Tissue-resident memory CD8+ T cells continuously patrol skin epithelia to quickly recognize local antigen

Silvia Ariotti, Joost B. Beltman, Grzegorz Chodaczek, Mirjam E. Hoekstra, Anna E. van Beek, Raquel Gomez-Eerland, Laila Ritsma, Jacco van Rheenen, Athanasius F. M. Marée, Tomasz Zal, Rob J. de Boer, John B. A. G. Haanen, and Ton N. Schumacher

Division of Immunology, The Netherlands Cancer Institute, 1066 CX, Amsterdam, The Netherlands; Theoretical Biology and Bioinformatics, Utrecht University, 3584 CH, Utrecht, The Netherlands; Department of Immunology, University of Texas, MD Anderson Cancer Center, Houston, TX 77054; Hubrecht Institute-Royal Netherlands Academy of Arts and Sciences and University Medical Center Utrecht, 3584 CT, Utrecht, The Netherlands; and *Computational and System Biology, John Innes Centre, Norwich NR4 7UH, United Kingdom

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Recent work has demonstrated that following the clearance of infection a stable population of memory T cells remains present in peripheral organs and contributes to the control of secondary infections. However, little is known about how tissue-resident memory T cells behave in situ and how they encounter newly infected target cells. Here we demonstrate that antigen-specific CD8+ T cells that remain in skin following herpes simplex virus infection show a steady-state crawling behavior in between keratinocytes. Spatially explicit simulations of the migration of these tissue-resident memory T cells indicate that the migratory dendritic behavior of these cells allows the detection of antigen-expressing target cells in physiologically relevant time frames of minutes to hours. Furthermore, we provide direct evidence for the identification of rare antigen-expressing epithelial cells by skin-patrolling memory T cells in vivo. These data demonstrate the existence of skin patrol by memory T cells and reveal the value of this patrol in the rapid detection of renewed infections at a previously infected site.

Cellular Potts Model | intravital imaging | HSV-1

Although it has been proposed that the control of local infections by resident memory T cells is due to the advantage conferred by the proximity to sites of viral entry (6), it has not been resolved how tissue-residing memory T cells mediate their protective effect. Here we address this question, through a combination of intravital microscopy and computational modeling. We demonstrate that the CD8+ T cells that stay behind in the epidermis actively patrol the skin, continuously migrating within the host tissue to contact surrounding cells. This uninterrupted movement, combined with the dendritic shape that memory T cells acquire upon epithelial residence, enables them to encounter infected cells in physiologically relevant timeframes. Thus, morphology and motility of tissue-resident memory T cells constitute a central component of their ability to provide enhanced local immune protection.

Results
Model to Dissect the Function of Skin-Resident Memory T Cells. To understand how tissue-resident CD8+ memory T cells guard against viral reinfections, we first set out to develop an experimental model in which the activity of skin-resident versus systemic CD8+ Tmem can be analyzed without potential confounding effects of virus-specific CD4+ memory T cells or antibodies. To this purpose, we transferred naïve CD8+ herpes simplex virus-1 (HSV)-specific (10), GFP+ T cells (GFP+gB) into C57BL/6j recipients and subsequently vaccinated the mice by skin DNA injection, using a DNA vaccine encoding the gB505 epitope that is recognized by gB T cells (Fig. 1A). The magnitude of vaccine-induced gB505-specific CD8+ T-cell responses in these mice (up to 5% of circulating CD8+ T cells at days 11–15 postvaccination; Fig. 1B) was comparable to that induced in mice that had not received adoptive transfer. However, the gB505-specific CD8+ T-cell response in recipients of GFP+ gB T cells fully consisted of GFP+ T cells (Fig. 1A), thus ensuring that the fluorescent cells observed during imaging represented the entire population of gB505-responsive T cells. Analysis of the vaccination site several weeks after treatment revealed the presence of large numbers of GFP+ cells that displayed a dendritic morphology (Fig. 1C). In agreement with previous literature (2), multiphoton analysis of the skin established that the GFP+ cells were exclusively restricted to the epidermal layers, including hair shafts (Fig. 1D and E). Flow cytometry demonstrated that the GFP+ cells extracted from the skin were uniformly CD8+TCRβ and K5gB505-stable, thereby qualifying them as bona fide HSV-specific CD8+ Tmem (Fig. 1F); furthermore, they expressed a high level of CD103 (integrin αβ) (Fig. 1G), an adhesion molecule implicated in T-cell retention within the epithelia (6). Consistent with published literature (6), the skin-resident memory T-cell pool that was induced by skin vaccination displayed a morphology and phenotype similar to that found after local skin infection with HSV, demonstrating that long-term tissue retention of CD8+ Tmem is not dependent on the presence of virus. To determine the contribution of the vaccine-induced CD8+ skin-resident memory T-cell pool to the control of authors.
secondary infections, we vaccinated recipients of GFP+gB T cells on one flank with the gB<sub>asg-505</sub> DNA vaccine, to create a localized CD8<sup>+</sup> memory T-cell pool. Three months after vaccination, mice were challenged with an HSV strain that expresses the td-tomato fluorescent protein (HSV<sub>TOM</sub>) at both the ipsilateral (i.e., previously vaccinated) and contralateral site, and infection growth was monitored over time (Fig. S1). Intravital imaging of the infected skin revealed the presence of similarly sized HSV-infected lesions early after infection (P = 0.4402). In contrast, whereas infections at the contralateral site continued to grow rapidly, HSV foci at memory CD8<sup>+</sup> T-cell sites were in large part contained (Fig. S1 C and D; comparison contra – ipsi, P < 0.001), establishing that skin-resident CD8<sup>+</sup> T cells play an important role in the control of local secondary HSV infections.

**Skin-Resident Memory T Cells Display a Continuous Migratory Behavior.**

To investigate how tissue-resident memory T cells can provide such local protection we performed long-term imaging of skin-resident GFP<sup>+</sup>CD8<sup>+</sup> Tmem at formerly infected sites. These experiments revealed that at all of the examined time points postinfection, these cells were sessile, but migrated productively within the epidermis, with mean speeds of about 1.3 μm/min (Movie S1). Whereas the speed of Tmem migration was relatively low, Tmem migration was characterized by long directional persistence, with cells moving in a similar direction for an average of ∼10.5 min. Combined, these speed and directionality parameters result in a high motility coefficient of ∼9 μm<sup>2</sup>/min, close to that of lymph node B cells (11). To address whether this steady-state migration could reflect continued low-level T-cell receptor (TCR) triggering due to the presence of residual antigen, we generated an experimental setting in which tissue-resident memory T cells reside in skin areas in which their cognate antigen was never introduced. To this purpose, naïve GFP<sup>+</sup>OTI T cells were transferred into C57BL/6j recipients and activated by application of a DNA vaccine encoding the OVA<sub>257–264</sub> epitope that is recognized by the OTI TCR to create a pool of effector GFP<sup>+</sup>OTI T cells. At this time point, mice were infected by epidermal HSV application, and entry and maintenance of the “bystander” GFP<sup>+</sup>OTI T cells at the site of HSV infection was evaluated. These analyses demonstrated that effector CD8<sup>+</sup> T cells also form long-term local T-cell memory at sites of former bystander infection (Fig. 2A). Importantly, these bystander tissue-resident memory T cells displayed a dendritic morphology (Fig. 2B) and showed a productive migration through the epidermis (Movie S2), indicating that both of these Tmem cell properties are antigen-independent. Thus, regardless of the presence of cognate antigen, CD8<sup>+</sup> T cells that enter the skin epithelium up-regulate the surface expression of CD103 and acquire dendritic morphology and constitutive motility.

**Migratory Behavior of Memory T Cells Allows Contact with a Large Number of Surrounding Cells.** The continuous movement of the epithelium-resident memory pool (Fig. 2C) suggests that in time CD8<sup>+</sup> T cells can cover long distances and as a consequence contact a large number of surrounding tissue cells. To visualize the cumulative skin area that can be patrolled by migrating populations of skin-resident CD8<sup>+</sup> Tmem over time, all consecutive images from a 4-h imaging session were overlaid (Fig. 2D). This analysis revealed that at the cell densities found within previously infected areas, the migratory behavior of CD8<sup>+</sup> Tmem is sufficient to allow the exploration of a large portion of skin within a period of hours. Constitutive steady-state migration of skin-resident Tmem was observed up to 18 mo after primary infection, the latest time point analyzed. As a control, and consistent with prior data (12), the cell body of skin-resident Langerhans cells (LCs) did not show detectable displacement (Fig. 2E). LCs, epidermal γδ T cells, and CD8<sup>+</sup> Tmem within the epidermis all have a dendritic shape. It has been established that LCs project their dendrites upward, toward the stratum corneum (14). However, the observation that skin-resident CD8<sup>+</sup> Tmem we analyzed showed a continuative process that in time CD8<sup>+</sup> Tmem show a continuous migration that allows the exploration of a large number of surrounding cells. To visualize the cumulative skin area that can be patrolled by migrating populations of skin-resident CD8<sup>+</sup> Tmem, all consecutive images from a 4-h imaging session were overlaid (Fig. 2D). This analysis revealed that at the cell densities found within previously infected areas, the migratory behavior of CD8<sup>+</sup> Tmem is sufficient to allow the exploration of a large portion of skin within a period of hours. Constitutive steady-state migration of skin-resident Tmem was observed up to 18 mo after primary infection, the latest time point analyzed. As a control, and consistent with prior data (12), the cell body of skin-resident Langerhans cells (LCs) did not show detectable displacement (Fig. 2E). LCs, epidermal γδ T cells, and CD8<sup>+</sup> Tmem within the epidermis all have a dendritic shape. It has been established that LCs project their dendrites upward, toward the stratum corneum (13), a property that allows them to capture antigens from the most superficial skin layers. Likewise, the dendrites of γδ T cells invariably project toward the stratum corneum (14). However, the observation that skin-resident CD8<sup>+</sup> Tmem show a continuous migration through the epidermis, together with the fact that the dendrites continuously change size, length, and direction (Movies S1 and S2), suggests that these dendrites could serve a different function. To contrast the morphology of dendrites from skin-resident γδ T cells, LC, and CD8<sup>+</sup> Tmem we analyzed these cell types in whole-mount stains. CD8<sup>+</sup> Tmem had fewer but thicker dendrites compared with γδ T cells; more importantly, whereas the dendritic extensions of Langerhans cells and epidermal γδ T cells pointed upward, the dendrites of CD8<sup>+</sup> Tmem were almost all parallel to the epidermis, consistent with a basal parallel to the epidermis (Fig. 3 and Movie S3). Thus, memory T cells residing in the epidermis differ from other immune cells in their migratory behavior.

**Fig. 1.** Morphology, localization, and phenotype of skin-resident memory CD8<sup>+</sup> T cells. (A) Recipients of GFP<sup>+</sup>gB T cells (day −1) were vaccinated with a DNA vaccine encoding the gB<sub>asg-505</sub> epitope (days 0, 3, and 6) and imaged 1 mo later. (B) The percentage of GFP<sup>+</sup>gB<sub>asg-505</sub> tetramer<sup>+</sup> cells was measured in blood over time (arrows indicate vaccination timepoints). (C) Top view confocal maximum intensity projections of GFP<sup>+</sup>gB<sub>asg-505</sub> T cells infiltrating skin 1 mo after local HSV<sub>TOM</sub> infection. Images are representative of five experiments. (Scale bars: Left, 50 μm; Middle and Right, 10 μm) (D and E) Areas of skin containing infiltrating GFP<sup>+</sup> gB<sub>asg-505</sub> T cells were imaged by intravital multiphoton microscopy 3 mo after infection; the position of the dermis was determined by exploiting second harmonic generation properties of collagen type I. Arrows indicate tissue direction, from outside to inside the animal. (F) Cell suspensions from skin biopsies 1 mo after local HSV<sub>TOM</sub> infection were analyzed for the indicated markers and GFP expression. Left plot is gated on live cells; right plot is gated on TCR<beta> live cells. GFP<sup>+</sup> cells are depicted in green and GFP<sup>−</sup> cells are depicted in black. (G) Surface expression of CD103 by TCR<beta>CD8<sup>+</sup>GFP<sup>+</sup> cells from previously infected skin (green), TCR<beta>CD8<sup>+</sup>GFP<sup>+</sup> splenocytes (gray), and TCR<beta>CD8<sup>+</sup>GFP<sup>+</sup> splenocytes (black line).
within the same environment with respect to their morphology and migration.

**Dendritic Shape of Skin-Resident Memory T Cells Is Independent of Skin Inflammatory State.** Effector CD8+ T cells, which are present in both the epidermis and dermis of HSV-infected mice, exhibit an amoeboid shape, whereas skin-resident memory CD8+ T cells, which are located exclusively in the epidermis (2; Fig. 1D and E), display a dendritic shape. To evaluate whether the shape difference between the two cell populations could be due to the skin layer in which they reside, we compared the shape of effector T cells (Teff) and Tmem in the same skin layer using LCs as an internal reference (Fig. S2). This analysis demonstrated that the shape difference between the two is not due to a simple difference in location. A second explanation for the “dendricity” acquired by memory T cells upon retention in the epidermis could be that this shape is imposed on CD8+ Tmem by the tight surrounding tissue after infection resolves. To investigate whether the dendritic shape displayed by the memory T-cell pool in the epidermis is solely imposed by the environment, we compared the shape of GFP+ OTI T cells present at sites of ongoing HSV infections, when either present as recently primed effector T cells or as locally resident memory T cells (Fig. 4A). Effector OTI T cells migrating within HSV-infected skin displayed the amoeboid, round shape that is also seen for antigen-specific effector T cells within effector sites (Fig. 4B). In contrast, skin-resident memory OTI T cells that infiltrated an HSV-TOM-infected area retained their dendritic, crawling behavior (Fig. 4B and C and Movie S4). Thus, the dendritic migration mode of CD8+ Tmem that are present in the epidermis does not merely stem from the physical constraints imposed by the environmental architecture, but at least partially reflects an adaptation of the memory T cells present at that site. The observation that skin-resident CD8+ Tmem that move within the epidermis continuously extend and contract dendrites along a 2D plane suggests that these cells migrate in between keratinocytes. To visualize the cellular environment of skin-resident CD8+ Tmem, we transferred naive Kaede’gB T cells (15) into Histone-2B-GFP mice (in which GFP marks the nuclei of all skin cells) and created a local skin-resident CD8+ Tmem pool. Several weeks postinfection, Kaede was locally photoconverted in vivo, to distinguish the transferred T cells from the GFP+ skin cells. Imaging of the photoconverted areas confirmed that skin-resident CD8+ Tmem extend and retract dendrites continuously in between skin cells while moving through the epidermis (Fig. 4D and Movie S5). To evaluate whether the differential behavior of Tmem and Teff correlated with phenotypic differences, the surface expression of a small number of T-cell markers was studied. CCR4 and NKG2D expression did not differ between Tmem and Teff (Fig. S3), making their contribution to the differential behavior of the two cell types unlikely. The chemokine receptor CXCR3, involved in T-cell migration to peripheral tissues (16), was detected on skin-resident memory T cells but was nearly absent on HSV-infected skin-infiltrating effector T cells (Fig. S3), conceivably reflecting down-regulation induced by ligand binding.
Subsequently, simulated cell encounters by these experimental data with that of simulated T cells that were either nonmotile or nondendritic indicate that T-cell motility is the primary factor that determines the efficiency of target cell encounter, with dendritic addition another 33% (Fig. S4C). As a side note, modeling also indicated that the dendritic (compared with amoeboid) morphology allowed migrating T cells to touch an 11% larger cell surface area of each contacted keratinocyte per time point (P < 0.001), a property that may conceivably increase the efficiency of scanning.

Skin Patrol by Tissue-Resident Memory T Cells Allows Rapid Recognition of Antigen-Expressing Cells in Vivo. To directly test the hypothesis that the epidermal migration by long-term resident memory T cells leads to antigen scanning and thereby allows the detection of antigen-expressing cells, we first established how antigen recognition by skin-resident memory T cells can be visualized in vivo. To this purpose, sites containing a GFP+gb memory T-cell pool were either left untreated or injected with the gB505 peptide, with control peptides (CMV pp65495 and Ova257-263), or with vehicle. In all cases, skin-resident Tmem displayed a transient increase in circularity and a decrease in migration speed following injection, likely reflecting skin trauma (Fig. 5 C and D and Fig. S5A). However, 3 h postinoculation, GFP+gb Tmem that had either been exposed to vehicle or to control peptides had recovered their dendritic shape and migratory behavior (Fig. 5 C–E and Movie S7). In sharp contrast, gB Tmem that had been exposed to gB505 peptide remained roundish and arrested for up to 9 h after peptide delivery (Fig. 5 C–E and Movie S8). This change in shape and motility was strictly antigen-dependent, because skin-resident OTI Tmem underwent similar changes upon Ova257-264 delivery, but not in the presence of pp65495 or gB505 control peptides (Fig. S5B). In an independent set of experiments, s.c. peptide delivery was also shown to result in T-cell rounding up and stalling in an antigen-specific manner (Fig. S6). These data demonstrate that antigen recognition by skin-resident Tmem can be visualized by alterations in both motility and morphology; the loss of motility is reminiscent of prior data on antigen recognition by naïve and effector T cells (23), and the large change in morphology is a characteristic that is unique to skin-resident memory T cells.

Having established both loss in dendricity and migration as experimental readouts to determine whether migrating Tmem can identify rare antigen-expressing cells, we created a situation in which only a small fraction of skin cells underwent de novo expression of an antigen recognized by the skin-resident memory T-cell population. To this purpose, we injected epidermis hosting a local GFP+gb memory T-cell pool with DNA encoding the Kutushka fluorescent protein as a genetic fusion to the gB505 peptide. Following DNA delivery, Kutushka-gB505-expressing cells were readily detectable amid a large number of antigen-negative cells (Movie S9). Remarkably, GFP+gb Tmem could be shown to continue migration and dendrite protrusion through the skin until they contacted an antigen-expressing target cell, after which they immediately stalled and rounded up (Fig. 6 A and B). Furthermore, stalling and rounding could also be observed for migrating Tmem even before antigen expression by the neighboring cells became detectable by microscopy (Fig. 6 C and Movie S9), suggesting that recognition of antigen expression during natural infection may occur before substantial pathogen replication can take place. Both stalling and rounding were fully antigen-dependent; Kutushka-expressing skin cells that lacked the gB505 epitope were completely ignored (Movie S9).

Quantification of the Efficiency of Target Cell Encounter by Computational Modeling. The above data demonstrate that memory T cells continuously migrate through the epidermal surface for long periods after infection and in the absence of antigenic stimulation. These observations raise the hypothesis that the combination of migratory behavior and morphology could represent a characteristic that is unique to skin-resident memory T cells. To explore to what extent the migration and morphology of Tmem would increase the contact and scanning of surrounding cells, we developed a 2D Cellular Potts Model (17), in which the density of keratinocytes (21) and T-cell migration and shape parameters closely match the experimentally determined values (SI Materials and Methods, Movie S6, Fig. 5A, and Fig. S4 A and B). Subsequently, simulated “target cells” and memory T cells were positioned randomly within an in silico field (size 0.54 mm²) and Tmem–target encounters were monitored over time. At the experimentally determined Tmem densities of about 430 cells per square millimeter, the majority of targets was detected within 1 h (Fig. S5B). Furthermore, even at T-cell densities that were up to fivefold below those determined experimentally, most targets were encountered within hours. If T-cell densities are reduced even further, the efficiency of steady-state target scanning no longer allows identification of each target cell with time periods of hours. However, even in this case viral outgrowth may potentially still be limited upon encounter of a small number of targets, through the “field effect” described by Müller et al. (22).

Comparisons of the number of target cell encounters by these simulated cells with that of simulated T cells that were either nonmotile or nondendritic indicate that T-cell motility is the primary factor that determines the efficiency of target cell encounter, with dendritic addition another 33% (Fig. S4C). As a side note, modeling also indicated that the dendritic (compared with amoeboid) morphology allowed migrating T cells to touch an 11% larger cell surface area of each contacted keratinocyte per time point (P < 0.001), a property that may conceivably increase the efficiency of scanning.

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Discussed

Taken together, the data presented here demonstrate that the CD8+ memory T cells that remain behind in areas of prior inflammation actively patrol the epidermis for antigen-expressing cells, independent of residual antigen or pathogen. The speed with which these cells migrate is much lower than that of lymph node T cells, perhaps explaining why this property has thus far not been appreciated (2). Importantly, though, we here demonstrate that, because of their high directional persistence, skin-resident memory T cells can efficiently patrol large portions of tissue, within time scales that are relevant to the time scale of viral replication. To carry out this patrol, tissue CD8+ Tmem extend dendrites in a 2D plane, thereby contrasting their morphology to that of sessile epidermal γδ T cells and Langerhans cells. Local memory T-cell patrol is likely to be of particular value in cases in which a tissue is prone to multiple attacks from the same infectious agent, such as during reactivation of herpes simplex or varicella zoster viruses (24). Thus, skin-resident CD8+ memory T cells can be considered an active first line of defense for secondary infections: Whereas during primary immune responses CD8+ T cells await the delivery of antigens that are brought to the draining lymph nodes by APCs, during local secondary responses in skin, memory T cells proactively search for antigen themselves. This observation raises the intriguing possibility that skin-resident memory T cells may be independent of local APCs for their protective function.
no contact
contact

A

B

C

Fig. 6. Patrolling skin-resident memory T cells can quickly identify rare targets. (A) Cell migration tracks of individual GFP^g8 Tmem either in contact or not in contact with an antigen-expressing target, normalized for their origin. (B) Circularity of GFP^g8 Tmem was analyzed from maximum intensity projections of individual cells. Cells were categorized based on contact with Katushka-gB^GFP-expressing cells at any time point during the imaging session, derived from one experiment. Bars indicate mean ± SEM. (C) Sequential images of migrating skin-resident memory T cells that either stop upon contact with specific target (asterisk) or keep migrating without target contact (arrow).

At present, it remains unclear which factors determine whether skin-resident T-cell memory is only formed locally or throughout the skin surface (2, 9). Nevertheless, the observation that locally patrolling T-cell memory can be induced by vaccination (Fig. 1A and B) but also at inflamed sites in which cognate antigen is absent (6; Fig. 2A and B) indicates that it should be possible to steer the formation of T-cell memory to defined epithelial areas. Based on the known protection provided by local immunity, this could be used to provide local immune defense against viruses that enter the body at well-defined epithelial sites.

Materials and Methods
Adoptive Transfer, HSV Infection, Peptide, and DNA Injection. Spleens from GFP^gB^T.1 or GFP^OTI mice were isolated and homogenized; after red blood cell lysis, CD8^ T cells were isolated and C57BL/6j mice were injected i.v. with 2.0 × 10^7 sorted naïve CD8^ T cells. Intraepithelial HSV infection was carried out on the shaved flank of anesthetized mice by tattooing a droplet containing ~2.5 × 10^6 pfu HSV_{OM} onto the skin using a sterile disposable needle bar mounted on a rotary tattoo device. For peptide delivery, 750 ng of peptide in PBS/10% (vol/vol) DMSO solution was tattooed in the flank of anesthetized mice. For skin cell transfection, 30 μg of the indicated DNA in PBS was applied onto the skin and injected using a sterile disposable needle bar mounted on a rotary tattoo device (25).

Confocal Microscopy. Anesthetized mice were placed in a custom-built chamber with the infected flank gently placed against a coverslip at the bottom side of the chamber. Images were acquired using an inverted Leica TCS SP2 confocal scanning microscope equipped with diode and argon lasers and enclosed in a custom-built environmental chamber that was maintained at 37 °C using heated air. Images were acquired using a 20×0.7 N.A. dry objective. GFP was excited at 488 nm wavelength and td-tomato at 561 nm. Typical voxel dimensions were 0.7–1.5 μm laterally × 1.5–2.5 μm axially. Three-dimensional stacks (typical size ~500 μm × ~500 μm × ~30 μm) were captured every 2 min for a period of up to 4 h.

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24. Diefenbach RJ, Miranda-Saksena M, Cunningham AL (2008) Transport of peptide in PBS/10% (vol/vol) DMSO solution was tattooed in the flank of an anesthetized mice. For skin cell transfection, 30 μg of the indicated DNA in PBS was applied onto the skin and injected using a sterile disposable needle bar mounted on a rotary tattoo device (25).
Supporting Information

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SI Materials and Methods

Mice. C57BL/6j, C57BL/6j ubiquitin-GFP, Histone-2B-GFP mice and C57BL/6j OTI TCR transgenic mice were obtained from Charles River Laboratories. gBp99-505 (SSIEFARL), Ova257-264 (SINFEKL), and CMV pp65,264-279 (NLVPMVATV) peptides were produced by standard Fmoc chemistry. MHC-tetramers loaded with indicated peptides were produced by UV-induced ligand exchange (1) and labeled with PE or APC (Invitrogen).

Reagents. Antibodies against MHC-II, CD4, CD11b, CD11c, TCR beta, CD103, CD3e (clone 17A2; BD Pharmingen). Tmem populations were identified based on GFP+ and CD3εlow signal. γδ T cells were identified as GFP+ and CD3εhigh (confirmed by anti-γδ TCR staining). To determine XY shape factors, cell areas were calculated by binarization of maximum intensity projections followed by convex hull transformation and automated calculation of a ratio of major axis to first minor axis in Slidebook 5.0 software (3i).

In Silico Simulations. In the Cellular Pott Model (CPM) formalism (5, 6), in silico cells are defined by multiple connected sites [with 2D coordinates i and j, identification number σ, and cell type τ(σ)] on a lattice. Sites that contact other cells or extracellular matrix (ECM) have a surface energy with the lattice positions they directly contact (i.e., at most eight). Changes in cell configurations occur over time, because during a simulation it is constantly attempted to extend cell sites into random neighboring positions. Such extensions are accepted with a probability that depends on the changes in the Hamiltonian:

$$ H = \sum_{\sigma} \sum_{i,j} J_{i\sigma,j\sigma} \left( (1 - \delta_{\sigma,\sigma'}) + \sum_{\sigma} \lambda (A_{\sigma} - A_\sigma)^2 \right), \quad [S1] $$

where the first term is the sum of all surface energies J, and the second term keeps cells of actual area a close to their target area A depending on the inelasticity λ. Furthermore, ε is the Kronecker delta and \( i \neq j \) sums over all eight neighbors in the 3 x 3 neighborhood. The change in H (i.e., \( \Delta H \)) upon a considered modification determines the acceptance probability of the extension, which is 1 if \( \Delta H < 0 \), and \( e^{-\Delta H/\lambda} \) otherwise (where \( \lambda \) is the amplitude of membrane fluctuations). The model and the extensions presented below were implemented in the C programming language.

Dendritic Memory T Cells. We modified our previously published simulations to describe T-cell and dendritic cell (DC) migration and their interactions using the CPM (7, 8) to model memory T-cell migration. To obtain simulated T cells with a dendritic shape and performing a persistent random walk, we explicitly defined multiple (N\(_{\text{dendrites}}\)) dendrites that are being extended and retracted. Memory T cells are endowed with an (initially random) target direction, which is updated (asynchronously between cells) every \( \Delta t \) seconds to become the displacement vector of the cell in the previous period. The initially preferred extension direction of new dendrites is biased into the current target direction of the cell according to a circular von Mises distribution with probability density function \( f(\theta) = e^{\kappa \cos(\theta)} \), where \( \theta \) is the initial direction of a new dendrite, \( \kappa \) is the modified Bessel function of order 0, \( \mu \) is the direction around which the distribution is clustered (i.e., equal to the current target direction), and \( \kappa \) is the concentration parameter (i.e., a large value for \( \kappa \) means that the initial orientation of new dendrites is strongly biased toward the target direction). Dendritic-still (nonmotile) \( T_{\text{stem}} \) are fixed in space because the starting position of new dendrites is restricted to subsequently dilated, closed, and eroded using the software package ImageJ. The 2D size of memory T cells was also estimated on the same maximum intensity projections using ImageJ.
a cubic area of five positions around the initial mean position of the cell. For dendritic-motile Tmem, new dendrites start growing at the current center of mass of a T cell (this is required to be at a position “inside” the cell). If the initial position of a new dendrite has failed to establish itself within the cell for 10 subsequent time steps because the center of mass of the cell is not part of the cell (e.g., due to a highly nonsymmetric shape), the initial position of the new dendrite is chosen from a random position inside that cell (note that the initially preferred extension direction of the dendrite is maintained in this case). After successful establishment of the initial position of a new dendrite, it will with probability $p_{\text{grow}}$ attempt to extend during the next maximally 300 time steps (if extension has failed for 50 subsequent time steps, retraction as explained below will start). To allow dendrites to grow in a slightly curved manner rather than exactly straight, the actual current extension direction of a dendrite is again chosen according to a circular von Mises distribution. However, $\mu$ now represents the currently preferred extension direction of the dendrite. Moreover, to limit the fluctuations in the dendrites’ extension direction over time the value for $\kappa$ within a dendrite ($\kappa_{\text{dendrite}}$) is much larger than the initially preferred direction of new dendrites of a cell that was discussed above ($\kappa_{\text{cell}}$). An extension into the chosen position is successful if that position is part of the cell, and otherwise another attempt to extend at the next time step (again with probability $p_{\text{grow}}$). The currently preferred extension direction of a dendrite is updated after each successful extension according to the latest actual extension direction. When a dendrite is outgrown, it will be retracted in reverse order with at most a single position per time step (occurring with probability $p_{\text{retract}}$ per time step). Upon complete withdrawal of a dendrite, a new dendrite starts to grow. To model pushing of growing dendrites against the T-cell membrane, the likelihood that membrane elements grow into positions adjacent to a dendrite is increased by extending $\Delta H$ with a term that decreases $\Delta H$ with $E_{\text{squeeze}}$ upon consideration of a membrane extension into a site that is the neighbor of a position that contains a dendrite located within the extending cell. To describe dendrites that realistically squeeze in between multiple in silico keratinocytes, $\Delta H$ is decreased even further, by $E_{\text{squeeze}}$ (which is bigger than $E_{\text{extend}}$), if the considered membrane extension is both adjacent to a position with a dendrite and touching at least two different keratinocytes. Breaking of dendrites is prevented by requiring membrane elements to adhere to the cell membrane (a dendrite is present to remain intact). The combination of a) dendrite growth in a preferred direction but in a slightly curved manner and b) dendrite pushing against the cell membrane (which is strongest in between keratinocytes) results in a persistent random walk during which pseudopods of T cells realistically embrace keratinocytes.

**Roundish Memory T Cells.** In *silico* memory T cells with a roundish shape (roundish-motile Tmem) were obtained without the explicit definition of dendrites: As above, cells are endowed with an (initially random) target direction, and additionally with a “directional propensity” $\gamma$. Together, these determine the likelihood that a membrane extension occurs, according to $\Delta H = -\cos(\alpha)$, where $\alpha$ is the angle between the current target direction and the vector given by the position considered to be modified and the mean position of the cell. This extra term in the Hamiltonian is only considered for positions that are being “retracted” (i.e., when another cell extends into the cell position under consideration). In this way, the cells more strongly keep a roundish shape. The target direction of roundish-motile Tmem is updated every $\Delta t$ time steps (asynchronously between cells) to become the displacement vector of the previous period.

**Simulation Parameters.** In our simulations we set the dimension of lattice sites equal to that measured experimentally (i.e., one site represents 732 $\mu$m $\times$ 732 $\mu$m). We consider a wrapped space (2D torus) of 1,000 $\times$ 1,000 pixels that is nearly completely packed with cells. Specifically, various combinations of memory T-cell and keratinocyte numbers were simulated: 20 Tmem with 8,820 keratinocytes, 50 Tmem with 8,780 keratinocytes, 100 Tmem with 8,720 keratinocytes, and 230 Tmem with 8,550 keratinocytes. Note that for presentation purpose, in the snapshots and movies shown, the simulations were performed in a reduced space (500 $\times$ 500 pixels) and cell numbers are scaled down accordingly (yet all presented statistics are based on the large 1,000 $\times$ 1,000 pixel simulations). In simulations with “infected targets” (Fig. 5B), 1,000 randomly chosen keratinocytes are considered to be infected and the time until those specific targets are detected is monitored. Cells are initialized at a random position as a 3 $\times$ 3 pixel block. Within a few simulated time steps they are close to their target areas, which are taken from experimental data (88.7 $\mu$m$^2$ for memory T cells according to estimates from our own imaging experiments, and 64.3 $\mu$m$^2$ for keratinocytes according to published estimates; ref. 21 in main text). We consider in *silico* memory T cells and keratinocytes that do not have a differential adhesion preference [i.e., their surface energies (6) are chosen such that they have a 0 surface tension]. Specifically, the surface energies used are as follows: $J_{\text{cell}} = 0, J_{\text{mem}} = 1,000, J_{\text{cell}} = 500, J_{\text{ker}} = 1,000, J_{\text{ker}} = 1,000, J_{\text{ker}} = 500$, where ECM indicates extracellular matrix, Tmem indicates memory T cell, and ker indicates keratinocyte. Parameters equal across all presented simulations: $T = 100$ (simulation temperature), $\lambda = 100$ (inelasticity), $A_{\text{ker}} = 120$ pixels (keratinocyte target area). Parameters for roundish memory T cells: $A_{\text{dendrites}} = 175$ pixels (T-cell target area), $\gamma = 2.875$ (directional propensity), $\Delta t = 240$ time steps (update time of target direction). Parameters for dendritic memory T cells: $A_{\text{dendrites}} = 165$ pixels, $\Delta t = 120$ time steps, $N_{\text{adapters}} = 10, E_{\text{extend}} = 1,000, E_{\text{squeeze}} = 10,000, p = 0.2$. With $p_{\text{grow}} = 0.01$ and $\kappa_{\text{dendrite}} = 1,000$ (which is bigger than $\kappa_{\text{cell}} = 5$), Tmem and stable dendritic memory T cells differ in the value of $\kappa_{\text{cell}}$ which is 5 and 0, respectively.

**Simulation Measurements.** A single Monte Carlo time step in the simulations is finished when all lattice positions have been considered for updating and corresponds to 1 s in real time. After an initial 5,000 s to allow time for the system to equilibrate, measurements are started and continued for multiple hours, as indicated in the figure legends. During this time period, mean T-cell positions are registered every 2 min (equal to the experimental data), and migration parameters are calculated as described above. Interactions between in *silico* memory T cells and keratinocytes are counted every second, and when there is at least one lattice point of the one cell in direct (nondiagonal) contact with a lattice point of the other cell. Contact areas between cells are estimated as the total number of shared direct, nondiagonal lattice points between two cells multiplied by the pixel dimension.

Fig. 51. Skin-resident memory CD8+ T cells contribute to local control of infection. (A) Recipients of GFP+gB T cells were vaccinated according to the scheme in Fig. 1A. Ten days after vaccination, the amount of gB<sub>(498-505)</sub>-MHC tetramer positive cells of endogenous origin (GFP−) or derived from the adoptive transfer was monitored in circulating blood. (B) Recipients of GFP+gB T cells were vaccinated on one flank with a DNA vaccine encoding the gB<sub>(498-505)</sub> epitope. Three months after local vaccination, mice were infected with HSV<sub>TOM</sub> on the same (ipsilateral) or opposite (contralateral) flank. (C) The size of red-fluorescent HSV foci of infection was monitored 24 and 72 h postinfection (18–37 foci from three different mice). The mean size of all contralateral foci at both 24 and 72 h postinfection was normalized to 100%; the size of the individual foci at the ipsilateral flank at the matching time point was calculated proportionally. Circles and triangles represent individual foci size. (D) Representative foci from contra- and ipsilateral sites at the indicated time points. GFP+gB T cells have been omitted for clarity. (Scale bar, 200 μm.)
Fig. S2. Effector CD8+ T cells infiltrating the epidermis and dermis of HSV-infected mice exhibit an amoeboid shape. Langerin-GFP mice were adoptively transferred with GFP+gB T cells and vaccinated by tattoo injection of gB(498-505) peptide-encoding DNA on days 0, 3, and 6. On day 10 postvaccination, mice were infected on the flank skin with HSV and the infected area was imaged 1 d post infection. The difference in GFP brightness of Langerin-GFP and GFP+gB T cells was used to discriminate the two cell types. (A) Snapshot of an HSV-infected area (red) surrounded by resident Langerhans cells (green) and infiltrating effector CD8+ T cells (white). (Scale bar, 50 mm.) (B and C) Using the Langerhans cells as reference, skin-infiltrating T cells were divided into superficial- and deep- tissue cells; the circularity of the cells was measured for both groups. (D and E) Mice were adoptively transferred with GFP+gB T cells and vaccinated by tattoo injection of gB(498-505) peptide-encoding DNA on days 0, 3, and 6. On day 10 postvaccination, mice were infected on the flank skin with HSV and the infected area was imaged 1 d postinfection. Another cohort of mice treated in the same way was imaged 1 mo after resolution of the infection to visualize the skin-resident memory T cells. (D) Mean fluorescence intensity plot of each z-slice within a z-stack reveals that whereas Tmem are present over a limited volume (the epidermis; Fig. 1E), effector T cells are present both in the superficial and deeper tissue. (E) Effector T cells, regardless of their depth, are more circular than memory T cells.
Fig. S3. Phenotypic characterization of skin-infiltrating effector and skin-resident memory CD8+ T cells. Mice were adoptively transferred with GFP+gB T cells and vaccinated by tattoo injection of gB_{498-505} peptide-encoding DNA on days 0, 3, and 6. On day 10 postvaccination, mice were infected on the flank skin with HSV_{TOM}. At 2 d (effector cells) or at least 4 wk (memory cells) postinfection, mice were killed and spleen and infected skin were collected and processed for flow cytometry.
Fig. S4. Simulation of the effect of steady-state migration and dendricity on the efficiency of skin patrol. Simulations (CPM) of Tmem cells in a field of keratinocytes were performed for 4 h in “memory T-cell time” (i.e., with the in silico cells covering a similar distance as Tmem in experimental measurements). See also Movie S7. (A) The circularity of simulated Tmem is similar to the circularity of in vivo skin-resident memory and effector T cells, respectively (compare with Fig. 4C). (B) (Left) The mean square displacement of in vivo-measured Tmem. The motility coefficient of Tmem is \( \sim 9 \, \mu \text{m}^2/\text{min} \); this is slower than that of lymph node T cells (67 \( \mu \text{m}^2/\text{min} \)) but similar to that of lymph node B cells (12 \( \mu \text{m}^2/\text{min} \)). (Right) In silico skin-resident Tmem plotted as a function of time, along with the best fit of \( M \) and \( P \) (solid lines) using Fürth’s equation. Fitted and measured migration parameters for dendritic-motile simulated T cells: \( M \sim 9.36 \, \mu \text{m}^2/\text{min} \) [95% confidence interval (CI) 9.21–9.51], \( P \sim 11.93 \, \text{min} \) (95% CI 11.34–12.53), mean measured speed 1.35 \( \mu \text{m}/\text{min} \); for roundish-motile Tmem: \( M \sim 9.19 \, \mu \text{m}^2/\text{min} \) (95% CI 8.96–9.43), \( P \sim 11.83 \, \text{min} \) (95% CI 10.91–12.82), mean measured speed 1.10 \( \mu \text{m}/\text{min} \); for dendritic-still Tmem: measured speed 1.11 \( \mu \text{m}/\text{min} \) (\( M \) and \( P \) not applicable). (C) The mean number of unique in silico keratinocytes scanned by the different types of simulated T cells over time. Dendritic-motile cells are displayed in blue, roundish-motile in pink, and dendritic-still in black (\( P < 0.0001 \) for all comparisons).
Dendricity and motility of skin-resident memory T cells allow skin patrol and are regulated by TCR triggering. (A) Mean circularity of Tmem cells was determined by imaging at the indicated time points after vehicle delivery or in an untreated control. The gray area indicates the SD of the control. (B) Recipients of GFP^{+}OTI T cells were infected with HSV_{F16D}. Three months after local infection, the skin was microinjected with gB_{498-505} peptide, CMV pp65_{495-503} peptide, or Ova_{257-264} Peptide. Time-lapse imaging of the area was conducted for 30 min, 9 h after peptide delivery. (Upper) Initial image of each time-lapse. (Lower) Superimposition of all images comprising the 30-min time-lapse imaging session. Representative of two experiments. (Scale bar, 50 μm.)
Fig. S6. Subcutaneous injection of cognate peptide alters skin-resident memory T-cell motility and shape. Recipients of GFP+gB T cells were infected with HSV1OM. Three months after local infection mice were injected s.c. with gB(498–505) or OVA(257–264) peptide. (A) Time-lapse imaging of the area was conducted for 30 min at the indicated time points. (Left) Initial image of each time-lapse. (Right) Superimposition of all images comprising the 30-min time-lapse imaging session. (B) The mean circularity of Tmem cells (±SEM) was determined by imaging at the indicated time points after local peptide delivery.

Movie S1. Skin-resident GFP+gB Tmem cells (green) migrating within the epidermis.
Movie S2. Skin-resident GFP+OTI (bystander) Tmem cells (green) migrating within the epidermis.

Movie S3. Three-dimensional visualization of the difference in dendrite polarity between epidermal Tmem (green), γδ T cells (DETC; red), and Langerhans cells (blue). The tissue structure in white is revealed by F-actin staining.

Movie S4. Effector GFP+OTI T cells (first segment) or GFP+OTI Tmem cells (second segment) patrolling an area infected with HSV_{103} (red).
Movie S5. Kaede$^*$gB Tnem cells (red) patrolling an area recovered from a previous HSV<sub>10m</sub> infection. The nuclei of surrounding skin cells are shown in green.

Movie S5

Movie S6. Four-hour cpm simulations of memory T cells (red) and keratinocytes (green). Contacted keratinocytes turn from green to light/dark blue, depending on the intensity of scanning. The video shows the behavior of dendritic-still, roundish-motile, and dendritic-motile simulated T cells. Time is indicated in hours:min:sec. (Scale bar, 100 μm.)

Movie S6

Movie S7. Skin-resident GFP$^+$gB T cells patrolling an area injected with control Ova peptide.

Movie S7
Movie S8. Skin-resident GFP⁺gB T cells stalling in an area injected with cognate gB peptide.

Movie S9. Skin-resident GFP⁺gB T cells exploring epidermis with rare antigen-expressing cells (red). First segment: transfected cells express Katushka and the cognate gB peptide. Second segment: transfected cells express Katushka and the control Ova peptide.