# Local advantage: skin DCs prime; skin memory T cells protect

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**How the immune system responds to local infection and establishes protective immunity in susceptible tissues remains unclear. Two new studies show that local tissue-resident dendritic cells prime cytotoxic T lymphocyte responses and that memory cytotoxic T lymphocytes remain in the tissue to provide antiviral immunity.**



Cytotoxic T lymphocytes (CTLs) are key effector cells that provide protection from viral infection. $CD8^+$ T cells bearing T cell antigen receptors specific for a given viral antigen are primed in the secondary lymphoid organs by dendritic cells (DCs). Exactly which subset of DCs primes CTLs in response to viral infection has been an area of intense debate. Many pathogens enter the host via a specific niche, most commonly the mucosal surfaces, where they establish local acute and chronic infection. Herpes virus family members represent a good example of such a pathogen. In particular, herpes simplex virus type 1 (HSV-1) enters the human host through the oral mucosa and establishes latency in the trigeminal ganglion. Reactivation of latent HSV-1 in the ganglion leads to anterograde transport of virus back to the skin, causing 'cold sore' lesions around the mouth. CTLs are important both in controlling HSV-1 reactivation[1] and in limiting viral replication in the peripheral site of replication[2]. Given the restricted nature of HSV-1 replication in the epithelial cells and latency in the innervating ganglia, which cells prime $CD8^+$ T cells in the draining lymph node and how protection is afforded by memory T cells are important unresolved issues. In this issue of *Nature Immunology*, two papers show that local tissue-resident cells do both: Heath and colleagues demonstrate that CTL priming is accomplished mainly by langerin-positive $CD103^+$ dermal DCs[3], whereas Carbone and colleagues report that memory CTLs in the skin remain in the tissue and maximize protective immunity to subsequent challenge with HSV-1 (ref. 4).

There are three DC subsets in the skin: Langerhans cells in the epidermis, and two subsets of dermal DCs, consisting of langerin-negative dermal DCs and the newly described langerin-positive $CD103^+$ dermal DCs[5–7] (**Fig. 1**). Traditionally, it has been thought that local tissue-resident DCs ingest microbial antigens by phagocytosis and migrate to the draining lymph node to prime naive T cells. However, that paradigm has been challenged by many studies showing that lymph node–resident DCs are the only cells that present antigens to T cells. As for the DCs involved in CTL priming, the importance of the lymph node–resident $CD8\alpha^+$ DCs has become well accepted[8]. $CD8\alpha^+$ DCs are the main antigen-presenting cells for CTLs that are generated after infection by HSV-1, influenza virus, vaccinia virus, lymphocytic choriomeningitis virus and *Listeria monocytogenes*. $CD8\alpha^+$ DCs can exclusively prime CTL responses whether HSV-1 is injected by needle into the footpad, by the intravenous route or by dermal abrasion[9] (**Fig. 1a**). In their study presented here, Bedoui *et al.* make the intriguing observation that reactivating HSV-1 causes a second phase of infection of the entire skin dermatome innervated by the infected ganglion[3] (**Fig. 1b**), which results in a second wave of antigen presentation in the lymph nodes draining the new site of viral replication. Reactivating HSV-1 replicates in the epithelium, notably in the absence of any artificial manipulation of the skin, allowing the authors to study the course of natural infection. Taking advantage of this system, they assess DC subsets for their ability to stimulate CTLs. They find that whereas cross-presentation of viral antigen during primary infection after scarification is mediated by the lymph node–resident $CD8\alpha^+$ DCs[3,9], antigen presentation after natural infection with HSV-1 in the skin during recrudescence is handled almost exclusively by the $CD103^+$ dermal DCs (**Fig. 1b**). These results are consistent with the fact that dermal DCs are the main antigen-presenting cells after natural infection of vaginal mucosa with HSV-2 (ref. 10) and are also supported by a study showing differences in the participation of migrant versus lymph node–resident DCs in CTL priming after natural mucosal infection versus skin abrasion with HSV-1, respectively[11].

Several intriguing questions arise from this study[3]. First, why are different DCs involved in CTL priming during the primary and secondary infection? Is it possible that scarification allows HSV-1 to be carried by the lymph, circumventing the requirement for presentation by migrant DCs? This is unlikely, as migrant DCs are still needed for the $CD8\alpha^+$ DCs to prime CTL immunity after scarification[9], which indicates that even if direct entry of the virus into the lymph node does occur, it is insufficient for priming by $CD8\alpha^+$ DCs. Because scarification causes considerable tissue damage, it is conceivable that the $CD103^+$ dermal DC functions may be suppressed by tissue-derived factors, rendering them unable to prime $CD8^+$ T cells. Langerin-positive dermal DCs are shown to be responsible for cross-presenting epidermally expressed self antigen[3] and are required for contact-hypersensitivity responses[7], which suggests that these cells are able to present a diverse set of antigens. In contrast, langerin-negative dermal DCs are the main antigen-presenting cell for $CD4^+$ T cells after scarification-induced HSV-1

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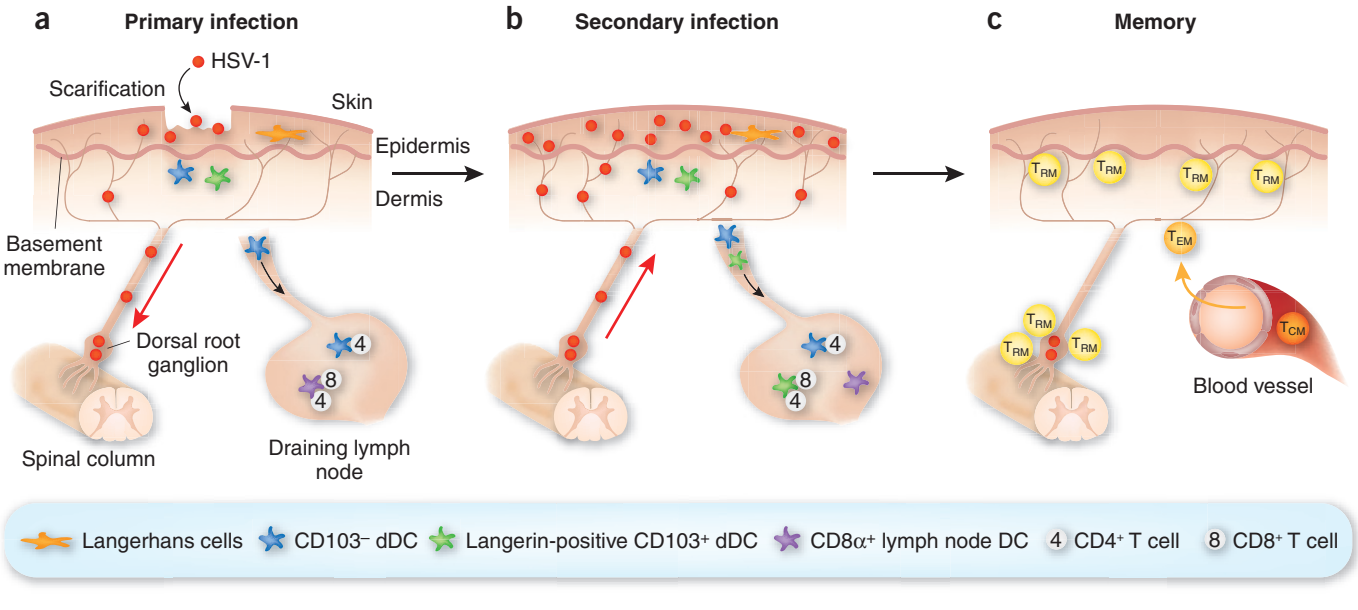


**Figure 1** The induction and execution of immune responses to HSV-1 are coordinated by local DCs and memory T cells. (**a**) Primary infection by HSV-1 is initiated by mechanical scarification of the superficial layer of the epidermis, which allows the virus to enter and replicate locally. HSV-1 infects the innervating ganglion by retrograde transport from the nerve endings in the skin and establishes latency. Virus introduced by this route is taken up by local skin-resident DCs, which, after migrating, present antigens to $CD4^+$ T cells. However, cross-priming of $CD8^+$ T cells is done uniquely by the lymph node–resident $CD8\alpha^+$ DCs. (**b**) Reactivation of latent virus in the ganglion results in anterograde migration of infectious virions to the skin and infection of epithelial cells throughout the dermatome innervated by the ganglion. After this natural route of reinfection, the viral antigens are cross-presented to $CD8^+$ T cells by langerin-positive $CD103^+$ dermal DCs, not $CD8\alpha^+$ DCs. (**c**) CTLs induced by DCs differentiate into three kinds of memory cells: central memory T cells ($T_{CM}$), effector memory T cells ($T_{EM}$) and tissue-resident memory T cells ($T_{RM}$). Only tissue-resident memory T cells take up residency in the skin (at the previous site of virus infection) and near the latently infected ganglion. After tertiary infection by HSV-1, tissue-resident memory T cells provide bulk of the protection, although effector memory T cells can also be recruited to the site from systemic circulation.



infection and after natural reactivation (**Fig. 1a,b**). Thus, it will also be important to understand the cellular and molecular mechanisms by which $CD103^+$ dermal DCs versus $CD103^-$ dermal DCs prime $CD8^+$ T and $CD4^+$ T cells. Second, which wave of T cell priming results in the establishment of protective immunity? Are the effector and memory CTLs induced by the first and second waves of HSV-1 infection quantitatively and qualitatively similar? The answers to these questions will not only be important for development of vaccines against HSV-1 but also provide key clues to the findings reported by Carbone *et al.*[4].

Once primed by DCs, the virus-specific $CD8^+$ T cells differentiate into at least two types of memory cells: central memory T cells home to lymphoid organs and have limited effector functions, whereas effector memory T cells home to peripheral tissues and rapidly secrete cytokines[12]. In their study presented here, Carbone and colleagues propose the existence of another type of memory T cells: tissue-resident memory T cells[4]. The authors carry out an elegant set of transplantation studies to demonstrate that these cells reside both near the latently infected ganglia and in the skin near the primary site of HSV-1 infection (**Fig. 1c**). Unlike central and effector memory T cells, tissue-resident memory T cells do not readily enter circulation once they establish residency in a given tissue and can proliferate locally after secondary viral challenge. Notably, tissue-resident memory T cells enter and remain in the tissue even in the absence of virus and, presumably, viral antigens. This is demonstrated by the finding that scarification alone induces the recruitment of these cells to damaged tissue and that skin containing these cells, when transplanted into a naive recipient, retains these cells for over 3 weeks even though it is separated from the latently infected ganglia. Most importantly, when secondary HSV-1 challenge is applied to previously infected flank (containing tissue-resident memory T cells) or to the opposite flank (able to recruit only effector memory T cells), the tissue containing tissue-resident memory T cells has 1% as much virus as is present in the site in which only the effector memory T cells are newly recruited. However, effector memory T cells still provide some protection relative to that afforded by unimmunized control by diminishing viral load to 1% as much as naive control. These data show that complete protection from a viral challenge requires not only systemic CTL memory but also that tissue-resident memory T cells be mobilized to the site of potential viral encounter before infection.

Tissue-resident memory T cells could represent a distinct lineage of memory cells that arise from the effector CTL pool, or they may differentiate from effector memory T cells once they arrive in the infected and/or damaged tissue. Tissue-resident memory T cells have high expression of integrin $\alpha_1\beta_1$ (VLA-1) and CD69 but are $CD62L^{lo}$ and $CD122^{lo}$. However, this expression pattern is also shared by effector memory T cells. If tissue-resident memory T cells are a distinct lineage of memory cells, what factors influence their development? Or if effector memory T cells do differentiate into tissue-resident memory T cells, this would indicate that the chemokines that recruit effector memory T cells are responsible for the eventual existence of these cells in a given tissue. The cues that are responsible for the conversion of effector memory T cells into tissue-resident memory T cells and how long this takes will be important areas of research. Another question that needs an answer is how tissue-resident memory T cells are retained in a particular tissue, given that viral antigen is not required. This is particularly relevant for autoimmune diseases in which

tissue-resident memory T cells may cause chronic tissue destruction.

Those questions aside, the findings reported by Gebhardt *et al.*[4] have important implications for vaccine development. Most pathogens gain a foothold in the host through specific portals of entry. For example, HIV-1 enters through the genital and rectal mucosa, whereas *Mycobacterium tuberculosis* gains access through the respiratory mucosa. Thus, provision of effective protection requires mobilization of tissue-resident memory T cells to the appropriate mucosal surfaces. Once again, the mechanism of the recruitment and retention of these cells in a given tissue needs to be investigated. One unconventional proposal would be to 'scratch and save (a life)': creating minor scarification to recruit these cells to the potential site of pathogen encounter after conventional parenteral immunization. Of course, not all surfaces are conducive to this type of manipulation. A more universal and powerful approach would be to immunize at the potential site of pathogen encounter. The development of safe mucosal vaccines able to establish tissue-resident memory T cells at the site of pathogen entry might be the way to prevent the transmission of deadly virus infection in humans.

In conclusion, these studies by the groups of Heath[3] and Carbone[4] provide important insight into the priming and execution of antiviral immunity to a local viral infection and open new avenues of investigation and, possibly, therapeutic approaches. With the development of a new *in vivo* approach for temporally and selectively depleting the skin of langerin-positive dermal DCs[7], future studies should identify the function of these cells in immune responses to a variety of antigens. In addition, elucidating the biology of tissue-resident memory T cells will provide clues about the generation of tissue-specific memory that is needed for vaccine development and for immune intervention in autoimmune diseases.


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# Gaining entry to an uninflamed brain

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**Little is known about how pathogenic T cells gain access to the uninflamed brain in multiple sclerosis and experimental autoimmune encephalomyelitis. A new study reports that interleukin 17–producing T helper cells enter the uninflamed central nervous system through the choroid plexus by a CCR6-CCL20–dependent mechanism.**

The blood-brain barrier is a protective barricade that limits the entry of large molecules and cells such as erythrocytes, platelets and lymphoid cells into the central nervous system (CNS) in normal conditions. Although there are well known 'windows' in this barrier, particularly adjacent to the hypothalamus, which allow cytokines such as interleukin 1 (IL-1), IL-6 and tumor necrosis factor to elicit fever[1], in general, the brain is very selective about allowing large molecules and cells to gain entry. Yet, how autoreactive T cells gain access to the uninflamed brain to initiate disease has remained unknown. In this issue of *Nature Immunology*, Reboldi and colleagues[2] demonstrate that autoreactive IL-17-producing T helper cells ($T_H$-17 cells) enter the CNS through a 'chink in the armor' of the blood-brain barrier. In a special location called the 'choroid plexus', these $T_H$-17 cells initiate the inflammatory cascade, causing demyelination through an interaction between the chemokine CCL20 and its receptor, CCR6.

For years, the brain has been considered a site of 'immune privilege', a place that tends to exclude members, both cells and molecules, of the immune community. 'Privilege' has its price, and of course, the immune system is any case skilled at outwitting protective 'covenants'. In multiple sclerosis, the blood-brain barrier is breached by a variety of immune cells, including macrophages, dendritic cells, B cells and autoreactive T cells[3]. T cells are thought to be the earliest sentinels that penetrate the blood-brain barrier, but data to support this idea are scant. So far, much has been reported on the cellular and molecular processes involved in the migration of lymphocytes to the brain through inflamed vessels. Research on homing through the inflamed blood-brain barrier has shown that the integrin $\alpha_4\beta_1$ is critical for this[3]. Such studies of the blood-brain barrier in inflammation have led to the development of the most potent drug so far approved for treatment relapsing remitting multiple sclerosis, natalizumab, a humanized monoclonal antibody specific for $\alpha_4\beta_1$.

The migration of $T_H$-17 cells to the CNS has been linked to the induction of inflammation in multiple sclerosis and in the animal model of experimental autoimmune encephalomyelitis (EAE)[4,5]. It has been shown in humans that $T_H$-17 cells 'preferentially' express CCR6 (ref. 6). Reboldi and colleagues now confirm that mice have a similar expression pattern in which CCR6 expression is restricted to $T_H$-17 cells and is not found on the surface of T helper type 1 ($T_H1$) or $T_H2$ cells[2]. Given those data, the authors next explore the contribution of this chemokine receptor to EAE.

The authors find that *Ccr6*$^{-/-}$ mice are completely resistant to EAE[2]. This effect is not due to a block in the differentiation of $T_H$-17 or $T_H1$ cells in the lymph nodes after induction of EAE but is associated with a lower capacity of $T_H1$ and $T_H$-17 cells to migrate to the CNS. EAE disease signs are restored in the *Ccr6*$^{-/-}$ mice by the transfer of myelin-specific T cells from *Ccr6*$^{+/+}$ 2D2 mice. Notably, in this transfer system, most of the T cells in the spinal cords and brain at the peak of disease are not of the *Ccr6*$^{+/+}$ donor origin but are almost all from the *Ccr6*$^{-/-}$ recipient. This finding indicates that the initial trigger of inflammation is caused by CCR6-dependent infiltration of the uninflamed CNS by autoreactive $T_H$-17 cells

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