Tissue-Resident Memory T cells in Cytomegalovirus Infection

Jenny Tosca Thom^a and Annette Oxenius^{a†}

Abstract

Herpesviruses establish life-long infection in their hosts and maintain latent reservoirs for sporadic reactivation at peripheral sites, such as skin and mucosae. For herpes simplex virus infection, experimental studies in mice revealed that immediate protection against local reactivation or superinfection events in the skin relies on tissue resident memory T cells (T_{RM}) rather than on their circulating counterparts. Recent evidence extends this notion to cytomegalovirus infection, which that potentially induces T_{RM} cells in both mice and humans particularly in mucosal tissues that constitute important viral sanctuaries and are relevant entry sites for challenge and superinfections. The discovery unravels promising opportunities to exploit cytomegalovirus based vaccine vectors for the specific induction of tissue resident T cell subsets.

Introduction

Herpesviruses share the extraordinary capacity to evade immunity, persist in tissues, and establish latent reservoirs, which allow for viral reactivation and transmission. Cytomegaloviruses (CMVs) are beta-herpesviruses that establish life-long latency in many organs including mucosal tissues. 60 – 90% of the world population is infected with human CMV (HCMV). While acute HCMV infection is largely asymptomatic in immunocompetent individuals, it is a frequent cause of morbidity and mortality among the immunocompromised, including AIDS patients, transplant recipients, and congenitally or perinatally infected infants. Although CMVs have co-evolved with their mammalian hosts and therefore exhibit strict species specificity, experimental infection of mice with murine CMV (MCMV) is considered a relevant model for human CMV infection in particular with respect to the functional and phenotypic characteristics of the elicited T cell responses. In both mice and humans CMV infection elicits substantial and sustained expansions of specific CD8⁺ T cells, a process known as memory “inflation” (reviewed in [1]). The MCMV model has been particularly useful in dissecting, on a mechanistic level, the parameters driving this atypical CD8⁺ T cell response. Furthermore, experimental MCMV infection has revealed that T cells are the principal effectors of the adaptive immune response. MCMV replication as well as T cell-mediated control follow tissue-specific patterns: while lytic MCMV replication is controlled within the first weeks of primary infection in visceral organs by IFN γ -producing CD8⁺ T cells, MCMV persists for months in mucosal sites fostering its transmission via mucosal secretions and specifically in the salivary glands (SG), virus replication is exclusively under the control of CD4⁺ and not CD8⁺ T cells [2,3]. The saliva is the most relevant vehicle for horizontal transmission of both HCMV and MCMV and the capacity of the virus to actively replicate in the SG for prolonged time periods supports its transmission efficiency. To achieve this, CMV

has evolved to encode a number of immune evasion genes that interfere with MHC I restricted antigen presentation. Particularly in the SG, the action of MCMV-encoded immune evasion genes completely deprives CD8⁺ T cells of their cognate antigen by exceptionally efficient MHC I downregulation in infected acinar glandular epithelial cells, the saliva producing cell type of the SG [4]. In consequence, the SG is the only organ where MCMV resists CD8⁺ T cell mediated control. This knowledge is based on studies conducted during primary MCMV infection and suggests that CD8⁺ T cells may also be incapable of curtailing viral reactivation or superinfection events during latency. However, the validity of this theory has never been tested and has in fact been called into question with the discovery of the SG's enormous potential to accommodate MCMV-specific tissue-resident memory CD8⁺ T (T_{RM}) cells [5,6].

In this review, we will set this new finding in context to the existing knowledge of herpesvirus-specific T_{RM} cells, elaborate their distinctive features in the SG, and discuss the implications for CMV immunity and vaccine strategies.

Tissue-resident memory T cells control viral replication and reactivation at mucosal sites

Classically, memory T cells are divided into central memory T cells (T_{CM}) and effector memory T cells (T_{EM}) based on specific recirculation patterns. T_{CM} cells circulate between lymphoid organs and blood and rapidly expand upon antigen re-encounter, while T_{EM} cells survey inflamed peripheral sites and exert immediate effector functions. As a result of technical shortcomings, it was assumed that memory T cells found in peripheral tissues constantly and uniformly recirculate. Important revisions of this long-standing concept of circulatory immune surveillance were initiated by studies demonstrating the existence of non-recirculating T_{RM} cells - a concept that is

particularly well studied for CD8⁺ T cells. The tissue-resident nature of this population was functionally shown by the lack of recirculation via parabiotic surgery (the vascular conjoining of a naïve parabiont with an immune parabiont that harbours memory T cells), tissue transplantation, and blockade of T cell recirculation [7-10]. Sizeable proportions of CD8⁺ and CD4⁺ T_{RM} cells express specific signature phenotypes, although recent studies indicate that these signatures are not sufficient for the identification of non-recirculating cells in tissues [11]. In the lung CD4⁺ T_{RM} cells co-express CD11a and CD69 [10], while CD8⁺ T_{RM} cells co-express CD103 and CD69 and have been found in many tissues such as the skin, the brain, sensory ganglia, the intestinal mucosa, and secretory glands [7,8,12-15]. The inductive cues for CD4⁺ and CD8⁺ T_{RM} generation and maintenance seem to be tissue- and context dependent. Antigen independent CD8⁺ T_{RM} induction is supported in the gut, the skin, the female reproductive tract as well as in the SG [5,6,15-17]. In contrast, CD8⁺ T_{RM} formation in the brain occurs dependent of local antigen [12]. In case of CD4⁺ T_{RM} formation, cognate local antigen was dispensable in the lung after influenza virus infection [10] but was critical in the SG after MCMV infection [5,6] and in the genital mucosa after HSV-2 infection [18].

CD4⁺ T_{RM} in the lung and in mucosal vaginal tissue and CD8⁺ T_{RM} cells in many tissues are long-lived and permanently positioned at peripheral sites that are prone to pathogenic invasion or reactivation, enabling them to respond most immediately to invading pathogens. In fact, the ability of circulating T_{EM} cells to protect against peripheral infections is very limited [9,16,19] and instead, T_{RM} cells substantially contribute to pathogen elimination at these peripheral sites as demonstrated in numerous infection models including influenza and *Mycobacterium tuberculosis*

infection of the lung or herpes simplex virus 1 (HSV-1) infection in skin and vaginal mucosa [8,10,16].

Herpesvirus infection generates protective CD4⁺ and CD8⁺ T_{RM} cells at peripheral sites of viral reinfection

The full benefit of T_{RM} cells manifests in the defense against herpesviruses that maintain latent reservoirs in absence of inflammation and sporadically reactivate in epithelial and neuronal tissues. Under non-inflammatory conditions, these peripheral sites are highly restricted to circulating T cells and the absence of T_{RM} cells would render these tissues particularly vulnerable to viral reactivation and superinfection. The alpha-herpesviruses HSV-1 and HSV-2 establish latent reservoirs in innervating neurons of the skin and vaginal epithelium, respectively [7,20]. HSV-1-specific CD8⁺ T_{RM} cells that reside at these sites are able to rapidly detect HSV-1 and provide protective immunity in a manner that is superior to their circulating counterparts [8,16,21,22]. While the protection against local HSV-1 infection appears to be an exclusive function of CD8⁺ T_{RM} cells, HSV-2 infection of the vaginal mucosa additionally establishes CD4⁺ T_{RM} cells that provide protection against local HSV-2 infection [18,23,24].

In humans, HCMV was described to preferentially establish latency in myeloid cells (reviewed in [25]) while in the murine model, the majority of the latent MCMV reservoir is found in non-hematopoietic cells and secretory glands including the SG [26]. During acute virus infection of the SG, CD4⁺ T cells are required to establish viral latency because MCMV suppresses the function of effector CD8⁺ T cells by highly efficient downregulation of MHC I molecules specifically in saliva producing epithelial cells [3,4,27]. Surprisingly, we recently demonstrated that while both CD4⁺

and CD8⁺ T cells in the SG can adopt the phenotypic features of T_{RM} cells (CD103 / CD69 co-expression for CD8⁺ T cells and CD11a / CD69 co-expression for CD4⁺ T cells) and are largely disconnected from the circulation (evidenced by absence of labelling by intravascularly administered antibodies and low level of equilibration between labelled cells within the SG and the vasculature), it is the CD8⁺ subset that protects against MCMV challenge infection in the SG [5], suggesting that these cells might also prevent viral reactivation from latency (Figure 1). Analysis of the cell types permissive for early MCMV infection in the SG indicated that MCMV targets largely non-epithelial cells in which MCMV fails to achieve complete MHC I downregulation, thereby providing a window of opportunity for readily positioned CD8⁺ T cells to detect viral antigen and control virus replication [5]. So far MCMV challenge experiments or CMV superinfection studies were mainly conducted using the subcutaneous route of reinfection, which is likely leading to a systemic viral challenge [28-31]. In challenge infection studies, systemic memory CD8⁺ T cells were shown to afford protection against lethal challenge in immunocompromised murine hosts [29,30] or to provide protection against systemic reactivation of HCMV in immunosuppressed organ transplant recipients [32-34]. However, in superinfection studies carried out in rhesus macaques, expression of CMV-encoded immune evasion genes was linked to the inability of CMV-specific CD8⁺ T cell immunity in restricting subcutaneous CMV superinfection [28]. Our own data, however, indicate that MCMV-encoded immune evasion genes do not compromise the ability of SG-resident CD8⁺ T_{RM} cells in protecting against intraglandular challenge infection with MCMV [5].

The mechanisms by which T_{RM} cells contain local infections are a field of active inquiry. CD8⁺ T_{RM} cells isolated from the brain, skin, and intestinal mucosa exert

enhanced effector functions, such as rapid production of IFN γ and target cell lysis [9,12,35,36], demonstrating that CD8⁺ T_{RM} cells are fully equipped to immediately control peripheral infection directly. Yet, the actions of CD8⁺ T_{RM} cells stretch far beyond the classical antiviral effects of cytotoxic T cells. By inducing local alterations of the tissue environment including the induction of antiviral and antibacterial gene expression, the activation of antigen-presenting cells and the recruitment of circulating immune cells, activated CD8⁺ T_{RM} initiate a state of antimicrobial defence that can mediate protection against heterologous infections. Specifically, in a manner dependent on IFN γ , TNF, and IL-2R, CD8⁺ T_{RM} activation initiates the maturation of dendritic cells, the activation of NK cells, as well as the recruitment of memory B and T cells to an extent that can generate near-sterilising immunity against antigen-unrelated pathogens [37,38]. Moreover, instead of killing infected neurons and causing immunopathology, CD8⁺ T_{RM} cells sustain HSV-1 latency by granzyme B mediated degradation of ICP4, an immediate early protein involved in viral replication [22].

Phenotypic equivalents of T_{RM} cells specific against herpesviruses exist in humans

The discovery of T_{RM} cells and their relevance in infection in the mouse model has prompted the search for equivalents of resident T cell populations in humans. These efforts are highly limited by technical constraints, and mainly restrict the analysis to phenotypic characterizations of T cell populations.

Extensive analyses of the memory T cell composition in human tissues performed by Sathaliyawala et al. [39] revealed that memory T cells exhibit tissue-specific compartmentalization throughout the human body, as well. Specifically, the vast

majority of memory T cells in lymphoid and mucosal sites expresses CD69, whereas circulating memory T cells are CD69 negative. CD8⁺ memory T cells in mucosal sites furthermore specifically express CD103. Evidence suggests that herpesvirus-specific CD8⁺ T_{RM} cells are also established in humans. For instance, CD103⁺ CD8⁺ T cells specific for Epstein Barr Virus (EBV) were found in human tonsils [40], and HSV-2 specific CD103⁺ CD8⁺ T cells were identified in human vaginal skin [41]. Turner et al. [42] reported that a significant proportion of HCMV-specific CD8⁺ T cells expresses CD69 in the lung and spleen, indicating that CMV infection promotes the establishment of T_{RM} populations in multiple tissues in humans. Yet, lung-resident memory CD8⁺ T cells specific for influenza virus and respiratory syncytial virus (RSV) predominantly express CD103, as opposed to HCMV-specific CD8⁺ memory T cells (reviewed in [43]), indicating that CD103 expression in the lung is a feature of memory CD8⁺ T cells specific for typical respiratory viruses. In line with this finding, we did not observe MCMV-specific CD103 expressing CD8⁺ T cells in murine lungs [5].

Importantly, in both mice and humans, memory CD4⁺ T cells outnumber memory CD8⁺ T cells in mucosal tissues [39,42], implying an evolutionary benefit from accommodating not only CD8⁺ but also CD4⁺ T cells in tissues. In mice, CD4⁺ T_{RM} cells readily produce effector cytokines and were shown to protect naïve recipients in adoptive transfer experiments, from lethal influenza lung infection [10], suggesting that CD4⁺ T_{RM} cells are as efficient as CD8⁺ T_{RM} cells in their ability to mount rapid recall responses and control localized infection. In HSV-2 infection, CD4⁺ T cells play a key role in protection, while CD8⁺ T cells appear dispensable [44] and CD4⁺ T_{RM} cells were shown to efficiently protect against HSV-2 challenge. Whether CD4⁺ T_{RM} cells also provide protection against human herpesvirus infection has not yet been

addressed. However, CD4⁺ T_{RM} cells appear to exist in humans as well: Walrath et al. report tissue-retained CD4⁺ T cells in the lungs of tuberculosis patients [45]. Purwar et al. describe a lung-resident population of CD4⁺ T_{RM} cells that are able to proliferate in response to stimulation with influenza antigen [46].

Requirements for T_{RM} induction are subset- and tissue-specific

The protective benefit of both CD4⁺ as well as CD8⁺ T_{RM} cell subsets in different models has encouraged efforts to specifically induce T_{RM} cells in the context of vaccination or therapeutic approaches. To this end, it is crucial to define and understand the different requirements for CD4⁺ and CD8⁺ T_{RM} generation and maintenance. In order to persist in a peripheral organ, T_{RM} cells must resist signals that induce tissue egress. Lymphocytes are guided to efferent lymphatics by a chemotactic gradient of sphingosine-1-phosphate (S1P), which is sensed by the S1P receptor 1 (S1PR1) and eventually enables tissue egress [47]. CD69 is expressed on the vast majority of T_{RM} cells and antagonises the action of the S1PR1 by interfering with its cell surface expression, thus desensitising the T_{RM} cell towards S1P-mediated egress stimuli [48]. Another mechanism of T_{RM} cell retention is integrin-mediated adhesion. The integrin CD103 tethers T_{RM} cells to E-Cadherin, which is highly expressed on epithelial cells [49]. In consequence, CD103-expressing CD8⁺ T_{RM} cells preferentially co-localize with epithelial structures [21]. Tissue retention of CD103 deficient CD8⁺ T cells is strikingly impaired in a variety of peripheral tissues [12,17,50]. However, not all T_{RM} cells rely on CD103 for their retention as indicated by the existence of CD103 negative CD8⁺ T_{RM} cells [11,12]. Moreover, CD103 expression in CD4⁺ T_{RM} cells is largely absent [51]. Apart from CD103, the integrin

VLA-1 interacts with collagen and laminin and its expression was found in CD8⁺ T_{RM} cells in the brain, gut, lung, and skin, as well as on CD4⁺ T_{RM} cells [8,50-53].

The requirements for T cells to take up residence and activate the required pathways for their retention in tissues are yet to be precisely defined. The impact of local cognate antigenic stimulation on the maintenance of CD8⁺ as well as CD4⁺ T_{RM} cells appears to depend on the tissue of residence. In a broad variety of peripheral tissues including gut, lung, and skin, ongoing antigenic stimulation is not required to induce CD103 expression [7,16]. However, CD103 expression on CD8⁺ T cells in neuronal tissue appears to be strictly dependent on cognate antigen in a model of VSV brain infection [12,17]. Teijaro et al. found that influenza-specific, lung-retained CD4⁺ T_{RM} cells acquired T_{RM} properties in a manner that is independent of antigen [10]. Our recent data indicate that SG-resident CD4⁺ T_{RM} cells rely on the presence of local antigen [5]. Taken together, these data clearly indicate that the tissue of residence decides upon the antigen dependence of T_{RM} formation and lodgement. Whether tissue-inherent factors outweigh subset-specific demands is not sufficiently elucidated, as studies that directly compare CD8⁺ and CD4⁺ T_{RM} formation within the same organ are scarce. Our data suggest that subset-specific demands outweigh tissue-intrinsic factors as CD4⁺ T_{RM} generation in the SG relied on local cognate antigen, while CD8⁺ T_{RM} generation was antigen-independent (Figure 1).

Implications for CMV-based vaccines

The protective benefit of both CD4⁺ as well as CD8⁺ T_{RM} cell subsets in different infection models has encouraged efforts to induce T_{RM} cells in the context of vaccination approaches.

CMV based vectors hold considerable potential in T cell vaccine development for several reasons: The asymptomatic, yet persistent and sporadically reactivating nature of CMV infection leads to the induction and maintenance of strong effector T cell responses, even if profoundly attenuated variants of CMV are used [54]. Furthermore, CMVs ability to superinfect allows for vaccination of CMV-immune individuals. These promising aspects were confirmed in rhesus macaques that were injected with recombinant rhesus CMV (RhCMV) expressing genes of simian immunodeficiency virus (SIV). This vaccine approach generated CD8⁺ T_{EM} cells which were able to restrict SIV replication at the site of infection and was superior to previously tested adenoviral vaccines that mainly induced T_{CM} responses [55,56].

The discovery that CMV infection is a potent inducer of both mucosa-resident CD4⁺ and CD8⁺ T_{RM} cells extends the scope of CMV-based vaccines even further, holding promising implications for vaccine strategies against pathogens that invade mucosae. Moreover, the efficient induction of a continuously growing population of inflationary memory CD8⁺ T cells induced specifically by CMV [57,58] (Figure 1) could be highly beneficial for the maintenance of large CD8⁺ T_{RM} populations. We have evidence that these inflationary cells, which are fueled by re-activated CD8⁺ memory T cells from LNs [26] and vascular sources [59] can provide low-level resupply of the CD8⁺ T_{RM} population in the SG [5,6] (Figure 1). This resupply might even render CD8⁺ T_{RM} cells independent of factors that are required for the maintenance of CD8⁺ T_{RM} cells in other organs, i.e. the integrin CD103, which is associated with T_{RM} cell retention in many other tissues [12,17,50].

Whether functional CD4⁺ T_{RM} cells can be generated by the same measures as CD8⁺ T_{RM} cells still requires further investigation. Insightful data were obtained from intravaginal HSV-2 infection, which leads to the lodgement of both CD8⁺ and CD4⁺

1 T_{RM} populations. As opposed to intravaginal HSV-2 infection, a conventional
2 parenteral vaccination strategy elicited systemic CD8⁺ and CD4⁺ T cell responses
3 ("prime") which could be attracted to the vaginal mucosal tissue by topical application
4 of chemokines (CXCL9/10) ("pull"). Interestingly, despite the initial recruitment of both
5 CD4⁺ and CD8⁺ T cells by the chemokine "pull", formation of a long-lived resident
6 population was restricted to CD8⁺ T cells [18]. These results indicate that CD4⁺ T
7 cells require additional factors locally provided by HSV-2 infection for long term
8 maintenance. Our recent findings indicate that CD4⁺ T_{RM} formation is strictly
9 dependent on the local presence of cognate antigen, thus corroborating the notion
10 that that these two subsets demand different requirements from the same organ for
11 T_{RM} generation [5]. These new insights should be taken into consideration for the
12 route and site of administration of vaccines that aim to induce functional CD4⁺ T_{RM}
13 populations.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

36 Conclusions

37 T_{RM} cells act as on-site guards of peripheral immunity that contain localized
38 reinfection of barrier tissues such as mucosae and skin. Their specific strength lies
39 within their ability to immediately purge infection when circulating cells are lagging
40 behind. In case of CMV infection, this benefit is highlighted by the ability of CD8⁺ T
41 cells to contain CMV infection in the SG when they are readily positioned before
42 CMVs immune evasion program achieves its maximal impact.
43
44
45
46
47
48
49
50
51
52

53 The T_{RM} concept appears universally favourable, given the ubiquity of T_{RM} cells in
54 various tissues. Yet, CD4⁺ and CD8⁺ T_{RM} cells require differential inductive cues from
55 the same tissues. Understanding how T_{RM} cells are recruited and maintained is
56
57
58
59
60
61
62
63
64
65

critically required to open promising opportunities for therapeutic and prophylactic intervention strategies that utilize the unique benefits of T_{RM} subsets.

Acknowledgements

We are grateful to the members of the Oxenius group for helpful discussions and critical reading of the manuscript. We would like to apologize that we could not comprehensively acknowledge the literature in this field due to limitations in the number of references. This work was supported by the ETH and the Swiss National Science Foundation (Grant No. 310030-146140 to AO).

References

- [1] Torti N, Oxenius A: **T cell memory in the context of persistent herpes viral infections.** *Viruses* 2012, **4**:1116-1143.
- [2] Lucin P, Pavic I, Polic B, Jonjic S, Koszinowski UH: **Gamma interferon-dependent clearance of cytomegalovirus infection in salivary glands.** *J Virol* 1992, **66**:1977-1984.
- [3] Walton SM, Mandaric S, Torti N, Zimmermann A, Hengel H, Oxenius A: **Absence of cross-presenting cells in the salivary gland and viral immune evasion confine cytomegalovirus immune control to effector CD4 T cells.** *PLoS Pathog* 2011, **7**:e1002214.
- [4] Lu X, Pinto AK, Kelly AM, Cho KS, Hill AB: **Murine cytomegalovirus interference with antigen presentation contributes to the inability of CD8 T cells to control virus in the salivary gland.** *J Virol* 2006, **80**:4200-4202.
- ** [5] Thom JT, Weber TC, Walton SM, Torti N, Oxenius A: The Salivary Gland Acts as a Sink for Tissue-Resident Memory CD8(+) T Cells, Facilitating Protection from Local Cytomegalovirus Infection.** *Cell Rep* 2015, **13**:1125-1136.
- First in vivo characterization of the phenotypical and functional properties of tissue-resident MCMV-specific CD4⁺ and CD8⁺ T cells in the salivary gland (SG) including the delineation of the inductive cues for their establishment. SG-resident CD8⁺ T cells were shown to confer protection against intraglandular MCMV challenge owing to recognition of MCMV infected target cells prior to full MCMV-mediated MHC class I downregulation.
- ** [6] Smith CJ, Caldeira-Dantas S, Turula H, Snyder CM: Murine CMV Infection Induces the Continuous Production of Mucosal Resident T Cells.** *Cell Rep* 2015, **13**:1137-1148.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Frist demonstration of a dynamic regulation of CD8⁺ T_{RM} populations in the SG after MCMV infection: after initial establishment of a pronounced CD8⁺ T_{RM} population in the SG, low numbers of new CD8⁺ T_{RM} could be recruited from the circulating pool of MCMV-specific CD8⁺ T cells throughout infection, resulting in comparable immunodominance within circulating and T_{RM} CD8⁺ T cell populations over time.

[7] Klonowski KD, Williams KJ, Marzo AL, Blair DA, Lingenheld EG, Lefrancois L: **Dynamics of blood-borne CD8 memory T cell migration in vivo.** *Immunity* 2004, **20**:551-562.

[8] Gebhardt T, Wakim LM, Eidsmo L, Reading PC, Heath WR, Carbone FR: **Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus.** *Nat Immunol* 2009, **10**:524-530.

[9] Jiang X, Clark RA, Liu L, Wagers AJ, Fuhlbrigge RC, Kupper TS: **Skin infection generates non-migratory memory CD8⁺ T(RM) cells providing global skin immunity.** *Nature* 2012, **483**:227-231.

[10] Teijaro JR, Turner D, Pham Q, Wherry EJ, Lefrancois L, Farber DL: **Cutting edge: Tissue-retentive lung memory CD4 T cells mediate optimal protection to respiratory virus infection.** *J Immunol* 2011, **187**:5510-5514.

** [11] Steinert EM, Schenkel JM, Fraser KA, Beura LK, Manlove LS, Igyarto BZ, Southern PJ, Masopust D: **Quantifying Memory CD8 T Cells Reveals Regionalization of Immunosurveillance.** *Cell* 2015, **161**:737-749.

Milestone report about present shortcomings in quantifying T cells in peripheral tissues, implicating the use of quantitative immunofluorescence microscopy and parabiosis experiments for accurate enumeration of tissue-resident memory CD8⁺ T cells as conventional methods for lymphocyte isolation fall short in quantitative recovery of tissue-resident T cells. Using quantitative immunofluorescence microscopy and parabiosis experiments T cells resident within non-lymphoid tissues

were found to vastly outnumber recirculating cells, further emphasizing their role in tissue surveillance.

[12] Wakim LM, Woodward-Davis A, Bevan MJ: **Memory T cells persisting within the brain after local infection show functional adaptations to their tissue of residence.** *Proc Natl Acad Sci U S A* 2010, **107**:17872-17879.

[13] Schon MP, Arya A, Murphy EA, Adams CM, Strauch UG, Agace WW, Marsal J, Donohue JP, Her H, Beier DR, et al.: **Mucosal T lymphocyte numbers are selectively reduced in integrin alpha E (CD103)-deficient mice.** *J Immunol* 1999, **162**:6641-6649.

[14] Kim SK, Reed DS, Heath WR, Carbone F, Lefrancois L: **Activation and migration of CD8 T cells in the intestinal mucosa.** *J Immunol* 1997, **159**:4295-4306.

[15] Hofmann M, Pircher H: **E-cadherin promotes accumulation of a unique memory CD8 T-cell population in murine salivary glands.** *Proc Natl Acad Sci U S A* 2011, **108**:16741-16746.

[16] Mackay LK, Stock AT, Ma JZ, Jones CM, Kent SJ, Mueller SN, Heath WR, Carbone FR, Gebhardt T: **Long-lived epithelial immunity by tissue-resident memory T (TRM) cells in the absence of persisting local antigen presentation.** *Proc Natl Acad Sci U S A* 2012, **109**:7037-7042.

[17] Casey KA, Fraser KA, Schenkel JM, Moran A, Abt MC, Beura LK, Lucas PJ, Artis D, Wherry EJ, Hogquist K, et al.: **Antigen-independent differentiation and maintenance of effector-like resident memory T cells in tissues.** *J Immunol* 2012, **188**:4866-4875.

[18] Shin H, Iwasaki A: **A vaccine strategy that protects against genital herpes by establishing local memory T cells.** *Nature* 2012, **491**:463-467.

1 [19] Bachmann MF, Wolint P, Schwarz K, Oxenius A: **Recall proliferation potential**
2 **of memory CD8+ T cells and antiviral protection.** *J Immunol* 2005, **175**:4677-
3
4 4685.

5
6 [20] Gebhardt T, Whitney PG, Zaid A, Mackay LK, Brooks AG, Heath WR, Carbone
7
8 FR, Mueller SN: **Different patterns of peripheral migration by memory CD4+ and**
9 **CD8+ T cells.** *Nature* 2011, **477**:216-219.

10
11 [21] Ariotti S, Beltman JB, Chodaczek G, Hoekstra ME, van Beek AE, Gomez-
12
13 Eerland R, Ritsma L, van Rheenen J, Maree AF, Zal T, et al.: **Tissue-resident**
14 **memory CD8+ T cells continuously patrol skin epithelia to quickly recognize**
15 **local antigen.** *Proc Natl Acad Sci U S A* 2012, **109**:19739-19744.

16
17 [22] Knickelbein JE, Khanna KM, Yee MB, Baty CJ, Kinchington PR, Hendricks RL:
18
19 **Noncytotoxic lytic granule-mediated CD8+ T cell inhibition of HSV-1**
20 **reactivation from neuronal latency.** *Science* 2008, **322**:268-271.

21
22 * [23] Iijima N, Iwasaki A: **T cell memory. A local macrophage chemokine network**
23
24 **sustains protective tissue-resident memory CD4 T cells.** *Science* 2014, **346**:93-
25
26 98.

27
28 Local protection against HSV-2 challenge was mediated by pre-existing CD4⁺ T_{RM} in
29
30 the genital mucosa. Importantly, local macrophages were essential in retaining CD4⁺
31
32 T_{RM} in the vaginal mucosa by providing a local CCL5 that supported formation and
33
34 maintenance of lymphocyte clusters containing HSV-2-specific CD4⁺ T_{RM}. These
35
36 results emphasize the establishment of T_{RM} cells in the context of vaccines against
37
38 sexually transmitted viral diseases.

39
40 * [24] Zhu J, Peng T, Johnston C, Phasouk K, Kask AS, Klock A, Jin L, Diem K,
41
42 Koelle DM, Wald A, et al.: **Immune surveillance by CD8αα+ skin-resident**
43
44 **T cells in human herpes virus infection.** *Nature* 2013, **497**:494-497.

One of the first studies associating HSV-2 control in human skin with the presence of long-term persisting, skin-resident antivirally active oligoclonal CD8 $\alpha\alpha^+$ T cells. Those cells lack chemokine receptor required for tissue emigration. Importantly, rapid HSV-2 control was associated with focal abundance of CD8 $\alpha\alpha^+$ T cells, suggesting a critical role in tissue surveillance and rapid containment of HSV-2 reactivation in human skin.

[25] Sinclair J: **Human cytomegalovirus: Latency and reactivation in the myeloid lineage.** *J Clin Virol* 2008, **41**:180-185.

[26] Torti N, Walton SM, Brocker T, Rulicke T, Oxenius A: **Non-hematopoietic cells in lymph nodes drive memory CD8 T cell inflation during murine cytomegalovirus infection.** *PLoS Pathog* 2011, **7**:e1002313.

[27] Jonjic S, Mutter W, Weiland F, Reddehase MJ, Koszinowski UH: **Site-restricted persistent cytomegalovirus infection after selective long-term depletion of CD4+ T lymphocytes.** *J Exp Med* 1989, **169**:1199-1212.

[28] Hansen SG, Powers CJ, Richards R, Ventura AB, Ford JC, Siess D, Axthelm MK, Nelson JA, Jarvis MA, Picker LJ, et al.: **Evasion of CD8+ T cells is critical for superinfection by cytomegalovirus.** *Science* 2010, **328**:102-106.

[29] Holtappels R, Simon CO, Munks MW, Thomas D, Deegen P, Kuhnappel B, Daubner T, Emde SF, Podlech J, Grzimek NK, et al.: **Subdominant CD8 T-cell epitopes account for protection against cytomegalovirus independent of immunodomination.** *J Virol* 2008, **82**:5781-5796.

[30] Bohm V, Podlech J, Thomas D, Deegen P, Pahl-Seibert MF, Lemmermann NA, Grzimek NK, Oehrlein-Karpi SA, Reddehase MJ, Holtappels R: **Epitope-specific in vivo protection against cytomegalovirus disease by CD8 T cells in the murine model of preemptive immunotherapy.** *Med Microbiol Immunol* 2008, **197**:135-144.

- [31] Reddehase MJ, Mutter W, Munch K, Buhning HJ, Koszinowski UH: **CD8-positive T lymphocytes specific for murine cytomegalovirus immediate-early antigens mediate protective immunity.** *J Virol* 1987, **61**:3102-3108.
- [32] Nauerth M, Weissbrich B, Knall R, Franz T, Dossinger G, Bet J, Paszkiewicz PJ, Pfeifer L, Bunse M, Uckert W, et al.: **TCR-ligand koff rate correlates with the protective capacity of antigen-specific CD8+ T cells for adoptive transfer.** *Sci Transl Med* 2013, **5**:192ra187.
- [33] Scheinberg P, Melenhorst JJ, Brenchley JM, Hill BJ, Hensel NF, Chattopadhyay PK, Roederer M, Picker LJ, Price DA, Barrett AJ, et al.: **The transfer of adaptive immunity to CMV during hematopoietic stem cell transplantation is dependent on the specificity and phenotype of CMV-specific T cells in the donor.** *Blood* 2009, **114**:5071-5080.
- [34] Bunde T, Kirchner A, Hoffmeister B, Habedank D, Hetzer R, Cherepnev G, Proesch S, Reinke P, Volk HD, Lehmkuhl H, et al.: **Protection from cytomegalovirus after transplantation is correlated with immediate early 1-specific CD8 T cells.** *J Exp Med* 2005, **201**:1031-1036.
- [35] Masopust D, Vezys V, Marzo AL, Lefrancois L: **Preferential localization of effector memory cells in nonlymphoid tissue.** *Science* 2001, **291**:2413-2417.
- [36] Hawke S, Stevenson PG, Freeman S, Bangham CR: **Long-term persistence of activated cytotoxic T lymphocytes after viral infection of the central nervous system.** *J Exp Med* 1998, **187**:1575-1582.
- ** [37] Ariotti S, Hogenbirk MA, Dijkgraaf FE, Visser LL, Hoekstra ME, Song JY, Jacobs H, Haanen JB, Schumacher TN: T cell memory. Skin-resident memory CD8(+) T cells trigger a state of tissue-wide pathogen alert.** *Science* 2014, **346**:101-105.

One of the first reports identifying the protective mechanism of CD8⁺ T_{RM} also in case of heterologous challenge infections. Activated CD8⁺ T_{RM} were found to rapidly induce transcription of various antiviral and antibacterial genes via secretion of IFN γ , including chemokines and cytokines involved in innate immune cell activation and recruitment. This local induction of a broad antiviral state provides a mechanistic explanation for the protection against antigenically heterologous virus challenge.

* [38] Schenkel JM, Fraser KA, Masopust D: **Cutting edge: resident memory CD8 T cells occupy frontline niches in secondary lymphoid organs.** *J Immunol* 2014, **192**:2961-2964.

First report identifying a population of CD8⁺ T_{RM} cells in secondary lymphoid organs: A proportion of virus-specific memory CD8⁺ T cells show phenotypic features of T_{RM} cells, are disconnected from the circulation and localize at entry points of secondary lymphoid organs (splenic marginal zone, red pulp, and lymph node sinuses), implying a role in surveilling entry ports of secondary lymphoid organs.

** [39] Sathaliyawala T, Kubota M, Yudanin N, Turner D, Camp P, Thome JJ, Bickham KL, Lerner H, Goldstein M, Sykes M, et al.: **Distribution and compartmentalization of human circulating and tissue-resident memory T cell subsets.** *Immunity* 2013, **38**:187-197.

First comprehensive analysis of human T cell subset distribution in circulation and tissues from deceased organ donors. This unique analysis revealed that effector memory CD4⁺ and CD8⁺ T cells were abundant in mucosal tissues whereas central memory subsets and naïve T cells were localized to lymphoid tissues. Interestingly, in non-lymphoid tissues - as opposed to the circulation - memory T cells expressed CD69, indicative for T_{RM} cells.

[40] Hislop AD, Kuo M, Drake-Lee AB, Akbar AN, Bergler W, Hammerschmitt N, Khan N, Palendira U, Leese AM, Timms JM, et al.: **Tonsillar homing of Epstein-**

Barr virus-specific CD8+ T cells and the virus-host balance. *J Clin Invest* 2005, 115:2546-2555.

[41] Zhu J, Hladik F, Woodward A, Klock A, Peng T, Johnston C, Remington M, Magaret A, Koelle DM, Wald A, et al.: **Persistence of HIV-1 receptor-positive cells after HSV-2 reactivation is a potential mechanism for increased HIV-1 acquisition. *Nat Med* 2009, 15:886-892.**

[42] Turner DL, Bickham KL, Thome JJ, Kim CY, D'Ovidio F, Wherry EJ, Farber DL: **Lung niches for the generation and maintenance of tissue-resident memory T cells. *Mucosal Immunol* 2014, 7:501-510.**

[43] van Aalderen MC, Remmerswaal EB, ten Berge IJ, van Lier RA: **Blood and beyond: properties of circulating and tissue-resident human virus-specific alphabeta CD8(+) T cells. *Eur J Immunol* 2014, 44:934-944.**

[44] Eriksson K, Bellner L, Gorander S, Lowhagen GB, Tunback P, Rydberg K, Liljeqvist JA: **CD4(+) T-cell responses to herpes simplex virus type 2 (HSV-2) glycoprotein G are type specific and differ in symptomatic and asymptomatic HSV-2-infected individuals. *J Gen Virol* 2004, 85:2139-2147.**

[45] Walrath J, Zukowski L, Krywiak A, Silver RF: **Resident Th1-like effector memory cells in pulmonary recall responses to *Mycobacterium tuberculosis*. *Am J Respir Cell Mol Biol* 2005, 33:48-55.**

[46] Purwar R, Campbell J, Murphy G, Richards WG, Clark RA, Kupper TS: **Resident memory T cells (T(RM)) are abundant in human lung: diversity, function, and antigen specificity. *PLoS One* 2011, 6:e16245.**

[47] Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V, Allende ML, Proia RL, Cyster JG: **Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* 2004, 427:355-360.**

- [48] Shiow LR, Rosen DB, Brdickova N, Xu Y, An J, Lanier LL, Cyster JG, Matloubian M: **CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs.** *Nature* 2006, **440**:540-544.
- [49] Cepek KL, Shaw SK, Parker CM, Russell GJ, Morrow JS, Rimm DL, Brenner MB: **Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the alpha E beta 7 integrin.** *Nature* 1994, **372**:190-193.
- [50] Mackay LK, Rahimpour A, Ma JZ, Collins N, Stock AT, Hafon ML, Vega-Ramos J, Lauzurica P, Mueller SN, Stefanovic T, et al.: **The developmental pathway for CD103(+)CD8+ tissue-resident memory T cells of skin.** *Nat Immunol* 2013, **14**:1294-1301.
- [51] Chapman TJ, Topham DJ: **Identification of a unique population of tissue-memory CD4+ T cells in the airways after influenza infection that is dependent on the integrin VLA-1.** *J Immunol* 2010, **184**:3841-3849.
- [52] Wakim LM, Woodward-Davis A, Liu R, Hu Y, Villadangos J, Smyth G, Bevan MJ: **The molecular signature of tissue resident memory CD8 T cells isolated from the brain.** *J Immunol* 2012, **189**:3462-3471.
- [53] Ray SJ, Franki SN, Pierce RH, Dimitrova S, Koteliansky V, Sprague AG, Doherty PC, de Fougères AR, Topham DJ: **The collagen binding alpha1beta1 integrin VLA-1 regulates CD8 T cell-mediated immune protection against heterologous influenza infection.** *Immunity* 2004, **20**:167-179.
- [54] Slavuljica I, Busche A, Babic M, Mitrovic M, Gasparovic I, Cekinovic D, Markova Car E, Pernjak Pugel E, Cikovic A, Lisnic VJ, et al.: **Recombinant mouse cytomegalovirus expressing a ligand for the NKG2D receptor is attenuated and has improved vaccine properties.** *J Clin Invest* 2010, **120**:4532-4545.
- [55] Hansen SG, Vieville C, Whizin N, Coyne-Johnson L, Siess DC, Drummond DD, Legasse AW, Axthelm MK, Oswald K, Trubey CM, et al.: **Effector memory T cell**

responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. *Nat Med* 2009, **15**:293-299.

[56] Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, Coyne-Johnson L, Whizin N, Oswald K, Shoemaker R, Swanson T, et al.: **Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine.** *Nature* 2011, **473**:523-527.

[57] Karrer U, Sierro S, Wagner M, Oxenius A, Hengel H, Koszinowski UH, Phillips RE, Kleenerman P: **Memory inflation: continuous accumulation of antiviral CD8+ T cells over time.** *J Immunol* 2003, **170**:2022-2029.

[58] Munks MW, Cho KS, Pinto AK, Sierro S, Kleenerman P, Hill AB: **Four distinct patterns of memory CD8 T cell responses to chronic murine cytomegalovirus infection.** *J Immunol* 2006, **177**:450-458.

[59] Smith CJ, Turula H, Snyder CM: **Systemic hematogenous maintenance of memory inflation by MCMV infection.** *PLoS Pathog* 2014, **10**:e1004233.

Figure legend

Figure 1 MCMV-specific T cell responses

Dynamics of systemic MCMV-specific CD4⁺ and CD8⁺ T cell responses (left). MCMV-specific CD4⁺ T cell responses (red line) and a subset of MCMV-specific CD8⁺ T cell responses (striped blue line) follow a conventional expansion – contraction – memory pattern. In contrast, a subset of MCMV-specific CD8⁺ T cell responses (blue line) continue to expand and establish high level of a stable population of activated T_{EM} cells (termed memory inflation).

In particular in the salivary gland a majority of MCMV-specific CD4⁺ and CD8⁺ T cells adopts a T_{RM} phenotype (middle). The pie charts indicate the relative proportion of MCMV-specific CD8⁺ and CD4⁺ T cells that co-express CD103 and CD69 (for CD8⁺ T cells) or CD11a and CD69 (for CD4⁺ T cells).

Factors that promote CD4⁺ and CD8⁺ T_{RM} induction in the salivary gland and protective function of MCMV-specific T_{RM} in the salivary gland (right). Induction of a T_{RM} phenotype relies on TGFβ signaling for CD8⁺ T cells and is independent of local antigen. In contrast, induction of the T_{RM} phenotype in CD4⁺ T cells depends on local antigen but is independent of TGFβ. MCMV-specific CD8⁺ T_{RM} cells can continuously be recruited from the pool of circulating cells to the salivary gland - also during viral latency. MCMV-specific CD8⁺ T_{RM} cells can protect against a local challenge infection with MCMV.

Figure Specific T cell response patterns

— inflationary CD8⁺
 - - - conventional CD8⁺
 — CD4⁺ T cells

T_{RM} phenotype*

■ *CD103⁺CD69⁺
 ■ *CD11a⁺CD69⁺

MCMV-specific T_{RM} cells in the salivary gland

MHC I MHC II T cell receptor T_{RM} cell