Cytomegalovirus (CMV)-specific CD8+ T cells in individuals with HIV infection: correlation with protection from CMV disease

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CD8+ cytotoxic T cells play a key role in immunological protection from clinical cytomegalovirus (CMV) disease. Numbers of CMV-specific CD8+ T cells are increased in untreated and antiretroviral-treated HIV patients compared with healthy controls. Accumulation of CMV-specific CD8+ T cells during HIV infection may reflect persistent reactivation of CMV owing to suboptimal immune control and/or oligoclonal expansion of the limited populations of CMV-specific CD8+ T cells present before antiretroviral therapy (ART). CD8+ T cells directed against the CMV immediate early (IE)-1 protein may play an important role in preventing CMV replication to pathogenic levels. However, immunological protection from CMV disease in HIV-infected individuals on ART does not appear to depend on total numbers of CMV-specific CD8+ T cells but rather on the presence of both effector-memory and effector CMV-specific CD8+ T cells that produce interferon-γ and/or perforin in response to CMV antigens.

Keywords: pp65, immediate early 1, perforin, interferon-γ

Introduction

Cytomegalovirus (CMV) is a human double-stranded DNA 230 kb β-herpesvirus that produces more than 200 proteins in three overlapping phases [immediate early (IE), early and late]. After primary infection, CMV is not eliminated from the host but remains in a latent state within granulocyte, macrophage and dendritic cell precursors.1,2 CD8+ cytotoxic T cells play a key role in immunological protection from clinical CMV disease, as delayed reconstitution of CMV-specific CD8+ T cells has been correlated with CMV disease in allogeneic bone marrow transplant recipients.3

Several methods are available to measure the number, diversity, differentiation and function of CMV-specific CD8+ T cells. These are predominantly flow cytometry techniques such as measurement of major histocompatibility complex class I–peptide tetramer binding, intracellular cytokine and perforin/granzyme expression in response to peptides or vaccinia virus constructs expressing individual viral genes,4 proliferation by dilution of the dye carboxyfluorescein succinimidyl ester5 and degranulation assays (CD107).6 ELISpot assays can also be used to measure cytokine production at the single cell level.

In healthy CMV-seropositive individuals, CD8+ T cell responses are directed towards multiple CMV antigens, predominantly pp65 and IE1, but also other structural, early/late antigens and immunomodulators (pp28, pp50, pp150, IE2 gH, gB, US2, US3, US6 and UL18).4,7,8 In individuals who carry HLA-A*02, NLVPMVATV (NLV) from pp65 and VLEETSVML (VLE) from IE1 have been identified as immunodominant epitopes for CD8+ T cell recognition.9,10 CMV can reactivate intermittently in healthy individuals,11 and the frequency and function of CMV-specific CD8+ T cells vary considerably among and within healthy subjects over time.12 Numbers of CMV-specific CD8+ T cells increase with age10 and CMV-seropositive individuals ≥65 years of age have increased numbers of CD28–CD45RA– effector cytotoxic T cells with reduced proliferative capacity in vitro compared with CMV-seronegative individuals of the same age.13 Factors involved in CMV latency and reactivation are not completely understood, but impairment of cell-mediated immunity, by immunosuppressive drug therapy in transplant recipients or infection with HIV, can result in reactivation of CMV and clinical disease.

Approximately 95% of HIV-infected individuals are infected with CMV. The introduction of combination antiretroviral therapy (ART) has reduced the incidence of clinical CMV end organ disease (EOD) in HIV-infected individuals. HIV patients receiving ART who are unable to control CMV replication have an increased risk of HIV disease progression and death,14 though not all HIV-infected individuals with CMV reactivation progress to EOD.15 Numbers of CMV-specific CD8+ T cells increase over

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time in untreated HIV patients, regardless of whether they progress to CMV EOD or remain asymptomatic. Numbers of CMV-specific CD8+ T cells also remain elevated in HIV patients on ART compared with healthy controls. The mechanisms underlying increased CMV-specific CD8+ T cell counts during HIV infection and the factors that determine the differentiation, diversity and antigenic repertoire of CMV-specific CD8+ T cells required to minimize CMV replication and provide protection from CMV disease remain largely unknown.

Expansion of CMV-specific CD8+ T cells during HIV infection

Numbers of CMV-specific CD8+ T cells increase in untreated and ART-treated HIV patients compared with healthy controls. Although CD8+ T cell responses are elicited against several CMV antigens, the proteins pp65 (structural) and IE1 (involved in viral reactivation) are major targets. Increased percentages of IE1-specific CD8+ T cells have been observed in HIV patients on ART compared with healthy controls, whereas percentages of pp65-specific CD8+ T cells were similar. As IE1 proteins are expressed before pp65 during CMV reactivation, increased numbers of IE1-specific CD8+ T cells may indicate persistent low-level CMV replication. Therefore, CMV reactivation may be occurring more frequently in immune reconstituted HIV patients than CMV-infected individuals without HIV infection. This may reflect persistent suboptimal immune protection in HIV patients who are severely immunodeficient before receiving ART. Detection of low-level, intermittent CMV reactivation in HIV-infected individuals may require sensitive PCR-based assays.

An alternative explanation for the increased populations of IE1-specific CD8+ T cells observed in HIV patients on ART is oligoclonal expansion of the limited populations of CMV-specific CD8+ T cells present prior to the commencement of therapy. The CD8+ T cell repertoire becomes less diverse during chronic HIV infection owing to reduced thymic output and the accumulation of memory CD8+ T cells expanded clonally as a consequence of prolonged antigenic stimulation. Long-term ART may not increase the diversity of T cell repertoires in HIV patients despite sustained suppression of HIV replication. The extent to which the CD8+ T cell repertoire is perturbed in HIV patients on ART may depend on the duration and severity of immunodeficiency before ART and on the ability of the thymus to replenish the naïve CD8+ T cell population.

Protection CD8+ T cell responses against CMV

In HIV patients, protection from clinical CMV disease does not correlate with CMV DNA levels, pp65 antigenaemia, CMV-specific IgG antibody titres, CMV-specific CD4+ T cell lymphoproliferation, anti-CMV NK-cell activity or numbers of CMV-specific CD8+ T cells. A recent study identified positive CMV-specific interferon (IFN)-γ ELISPOT assay results as a marker of protective immunity against CMV viraemia and EOD in HIV patients after ART. There is also increasing evidence that the antigenic repertoire, diversity and function of CMV-specific CD8+ T cells may predict patients at risk of CMV disease.

Antigenic repertoire and diversity

HIV-infected individuals who control CMV infection on ART, either spontaneously or after recovery from acute CMV EOD, develop a broader repertoire of CMV-specific CD8+ T cells against pp65 and IE1 than do HIV-negative subjects and severely immunodeficient patients who are unable to control CMV infection. Restoration of protective immunity to CMV in HIV patients with previous CMV EOD receiving ART is focused on IE1 in the first 2 years of treatment but switches towards pp65 after 5 years on ART, and becomes similar to the CMV-specific immunity seen in long-term non-progressors. In contrast, IE1-specific CD8+ T cells were still dominant after 4 years of ART in HIV patients with nadir CD4+ T cell counts <50 cells/mm³, most of whom had not had CMV EOD. High frequencies of IE1- but not pp65-specific CD8+ T cells also correlated with protection from CMV disease in a cohort of 27 heart and lung transplant recipients. Therefore, high numbers of IE1-specific CD8+ T cells in immunocompromised individuals with high rates of CMV reactivation might prevent the development of CMV disease. However, it is unclear whether and when the dominance of IE1-specific CD8+ T cells declines. In addition, the presence and potential protective role of CD8+ T cells reactive against CMV proteins other than IE1 and pp65 have not been assessed in HIV patients on ART.

Differentiation and function

Two prototypic types of CMV-specific CD8+ T cells can be found in latently infected individuals: CD8+CD27+CD28+CCR7- effector-memory cells that do not abundantly express perforin or granzyme B and CD8+CD27−CD28−CCR7- effector cells that express both perforin and granzyme B and execute direct ex vivo cytolyis. In HIV patients on ART, CMV-specific CD8+ T cells with an effector phenotype predominate. These cells may contribute to the maintenance of latency by directly killing virus-expressing cells and generating abundant effector T cells in situations of virus reactivation or reinfection. During acute CMV EOD, CMV-specific effector-memory CD8+ T cells are almost undetectable. However, during long-term recovery from CMV EOD (>5 years on ART), numbers of CMV-specific effector-memory CD8+ T cells were significantly elevated, suggesting that the presence of both effector-memory and effector CD8+ T cells are important for responding to periodic CMV reactivation and hence for protection from clinical disease.

The most important antiviral effector functions of CD8+ T cells are secretion of antiviral cytokines (IFN-γ, macrophage inflammatory protein-1β and tumour necrosis factor-α) and lysis of virus-infected cells through secretion of perforin and granzymes. In untreated HIV patients, increases in the CMV DNA level correlate with increasing CMV-specific CD8+ T cell counts and percentages of CMV-specific CD8+ T cells expressing perforin and granzyme B, but with decreasing percentages of CMV-specific CD8+ T cells producing IFN-γ. Therefore, CMV-specific CD8+ T cells producing IFN-γ, rather than perforin or granzyme B, appear to restrict CMV replication during untreated HIV infection. Similar results have been observed in HIV-positive children, where low numbers of CMV-specific IFN-γ-producing CD8+ T cells were associated with an inability to suppress CMV replication completely in 41% of subjects. This was observed despite increases in CMV-specific IgG antibody titres and higher numbers of CD8+ effector T cells on ART.
CD8+ T cell IFN-γ responses to ex vivo stimulation with overlapping peptide pools that span pp65 and IE1 also correlated with clinical evidence of protective immunity in HIV patients on ART. However, a significantly higher proportion of IE1-specific CD8+ T cells expressed perforin than pp65-specific CD8+ T cells in HIV patients on ART. Therefore, perforin-expressing IE1-specific CD8+ T cells may also play a role in preventing CMV replication to pathogenic levels in HIV patients receiving ART.

In summary, immunological protection from CMV disease in HIV-infected individuals on ART is enhanced by the presence of CD8+ T cells that (i) are directed against the IE1 protein, (ii) are from all stages of differentiation (both effector-memory and effector T cells) and (iii) produce IFN-γ and perforin.

Potential clinical applications

Current guidelines indicate that ART may be deferred in asymptomatic HIV patients until their CD4+ T cell count declines to 200 cells/mm³. Commencement of ART before the CD4+ T cell count declines to 200 cells/mm³ prevents the development of CMV EOD, but the effect this level of immunodeficiency might have on control of CMV replication is not known. One concern is that CD8+ T cell clonality could increase as a result of suboptimal control of CMV replication and compound the increased clonality of old age, which could impair immune competence. This concern will become more relevant as the longevity of HIV patients increases. Knowledge of the characteristics of CMV-specific CD8+ T cells that provide protection from CMV EOD and potentially limit CMV replication, and improvement of methods to quantify these cells, may therefore be used as an additional guide on when to commence ART. Similarly, analysis of CMV-specific CD8+ T cells might also be considered in the decision to cease maintenance therapy for CMV infection in HIV patients undergoing immune reconstitution on ART, thus reducing the cost and toxicity associated with prophylaxis. In addition, it is unknown whether some anti-CMV agents facilitate reconstitution of protective CMV-specific CD8+ T cell subsets better than others. Finally, a small proportion of HIV patients receiving ART do not restore protective CMV-specific T cell responses even though CD4+ T cell counts are increased. At present, the extent to which impaired CMV-specific CD8+ T cell responses contribute to this problem is unclear, but this could now be investigated with a view to using adoptive allogeneic cellular immunotherapy from CMV-infected donors.

Transparency declarations

None to declare.

References


