. Infect. Dis.* 183, 197–205
- 40 Ahmed, R. and Gray, D. (1996) Immunological memory and protective immunity: understanding their relation. *Science* 272, 54–60
- 41 Lieberman, J. *et al.* (2002) Avoiding the kiss of death: how HIV and other chronic viruses survive. *Curr. Opin. Immunol.* 14, 478–486
- 42 Matloubian, M. *et al.* (1994) CD4⁺ T cells are required to sustain CD8⁺ cytotoxic T-cell responses during chronic viral infection. *J. Virol.* 68, 8056–8063
- 43 Battagay, M. *et al.* (1994) Enhanced establishment of a virus carrier state in adult CD4⁺ T-cell deficient mice. *J. Virol.* 68, 4700–4704
- 44 von Herrath, M.G. *et al.* (1996) CD4-deficient mice have reduced levels of memory cytotoxic T lymphocytes after immunization and show diminished resistance to subsequent virus challenge. *J. Virol.* 70, 1072–1079
- 45 Cardin, R.D. *et al.* (1996) Progressive loss of the CD8⁺ T cell-mediated control of a γ -herpesvirus in the absence of CD4⁺ T cells. *J. Exp. Med.* 184, 863–871
- 46 Hasenkrug, K.J. *et al.* (1998) Critical role for CD4(+) T cells in controlling retrovirus replication and spread in persistently infected mice. *J. Virol.* 72, 6559–6564
- 47 Rooney, C.M. *et al.* (1998) Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. *Blood* 92, 1549–1555
- 48 Brodie, S.J. *et al.* (1999) *In vivo* migration and function of transferred HIV-1-specific cytotoxic T cells. *Nat. Med.* 5, 34–41
- 49 Becker, T.C. *et al.* (2002) Interleukin-15 is required for proliferative renewal of virus-specific memory CD8 T cells. *J. Exp. Med.* 195, 1541–1548
- 50 Doherty, P.C. and Christensen, J.P. (2000) Accessing complexity: the dynamics of virus-specific T-cell responses. *Annu. Rev. Immunol.* 18, 561–592
- 51 Catalina, M.D. *et al.* (2002) Phenotypic and functional heterogeneity of EBV epitope-specific CD8⁺ T cells. *J. Immunol.* 168, 4184–4191
- 52 Hislop, A.D. *et al.* (2002) Epitope-specific evolution of human CD8⁺ T-cell responses from primary to persistent phases of Epstein-Barr virus infection. *J. Exp. Med.* 195, 893–905
- 53 Azuma, M. *et al.* (1993) CD28⁺ T lymphocytes. antigenic and functional properties. *J. Immunol.* 151, 1147–1159