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Skin-resident memory CD8+ T cells trigger a state of tissue-wide pathogen alert

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After an infection, pathogen-specific tissue-resident memory T cells (T_{rm}) persist in nonlymphoid tissues to provide rapid control upon reinfection, and vaccination strategies that create T_{rm} pools at sites of pathogen entry are therefore attractive. However, it is not well understood how T_{rm} provide such pathogen protection. Here, we demonstrate that activated T_{rm} in mouse skin profoundly alter the local tissue environment by inducing a number of broadly active antiviral and antibacterial genes. This "pathogen alert" allows skin T_{rm} to protect against an antigenically unrelated virus. These data describe a mechanism by which tissue-resident memory CD8+ T cells protect previously infected sites that is rapid, amplifies the activation of a small number of cells into an organ-wide response, and has the capacity to control escape variants.

Tissue-resident memory CD8+ T cells (T_{rm}) are a subtype of memory lymphocytes (*1*) that permanently reside in nonlymphoid tissues in mice and humans (*2–11*). Analysis of herpes simplex virus (HSV)-1 and HSV-2 shedding episodes in infected human mucosa has shown that emerging lesions are often controlled within 6-12 hours (*12*). Furthermore, the severity of viral lesions during reactivation is likely determined by local immune control, and data in mouse models suggest that such tissue protection can be mediated by locally residing memory CD8+ T cells (*13–15*).

The mechanisms by which a small number of local memory cells can protect a peripheral tissue have not been established and, given the low numbers of T_{rm} in tissues, unlikely to solely involve the direct killing of target cells (*16*). In addition, while T_{rm} are able to recruit circulating memory CD8+ T cells to the peripheral tissue within 48 hours of activation (*17*), such recruitment is unlikely to achieve early pathogen control (*12*).

To investigate how small numbers of tissue-resident memory T cells confer rapid protection of local tissue, we created a pool of T_{rm} by intradermal DNA vaccination of mice that had received small numbers of gBT-GFP cells specific for the HSV-1-derived glycoprotein B peptide ($gB_{498-505}$) (fig. S1) (*9, 18*). Weeks later, skin areas harboring T_{rm} were challenged with HSV-1 or $gB_{498-505}$ peptide. Immunohistochemical analysis of HSV-1 infected or $gB_{498-505}$ peptide-challenged skin tissue of gBT-GFP T_{rm} memory mice and naive mice at nine hours post infection/peptide administration did not reveal any difference in the infiltration of macrophages, gBT-GFP memory T cells, or CD3 cells. A moderate increase in the number of neutrophils was only observed upon peptide administration (fig. S2, A to F). To evaluate other possible effects of tissue-resident memory CD8+ T cell activation on the surrounding tissue, we obtained transcriptional profiles from the entire skin tissue at the same early time point following in situ triggering of T_{rm}. Comparison of the transcriptional profiles in skin exposed to control ($OVA_{257-264}$) peptide or cognate ($gB_{498-505}$) peptide revealed differential expression of a large number of genes (cut-offs: FDR < 0.05; $\log_2$ fold change > ±1.2) (Fig. 1A and fig. S3). To distinguish between noise caused by variation in tissue composition and signal due to T_{rm}-triggering, transcriptional profiling was performed on a second cohort of mice (Fig. 1B and fig. S3). Genes of which expression was altered at a comparable magnitude in both data sets (difference in magnitude of induction <1.5; blue in fig. S3B), were retained for further analysis.

To test whether the observed changes in gene expression were due to T cell receptor (TCR) recognition of antigen, cohorts of mice harboring T_{rm} specific for the $OVA_{257-264}$ epitope were challenged with either $gB_{498-505}$ or $OVA_{257-264}$ antigen. In this setup, activation of the OTI-GFP T_{rm} by cognate $OVA_{257-264}$ resulted in a reproducible change of the skin transcriptional profile (Fig. 1, C and D). Furthermore, changes in gene expression in gBT-GFP T_{rm} skin challenged with cognate $gB_{498-505}$ peptide and OTI-GFP T_{rm} challenged with cognate $OVA_{257-264}$ peptide were highly correlated (Fig. 1E). Thus, triggering of T_{rm} harboring skin with peptide antigen leads to a rapid alteration in the transcriptome that is visible at the level of the entire tissue before substantial influx of immune cells is seen.

Combination of all 4 data sets resulted in a list of 89 genes that are differentially expressed (all increased) upon specific triggering of tissue-resident CD8+ memory T cells (table S1 and Fig. 1E). Induction of part of this gene set was already observed 3 hours after antigen administration, and induction was essentially complete after 6 hours (fig. S4). Supporting the immunohistochemical results, T-cell specific genes did not show any significant difference between T_{rm}-harboring skin treated with specific or control peptide (fig. S5). Independent full transcriptome gene ontology analyses (*19*) of the four data sets indicated inflammation and immunity as dominant signatures of all data sets (table S2).

Transcriptome analysis of two independent experiments in which gBT-GFP T_{rm} skin was challenged with HSV-1 or control showed a similar pattern of gene induction. For most genes within the gene set (table S1), magnitude of induction was larger upon peptide triggering, possibly because a greater number of T_{rm} can encounter antigen early after peptide administration (group 1 in Fig. 1F). In addition, a second group of genes, including a large number of chemokines and cytokines involved in innate immune cell movement was more strongly or only upregulated upon virus infection (group 2 in Fig. 1F). Together, these data show that antigen-specific activation of tissue-resident memory T cells is sufficient to initiate an early response that is visible at the level of the entire tissue.

Strikingly, many of the genes that were induced upon peptide administration were expressed at levels >10 to >100-fold the level of T-cell specific genes (Fig. 1G). Furthermore, analysis of the identified gene set revealed the induction of a broad spectrum antipathogen response. To dissect whether this rapid tissue response depends upon systemic antigen-specific memory T cells, or only requires the tissue-resident

memory T cell population, OTI-GFP cells from male donors were transferred into syngeneic female recipients and activated by vaccination. In this setting, the systemic memory T cell pool (central memory + effector memory; T_{cm}+T_{em})—but not the tissue-resident memory pool—is cleared (*5*) (fig. S1). Comparison of the transcriptional profile in skin of recipients harboring either $T_{rm,}$ or both T_{rm} and T_{cm}+T_{em} indicates that activation of the T_{rm} pool is sufficient to induce expression of the large series of genes within skin (Fig. 2A).

Upstream regulator analysis of the induced gene signatures by Ingenuity Pathway Analysis indicated that the cytokine interferon (IFN)γ is the most likely factor controlling the transcriptional alterations seen in T_{rm} conditioned skin (table S3), and prior work has shown that $CD8^+$ T_{rm} rapidly re-express IFNγ after local antigen re-challenge (*17*). Analysis of full thickness skin 9 hours after triggering of a population of wild type or *Ifng*$^{-/-}$ T_{rm} cells revealed that a large part of the transcriptional alterations seen upon T_{rm} triggering are dependent on T_{rm}–derived IFNγ (Fig. 2B). Furthermore, this IFNγ acts on skin cells other than T_{rm} themselves, as the tissue response is also largely lost in *Ifngr1*$^{-/-}$ recipient mice in which only T_{rm} express IFNγ receptor 1 (Fig. 2C). These data suggest that shortly after T cell receptor (TCR) triggering, activated T_{rm} express IFNγ to enhance expression of proteins involved in pathogen control within the surrounding tissue. To test this hypothesis, we analyzed the expression pattern of IFITM3 (Interferon-Induced TransMembrane protein 3; blue in Fig. 1G), a protein with broad-spectrum antiviral activity (*20*), and one of the transcripts induced by T_{rm} triggering (Fig. 1 and table S1). Within 6 hours of T_{rm} activation by cognate antigen, most epidermal and dermal cells expressed IFITM3, with maximal levels at 9-18 hours post conditioning (Fig. 3). By 36 hours, IFITM3 expression was largely restricted to the outer layers of the epidermis, indicating that local T_{rm} activation leads to a transient change in the skin transcriptome that is still visible in aging keratinocytes by the time newly formed keratinocytes have returned to steady state (Fig. 3B).

In most models of infection control by $CD8^+$ T cells, both the initial T cell activation and the final output signal (e.g., cytolysis) are dependent on recognition of cognate antigen. In contrast, the above-described tissue conditioning by T_{rm} requires antigen as input signal, but generates an output signal -the up-regulation of genes involved in broad-spectrum defense- that does not rely on antigen recognition, a mechanism reminiscent of that of effector $CD4^+$ T cells (*21*). To test the potential relevance of this state of T_{rm}–induced "pathogen alert," we analyzed whether T_{rm} activation could lead to control of an antigenically unrelated pathogen in vivo. Skin resident OTI-GFP T_{rm} were activated by local injection of cognate peptide, and 9 hours later the same area was infected with antigenically unrelated HSV-1. At two time points post-virus administration, progression of HSV-1 infections was scored microscopically (day 1) and macroscopically (day 3). As expected, disease progression in naïve mice was not influenced by $OVA_{257-264}$ peptide administration (Fig. 4A). In contrast, in mice harboring skin OTI-GFP T_{rm}, application of cognate $OVA_{257-264}$ peptide resulted in a strong reduction in HSV-1 disease severity relative to control conditions (Fig. 4A). Analysis of HSV-1 challenged skin tissue by anti-HSV staining showed that OTI-GFP T_{rm} activation resulted in a substantial reduction of both tissue necrosis and lateral spreading of herpetic lesions (Fig. 4B). In line with this, viral DNA levels were reduced in skin of mice harboring activated OTI-GFP T_{rm} at the time of infection (Fig. 4C, $P < 0.0001$). Taken together, these data demonstrate that the tissue conditioning that is induced by T_{rm} activation leads to enhanced pathogen control that is independent on the antigenic identity of this pathogen.

Three aspects of T_{rm}-mediated tissue conditioning are noteworthy. First, tissue conditioning is almost immediate. This property is likely to be of major relevance as, at least in case of HSV-2, early immune control is the major determinant of episode severity (*14*). Second, tissue conditioning forms an effective amplification system, in which activation of a rare cell type leads to a tissue-wide response. Third, T_{rm}-mediated tissue conditioning results in protection that is ultimately antigen independent: while initial T_{rm} activation requires recognition of antigen, the genes that are up-regulated in response display activity toward a wide array of pathogens.

From a conceptual point of view, these data place T_{rm} as a bridge between the adaptive and innate immune system, in which the TCR in T_{rm} has a function similar to that of Toll-like receptors in innate immune cells. From a practical point of view, the fact that T_{rm} triggering leads to an output signal that no longer requires antigen recognition may also help counteract viral escape. Recently, strategies have been put forward to create T_{rm} populations at sites of potential pathogen entry (*10*, *22*, *23*). The current data not only help to provide a mechanistic explanation for the effects of such vaccines but also suggest that in case of pathogens that exist as quasispecies, protection may conceivably be provided not only against the vaccine-encoded sequence but also against viral variants that are transferred in parallel.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1254803/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S3
Reference (*24*)

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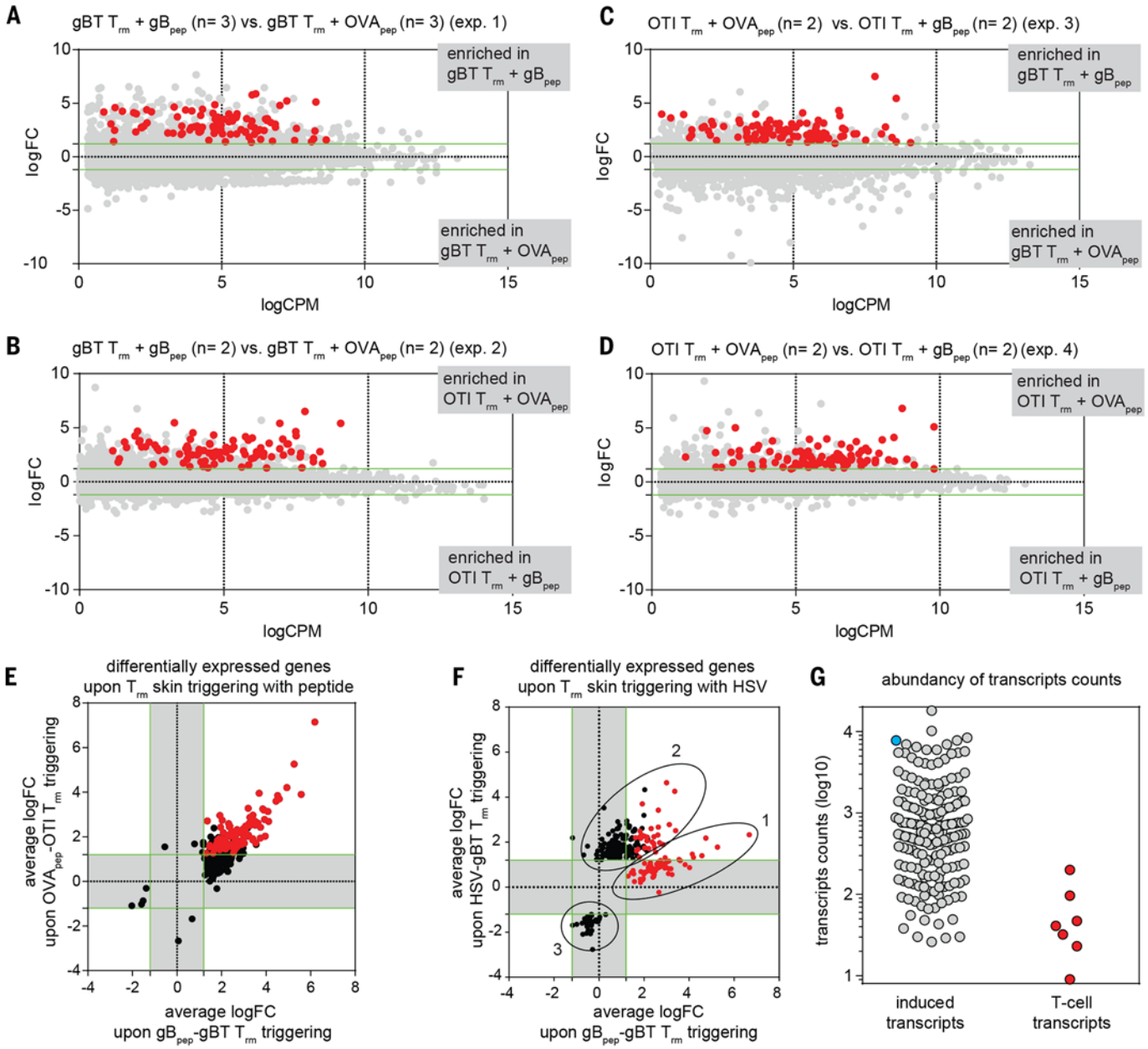

Fig. 1. Memory CD8$^+$ T cell triggering alters tissue-wide gene expression profiles. (**A** to **D**) Transcriptome analysis of full-thickness skin from mice harboring gBT-GFP T$_{rm}$ (A and B) or OTI-GFP T$_{rm}$ (C and D) upon local administration of either gB$_{498-505}$ or OVA$_{257-264}$ peptide. Relative abundance is plotted for averaged normalized read counts. All detected nondifferentially expressed genes are depicted in grey. Genes that are differentially expressed in all four comparisons (logFC > ±1.2; FDR < 0.05, difference in magnitude between replicate experiments <1.5, fig. S3) are depicted in red. In these and further plots, horizontal green lines represent logFC limits for significance ±1.2). (**E**) Average logFC for gBT T$_{rm}$ and OTI T$_{rm}$ harboring skin upon triggering with cognate peptide. Genes listed in table S1 (differentially expressed upon T$_{rm}$ triggering) are shown in red, genes that were only up-regulated in one of the T$_{rm}$ groups in black. (**F**) Average logFC for skin harboring gBT T$_{rm}$ upon triggering with either HSV-1 or cognate peptide. Genes listed in table S1 are shown in red, genes specifically up-regulated upon HSV-1 infection are depicted in black. Group 1: correlated behavior between both triggers, enriched in interferon-responsive genes; Group 2: Preferentially or only induced by HSV, enriched in secreted molecules; Group 3, Reduced by HSV, too small for pathway analysis. (**G**) Comparison of normalized transcript counts of the differentially expressed gene set in table S1 ("induced transcripts") with normalized transcript counts of a set of T-cell specific genes ("T-cell transcripts"). Amongst "induced transcripts," IFITM3 is depicted in blue; the "T cell transcripts" gene set (IFNγ, CD2, zap70, CD5, CD69, CD8a, and CD8b1) is depicted in red. Values representative of eight comparisons.

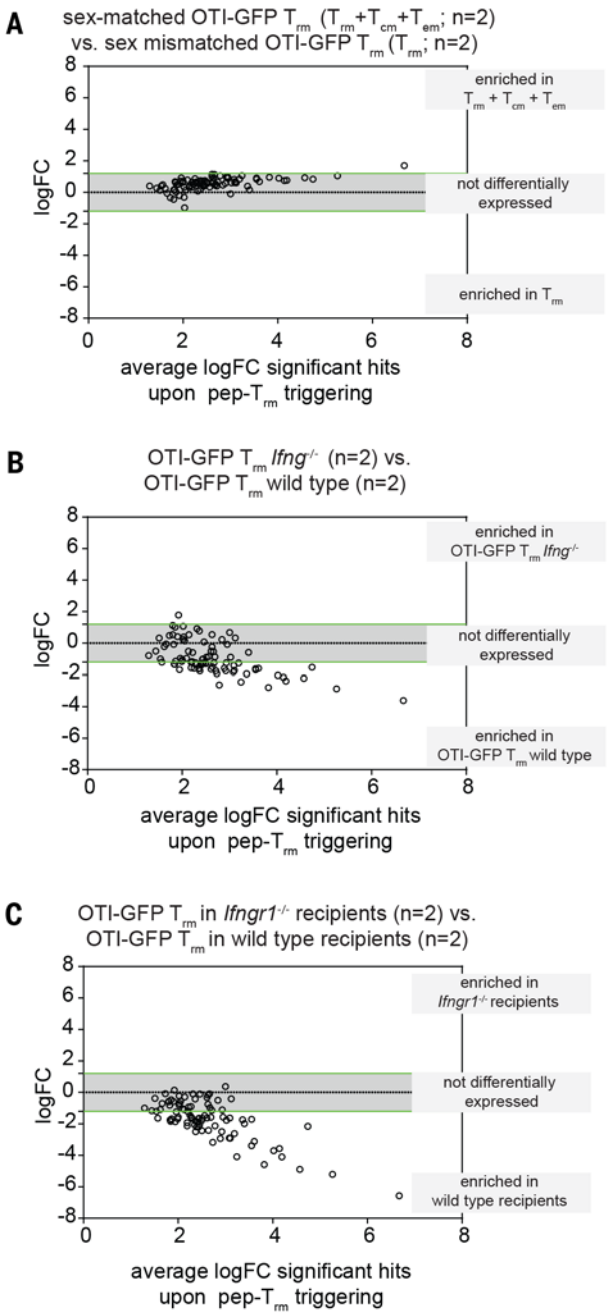

Fig. 2. Skin-resident memory CD8$^+$ T cells mediate the induction of an antiviral state through IFNγ. (**A**) Female recipients of either sex-matched or mismatched OTI-GFP$^+$ cells were vaccinated to induce skin T_{rm}. Skin areas harboring T_{rm} were treated locally with $OVA_{257-264}$ peptide, and sacrificed 9 hours later. Note that the absence of systemic memory T cells (see fig. S1) does not significantly reduce tissue conditioning by peptide triggering. On average, induction of the identified gene set was slightly more pronounced in sex-matched recipients (1.15 fold, n.s.), which could either reflect a limited contribution of the circulating memory T cell pool, or the slight reduction in T_{rm} numbers in skin of mismatched recipients (fig. S1F). (**B**) Recipients of OTI-GFP$^+$ T_{rm} derived from either wild type or IFNγ deficient donors were vaccinated to induce skin T_{rm}, and the effect of T_{rm} triggering was then analyzed as in (A). (**C**) IFNγR proficient or deficient recipients of OTI-GFP$^+$ T_{rm} were vaccinated to induce skin T_{rm}, and the effect of T_{rm} triggering was then analyzed as in (A).

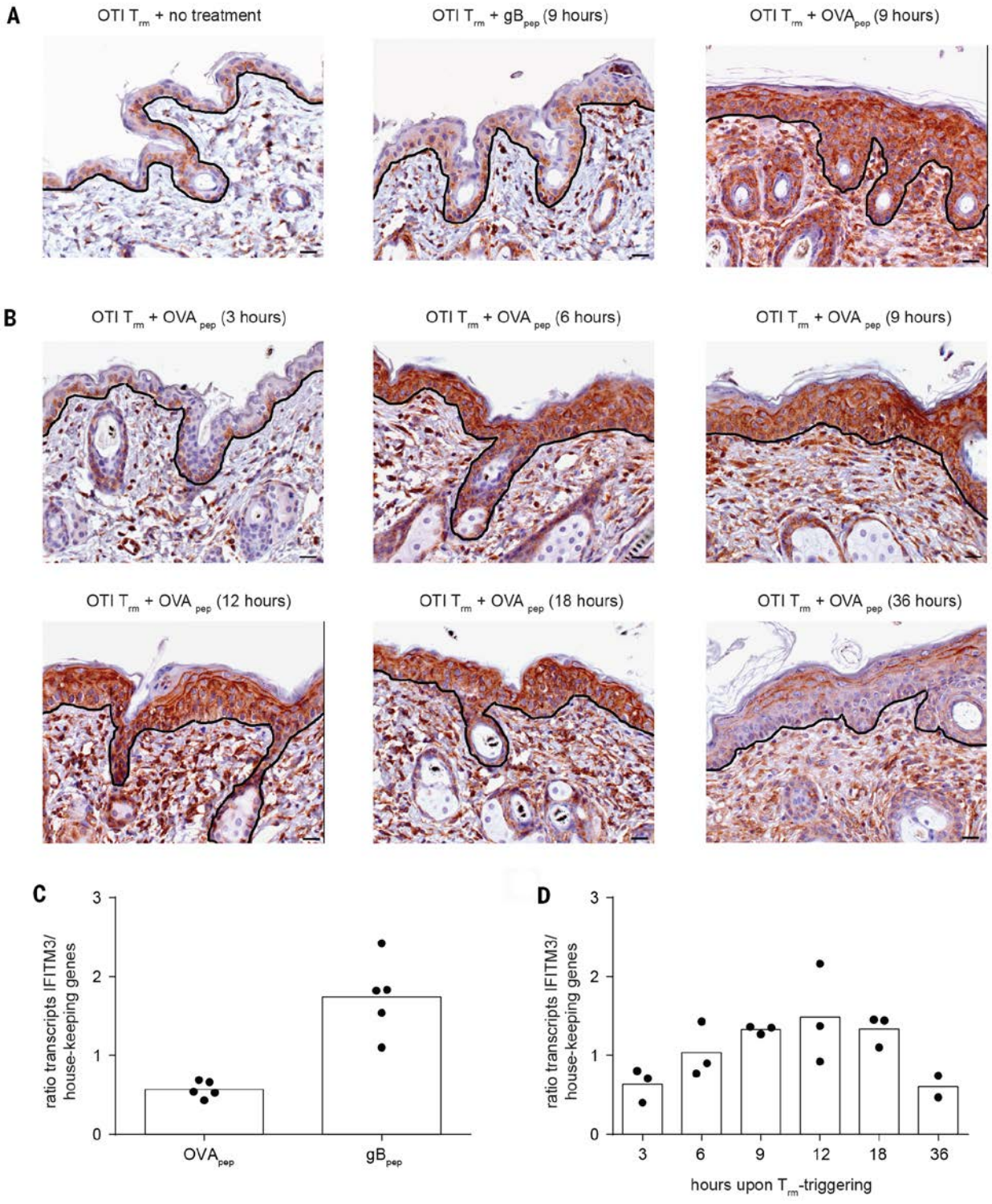

Fig. 3. Tissue conditioning by skin-resident memory CD8$^+$ T cells results in the induction of an antiviral state in large numbers of surrounding cells. (**A**) Immunohistochemical detection of IFITM3 in the skin of mice harboring OTI-GFP$^+$ T_{rm}, analyzed at steady state or 9 hours after treatment with either cognate $OVA_{257-264}$ or control $gB_{498-505}$ peptide (representative of 3 mice per group). (**B**) Immunohistochemical detection of IFITM3 in the skin of mice harboring OTI-GFP$^+$ T_{rm} at the indicated time points after treatment with $OVA_{257-264}$ peptide (representative of 3 mice per time point). The boundary between epidermis (top in all images) and dermis is highlighted by a black line. Bar is 20 µm. (**C** and **D**) Naive gBT-GFP$^+$ cells were transferred into recipients that were subsequently tattoo-vaccinated with DNA encoding TTFC-gB_{pep} in order to create a population of resident T_{rm}. Several weeks after tattooing, skin harboring T_{rm} was injected with either gB_{pep} or OVA_{pep} and processed for transcriptome analysis 9 hours later. (C) Ratio between IFITM3 counts and the median counts of 20 house-keeping genes. (D) The same analysis is depicted for samples in which skin harboring OTI T_{rm} was injected with OVA_{pep} and analyzed at the indicated time points.

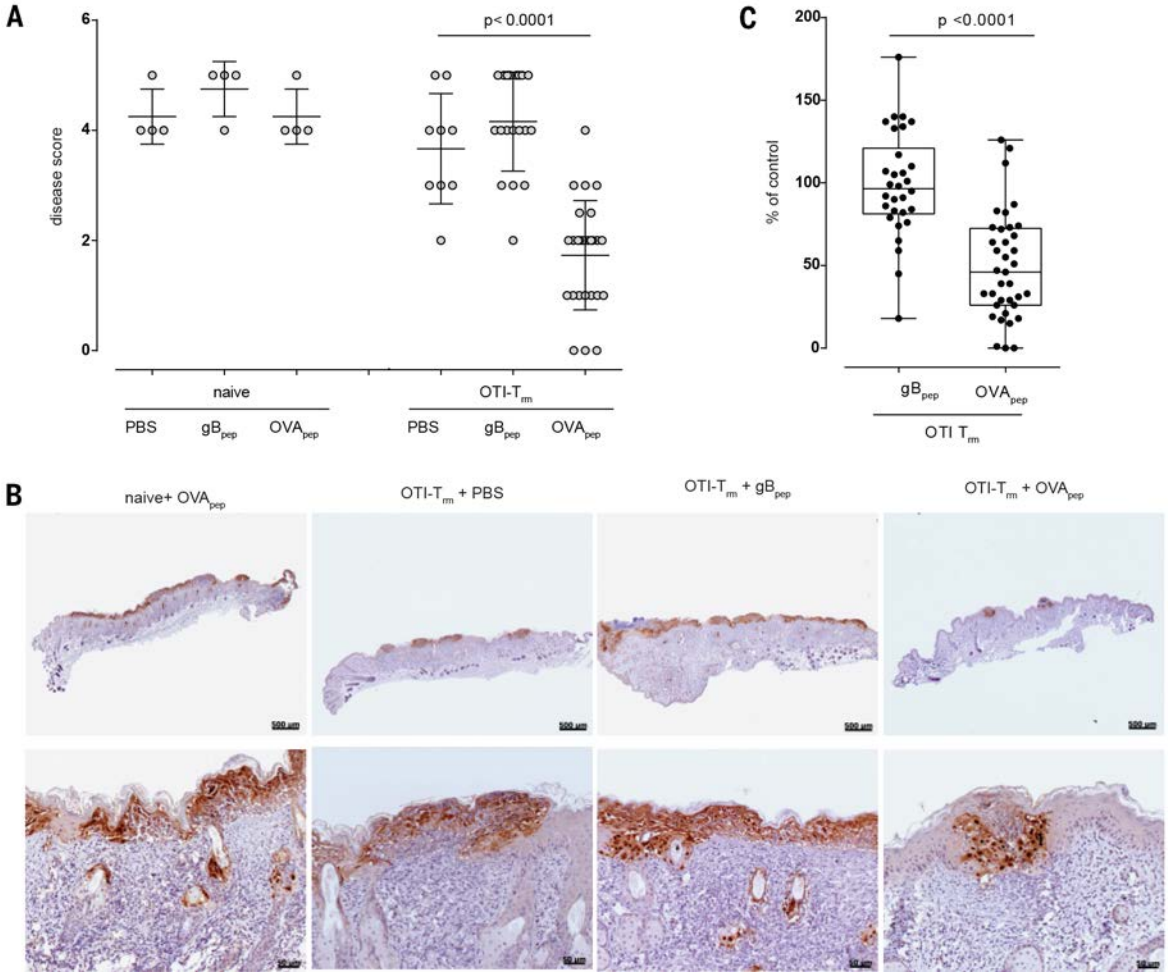

Fig. 4. CD8$^+$ T_{rm} triggering provides cross-protection against an antigenically unrelated pathogen. (**A**) Naïve mice or mice harboring OTI-GFP$^+$ T_{rm} (both hind legs) were injected locally with PBS, cognate $OVA_{257-264}$ peptide, or control $gB_{498-505}$ peptide and locally infected with HSV-1 9 hours later. Sixty hours after infection, the extent of each infection was scored by visual inspection by an observer blinded to experimental group (all naïve groups: n = 2; OTI-T_{rm}+ PBS: n = 5; T_{rm}+ $gB_{498-505}$: n = 10; T_{rm}+ $OVA_{257-264}$: n = 13; both legs analyzed separately). (**B**) Mice harboring OTI-GFP$^+$ T_{rm} (both hind legs) were injected locally with cognate $OVA_{257-264}$ peptide or control $gB_{498-505}$ peptide and were locally infected with HSV-1 9 hours later. After 60 hours, the amount of viral DNA in infected skin was measured. Data are representative of four independent experiments with at least five mice per group. To allow comparison between experiments, the amount viral DNA in the OTI-T_{rm}+ $gB_{498-505}$ group was set to 100% for each experiment. (**C**) Immunohistochemical detection of HSV-1 infection in naïve mice that received a local injection with $OVA_{257-264}$ peptide (B) or mice harboring OTI-GFP T_{rm} triggered with cognate $OVA_{257-264}$ peptide, control $gB_{498-505}$ peptide, or PBS (C). For each condition, two different magnifications of the same sample are shown. Data are representative of 2 (naïve group), 5 (T_{rm}+ PBS), 10 (T_{rm}+ $gB_{498-505}$) and 13 (T_{rm}+ $OVA_{257-264}$) mice per group.

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