The avid competitors of memory inflation

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ABSTRACT:

Cytomegaloviruses (CMV) coevolve with their hosts and latently persist in the vast majority of adult mammals. Therefore, persistent T-cell responses to CMV antigens during virus latency offer a fascinating perspective on the evolution of the T-cell repertoire in natural settings. We addressed here the life-long interactions between CMV antigens presented on MHC-I molecules and the CD8 T-cell response. We present the mechanistic evidence from the murine model of CMV infection and put it in context of clinical laboratory results. We will highlight the remarkable parallels in T-cell responses between the two biological systems, and focus in particular on memory inflation as a result of competitive processes, both between viral antigenic peptides and between T-cell receptors on the host's cytotoxic lymphocytes.

Introduction

Cytomegalovirus (CMV) is the paradigmatic exponent of the ß-subfamily of herpesviruses. CMV is a globally ubiquitous herpesvirus with a prevalence about 50% in most developed countries and 80% or higher in developing ones [1]. While the primary infection in most immunocompetent hosts is mild or asymptomatic, CMV may cause diverse clinical conditions in the susceptible host. Infections that occur during pregnancy are the most common infectious cause of severe and lasting damage to the developing embryo, including deafness, blindness and learning disabilities [2]. Additionally, CMV may cause life-threatening disease in immunocompromised adults, such as transplant recipients or AIDS patients [3]. A life-long latent persistence in the infected host is a defining characteristic of herpesviruses. Latency is defined as a dormant state, in which the virus maintains minimum gene expression, but can reactivate its lytic replication cycle and cause disease. If the immune system of a host is suppressed, either naturally or iatrogenically, CMV may reactivate, causing interstitial pneumonia, gastroenteritis, transplant rejections or graft versus host disease [3]. The control of CMV infection is mediated by adaptive Immune responses, and in particular the T cell response. Namely, transfer of CMV specific T-cells to immunocompromised patients was sufficient to control the virus and subdue clinical symptoms in numerous studies [4-6]. On the other hand, a vaccine against CMV is still not available [7], despite numerous efforts [8]. In this review, we would like to summarize the latest inputs regarding the dynamics of T cell responses upon CMV infection, and the forces shaping the expansion of T-cell clones in competition with each other.

T cell responses upon CMV infection in human and mice

The human cytomegalovirus (HCMV) induces a T-cell response that is sustained for life and formidably strong [9,10]. In HCMV seropositive healthy people, the fraction of HCMV specific cells may exceed 10% of the memory compartments [10], indicating that a strong T-cell response may be necessary to prevent virus reactivation. Therefore, it is reasonable to assume that only a vaccine that elicits robust T-cell responses in combination with humoral immunity might provide sufficient immune protection against HCMV. Most of the approved vaccines induce antibody responses, which target the pathogens in the intercellular space and not within infected cells. However, some viruses, including HCMV, can also spread between cells through cell-cell contact without ever leaving a cell [11]. Therefore, antibody responses against HCMV may not be enough, because only a vaccine that efficiently targets HCMV in infected cells by cellular immunity would provide sufficient immune control of HCMV. In that case, understanding what is required for the induction of the uniquely strong T-cell responses against HCMV would be crucial for the successful development of any HCMV vaccine.

The mechanistic study of T-cell responses, their dynamics and cause-effect relationships requires an experimental model. Unfortunately, HCMV cannot be studied directly in an animal host, because its replication is restricted to human cells due to millions of years of coevolution. Therefore, CMV infection has been experimentally studied by analyzing the infection of animals with coevolving CMV species that naturally infect them [12]. The mouse cytomegalovirus (MCMV) model of infection is a well-established system that has been used for decades to study the mechanisms of the immune response to CMV infection [13,14]. Functional and phenotypical similarities of the T-cell response to HCMV and MCMV have been documented in numerous studies. For instance, CMV-specific CD8 T cell populations in humans and mice show an expansion of two main primed T cell subsets, effector and central memory cells [15], during the acute and latent infection [16,17]. Both HCMV and MCMV specific CD8 T cells display a terminally

differentiated effector phenotype in latently infected hosts [18,19], characterized by a loss of CD62L, CD27 and CD28 from the cell surface with a concomitant expression of KLRG1 [19-22]. Likewise, in both HCMV and MCMV infection, these effector cells retain some functionality, as they are able to respond to antigen restimulation by cytokine secretion [21,23] and thus they are clearly distinguishable from exhausted T-cells induced by other chronic infections [24]. Finally, CMV-specific effector T cells show poor proliferative responses upon restimulation, and this also is a shared trait of mice and men [20,25]. In conclusion, the CD8 T cells induced by MCMV show sufficient similarities to the HCMV situation to warrant the use of the MCMV model to study the mechanisms of T-cell induction and maintenance upon CMV infection.

Phenotype of CMV specific T cell responses

Memory inflation is an ongoing accumulation of antigen-specific CD8 T cells upon CMV infection [26-28], which was first identified in the lungs of latently infected mice [18], and later documented by dynamic follow-up of T-cell responses in the peripheral blood [29]. Importantly only some MCMV-specific cells are inflationary, whereas other ones induce conventional responses that contract upon the resolution of the acute infection [17,21]. The difference in the long-term outcome of these two subsets is not only a difference in population size, but also in the expression pattern of surface receptors, cytokines and transcription factors [30]. While the inflationary and the conventional T-cells show distinct outcomes in the long-term, the initial responses are remarkably similar. Upon naïve cell priming, both the inflationary and the non-inflationary cells expand to form populations of short-lived effectors and memory-precursor effector cells, which both contribute to virus clearance [21,31,32]. It is only after the resolution of the primary infection, that the differences between inflationary and conventional cells become obvious. The population of conventional T cells contracts, leading to the generation of a small pool of antigen specific cells with the classical central-memory phenotype ($CD62L^{hi}$, $CCR7^{hi}$, $Bcl\text{-}2^{hi}$, $CD27^{hi}$, and $KLRG1^{lo}$). On the other hand, the inflationary cells retain a spectrum of phenotypes, from the central-memory, via transitional ones (e.g. $CD62L^{lo}CD27^{hi}$) to terminally differentiated short-lived effector cells ($CD62L^{lo}$, $CD27^{lo}$, $CD28^{lo}$, $Bcl2^{lo}$, $CD127^{lo}$, $KLRG1^{hi}$), where the more mature subsets are increasingly more abundant (Figure 1). A similar distribution of CD27 and CD28 phenotypes is also observed in CD8 T cells recognizing immunodominant HCMV antigens in healthy people [33] or in transplant recipients [34]. Likewise, CD8 T cells against defined immunodominant HCMV epitopes are more abundant with age [9,35], although the broad response to HCMV peptide pools did not show this increase [36]. The early dynamics of T-cell responses to HCMV remain largely unknown due to the lack of specific symptoms, but the available evidence points to substantial overlaps in the phenotype, function and dynamics of cellular immune responses to MCMV and HCMV in the chronic phase. Therefore, the mechanistic insights from the mouse model may inform us about the dynamics and the maintenance of human T cell response to HCMV.

Role of antigen competition during memory inflation

The overall size of inflationary responses is determined by inoculum size during primary infection [37] and robust virus replication during primary infection results in a larger latent MCMV load [38]. Furthermore, non-hematopoietic cells are a major site of MCMV latency [32,39] and memory inflation, as opposed to the primary response, depends on antigen that is presented on non-hematopoietic cells [40,41]. Taken together, the evidence strongly suggests that antigens from latently infected cells fuels the memory inflation [42,43]. Memory inflation is strongly influenced by a plethora of factors, including cytokine signaling or the balance between cell proliferation and survival, just to name a few, but for reasons of space and focus, this review will specifically

contemplate the effects of pMHC-TCR interaction. This interaction is key to understand memory inflation, which is not an overall ongoing expansion of CMV specific T cells, but rather the expansion of T-cells recognizing a few optimal antigenic peptides at the expense of other, subdominant ones [44,45]. Competition for antigen between inflationary CD8+ T cells upon MCMV infection has been described in several studies. Infecting mice with recombinant MCMV viruses encoding immunodominant exogenous epitopes leads to strong inflationary responses against these epitopes and a reduction of inflationary T cells recognizing endogenous MCMV antigens [46-48]. Likewise, MCMVs with mutated immunodominant peptides induce stronger T cell responses against subdominant epitopes or responses to novel epitopes that are not immunogenic in wild-type MCMV infection [49,50]. However, antigen competition does not occur in all experimental systems. A recombinant MCMV expressing the immunodominant epitope SIINFEKL did not reduce memory inflation of CD8+ T cells specific for endogenous MCMV epitopes when used in coinfection with MCMV WT (lacking the SIINFEKL epitope) [48]. This observation argues that competition for T cell responses occurs only if antigens are present in the same virus genome, and likely in the same latently infected cell, where the most robust antigenic peptides become the agents of memory inflation.

So what exactly determines the robustness of an antigen in the induction of memory inflation? The epitope's availability for processing by the constitutive proteasome is a key requirement for memory inflation [51,52], likely because the latently infected non-hematopoietic cells do not express the immunoproteasome, unless activated by interferons. Another major factor is the binding affinity of T-cell receptors (TCR) to peptide-MHC complexes (pMHC) on target cells, evidenced by stronger inflationary responses to high-affinity than to low-affinity epitopes, if expressed at the exactly same position [53]. These effects are intuitive. A pMHC that is firmly bound by the cognate TCR will induce T-cells more efficiently at each binding than an epitope that TCRs rapidly disengage from. Finally, the context of gene expression defines the size of responses, because the same epitope induces stronger inflationary responses when expressed in the context on an immediate-early (IE) gene, than in the context of genes expressed later in the virus cycle [47,51,53]. The early gene expression as a determinant of immunodominance is likely a reflection of peptide abundance on pMHC. IE transcripts are more common during latency [54]. Therefore, T-cells recognizing an IE epitope will be engaged more frequently, which will select them over antigen specific T cells recognizing epitopes expressed in early or late genes.

Role of TCR competition during memory inflation

Numerous host factors determine the size of the T-cell response as well. TCR diversity is a result of somatic recombination and selection during T-cell maturation in the thymus [55]. Therefore, clonally different CD8 T cells may still recognize the same pMHC complex, albeit with different affinities of binding. Early upon infection, a polyclonal TCR repertoire is recruited, in part to the same epitope. The frequency of naïve precursor T cells defines the hierarchy of primary CD8 responses to MCMV, but it does not determine which epitopes become inflationary later on [56]. Therefore, memory inflation depends on factors that occur after priming. A robust response of early primed KLRG1^{lo} cells defines the size of the inflationary T cell pool [31], as these cells generate a robust pool of memory cells, which later feed the inflationary population [20,57]. Multiple clonotypes simultaneously undergo Ag-driven proliferation during latent MCMV infection, resulting in stable CD8+ T cell repertoires, which appear to be dominated by persistent clonotypes [58]. However, a more detailed analysis of responding T-cells identifies a highly dynamic evolution of the responding repertoires based on TCR avidity. TCRs with higher affinity of binding prevail

over those with lower affinity of response in the initial months post infection [31,59], because a higher affinity leads to higher functional avidity of responses. Therefore, cells that react to less peptide are naturally selected to proliferate over those that need more target peptides to become activated. However, long-term monitoring of inflationary T-cell responses at single cell level paints a different picture. Over time, the lower avidity cells expand in the population, whereas high-avidity T cells assume more mature phenotypes [59]. It has been proposed that this is due to an earlier onset of terminal differentiation and senescence of inflationary T-cell clones with high-avidity responses [59]. Therefore, the long-term outcome of memory inflation is a reverse evolution of the repertoire, first towards a higher affinity repertoire, and then away from it [59,60].

It is intriguing that the concept of reverse evolution is also supported by some observations of clinical samples, where large populations of inflationary T-cells in a patient are typically characterized by low avidity responses, whereas people with smaller populations of T-cells recognizing the same epitope have typically higher avidities of responding cells [59]. On the other hand, the emergence of high-affinity TCR clones against an immunodominant HCMV epitope has been described to occur specifically in older people, contrasting the idea of reverse evolution in clinical conditions [61]. Likewise, functional avidity of dominant TCR clones was in general higher than the avidity of subdominant clones in another study [62]. Therefore, we would like to propose that the low avidity of large responder populations does not necessarily reflect reverse evolution of low-avidity T cells, because it may also be a reflection of viral peptides competing for T-cell attention. In this scenario, in a patient with an HLA-I haplotype that provides a definite advantage to T-cells recognizing one HCMV peptide and little competition against other populations, these cells will strongly expand, and even TCRs with lower avidities will be recruited to the inflationary pool. However, if another patient has an HLA-I constellation where the same epitope can be recognized, but needs to compete for inflationary responses with co-dominant epitopes, only T-cells clones with higher affinity of TCR binding to pMHC will be selected. Therefore, the overall population of responding cells to this epitope will be smaller, but will consist of T cells with higher affinity TCRs (Figure 2). The result would then be an inverse correlation of TCR avidity and inflationary population size, in line with the clinical observation [59]. While this has not been formally shown in an experimental system, the second scenario may provide an additional explanation for the clinically observed phenomenon.

Conclusion

While CMV infection alters permanently the composition of the T-cell compartment in a latently infected host [45], the effects on its functionality remain remarkably modest [63,64] . This phenomenon is likely a reflection of virus-host coevolution, where the presence of a latent CMV may even result in improved immune function [65,66]. Therefore, the complex dynamics of T-cell responses to this virus may serve to inform us on requirements for a healthy immune response and persistence of functional antigen specific cells. This is not merely a question of academic interest. Understanding such requirements would pave the way to the design of better vaccines, which may optimally induce cellular immune responses and T-cell memory. Whether CMV should be the vector used in such approaches [67] is an open question, but the tremendous advances of our understanding of T cell immunity achieved by studying CMV infection remain indisputable.

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The authors declare that they have no conflicts of interest.

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Figure 1

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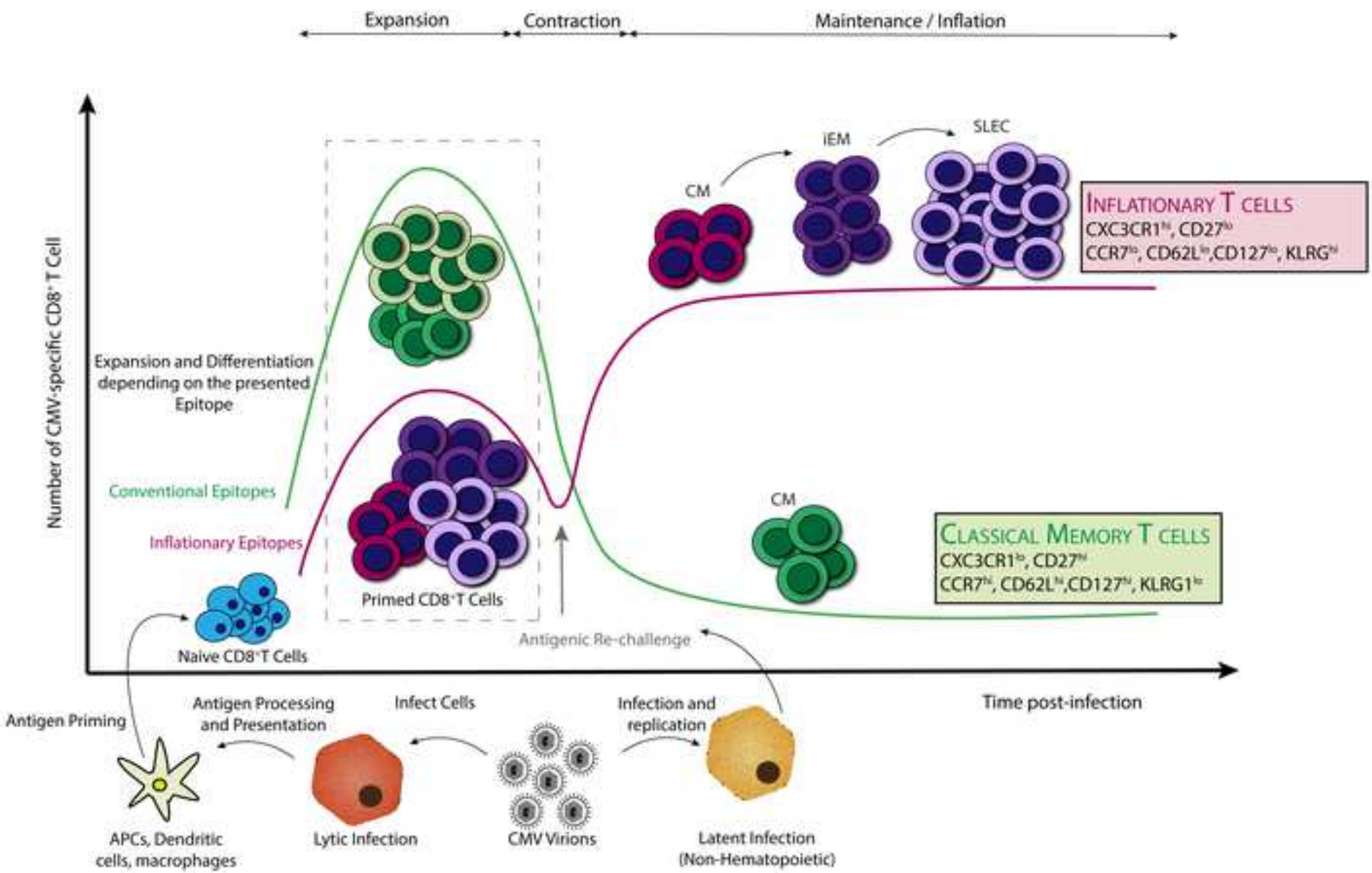
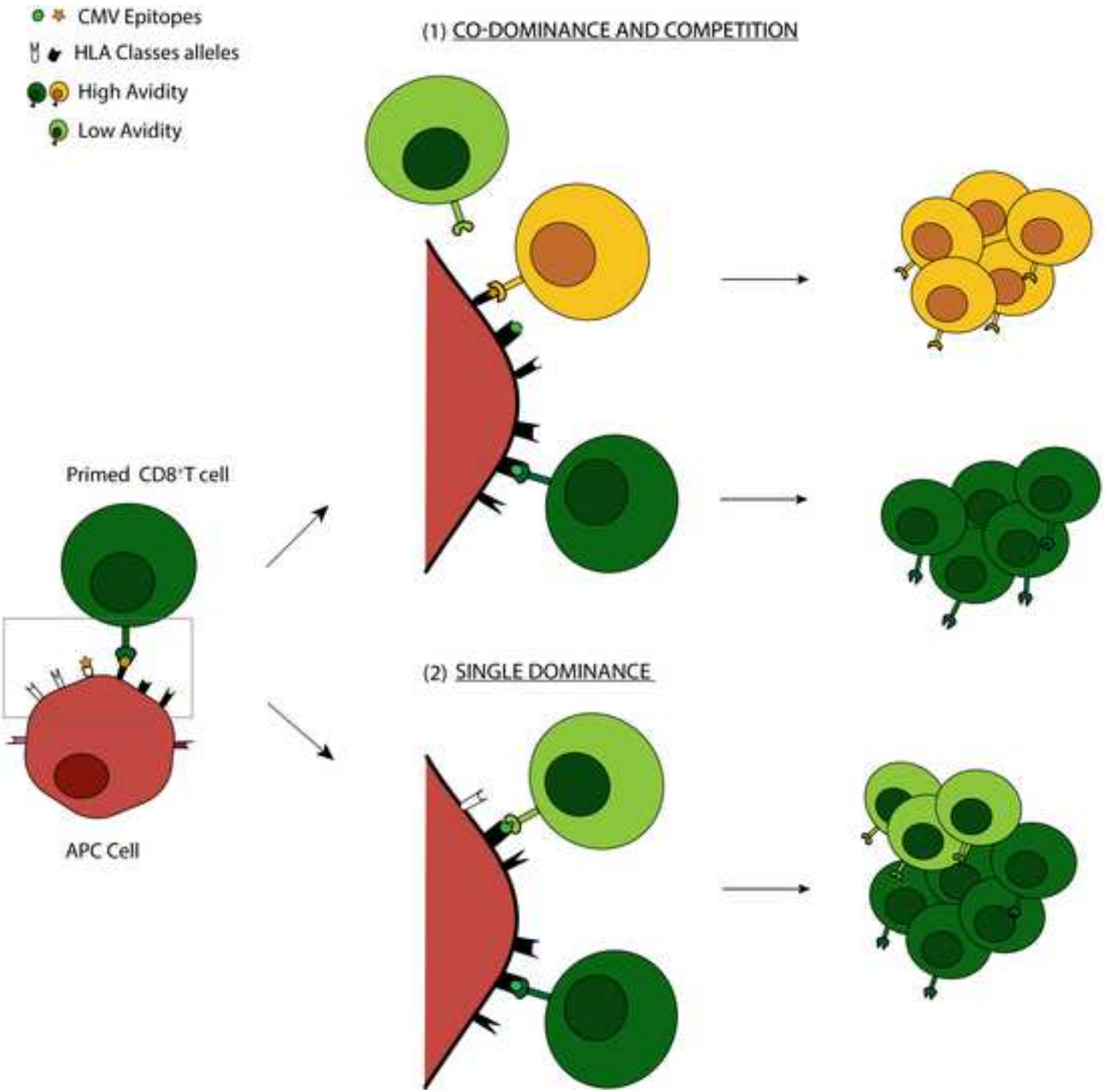


Figure 2



We added to the reference list the descriptions to the referenced literature indicated by one or two stars.