

Asymptomatic memory CD8⁺ T cells

From development and regulation to consideration for human vaccines and immunotherapeutics

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Generation and maintenance of high quantity and quality memory CD8⁺ T cells determine the level of protection from viral, bacterial, and parasitic re-infections, and hence constitutes a primary goal for T cell epitope-based human vaccines and immunotherapeutics. Phenotypically and functionally characterizing memory CD8⁺ T cells that provide protection against herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2) infections, which cause blinding ocular herpes, genital herpes, and oro-facial herpes, is critical for better vaccine design. We have recently categorized 2 new major sub-populations of memory symptomatic and asymptomatic CD8⁺ T cells based on their phenotype, protective vs. pathogenic function, and anatomical locations. In this report we are discussing a new direction in developing T cell-based human herpes vaccines and immunotherapeutics based on the emerging new concept of “symptomatic and asymptomatic memory CD8⁺ T cells.”

Introduction

Following repeated infections, various populations of memory CD8⁺ T cells develop and might be separated into major sub-populations that differ in their phenotype, effector functions, proliferative capacity, anatomical locations, and long-term fates.^{1–4} However, the continuous evolution of the CD8⁺ T cell population over time following an infection often makes it difficult to track the developmental lineage of memory CD8⁺ T cells. Several research groups are presently studying the mechanisms of establishment, persistence, and regulation of local memory CD8⁺ T cells (i.e., tissue resident memory CD8⁺ T cells [T_{RM}]) and effector memory CD8⁺ T cells [T_{EM}]). Local T_{RM} and T_{EM} cells reside within infected mucocutaneous tissues and are relatively important, compared with central memory CD8⁺ T cells (T_{CM}), in providing immediate protection against subsequent re-infection and reactivation.⁵ However, until our recent work on HSV-1

and HSV-2-specific memory CD8⁺ T cells,⁶ the segregation of memory CD8⁺ T cells into asymptomatic (ASYMP) memory CD8⁺ T cell sub-population (i.e., those memory CD8⁺ T cells that actually lead to protection against herpes) and symptomatic (SYMP) memory CD8⁺ T cell sub-populations (i.e., those memory CD8⁺ T cells that, in contrast, lead to immunopathology and perhaps even lead to the exacerbation of herpetic disease) has not been reported.

Ocular HSV-1 infection is the leading cause of viral induced corneal blindness in the United States.^{6–16} Genital HSV-2 infection is among the most common sexually transmitted viral infections.^{8,9,17–19} The prevalence of HSV seropositive adults, 15 y and older, is estimated to be at least 61 million within the United States, and over half billion worldwide.^{20–22} After the initial viral exposure, HSV-1 and HSV-2 replicate in mucosal epithelial cells causing primary clinical inflammatory manifestations characterized by painful mucocutaneous vaginal blisters, corneal disease, and oro-facial herpes (cold sore).^{23–29} Newly infected seronegative pregnant women can vertically transmit the virus to their newborns, causing encephalitis and death.^{19,30,31} Once the primary infection is cleared the HSV establishes life-long latency in sensory neurons of dorsal and trigeminal ganglia.^{32,33} Several factors, including stress, elevated body temperature, UV exposure, and hormonal changes trigger HSV-1 and HSV-2 reactivation from latently infected sensory neurons. The reactivated virus travels back and re-infects the primary site of infection resulting in recurrent painful inflammatory lesions.³² The infection process and the therapeutics currently used against HSV-1 and HSV-2 replication and fusion have been recently reviewed.³⁴ Infected individuals must rely on sustained or intermittent antiviral drugs (Acyclovir and derivatives), sexual behavior education and barrier methods to limit the spread of HSV-1 and HSV-2.^{35,36} The effectiveness of antiviral therapy is sometimes limited by the development of antiviral resistance. Controlling the spread of herpes through vaccine or immunotherapeutic approaches still remains a challenge.^{18,22,37–39} It is generally known that: (1) Natural immunity does not efficiently protect against new or recurrent herpes infection or disease,^{38,40–45} suggesting that an effective vaccine will have to induce a more vigorous immune response than sub-optimal natural immunity. (2) The immune

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system causes the majority of mucocutaneous herpetic lesions.⁴⁶ (3) Recurrent herpetic corneal disease is mediated by T cells⁴⁷⁻⁵² and in mice CD4⁺ and CD8⁺ effector T cells can either protect against or potentiate herpetic corneal disease.⁵³⁻⁵⁶ (4) Inducing local HSV-specific CD8⁺ T cell immunity at the sites of infection (i.e., the ocular, oral, and vaginal muco-cutaneous tissues, and sensory ganglia) or in the draining lymph node is necessary to prevent virus transmission/reactivation and/or limit the severity of ocular herpes.^{16,57-60}

This review: (1) describes our novel concept of “development of symptomatic/asymptomatic CD8⁺ T cell sub-populations”; (2) describes the spatial and temporal position of the new symptomatic and asymptomatic memory CD8⁺ T cell sub-populations within the widely known T_{EM}⁺, T_{RM}⁺, and T_{CM}⁺ CD8⁺ T cell populations; (3) sheds the light on the mechanisms of induction, establishment, persistence, and regulation of symptomatic and asymptomatic memory CD8⁺ T cells in ocular, oral, and genital herpes infection models; and (4) discusses how the new “symptomatic/asymptomatic CD8⁺ T cell sub-populations” concept provides new immunological insights and a new direction for the design of efficient and safe T cell-based human vaccines and immunotherapeutics.

Heterogeneity of Symptomatic and Asymptomatic Memory CD8⁺ T Cells

After the clearance of pathogens, the vast majority (often as much as 90 to 95%) of effector CD8⁺ T cells die, leaving behind only 5 to 10% of effector CD8⁺ T cells to differentiate into a heterogeneous pool of memory CD8⁺ T cells.⁶¹⁻⁶⁵ The level of heterogeneity of memory CD8⁺ T cells is an important aspect that has come to light over the past few years. Tracking the developmental lineage of CD8⁺ T cells between effector and memory stages is complicated by the continuously evolving memory CD8⁺ T cell sub-populations that develop long after the infections are cleared.⁶⁶⁻⁷³ These diverse populations of memory CD8⁺ T cells can at times be categorized into sub-populations that differ in their phenotype, effector functions, proliferative capacity, anatomical locations, and long-term fates.^{61,74}

Phenotypic markers

Several phenotypic markers are used to separate diverse sub-populations of memory CD8⁺ T cells include: CD62L, IL-7R (CD127), CD69, CD11a, CCR5, CCR7, and CD103⁺ (known as $\alpha 4\beta 7$ integrin) (anatomical localization), IL-2/IFN- γ /TNF- α , perforin, granzymes A/B/C/K, programmed death-1 (PD-1) (effector functions/dysfunction), Bcl-2, CD122, CD28, CD57, CD27, KLRG1, CXCR3, and CD43 (survival and/or proliferative capacity).⁷⁵⁻⁷⁷ Various factors affect the expression of these markers, including: (1) the type and duration of infection; (2) inflammatory cytokines; (3) antigen specificity; (4) naïve T cell precursor frequency; and (5) anatomic location within the host's body.⁹ Thus, CD8⁺ T cells may exhibit a range of differentiated phenotypes spanning from IL7R^{low}KLRG1^{high} short-lived effector cells (SLECs) to IL7R^{high}KLRG1^{low} memory precursor effector

cells (MPECs) that are capable of generating long-lived memory CD8⁺ T cells,^{71,78-80} T_{CM}⁺, T_{EM}⁺, and T_{RM}⁺ cells.^{81,82}

In addition to the 5 factors above, we recently found that symptomatic and asymptomatic epitope stimulations are among the major factors that affect the development of memory CD8⁺ T cell sub-populations and hence, they define the fate of antigen specific symptomatic and asymptomatic memory CD8⁺ T cells post-infection. For instance, following intravaginal infection with HSV-1 or HSV-2, asymptomatic epitopes appear to preferentially induce effector memory CD8⁺ T_{EM}⁺ cells (Fig. 1). These asymptomatic CD8⁺ T_{EM}⁺ cells are destined to survive and become long-lived CD8⁺ T cells that reside within the vaginal mucosal tissues, which are the sites of recurrent infections and disease (Fig. 1). In contrast, symptomatic epitopes appear to preferentially induce central memory symptomatic CD8⁺ T cells that are not destined to survive within the mucosal tissues, but rather reside in the lymphoid organs (Fig. 1).

Functional assays

Functional assays, such as cytokine production, are combined with IL7R (CD127) and KLRG1 phenotyping to assist discrimination between MPECs and SLECs subsets. For instance, the IL-6 receptors is used to help distinguish memory precursor cell subsets, as the IL-6R α ^{high}IL7R^{high} pre-memory CD8⁺ T cells survive after priming and contribute to the majority of functional memory CD8⁺ T cells after the contraction phase.⁸³ Compared with mono-functional CD8⁺ T cells that only produce one cytokine; poly-functional CD8⁺ T cells produce IFN- γ , TNF- α , and IL-2 and preferentially survive and develop into MPECs. While MPECs can give rise to long-lived T_{CM}⁺, some SLECs can give rise to a terminally differentiated population of T_{EM}⁺ cells.^{71,84,85} We recently showed that induction of polyfunctional CD8⁺ T cells is more protective against ocular and genital herpes than mono-functional CD8⁺ T cells.^{61,17} These poly-functional CD8⁺ T cells appeared to be a differentiated population of T_{EM}⁺ cells. It remains to be determined whether symptomatic and asymptomatic epitope stimulation can lead to vaginal mucosa (VM)-resident CD8⁺ T cells differentiation into MPECs or SLECs.

Lectins and integrins

Lectins and integrins also contribute to heterogeneity of memory CD8⁺ T cells in the mucocutaneous tissues.^{17,86} High levels of $\alpha 4\beta 7$ integrin (CD103) are expressed on mucosal tissue resident memory CD8⁺ T cells. CD103 binds to epithelial cadherin (E-Cadherin) expressed by epithelial cells. We found that compared with CD8⁺ T cells from HSV-seropositive symptomatic individuals, CD8⁺ T cells from HSV-seropositive asymptomatic individuals express high levels of CD103 (CD103^{high}) (Fig. 1). Thus, CD103 and E-Cadherin interaction is crucial for retention of asymptomatic memory CD8⁺ T_{EM}⁺ cells in the epithelium. The rapid upregulation of CD103 on asymptomatic CD8⁺ T_{RM}⁺/T_{EM}⁺ cells is mediated by TGF- β , which plays a critical role during T_{RM}⁺/T_{EM}⁺ differentiation and rapid control of infection in barrier tissues, such as skin and mucosa.^{87,88} We are currently investigating the underlying mechanisms of retention of asymptomatic memory CD8⁺ T_{RM}⁺/T_{EM}⁺ cells within the ocular and vaginal mucosal tissues as well as the possible regulation by TGF- β of

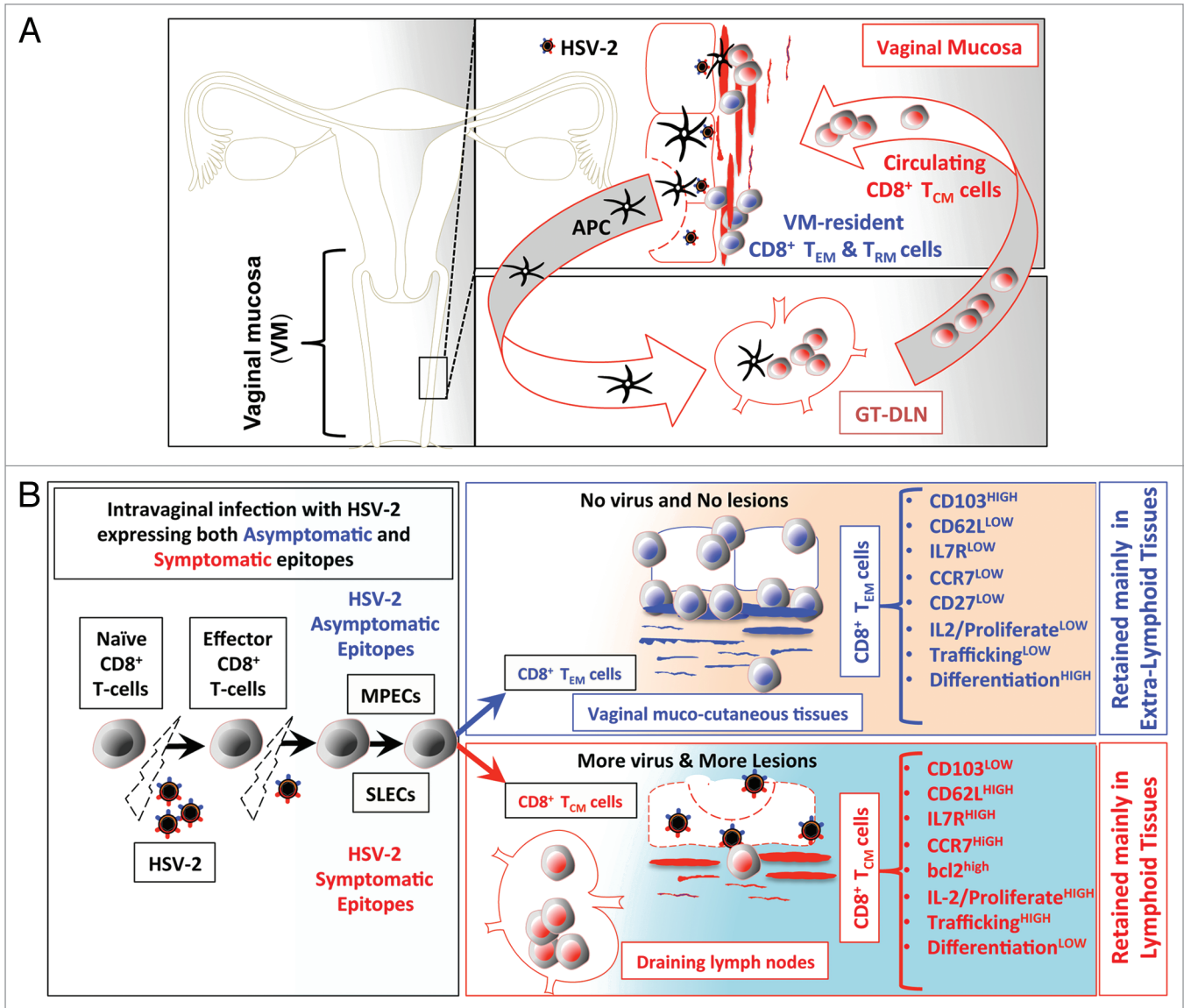


Figure 1. (A) Following intravaginal infection with HSV-2; stimulation with pathogenic symptomatic and protective asymptomatic human epitopes, expressed by the virus, greatly contributes to development of HSV-specific memory CD8⁺ T cell subsets, with either VM-resident effector memory (T_{EM}) and tissue-resident memory (T_{RM}) or lymphoid resident central memory CD8⁺ T cells (T_{CM}). (See text for details). We hypothesize that virus derived symptomatic epitopes boost primarily CD8⁺ T_{CM} that reside primarily in the secondary lymphoid tissues, such as GT-DLN, which, once re-activated, re-circulate through the bloodstream into the vaginal mucosa (VM). In contrast, virus derived asymptomatic epitopes boost primarily VM-resident CD8⁺ T_{EM}/T_{RM} that are retained in the mucosal site of viral entry. **(B).** Anatomic-distribution, phenotypic, and functional characteristics of HSV-specific symptomatic central memory CD8⁺ T cells (T_{CM}) vs. asymptomatic effector memory (T_{EM}) CD8⁺ T-cells induced following intravaginal infection with HSV-2. Intra-vaginal (VAG) infection with HSV-2 bearing both symptomatic or asymptomatic epitopes prime naïve T cells into effector T cells to clear the virus infection while simultaneously generating memory CD8⁺ T cells to protect against subsequent encounter with the virus following re-infection or reactivation from latently infected sensory ganglia. We hypothesize that after the clearance of the virus, there is an optimum equilibrium between SLECs (IL7R^{high}KLRG1^{low}) and MPECs (IL7R^{high}KLRG1^{low}) cells derived from an early effector cells (EECs, IL7R^{low}KLRG1^{low}), which then transit to 2 main categories of memory CD8⁺ T cells: T_{CM} CD8⁺ T cells specific to symptomatic epitopes and T_{EM} CD8⁺ T cells specific to asymptomatic epitopes. T_{EM} CD8⁺ T-cells are mainly retained in the vaginal mucosa (see text for detail).

CD103 expression on ocular and vaginal tissue-resident HSV-specific memory CD8⁺ T_{RM}/T_{EM} cells.

Cytokines

The progression of the anti-viral CD8⁺ T cell response is controlled by cytokine milieu.⁸⁹⁻⁹⁴ The decision of an effector CD8⁺ T cell to develop into SLECs or MPECs appears to be regulated by the amount of inflammatory cytokines (i.e., IL-6, IL-8, and

IL-12) present during T cell priming.⁷¹ Memory T cell survival depends on IL-15, however, this alone is not sufficient for long-term maintenance of these cells.^{69,71,95} The reliance of CD8⁺ T cell expansion on inflammatory cytokines (IL-12, IFN- α/β , and IFN- γ) or on IL-2, depends on the tissues in which they reside.⁹⁵⁻⁹⁹ In fact, a handful of inflammatory cytokines have been found to influence CD8⁺ T cell transition from the effector

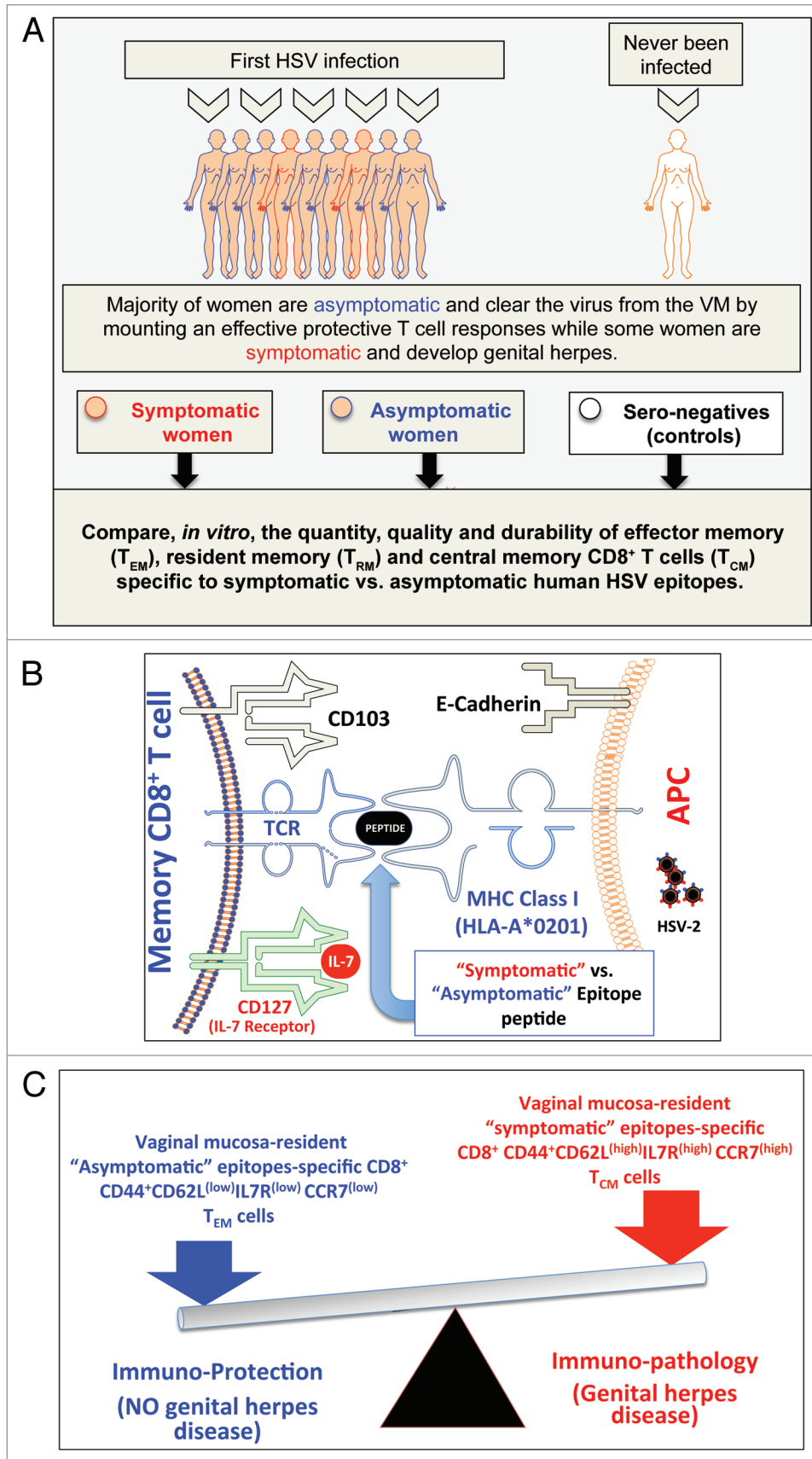


Figure 2. For figure legend, see page 949.

Figure 2. A proposed model of phenotypic and functional characteristics of HSV-specific CD8⁺ T cells in HSV-seropositive symptomatic vs. asymptomatic individuals: **(A)** Majority of women are asymptomatic and clear the virus from the VM by mounting a protective T cell responses while some women are symptomatic and develop genital herpes. Significant differences in the quantity, the quality and the durability of effector memory (T_{EM}), tissue-resident memory (T_{RM}) and central memory (T_{CM}) CD8⁺ T cells specific to symptomatic vs. asymptomatic human HSV-2 epitopes are detected in symptomatic and asymptomatic women. **(B)** Potential interactions between an APC, presenting an asymptomatic vs. symptomatic epitope, (right) and a memory CD8⁺ T cell (left) occur in HSV-2 infected vaginal mucosa, and appear to be mediated by TCR/MHC, CD103/E-Cadherin, and CD127-IL7 pathways. **(C)** The balance between the asymptomatic vs. symptomatic epitopes will determine herpes protection vs. immunopathology.

state to the memory state.⁹ TGF-β also affects differentiation of memory T cells within the mucosa.¹⁰⁰ The mechanisms by which cytokine milieu controls the heterogeneity of symptomatic and asymptomatic memory CD8⁺ T cell subsets remain to be fully elucidated.

Intrinsic (transcription) factors

Many survival and transcriptional factors are involved in the regulation of effector state to memory state transition.^{71,101} These include Bcl2, Eomes, Tcf1, Blimp1, Id2, Id3, and T-bet.¹⁰² T-bet is a key lineage-determining transcription factor that controls the fate SLECs/MPECs during infection.^{68,71,103} Depending on the amount of inflammation, a gradient of T-bet is created in which high T-bet promotes SLECs and low T-bet promotes MPECs.^{71,104} The control of intrinsic (transcription) factors in the heterogeneity of symptomatic and asymptomatic memory CD8⁺ T cells also remain to be elucidated.

Anatomical locations

Anatomical locations play a crucial role in the distribution of memory CD8⁺ T cell subsets in different organs. Mucosal surfaces, that constitute an impressive and powerful first-line of defense system that is frequently exposed to an array of exogenous antigens and pathogens, contain mainly CD8⁺ T_{RM}/T_{EM} cells.^{6,9} The mucosal immune system is largely separate and distinct from the systemic immune system. Several infectious diseases with the highest rates of morbidity and mortality begin primarily as local infections at one of the mucosal barrier sites. CD8⁺ T_{RM}/T_{EM} cells from muco-cutaneous tissues, such as the skin or mucosal lining of the respiratory tract, gut, and genital tract, defend against pathogen invasion. As illustrated in **Figures 1 and 2**, with respect to symptomatic/asymptomatic memory CD8⁺ T cells, most asymptomatic CD8⁺ T cells appear to reside in non-lymphoid mucosal tissues. Additionally, they have a decreased ability to traffic to lymphoid tissues and appear more terminally differentiated due to a lower proliferation capacity in response to Ag or homeostatic cytokines (IL-15 and IL-7).^{95,105-107} In contrast, most symptomatic CD8⁺ T cells that are traditionally defined by the presence of CD62L and/or CCR7 lymph-node-directing molecules and low expression of chemokine receptors, such as CCR5, appear to be associated with homing to inflammatory sites.¹⁰⁸

Establishing HSV-Specific Asymptomatic Memory CD8⁺ T Cell Responses at the Mucosal Tissues

Based on T cell circulation, tissues are characterized into 3 classes: permissive tissues, effector permissive tissues and restrictive tissues.⁹

Permissive tissues

Permissive tissues are readily accessible by both effector and CD8⁺ T_{EM} cells, even in the absence of local inflammation or antigens. These constitute the spleen, lung, liver, kidney, and adipose tissue.¹⁰⁹⁻¹¹¹

Effector permissive tissues

Effector permissive tissues are attainable by effector CD8⁺ T cells, but not by asymptomatic memory CD8⁺ T_{EM} and T_{CM} cells. These tissues are generally colonized by CD8⁺ T cells early during the effector phase and consist of the gut, brain, and peritoneal cavity. Though the presence of antigens or inflammatory factors may enhance recruitment, migration of effector CD8⁺ T cells to these tissues does not require direct infection. These tissues also become inaccessible to circulating symptomatic memory CD8⁺ T cells after resolution of infection.^{72,112-114}

Restrictive tissues

Restrictive tissues are inaccessible by either effector or memory CD8⁺ T cells at steady-state of infection and are only accessible to effector CD8⁺ T cells when there is a local inflammation. These tissues include the skin epidermis, vaginal epithelial layer, salivary glands, lung airways, cornea, and the sensory ganglia—all of which lack tissue tropic chemokines or adhesion molecules. Thus, systemic and local immunization at distal sites may often result in a diminished level of access for effector T cells into restrictive tissues.^{72,112-114}

Many infections, including HSV-1 and HSV-2, occur at the ocular, oral and vaginal mucosal tissues.^{17,18,115,116} At the steady-state, the mucosal epithelial layer and the sub-mucosa are protected by innate leukocytes and lymphocytes. The restrictive mucosal tissues are inaccessible to effector and memory CD8⁺ T cells at a steady-state but can be reached by effector CD8⁺ T cells when there is a local inflammation induced, for example, by primary or recurrent herpes infection.¹¹⁵⁻¹¹⁷ Thus, the female genital tract is an immunologically restrictive tissue that normally prevents entry of activated T cells in the absence of inflammation or infection.¹¹⁵ Recruitment of HSV-specific CD8⁺ T cells to the vaginal mucosal (VM) tissues is therefore thought to be restricted because effector and memory CD8⁺ T cells does not circulate through the genital tract.¹¹⁵⁻¹¹⁷ However, while the VM tissue is considered mainly an effector site for CD8⁺ T cells, recent data demonstrates that the VM tissue may also be an inductive site for CD8⁺ T cells (Wang Y., AAI 2013, Abstract #3186). CD8⁺ T cells persist in the VM tissue at the dermal-epidermal junction (DEJ)—the portal of release of viral particles that reactivate from latently infected neurons of sensory ganglia—for prolonged periods of time after herpes lesions are cleared.^{118,119} However, the mechanisms by which vaginal DEJ-resident memory CD8⁺ T cells are generated and maintained remain to be fully elucidated.

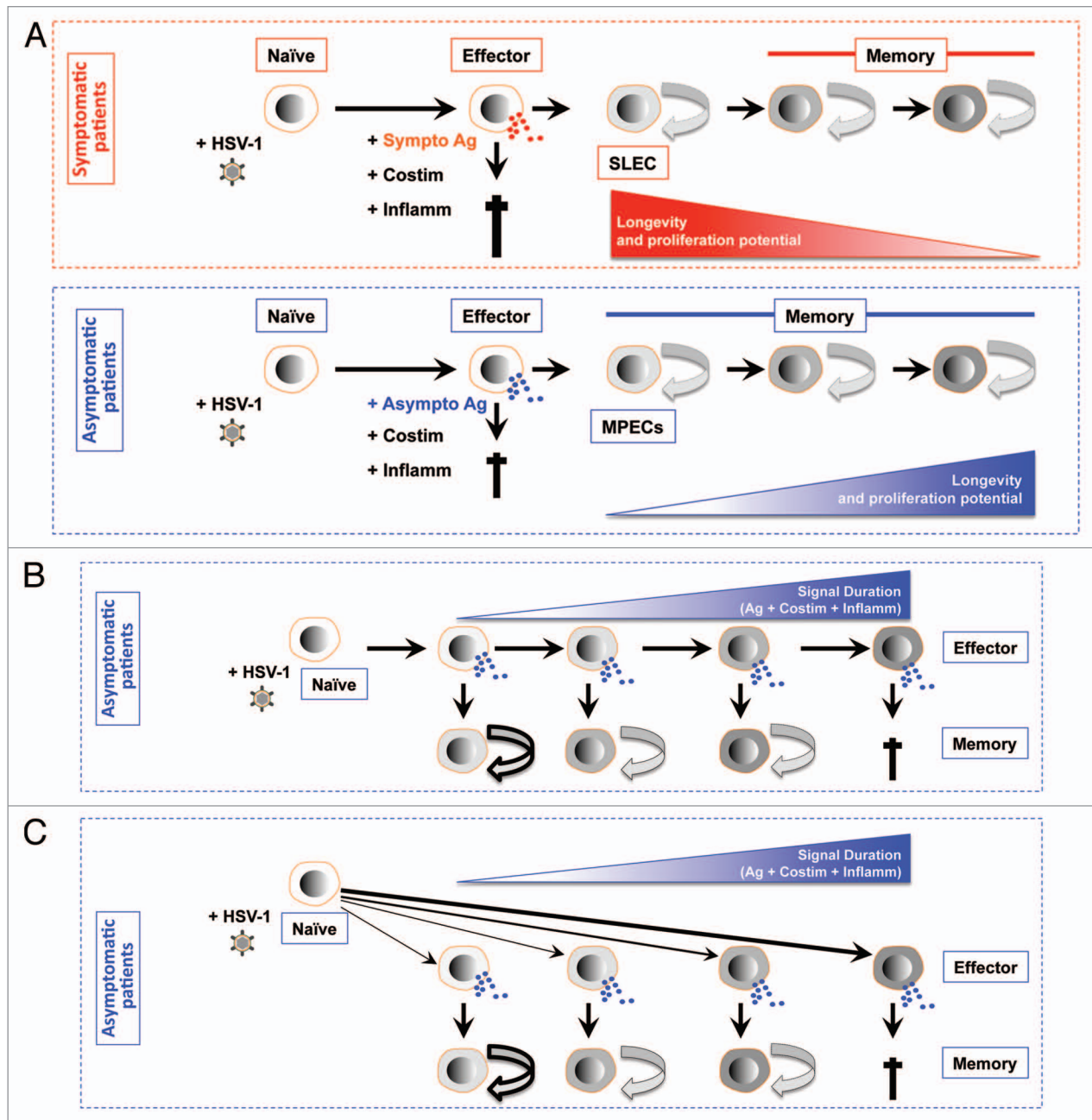


Figure 3. Models for generating diverse differentiated states of effector and memory CD8⁺ T cells following stimulation by symptomatic vs. asymptomatic epitopes. **(A)** First working model of de-differentiation for generating CD8⁺ T cell diversity proposes that soon after symptomatic or asymptomatic activation daughter naïve CD8⁺ cells become fully differentiated and functional by producing cytokines and exhibiting cytotoxic activity. The majority of effector CD8⁺ T cell then die. While a minority of asymptomatic epitope specific CD8⁺ T cells will de-differentiate into MPEC and long-lived memory CD8⁺ T cells. **(B)** Second working linear model for generating CD8⁺ T cell diversity proposes that degree of effector cell differentiation is regulated by the duration of exposure of daughter cells to symptomatic and asymptomatic epitopes following repetitive reactivation of HSV-2 from latency. The majority of terminally differentiated effector CD8⁺ T cell will die following cumulative encounter with the virus. While a minority of asymptomatic epitope specific CD8⁺ T cells which did not reach the end stage of differentiation will develop into MPEC and long-lived memory CD8⁺ T cells. The memory CD8⁺ T cell phenotypes vary according to the differentiation state of the effector cells from which they descended; curved arrows with bold, thin, or dashed lines indicate a high, medium, or low degree of proliferative potential and longevity. **(C)** Third working divergent model for generating CD8⁺ T cell diversity is similar to B, except that the strength of symptomatic vs. asymptomatic signal (instead of the cumulative symptomatic vs. asymptomatic stimulation) will determine the degree of effector cell differentiation and formation MPEC and long-lived memory CD8⁺ T cells.

Recently, it has been reported that CD8 $\alpha\alpha$ ⁺ T cells resident in DEJ tissue play an important role in immune surveillance and in initial containment of HSV-2 reactivation in human VM

tissue.¹¹⁹ Generation of long-term VM-resident memory CD8⁺ T cell immunity against sexually transmitted pathogens depends on the formation of long-lasting asymptomatic memory T_{EM} and

T_{RM} CD8⁺ cells.^{68,71,95,120,121} Compared with other mucosal tissues, such as the gut, induction of VM-resident CD8⁺ T cell immunity has received much less attention. In general, parenteral immunization induces systemic but not mucosal immune responses, while mucosal immunization induces both systemic and mucosal immune responses.^{9,17,122-124} We have recently described, for the first time, a Lipo/rAdv5 prime/boost mucosal vaccine delivered intravaginally (IVAG) that induced robust and long-lived HSV-specific asymptomatic CD8⁺ T cell responses which protect against genital herpes infection and disease.^{17,18} The induced long-lasting memory CD8⁺ T cell responses persisted in both the VM and GT-DLN for up to 8 mo post immunization. To avoid exacerbation of vaginal mucosal disease it is imperative to avoid inducing pathogenic symptomatic CD8⁺ T cells. Our lab is currently exploring the determining factors that regulate the induction, persistence, and retention of asymptomatic CD8⁺ T_{EM} cells within the ocular and genital mucosal tissues, the sites of primary and recurrent HSV-1 and HSV-2 infections, respectively (Fig. 1). Additionally, the development of asymptomatic CD8⁺ T cells within the ocular and genital mucosal tissues most likely displays a more profound complexity and is more uniquely regulated in comparison to the development on other mucosal surfaces.^{115,125}

In the following paragraphs we will discuss our novel concept of “symptomatic/asymptomatic CD8⁺ T cell development” within the widely known T_{EM} , T_{RM} , and T_{CM} CD8⁺ cell populations. It will also shed some light on the mechanisms of induction, establishment, persistence, and regulation of “asymptomatic” memory CD8⁺ T cells at the ocular and genital mucocutaneous tissues and the new direction for the design of efficient and safe “asymptomatic” T cell-based human vaccines and immunotherapeutics.

Contribution of Memory Precursor Effector Cells (MPECs) and Short-Lived Effector Cells (SLECs) in the Development of Symptomatic vs. Asymptomatic Memory CD8⁺ T Cells

T cell responses to a primary viral infection are optimally designed to generate large numbers of effector CD8⁺ T cells that clear the virus infection while simultaneously generating memory CD8⁺ T cells that protect against subsequent encounter with the virus following re-infection or reactivation. During a primary T cell response, the effector T cell pool contains 2 cellular subsets: short-lived effector cells (SLECs), a majority of which undergo apoptosis, and the memory precursor effector cells (MPECs) that differentiate into memory cells.¹²⁶⁻¹²⁹ To ensure the balance between these equally essential processes, CD8⁺ T cells integrate multiple signals and undergo an exponential increase in the number of T cells that is associated with a dynamic CD8⁺ T cell differentiation process.¹³⁰ Such regulation results in the equilibrium between SLECs (IL7R^{low}KLRG1^{high}) and MPECs (IL7R^{high}KLRG1^{low}) that are derived from early effector cells (EECs, IL7R^{low}KLRG1^{low}).¹³⁰ What properties distinguish terminally differentiated CD8⁺ T cell sub-populations and how this

process influences HSV-specific memory CD8⁺ T cell developments in the ocular, oral, and genital mucosal tissues are important questions that remain to be answered. Interestingly, recent results from our laboratory suggest that CD8⁺ T cells specific to herpes asymptomatic epitopes tend to have mainly a SLECs phenotype while CD8⁺ T cells specific to herpes symptomatic epitopes tend to have mainly a MPECs phenotype (Khan et al., unpublished data).

In general, after several rounds of cell division, the proliferative potential of effector CD8⁺ T cells declines and they become highly sensitive to apoptosis and death.^{95,131} In contrast, memory CD8⁺ T cells are multi-potent and, thus, are less terminally differentiated (they can remain resting memory CD8⁺ T cells or re-differentiate into effector CD8⁺ T cells at any time), can self-renew, have a high proliferative potential and increased longevity.^{71,95,131-135} Recent genomic and proteomic studies revealed many differences between gene expression profiles of memory and effector CD8⁺ T cell.^{136,137} During early infection, virus-specific effector cells that are generated from SLECs, (KLRG1^{high}IL7R^{low}) rarely persist into “memory” time points as they often die from apoptosis once the acute infection is cleared. In contrast, memory CD8⁺ T cells generated from MPECs (KLRG1^{low}IL7R^{high}) often develop into a mixture of T_{EM} , T_{RM} , and T_{CM} cells.^{71,138-140} We hypothesize that the delicate balance of MPEC vs. SLEC and the T_{CM} vs. T_{RM}/T_{EM} cell lineage decisions is strongly influenced by the repertoire of TCR signals that are generated by symptomatic and asymptomatic epitopes displayed by a viral pathogen. Specifically, TCR recognition of asymptomatic epitopes will drive development of CD8⁺ T cells with preferentially SLEC phenotype, while recognition of symptomatic epitopes will drive preferentially MPEC phenotype. The challenge we now face is to determine the molecular mechanisms that lead symptomatic and asymptomatic epitopes-specific effector CD8⁺ T cell development to ultimately choose the MPEC vs. SLEC fate following an infection.¹⁴¹ A better knowledge of MPECs and SLECs regulation and its association with symptomatic and asymptomatic T cells responses should help develop a safe and effective herpes vaccine.¹²⁶⁻¹²⁹

Following CD8⁺ T cell responses to a primary viral infection, the majority of SLECs undergo apoptosis, while the MPECs differentiate into memory cells.¹²⁶⁻¹²⁹ The equilibrium between MPEC vs. SLEC and the T_{CM} vs. T_{EM} cell lineage are integrated by multiple external and internal signals associated with an exponential increase in the number of cells and a dynamic CD8⁺ T cell differentiation process.¹³⁰ This review highlights how symptomatic and asymptomatic immune responses contribute in shaping the MPEC vs. SLEC and the T_{CM} vs. T_{EM} cell fate. The symptomatic and asymptomatic mechanisms that lead to terminally differentiated CD8⁺ T cells and how this process influences memory CD8⁺ T cell developments in the ocular and genital mucosal tissues are important questions that remain to be answered. We hypothesize that the delicate balance of MPEC vs. SLEC and the T_{CM} vs. T_{EM} cell lineage decisions is likely influenced by the repertoire of TCR signals that are generated by “symptomatic” and “asymptomatic” epitopes displayed by a viral pathogen.

Anatomic-Distribution, Phenotypic, and Functional Characteristics of Various Subsets of Symptomatic and Asymptomatic Memory CD8⁺ T Cells

Although memory CD8⁺ T cell sub-populations are widely heterogeneous in terms of their phenotype, function, and anatomic distribution, they can be divided into 3 major subtypes: (1) the effector memory CD8⁺ T cells (T_{EM}); (2) the central memory CD8⁺ T cells (T_{CM}); and (3) the tissue-resident memory CD8⁺ T cells (T_{RM}).¹⁴² These 3 major populations of memory T cells play cooperative and complementary roles in protecting the host from re-infection.^{143,144} As illustrated in **Figure 1**, these 3 populations differ in their anatomic-distribution, phenotype, and function.

Anatomic distribution

T_{CM} cells circulate in blood and reside mainly in lymphoid tissues. T_{EM} and T_{RM} cells are retained in tissues within the portal entry sites of potential invading pathogens, such as the ocular, oral and vaginal muco-cutaneous tissues. They are rapidly mobilized (within hours of re-infections). While CD8⁺ T_{EM} cells are also present in blood and splenic red pulp, they are the dominant T cell sub-population in extra-lymphoid tissues.^{88,145} T_{EM} are sequestered in epithelia and underlying stroma,⁸⁸ and protect these tissues.^{144,146} CD8⁺ T_{EM} cells have altered T cell trafficking patterns due to: (1) downregulation of T cell homing molecules (CD62L, CCR7)^{147,148}; and (2) constitutive upregulation of non-lymphoid homing lectins, integrins, and chemokine receptors. Unique skin-resident HSV-specific CD8⁺ T_{EM}/T_{RM} cell subset have recently been reported to persist up to 8 mo after clearance of skin infection.^{17,86} They express high level of α E integrin (CD103). CD103 binds to epithelial cadherin (E-Cadherin) in epithelial cells. CD103-E-Cadherin binding is critical for the retention of CD8⁺ T_{EM}/T_{RM} cells within the epithelium. The role of T_{EM}/T_{RM} cells in protection against ocular, oral, and genital herpes, as often seen in asymptomatic individuals, remains to be determined.

Phenotype

CD8⁺ T_{CM} cells are CD8⁺CD103^{low}CD62L^{high}CCR7^{high}. CD8⁺ T_{EM} cells are CD8⁺CD103^{low}CD62L^{low}CCR7^{low}. CD8⁺ T_{RM} cells are CD8⁺CD103^{high}CD62L^{low}CCR7^{low}. Markers other than CD103, CD62L, and CCR7 that are used to identify the CD8⁺ T_{RM} cell sub-population include a high expression of CD11a, CD49a, and CD69.^{142,149-151}

Function

CD8⁺ T_{CM} cells have high proliferation potential and high capacity to secrete IL-2 upon re-stimulation.^{88,108,152} CD8⁺ T_{CM} cells must undergo differentiation for effector functions (e.g., cytotoxic and cytokine production).^{88,153,154} In contrast, CD8⁺ T_{EM}/T_{RM} cells that reside in extra-lymphoid tissues are already differentiated and poised for immediate effector function.¹⁴³ Tissue resident CD8⁺ T_{RM} cells are potent effectors that provide rapid long-term protection against tissue re-infection, express constitutively high levels of granzyme B, and eliminate infected target cells with secreted perforin.^{82,155-158} After clearance of epithelial HSV-1 and HSV-2 infections, CD8⁺ T_{RM} cells resident in the skin contain lower perforin and granzyme transcripts as compared with the effector phase.¹⁵⁹⁻¹⁶¹ However, HSV-specific

CD8⁺ T cells in the sensory ganglia express high level of granzymes and perforin due to TCR engagement by latently infected neurons, which are critical for suppressing reactivation of HSV-1 and HSV-2.^{82,158-160} Tissue resident CD8⁺ T_{RM} cells produce high levels of effector IFN- γ , TNF- α , IL-22, and IL-17 cytokines and chemokines such as MIP-1, upon viral antigen re-encounter.¹⁶² Tissue resident CD8⁺ T_{RM} cells efficiently and immediately interfere with virus replication in peripheral non-lymphoid tissues, such as the skin. Tissue resident CD8⁺ T_{RM} cells express cytotoxic granules to provide rapid cytotoxic response against viral infections.^{88,163} We have recently showed that vaginal mucosa-resident HSV-specific memory CD8⁺ T cells induced by our intravaginal Lipo/rAdv prime-boost vaccine is correlated with protection against genital herpes infection and disease ($R^2 = 0.7836$; $P < 0.0001$).¹⁷ However, the relative contribution of T_{EM}/T_{RM} vs. T_{CM} in long-term protective memory against genital herpes remains to be elucidated.

Development of Central Memory (T_{CM}), Effector Memory (T_{EM}), and Tissue-Resident Memory (T_{RM}) CD8⁺ T Cells in Symptomatic vs. Asymptomatic Settings

Understanding the molecular mechanisms by which memory CD8⁺ T cells are established and maintained within the tissues will allow us to develop new vaccine and immunotherapeutic approaches to induce antigen-specific activation vs. tolerance depending on patient's clinical needs. Memory CD8⁺ T cells can survive long-term in the absence of antigens (over 2 y in mice and over 50 y in humans).^{68,71,95,120,121} As mentioned above, there are several subsets of memory T cells, including central memory (T_{CM}), effector memory (T_{EM}), and tissue-resident memory (T_{RM}) cells (based on CD62L, IL7R, CCR7, CD11a, and CD103 expression).⁹⁵ T_{CM} cells are mainly CD8⁺CD103^{low}CD62L^{high}CCR7^{high}. T_{EM} cells are mainly CD8⁺CD103^{low}CD62L^{low}CCR7^{low}. Another sub-population, has been recently described as permanently residing in peripheral tissues, is called tissue-resident memory CD8⁺ T cells (T_{RM} cells)^{142,149-151} and is CD8⁺CD103^{high}CD62L^{low}CCR7^{low}. T_{RM} cells are also CD11a^{high}, CD49a^{high}, and CD69^{high}. Although central memory CD8⁺ T cells (T_{CM}) appear to provide some protection against systemic infection, T_{EM} and T_{RM} cells have special features that make them well suited to respond quickly and effectively when infection is localized to peripheral compartments, such as the vaginal, the oral, and the ocular mucosal surfaces. As mentioned above, CD8⁺ T_{CM} vs. CD8⁺ T_{EM}/T_{RM} cell lineage decision is influenced by the nature and strength of TCR signaling and IL-2 in addition to IL-15 and other exogenous and endogenous factors.^{164,165} HSV-specific T_{RM} cells are preferentially retained in close proximity to the epidermis and peripheral nerves in vaginal muco-cutaneous tissues, following HSV-1 and HSV-2 infections, whereas clusters of neuronal T_{RM} cells are retained in areas of previous infections for at least several weeks.^{82,151,155-159,161,166} Our lab is actively engaged in determining the relative contribution of HSV symptomatic vs. asymptomatic epitopes in the induction of CD8⁺ T_{CM}, T_{EM}, and T_{RM} cell

sub-populations and their homing in lymphoid vs. the mucocutaneous tissues. Additionally, our lab is investigating the role of symptomatic vs. asymptomatic CD8⁺ T cell sub-populations in the protection against herpes at the ocular (HSV-1), oral (HSV-1) vaginal (HSV-1/HSV-2) mucocutaneous sites of infection. The project involves in vitro studies in symptomatic vs. asymptomatic humans as well as in vivo studies using our novel susceptible “humanized” HLA transgenic mouse, guinea pig, and rabbit models of ocular, oral, and genital herpes. Determining how CD8⁺ T_{CM}, T_{EM} and T_{RM} cell sub-populations develop and protect against infections and diseases might tightly depend on the models by which symptomatic vs. asymptomatic epitope-specific memory CD8⁺ T cells develop.

Models for Memory CD8⁺ T Cells Development Within Symptomatic and Asymptomatic Memory CD8⁺ T Cells Concept

After an acute infection, the memory CD8⁺ T cell population evolves progressively over time into sub-populations that are enriched with cells with higher proliferative capacity, greater longevity, and with slight alterations during latent, chronic, and persistent phases of infections.^{149,167-169} How effector CD8⁺ T cell differentiation is balanced to permit the formation of effector cell properties in the MPECs and yet still prevents the MPECs from acquiring a terminal SLECs state is still controversial. More so, it is also unclear whether naïve (N) and effector (E) CD8⁺ T cells specific to symptomatic vs. asymptomatic epitopes follow a different path of development into memory (M) cells (i.e., designated in this review as symptomatic vs. asymptomatic N > > E > > M transitions). Nevertheless, based on the studies from many murine models of persistent infections, 3 major models have been proposed to explain naïve to effector to memory CD8⁺ T cell transitions:

Model 1: De-differentiation model

De-differentiation model implies that after antigenic activation most naïve CD8⁺ T cells reach a terminally differentiated effector stage, where the cells become fully functional effector CD8⁺ T cells that have cytotoxic activity and produce cytokines. However, some effector CD8⁺ T cells are capable of de-differentiating into memory CD8⁺ T cells that gain longevity and a high proliferative potential (Fig. 3A). During an effector to memory transition, MPECs gradually acquire proliferative and survival capacity and produce IL-2, Bcl-2, and CD62L.¹⁷⁰⁻¹⁷⁶ The underlying mechanisms behind this functional maturation remain to be fully elucidated. However, the exposure to IL-2 during infection, the presence of CD4⁺ T helper cells, and the maintenance of lower level of expression of T-bet appear to be important factors for establishing a memory CD8⁺ T cell population with a higher proliferative capacity.^{95,105,137,165,177-180} The de-differentiation model would elegantly fit into the ocular, oral, and genital herpes asymptomatic infections. In theory, after activation by both symptomatic and asymptomatic epitopes, most CD8⁺ T cells would reach a terminally differentiated effector stage but only asymptomatic epitopes-specific CD8⁺ T cells would be able to

de-differentiate into memory CD8⁺ T_{EM}/T_{RM} cells, as illustrated in Figure 3A. We are currently trying to test the de-differentiation model in ocular, oral, and genital herpes asymptomatic infections and to understand the contribution of symptomatic and asymptomatic stimulations in the phenotypic and functional maturation of CD8⁺ T cells during the naïve to effector to memory transitions.

Model 2: Decreasing potential or linear model

Decreasing potential or linear model implies that after antigenic stimulation, naïve CD8⁺ T cells differentiate into a stepwise manner and, in doing so, progressively acquire a more terminally differentiated phenotype. Thus, CD8⁺ T cells that become MPECs do not progress as far as the SLECs (Fig. 3B). In this model, the progression of differentiation may be controlled by successive antigenic stimulations of CD8⁺ T cells.^{95,181,182} This model would also fit ocular, oral, and genital herpes symptomatic and asymptomatic concept. In theory, during the latency/reactivation cycles, spontaneous intermittent reactivation of the virus from latency leads to successive antigenic stimulation of CD8⁺ T cells with symptomatic and asymptomatic epitopes expressed by the virus proteins. Thus, the differentiation state of an effector CD8⁺ T cell would reflect the number of times in which CD8⁺ T cells encountered either symptomatic or asymptomatic epitopes during infection/reactivation. This linear model would provide a likely mechanism for generating a spectrum of symptomatic or asymptomatic effector and memory CD8⁺ T cells.^{95,108,181,183,184} In the case of HSV latent infection, the successive reactivation of the virus and re-stimulation of CD8⁺ T cells with symptomatic vs. asymptomatic epitopes will contribute to dictating phenotypical and functional maturation of CD8⁺ T cells throughout the naïve to effector to memory transition, as illustrated in Figure 3B. The successive stimulation of CD8⁺ T cells with asymptomatic epitopes would lead to the formation of more T_{EM}/T_{RM} cells and more CD8⁺ T cell memory to effector transitions. In contrast, a successive stimulation of CD8⁺ T cells with symptomatic epitopes will lead to T_{CM} and less memory to effector transitions.

Model 3: Divergent model

Divergent model for generating CD8⁺ T cell diversity proposes that soon after antigenic activation, daughter CD8⁺ T cells are instructed to generate either SLECs or MPECs subsets, as illustrated in Figure 3C. Although the divergent model is asymmetric and produces only 2 cell types, it also incorporates a range of “in-between” differentiated states that depend on the overall nature and strength of symptomatic vs. asymptomatic epitopes stimulation encountered by a T cell at or near the time of priming.^{95,185-188} Following a first encounter with an antigen, dividing CD8⁺ T cells exhibit unequal partitioning of symptomatic and asymptomatic epitopes that mediate signaling, cell fate specification, and an asymmetric cell division and lead to the differentiation toward either an effector or memory T cell lineage.¹⁸⁹ This model would also suit the scenario where a primary ocular, oral, or genital herpes infection would lead to various strengths of CD8⁺ T cell stimulations depending on whether HSV symptomatic or asymptomatic epitopes were encountered. The differentiation state of an effector CD8⁺ T cell would then denote the strength of symptomatic or asymptomatic T cell epitope signal during the priming

of naïve CD8⁺ T cells, as illustrated in **Figure 3C**. Specifically, a strong stimulation of naïve CD8⁺ T cells with asymptomatic epitopes would lead to the formation of more T_{EM}/T_{RM} and more CD8⁺ T cell memory to effector transitions. In contrast, a robust stimulation of CD8⁺ T cells with symptomatic epitopes would lead to T_{CM} and less memory to effector transitions.

The Role Of Cytokines In Symptomatic Vs. Asymptomatic Memory CD8⁺ T Cell Development

As mentioned above, a handful of inflammatory cytokines influence CD8⁺ T cell transition from effector to memory state.¹⁹⁰ IL-12, IFN- α/β , and IFN- γ inflammatory cytokines strongly enhance the expansion of effector CD8⁺ T cells, their cytotoxic activity, and the production of antiviral cytokines.^{95-98,103,131,191-194} Optimal effect of IL-12, IFN- α/β , and IFN- γ inflammatory cytokines appear to occur particularly when effector CD8⁺ T cells are activated by weak stimuli or following a cross-presentation of antigens. However, these inflammatory cytokines may also act as a double-edged sword: (1) on one hand, they stimulate effector CD8⁺ T cell expansion and function; (2) on the other hand, they drive the terminal maturation of effector CD8⁺ T cells, hence, limiting the potential transition of effector to memory state. Among the IL-12, IFN- α/β , and IFN- γ inflammatory cytokines, the role of IFN- γ in the transition of effector to memory still remains controversial. A recent work has shown that IFN- γ promotes effector CD8⁺ T cell contraction and downregulation of IL7R.^{95,193} Yet, another recent report showed that IFN- γ receptor (IFN- γ R) is needed for normal memory CD8⁺ T cell formation.⁹⁵ In IL12^{-/-} mice, a higher frequency of IL7R^{high} MPECs and memory CD8⁺ T cells form following infection,^{95,97,103,194} suggesting that IL-12 plays a critical role in the effector CD8⁺ T cell fate decisions. Our recent findings showed that stimulation of CD8⁺ T cells with HSV-1 asymptomatic epitopes preferentially induced poly-functional T cells that produce IL-12, TNF- α , and IFN- γ inflammatory cytokines.⁶ In contrast, stimulation of CD8⁺ T cells with HSV-1 symptomatic epitopes preferentially induced mono-functional T cells.⁶ Moreover, we found that HSV-1 asymptomatic epitopes preferentially induced CD8⁺ T_{EM} cells (Khan et al., unpublished data). In contrast, HSV-1 symptomatic epitopes favor induction of CD8⁺ T_{CM} cells (Khan et al., unpublished data). We, therefore, hypothesize that the delicate balance of T_{CM} vs. T_{EM} cell lineages decision is strongly influenced by the profile of cytokines generated following stimulation with symptomatic vs. asymptomatic epitopes. Specifically, the recognition of asymptomatic epitopes will drive the development of tissue-resident memory CD8⁺ T cells with preferentially a T_{EM}/T_{RM} phenotype, while the recognition of symptomatic epitopes will drive preferentially a T_{CM} phenotype.

Homeostatic cytokines IL-7 and IL-15 are also critical for the long-term survival and turnover of memory CD8⁺ T cells.^{85,95,195} Prolonged deprivation of IL-7 and IL-15 homeostatic cytokines has considerable consequences on the formation and maintenance of memory CD8⁺ T cells.^{95,121,177,196-199} Formation of KLRG1^{high}IL-7R^{low} SLECs depends on production of IL-15, but this alone is

not sufficient to maintain these cells for a long-term period.^{69,71,95} Once T cells enter peripheral tissues, various molecules from tissue microenvironment may further influence their effector heterogeneity. For example, TGF- β have various tissue-specific effects on the differentiation of T cells in the intestinal mucosa,¹⁰⁰ but its effect on ocular, oral, and vaginal mucosa-resident CD8⁺ T cells remains to be determined. In general, a rapid upregulation of CD103, an integrin that is expressed by T cells and required for tissue retention, is mediated by TGF- β 1.⁸⁷

Given the importance of tissue-resident memory CD8⁺ T_{EM}/T_{RM} cells in providing local protection against subsequent HSV re-infection and reactivation, the factors that regulate memory CD8⁺ T cell retention within the ocular, oral, and vaginal mucosa where protection is required is not yet known. To date, virtually no results are available on the mechanisms involved in the retention of memory CD8⁺ T cells within the ocular, oral, and vaginal mucosa. We have recently demonstrated^{17,18} that: (1) targeting the vaginal mucosa (VM) with a lipopeptide/recombinant adenovirus (Lipo/rAdv) heterologous prime/boost vaccine, containing a mouse CD8⁺ T cell epitope, induced a robust and durable VM-resident CD8⁺ T cells that protects against genital herpes infections and disease; and (2) VM-resident IFN- γ -producing CD8⁺ T cell responses correlated positively with protection ($R^2 = 0.7836$; $P < 0.0001$). Moreover, we found a higher expression of CD103 on asymptomatic CD8⁺ T cells vs. symptomatic CD8⁺ T cells, responding to HSV-2 infection (unpublished data). These results suggest that priming conditions dominated by asymptomatic viral epitopes stimulation may increase CD103 expression on memory CD8⁺ T cells and, thus, might promote long-lasting retention of memory CD8⁺ T cells within the VM (**Figs. 1 and 2**). Even so, the mechanisms that regulate the retention of HSV-specific memory CD8⁺ T cells in the ocular and mucosal tissues; and particularly (1) the role of CD103 in the retention of ocular and vaginal mucosa-resident HSV-specific CD8⁺ T cells; (2) whether the expression of CD103 on ocular, oral, and vaginal mucosa-resident HSV-specific memory CD8⁺ T cells are affected with TGF- β ; and (3) whether stimulation with symptomatic vs. asymptomatic epitopes would affect the expression of CD103, and the production of inflammatory and homeostatic cytokines by memory CD8⁺ T cells and hence affect memory CD8⁺ T cells development and retention within the peripheral tissues, such as the ocular, oral, and vaginal mucosal tissues remain largely unexplored.

The Role of Cell Intrinsic (Transcription) and Epigenetic Factors in the Development of Symptomatic Vs. Asymptomatic Memory CD8⁺ T Cells

As mentioned above, modulation of many cell-intrinsic factors, in part achieved through epigenetic modifications of DNA and histones, are involved in the regulation of effector to memory transition. Transcription factors that regulate effector T cell differentiation, such as T-bet, controls SLEC vs. MPEC fate decisions through its level of expression.^{69,71,103,194} Higher expression of T-bet

favors the formation of KLRG1^{high}IL7R^{low} SLECs while lower expression of T-bet promotes KLRG1^{low}IL7R^{high} MPECs.^{69,71,95} Similar to T-bet, the transcriptional regulator ID2 (inhibitor of differentiation 2) plays a critical role in effector to memory CD8⁺ T cell transition.^{95,200} ID2-deficient CD8⁺ T cells have a more IL7R^{high}CD27^{high} “MPEC-like” phenotype.^{95,200} Another transcription factor, Blimp-1 (prdm1) is expressed at high levels by KLRG1^{high}IL7R^{low} SLECs.¹⁸⁰ CD8⁺ T cells in Blimp1-deficient mice have an activated and highly proliferative phenotype, suggesting that Blimp1 expression is anti-proliferative.^{95,201,202} Moreover, the potential antagonists to Blimp-1, Bcl-6, and its homolog, Bcl-6b appear to promote the development of memory CD8⁺ T cells and thus, increasing their proliferation.^{95,203-205} Furthermore, recent evidence indicates that the apparent reduction in the proliferative potential of KLRG1^{high} CD8⁺ T cells is also regulated by an increase in the expression of p27kip, a cell cycle inhibitor⁹⁵ and a decrease in the expression of Bmi-1, a transcriptional repressor, both promoting T cell proliferation.^{95,206} Undoubtedly, as more genes are discovered and examined in epigenetic studies, the list of intrinsic factors involved in this process will continue to grow in the next few years. Increasing the expression of Bcl-2 and Bcl-XL or blocking their actions in effector CD8⁺ T cells does not greatly affect the normal rate of effector cell death following infection.^{72,85,95,195,196,199} Among the molecules that have been found to promote and prevent effector CD8⁺ T cell contraction are the pro-apoptotic Bim and the serine protease inhibitor 2A (Spi2A), respectively.^{95,207-211} Inflammatory cytokines can also modulate the expression of key transcription factors that regulate effector T cell differentiation, such as T-bet and eomesodermin (eomes).^{95,212} IL-12 augments T-bet expression and diminishes eomes expression in activated CD8⁺ T cells.^{69,71,103,194} Our current understanding of epigenetic mechanisms that regulate the on-off expression of CD8⁺ T cell effector molecules at the naïve-effector-memory stages of differentiation, and how modifications to the genome/epigenetic factors in coming years, will likely serve as novel targets to regulate naïve-effector-memory CD8⁺ T cell transitions.²¹³⁻²¹⁶

The Novel “Symptomatic/Asymptomatic” Concept: Considerations for Herpes Vaccines and Immunotherapeutics

The memory CD8⁺ T cells, designated as protective or asymptomatic memory CD8⁺ T cells, can induce lifelong protection from re-infections with a wide variety of viral, bacterial, and parasitic pathogens. Conversely, the memory CD8⁺ T cells, designated as pathogenic or symptomatic CD8⁺ T cells, can cause immunopathology when not properly regulated.^{15,217-221} The in vivo immunogenicity and protective efficacy of human CD8⁺ T cell epitopes found to be antigenic in vitro is crucial in vaccines design.²²²⁻²²⁵ Up to 50% of HSV-1 and HSV-2 CD8⁺ epitopes recently reported as recognized by human CD8⁺ T cells in vitro, may not be protective as expected.^{118,226-232} Moreover, some of the HSV-1/HSV-2 human T cell epitopes may actually be pathogenic and contribute to exacerbation of disease.²¹⁸ A human

CD8⁺ T cell epitope derived from HSV-1 gK caused a significant increase in virus replication in the eyes of HLA-A*0201 transgenic mice associated with increased acute corneal scarring.²¹⁷⁻²²¹ The gK CD8⁺ T cell epitope also exacerbated ocular disease in HLA-A*0201 transgenic rabbits (BenMohamed, unpublished data). This suggests that gK CD8⁺ T cell epitope might be clinically harmful and as such is considered as a symptomatic epitope and thus must be excluded from future asymptomatic vaccine. The mechanisms by which symptomatic CD8⁺ T cell epitopes exacerbate ocular, oro-facial, and genital herpetic diseases remain to be fully elucidated (see our recent review²³³). Regardless of the nature of that mechanism, for symptomatic epitopes are associated with recurrent herpetic disease, it is logical to exclude them from future herpes vaccines and immunotherapeutics, since they may actually exacerbate, rather than protect from, recurrent herpetic disease.^{218,234-238} Thus following a primary mucosal infection with a pathogen, such as HSV-1 or HSV-2, virus-specific asymptomatic epitopes presented by infected antigen presenting cells (APCs) would induce activation, clonal expansion, and differentiation of naive asymptomatic epitope-specific CD8⁺ T_{EM}/T_{RM} cells into a pool of “protective” effector cytotoxic T lymphocytes. This is necessary for the clearance of the virus. In contrast, virus specific symptomatic epitopes presented by infected APCs to naive symptomatic epitope-specific CD8⁺ T_{CM} cells would instead induce a “pathogenic” T cell population and may exacerbate the viral disease.^{9,38,122,123,239-242} Therefore, the central hypothesis of our novel “symptomatic vs. asymptomatic T cells” concept is that, following intravaginal infection by a pathogen, symptomatic and asymptomatic activation phenotype would recall memory CD8⁺ T cells, with either a central or effector memory phenotype.

In the most recent clinical herpes vaccine trials, therapeutic vaccination with recombinant herpes proteins that presumably contained both asymptomatic and symptomatic T cell epitopes, only led to moderate and transient protection.^{6,122,239} Based on our “symptomatic vs. asymptomatic T cells” concept, a human vaccine containing asymptomatic (protective) CD8⁺ T cell epitopes, but not symptomatic (pathogenic) CD8⁺ T cell epitopes, would foster better protective and long-lasting CD8⁺ T_{EM}/T_{RM} cells against HSV re-infections and reactivations. The immune mechanisms by which HSV-specific asymptomatic CD8⁺ T cells control herpes infection and disease, and by which HSV-specific symptomatic CD8⁺ T cells may exacerbate herpes infection and disease, remain to be fully elucidated. Following an asymptomatic vaccination, protective asymptomatic HSV-specific CD8⁺ T_{EM}/T_{RM} cells would be profoundly localized to sites of primary and recurrent infections such as, the ocular, oral, and vaginal muco-cutaneous surfaces, the skin, the cervix, and the eye, along with sites of latent infection, in the sensory ganglia, such as the trigeminal ganglia and the dorsal sacral ganglia. Although HSV-1/2 infections co-exist with brisk T-cell responses, pathogenic symptomatic HSV-specific CD8⁺ T_{CM} cells would be associated with worsened recurrent infection.^{6,122,239} Hence, induction of protective asymptomatic T cells, and suppression of pathogenic symptomatic T cells, are essential futures for a safe and efficient herpes vaccinations. Therefore, identifying HSV-1 and HSV-2

asymptomatic and symptomatic T cell epitopes from all the 84+ herpes proteins is critical for a rational design of asymptomatic and effective ocular, oral, and genital herpes vaccines.

Evidence from B6 mice suggests that CD8⁺ T cells, specific to the immunodominant HSV-1 and HSV-2 glycoprotein B (gB) H2^b-restricted epitope (gB_{498–505}), protect against ocular and genital herpes infection and diseases. However, the possible role of human CD8⁺ T cells, specific to HLA-restricted gB epitopes, in protective immunity seen in HSV-1 and/or HSV-2 seropositive asymptomatic healthy individuals (who have never had clinical herpes) remains to be determined. In our recent study,⁶ 6 out of 10 potential HLA-A*02:01-restricted CD8⁺ T cell epitopes from the HSV-1 gB amino acid sequence exhibited high binding affinity to HLA-A*02:01 molecules. In 10 sequentially studied HLA-A*02:01 positive and HSV-1 seropositive asymptomatic individuals, the most frequent, robust, and polyfunctional CD8⁺ T-cell responses, as assessed by a combination of tetramer, IFN- γ -ELISpot, CFSE proliferation, CD107_{a/b} cytotoxic degranulation and multiplex cytokine assays, were directed mainly against epitopes gB_{342–350} and gB_{561–569}. In contrast, in 10 HLA-A*02:01 positive, HSV-1 seropositive symptomatic individuals (with a history of numerous episodes of recurrent clinical herpes disease) resulted in frequent, but less robust, CD8⁺ T-cell responses directed mainly against non-overlapping epitopes (gB_{183–191} and gB_{441–449}). Asymptomatic individuals had a significantly higher proportion of HSV-gB-specific CD8⁺ T cells expressing CD107_{a/b} degranulation marker and produced more effector cytokines IL-2, IFN- γ , and TNF- α compared with symptomatic individuals. Furthermore, immunization of a novel herpes susceptible HLA-A*02:01 transgenic mouse model with asymptomatic epitopes, but not with symptomatic epitopes, promoted strong CD8⁺ T cell-dependent protective immunity against ocular herpes infection and disease. This study is first to directly confirm the new “symptomatic/asymptomatic CD8⁺ T cell development” concept. As per our new “symptomatic/asymptomatic” concept, inflammatory corneal lesions are not a direct consequence of the damage caused by the virus or by auto-reactive or bystander T cells, but rather a result of an immunopathological T cell reaction caused by symptomatic HSV-1 epitopes. In contrast, T cell responses specific to asymptomatic HSV-1 epitopes would result in immuno-protection. Regardless of the mechanisms involved, if symptomatic individuals tend to generate CD8⁺ T cells that recognize symptomatic epitopes, it would therefore be rational to exclude such symptomatic epitopes from future herpes vaccines, based on the grounds that they may exacerbate rather than cure recurrent herpetic disease.

Concluding Remarks

Tracking the developmental lineage of CD8⁺ T cells between effector and memory stages is complicated by the continuously evolving memory CD8⁺ T cell sub-populations that develop long after infections are cleared. With respect to symptomatic and asymptomatic memory CD8⁺ T cell sub-populations, most asymptomatic CD8⁺ T cells appeared to be of the CD8⁺ T_{EM}/

T_{RM} cell phenotype, reside in non-lymphoid mucosal tissues, have a reduced ability to traffic to lymphoid tissues and appear more terminally differentiated due to a lower proliferation capacity in response to antigen or homeostatic IL-15 and IL-7 cytokines.^{95,105–107} In contrast, symptomatic epitope stimulations likely induce memory CD8⁺ T_{CM} cells.

During early infection, virus-specific effector cells that are generated from SLECs, (KLRG1^{high}IL7R^{low}) rarely persist into “memory” time points and often die from apoptosis once the acute infection is cleared. In contrast, memory CD8⁺ T cells generated from MPECs (KLRG1^{low}IL7R^{high}) often develop into a mixture of T_{EM}/T_{RM} and T_{CM} cells.^{71,137,139,140} It is likely that the delicate balance of MPEC vs. SLEC and the T_{EM}/T_{RM} vs. T_{CM} cell lineages decision is strongly influenced by the repertoire of TCR signaling that is generated following symptomatic vs. asymptomatic epitopes stimulation. Specifically, recognition of asymptomatic epitopes will mainly drive the development of tissue-resident memory CD8⁺ T cells with preferentially an SLEC and T_{EM}/T_{RM} cell phenotype, while recognition of symptomatic epitopes will mainly drive preferentially a MPEC and T_{CM} cell phenotype.

The above remark is supported by our recent findings showing that stimulation of CD8⁺ T cells with asymptomatic epitopes preferentially induce polyfunctional T cells that produce IL-12, TNF- α , and IFN- γ inflammatory cytokines. In contrast, stimulation of CD8⁺ T cells with symptomatic epitopes preferentially induce monofunctional T cells. Moreover, we found that asymptomatic epitopes favorably induce T_{EM} cells. In contrast, symptomatic epitopes favorably induce T_{CM} cells. It is likely that the delicate balance of T_{CM} vs. T_{EM} cell lineages decision is strongly influenced by the profile of cytokines generated following stimulation with symptomatic and asymptomatic epitopes. Specifically, recognition of asymptomatic epitopes will mainly drive development of tissue-resident memory CD8⁺ T cells with preferentially a T_{EM}/T_{RM} phenotype, while recognition of symptomatic epitopes will mainly drive preferentially a T_{CM} phenotype.

While developing an effective herpes vaccine is scientifically feasible, virologists, and immunologists still remain puzzled by the relationship between the effector/memory T cell sub-populations and the infection/disease process.⁶ The key for an ultimate development of an effective herpes vaccine can be drawn from studies of T cells from HSV-seropositive asymptomatic individuals who manage to “naturally” and continuously control recurrent herpetic disease to clinically undetectable levels.⁶ One ongoing research goal in our laboratory is to explore the underlying mechanisms of “symptomatic vs. asymptomatic T cells” concept with respect to (1) CD103 expression promoting the long-term persistence of memory CD8⁺ T_{EM}/T_{RM} cells at the ocular, oral, and genital muco-cutaneous site of HSV-1 and HSV-2 infections^{118,119}; (2) CD103 regulation of the retention of a mucosal tissues-resident asymptomatic CD8⁺ T cells; and (3) TGF- β regulation of CD103 expression on mucosal tissues-resident memory CD8⁺ T cells that promote their adhesion to the extracellular matrix.²⁴³

The immune mechanism(s) by which HSV-specific asymptomatic CD8⁺ T cells control herpes disease and HSV-specific

symptomatic CD8⁺ T cells remain to be fully elucidated in humans. Following an asymptomatic vaccination, protective asymptomatic HSV-specific CD8⁺ T_{EM}/T_{RM} cells would localize to sites of primary and recurrent infections, such as the ocular, oral, and vaginal mucosal surfaces, the skin, the cervix, and the eye, as well as to sites of latent infection in the sensory ganglia. Although HSV-1/2 infections co-exist with brisk T-cell responses, pathogenic symptomatic HSV-specific CD8⁺ T_{CM} cells would be associated with worsened recurrent infection.^{6,122,239} Hence, positive selection of protective asymptomatic T cells and negative selection of pathogenic symptomatic T cells through a molecular-based approach is essential for future herpes vaccines. Identifying viral asymptomatic and symptomatic T cell epitopes from all the 84+ herpes proteins is a good starting point and critical for a rational design of an effective ocular and genital herpes vaccine.

We recently reported, for the first time, that a Lipo/rAdv5 prime/boost mucosal vaccine delivered intravaginally, induced a robust and long-lasting HSV-specific asymptomatic CD8⁺ T cells that protect against genital herpes infection and disease.^{17,18} The induced long-lasting memory CD8⁺ T cell responses persisted in both the VM and GT-DLN for up to 8 mo post immunization. To avoid exacerbation of vaginal mucosal disease, it is imperative to avoid inducing symptomatic CD8⁺ T cells. We also found that immunization of a novel herpes susceptible HLA-A*02:01 transgenic mouse model with asymptomatic epitopes, but not with symptomatic epitopes, induced strong CD8⁺ T cell-dependent protective immunity against ocular herpes infection and disease.

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This study is first to directly confirm our new "symptomatic/asymptomatic CD8⁺ T cell development" concept. As per this concept, inflammatory corneal lesions are not a direct consequence of the damage caused by the virus or by auto-reactive or bystander T cells, but rather a result of the balance between immunopathological T cell responses specific to symptomatic HSV-1 epitopes and immuno-protective T cell responses specific to asymptomatic HSV-1 epitopes.

The new "symptomatic/asymptomatic CD8⁺ T cell" concept provides new immunological insights and a new direction for the design of an efficient and safe T cell-based human vaccines and immunotherapeutics, not only for herpes but for other infectious diseases. Regardless of the mechanisms involved, if symptomatic individuals tend to generate CD8⁺ T cells that recognize symptomatic epitopes, it would be rational to exclude such symptomatic epitopes from any future vaccines on the grounds that they may exacerbate, rather than cure, recurrent infectious disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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