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Resident memory CD8 T cells trigger protective innate and adaptive immune responses

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Abstract

The pathogen recognition theory dictates that upon viral infection, the innate immune system first detects microbial products, and then responds by providing instructions to adaptive CD8 T cells. Here, we show in mice that resident memory CD8 T cells (T_{RM}), non-recirculating cells located at common sites of infection, can achieve near sterilizing immunity against viral infections by reversing this flow of information. Upon antigen re-sensitization within the mouse female reproductive mucosae, CD8+ T_{RM} secrete cytokines that trigger rapid adaptive and innate immune responses including local humoral responses, maturation of local dendritic cells, and activation of natural killer cells. This provided near sterilizing immunity against an antigenically unrelated viral infection. Thus, CD8+ T_{RM} rapidly trigger an antiviral state by amplifying receptor-derived signals from previously encountered pathogens.

CD8 T cells control viral infections. To be licensed with effector functions, naïve CD8 T cells must first be activated by specialized members of the innate immune system that have been alarmed by danger signals in the form of recognition of broadly conserved microbial associated molecular patterns (such as dsRNA or LPS)(1). Paradigmatically, CD8+ T cells act very locally by contacting infected host cells for both the detection of pathogen-associated peptides presented by major histocompatibility complex class I (MHCI) and target-cell specific delivery of toxic effector molecules(2). After clearance of infection, memory T cells remain. Central, effector, and resident memory T cell subsets (T_{CM}, T_{EM}, and T_{RM}, respectively) occupy different anatomic niches where they fulfill distinct roles in protective immunity(3, 4). The most recently described subset, T_{RM}, are non-recirculating memory T cells that remain positioned at common portals of re-infection, including barrier tissues such as the mucosae and skin(5-10). When present at the site of re-infection, T_{RM} accelerate protection against homologous re-infections, although the mechanism remains obscure(10-15). We recently demonstrated that reactivation of T_{RM} results in bystander recruitment of recirculating memory T cells to the site of anamnestic Ag exposure in a

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manner dependent on the cytokine interferon-γ (IFNγ) (16). This suggested that T_{RM} serve a sentinel and alarm function in addition to more prototypical CD8 T cell functions.

We wanted to further understand the mechanism by which CD8+ T_{RM} communicated local pathogen-associated antigen re-encounter to recirculating memory CD8 T cells located outside the tissue. OT-I or P14 chimeric mice, which contained OVA-derived SIINFEKL or gp33 peptide specific CD8+ T_{RM}, respectively, were established in the FRT as follows: naïve OT-I or P14 CD8 T cells were transferred i.v. to naïve C57Bl/6J mice, and mice were infected one day later with recombinant vaccinia virus expressing OVA (VV-OVA) or lymphocytic choriomeningitis virus (LCMV), respectively (Figs. S1A&B). FRT T_{RM} were then reactivated locally by depositing cognate SIINFEKL or gp33 peptide into the cervical lumen (transcervical, t.c.), as described(17). Here, we found that T_{RM} reactivation with either peptide or recombinant vaccinia virus expressing cognate peptide induced expression of the cell adhesion molecule VCAM-1 (vascular cell adhesion molecule-1) on local vascular endothelium (Fig. 1A-C). IFNγ is produced by reactivated T_{RM} in vivo(16). We found that exogenous t.c. deposition of IFNγ into naïve mice was sufficient to induce local VCAM-1 expression (Fig. S2). Further experiments revealed that when OT-I *Ifng*$^{-/-}$ T_{RM} were reactivated in wild type mice, VCAM-1 was no longer upregulated, demonstrating that T_{RM} induce local VCAM-1 in a cell autonomous manner by secreting IFNγ upon antigen re-sensitization (Fig. 1A&B). Additionally, when circulating P14 memory CD8 T cells were depleted (while preserving T_{RM} in the FRT) by injecting 1μg HIS51 anti-Thy1.1 depleting antibody(16), peptide reactivation resulted in similar levels of VCAM-1 on endothelial cells, suggesting that VCAM-1 expression is driven by local CD8 T cells (Fig. 1D).

VCAM-1 is the ligand for α4β1 integrin, which plays a role in lymphocyte migration(18). Bystander OT-I CD8 T cell recruitment in response to gp33-specific T_{RM} reactivation was inhibited when either VCAM-1 or α4β1 (CD49d) was blocked with neutralizing antibodies (Fig. 1E). As CD8+ T_{RM} communicate antigen sensitization to recirculating CD8 T cells through an IFNγ-VCAM-1 axis, we asked whether other lymphocyte lineages might also be recruited. B cells accumulated within the FRT within 12 hours of local CD8+ T_{RM} reactivation, and increased >100-fold by 48h (Fig. 1F&G). Similar results were observed when recombinant gp33-expressing vaccinia virus was used to reactivate T_{RM} instead of peptide (Fig. 1H), and in LCMV immune mice that did not receive a transfer of P14 CD8 T cells (Figs. 1I and S1C). Moreover, depletion of recirculating P14 CD8 T cells with a-Thy1.1 depleting antibody (which preserves P14 T_{RM} within the FRT) did not impair B cell recruitment upon t.c. gp33 peptide challenge, demonstrating that B cell recruitment was driven by locally reactivated CD8 T cells figure (Fig. 1J). However, B cell recruitment was critically dependent on local CD8 T cell derived IFNγ and VCAM-1 induction (Figs. 1K&L).

These observations indicated that reactivated T_{RM} may broadcast detection of pathogen associated peptides to other cell types through cytokines. While most microbes are cleared by the innate immune system without priming adaptive responses, professional pathogens require adaptive immunity for clearance. Dendritic cells (DCs) are principal messengers between the innate and adaptive immune systems. Detection of broadly conserved microbial associated molecular patterns instructs DC maturation, which in turn initiates T cell

activation(1, 19, 20). As T_{RM} recognition of peptides indicates re-exposure to a pathogen that caused sufficient infection to elicit an adaptive immune response we asked if CD8+ T_{RM} reactivation could trigger DC maturation. When either transgenic or endogenous cognate-antigen specific CD8+ T_{RM} were established, local exposure to either pathogen associated peptide or recombinant vaccinia virus expressing cognate peptide resulted in DC maturation within only 12h, as indicated by the induced expression of co-stimulatory molecules CD80, CD86, CD40, as well as the lymph node homing chemokine receptor CCR7 (Figs. 2A-C & S3). Depletion of circulating P14 CD8 T cells did not alter DC maturation after peptide re-sensitization, suggesting that CD8 T_{RM} activation was sufficient for DC maturation (Fig. 2D). These data indicate that CD8+ T_{RM} ligands also serve as potent inducers of innate immune responses. Intracellular cytokine staining indicated that CD8+ T_{RM} expressed the cytokine TNFα in vivo within 12h of local reactivation (Fig. 2E), and TNFα was essential for DC maturation (Figs. 2F&G).

Natural killer (NK) cells are innate lymphoid cells that play essential roles in control of viral infections(21, 22). They have advantages over the adaptive immune system because they are constitutively abundant and broadly distributed(21). When activated, they kill host cells showing signs of stress, but lack highly specific antigen receptors for detecting pathogen associated peptides(22). Thus viral infections also require the presence of CD8 T cells for clearance. T_{RM} reactivation with either peptide or recombinant vaccinia virus expressing cognate peptide resulted in granzyme B upregulation among NK cells within the FRT within 12h and this event also occurred when circulating memory P14 CD8 T cells were depleted (Fig. 3A-C). Contemporaneously, local memory CD8 T cells of irrelevant specificities also increased expression of granzyme B (Fig. 3A). These data suggested that in the event of local T_{RM} reactivation, NK cells and memory CD8 T cells receive signals to become poised for cytolysis of infected host cells(2). T_{RM} expressed the cytokine interleukin (IL)-2 in vivo within 12h of local reactivation (Fig. 3D), and blocking IL-2 receptor β (IL-2Rβ) abrogated granzyme B upregulation on both NK cells and bystander memory CD8 T cells (Figs. 3E&F).

These data established that T_{RM} reactivation induced broad activation of innate and adaptive immune components. This observation provided impetus for the hypothesis that T_{RM} may protect the host in part by 1) acting as sensory cells for previously encountered peptides that are associated with intracellular pathogenic infections, 2) communicating re-sensitization to abundant non-specific immune effectors, and thus 3) triggering an organ-wide antiviral state. To test this hypothesis, we established P14 chimeric mice with gp33 peptide-specific T_{RM} within the FRT (Fig. 4). Mice were subsequently challenged with a recombinant vaccinia virus (VV-OVA) for which memory T cells had not been established. As expected, infection resulted in abundant tissue viral load within two days of transcervical exposure in both naïve mice and in mice containing gp33-specific T_{RM}. However, if gp33 peptide was included in the inoculum in order to reactivate local T_{RM} at the time of viral challenge, we failed to detect infection in 9 of 11 mice, coinciding with a $\sim 10^4$-fold reduction in viral load (Fig. 4A). Similar results were observed when gp33 peptide was delivered 12h prior to viral challenge (Fig. 4B) and in mice that harbored only endogenous gp33-specific T_{RM} (i.e. LCMV immune mice that did not receive a transfer of P14 CD8 T cells, Fig. 4C and S1C). These data suggest that T_{RM} reactivation, which was associated with widespread activation

of the local immune surveillance network, induced an anti-viral state at the site of infection. This near sterilizing protection was completely abrogated when IFNγ, TNFα, and IL-2Rβ–dependent cytokines were blocked (Fig. 4A).

CD8 T cells are classically thought to control viral infections by contact dependent interactions between antigen-specific CD8 T cells and each infected host cell, followed by directed target cell killing(2). These data support an expanded range of functions by which T_{RM} mediate protective immunity. Here we show that T_{RM} reverse the flow of information from innate to adaptive immune systems by demonstrating that adaptive T_{RM} sensitization initiates broad local immune activation. In this light, our data suggest that T_{RM} could be viewed as a pathogen recognition entity that more sensitively engages innate immune functions than what is accomplished by innate recognition of conserved microbial associated molecular patterns. The T_{RM} functions described here were dependent upon IFNγ, TNFα, and IL-2Rβ–dependent cytokines, which may help explain why memory CD8 T cell populations that are competent to produce each of these cytokines (referred to as polyfunctional memory CD8 T cells) are often best associated with protective immunity(23).

It may be possible to leverage the functions of T_{RM} described here for therapeutic purpose. For instance, local reactivation of established T_{RM} with peptide vaccines could be employed to increase local immunity against unrelated pathogens. The potent inflammatory effects of T_{RM} reactivation may also have pathological consequences, and may contribute to the observed association between viral infections and exacerbation of tissue specific autoimmune or inflammatory diseases. Principally, our results indicate how sensitization of relatively small numbers of T_{RM} may result in an amplified signal to more abundant members of the innate immune system in order to trigger an organ-wide antiviral state.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The data presented in this manuscript are tabulated in the main paper and in the supplementary materials. This work was funded by R01AI084913-01 (D.M.), the NIH Director's New Innovator Award Program DP2-OD-006467 (D.M.) and T32AI007313 and F30DK100159-01 (J.M.S.).

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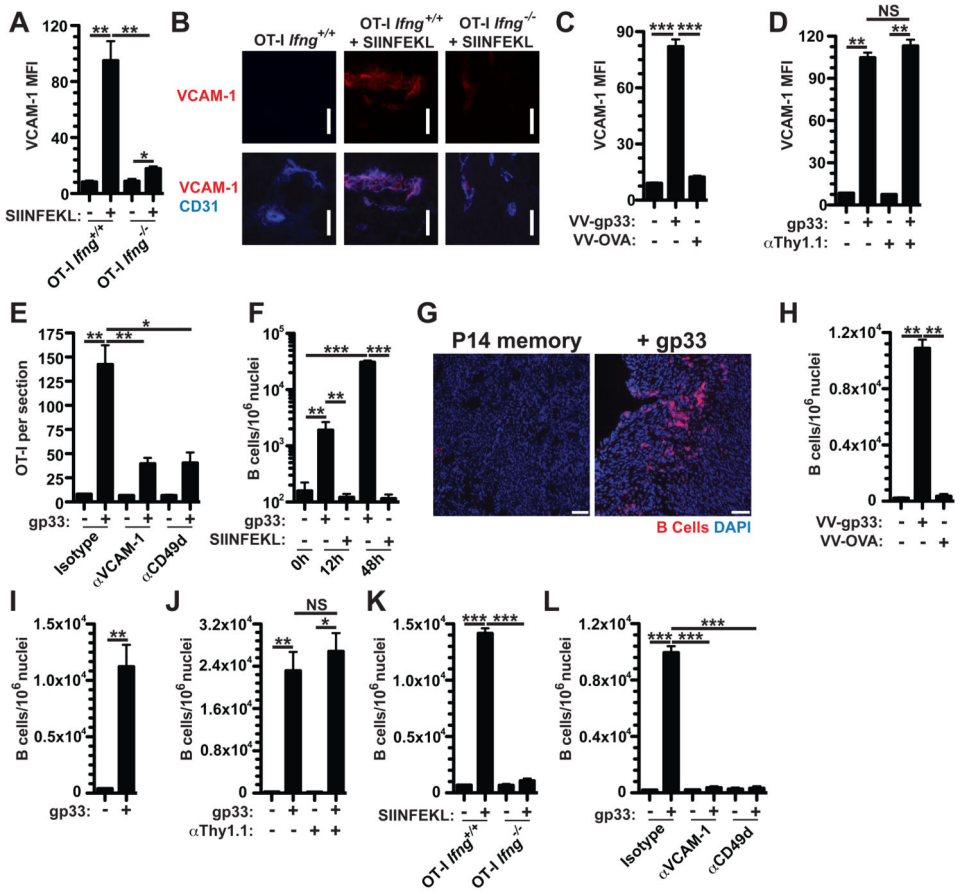

Fig 1. T_{RM} reactivation induces memory CD8 T cell and B cell recruitment through IFNγ-dependent VCAM-1 upregulation

(A) VCAM-1 mean fluorescence intensity (MFI) on CD31+ vascular endothelium in the FRT 12h after OT-I *Ifng*$^{-/-}$ or OT-I *Ifng*$^{+/+}$ T_{RM} were reactivated by SIINFEKL peptide deposited t.c. **(B)** Representative images of VCAM-1 (red) and CD31 (blue) staining. 12 hours after t.c. challenge, VCAM-1 expression was quantified on FRT vascular endothelium after **(C)** P14 immune chimeras were t.c. challenged with either VV-gp33, VV-OVA or left untreated or **(D)** when P14 immune chimeras were injected with anti-Thy1.1 antibody or left untreated 5 days prior to t.c. deposition of gp33 peptide. **(E)** OT-I CD8 T cells were transferred i.v. into P14 immune chimeras that were treated with anti-VCAM-1, anti-CD49d or isotype control antibody and were then challenged with gp33 t.c. 48h later, OT-I T cells per coronal section were enumerated. **(F)** B cells in the FRT were enumerated 48h after t.c. gp33 peptide challenge of P14 immune chimeras, and **(G)** shows a representative image. B cells in the FRT were also enumerated in **(H)** P14 immune chimeras that were t.c. challenged with either VV-gp33, VV-OVA or left untreated, in **(I)** LCMV immune mice that never received P14 cells, and **(J)** in P14 immune chimeras that were injected with anti-Thy1.1 antibody 5d prior to t.c. gp33 peptide challenge. **(K)** B cells within the FRT of OT-I *Ifng*$^{-/-}$ or OT-I *Ifng*$^{+/+}$ immune chimeras were enumerated 48h after t.c. SIINFEKL challenge. **(L)** B cells in the FRT were quantified 48h after P14 immune chimeras were challenged t.c. with gp33 peptide in the presence of VCAM-1 or CD49d blocking

antibodies. Each experiment shown includes 3-6 mice and data is representative of 2-3 independent experiments. Scale bars=20μm. *=p<0.05, **=p<0.01, ***=p<.001, unpaired two-tailed *t*-test, error bars indicate mean±SEM.

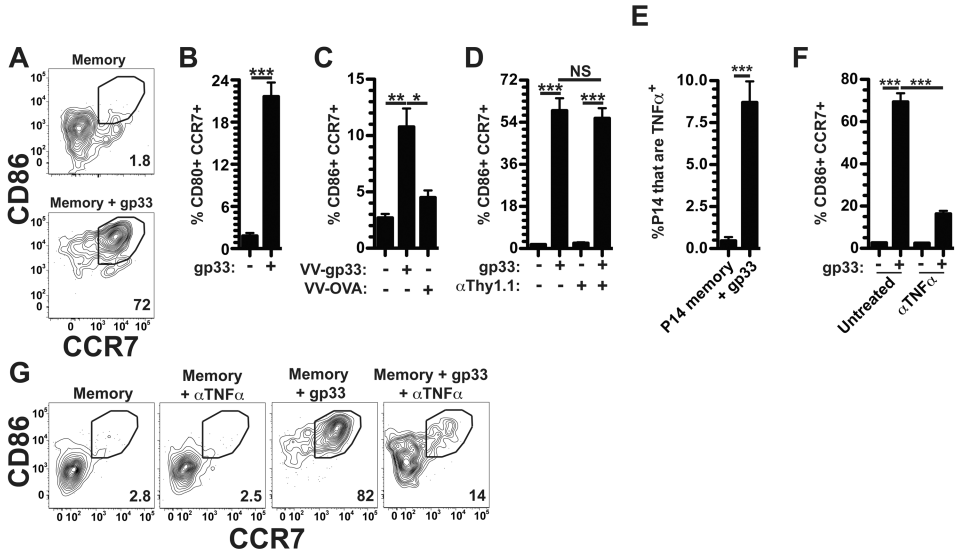

Fig 2. T_{RM} reactivation induces DC maturation

CD86 and CCR7 expression was evaluated on $CD11c^{+}$/$MHC\text{-}II^{+}$ DC in the FRT 12h after t.c challenge of **(A)** P14 immune chimeras challenged with gp33 peptide, **(B)** LCMV immune mice (that never received P14 cells) challenged with gp33 peptide, **(C)** P14 immune chimeras that were t.c. challenged with either VV-gp33 or VV-OVA, or **(D)** P14 immune chimeras that were injected i.p. with 1 μg of anti-Thy1.1 antibody five days prior to gp33 challenge. **(E)** Intracellular TNFα expression was evaluated in P14 CD8 T cells from the FRT by flow cytometry 12h after t.c. gp33 challenge. **(F&G)** DC phenotype was evaluated as in **(A)**, but the indicated mice were pretreated with TNFα blocking antibody. Representative of 2-3 experiments totaling 6-14 mice/group. *=p<0.05, **=p<0.01, ***=p<.001, unpaired two-tailed *t*-test, error bars indicated mean±SEM.

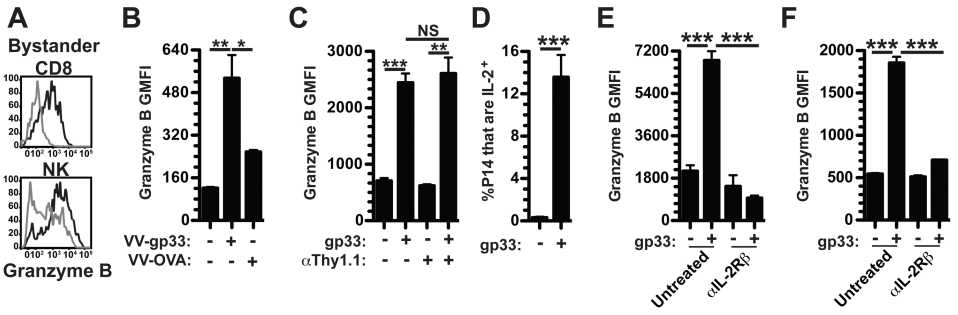

Fig 3. T_{RM} reactivation induces NK cell activation

(A) P14 immune chimeras were challenged t.c. with gp33 to reactivate T_{RM}. 12h later, intracellular granzyme B expression was evaluated in bystander CD8 T cells (P14 CD8 T cells were excluded from analysis) and NK cells isolated from the FRT (grey line=without gp33 challenge, black=gp33 challenge). Intracellular granzyme B expression within NK cells isolated from the FRT was evaluated 12h after **(B)** P14 immune chimeras were t.c. challenged with either VV-gp33 or VV-OVA or left untreated or **(C)** P14 immune chimeras were previously treated with anti-Thy1.1 antibody before t.c. gp33 peptide challenge. **(D)** 12h after t.c. gp33 peptide challenge, intracellular IL-2 expression by P14 CD8 T cells isolated from the FRT of P14 immune chimeras was evaluated. Intracellular granzyme B expression by **(E)** NK cells and **(F)** CD8 T cells isolated from the FRT of P14 chimeras 12h after gp33 peptide challenge when mice were pre-treated with IL-2Rβ blocking antibody. n=3, representative of 3 experiments. *=p<0.05, **=p<0.01, ***=p<.001,unpaired two-tailed *t*-test,mean±SEM.

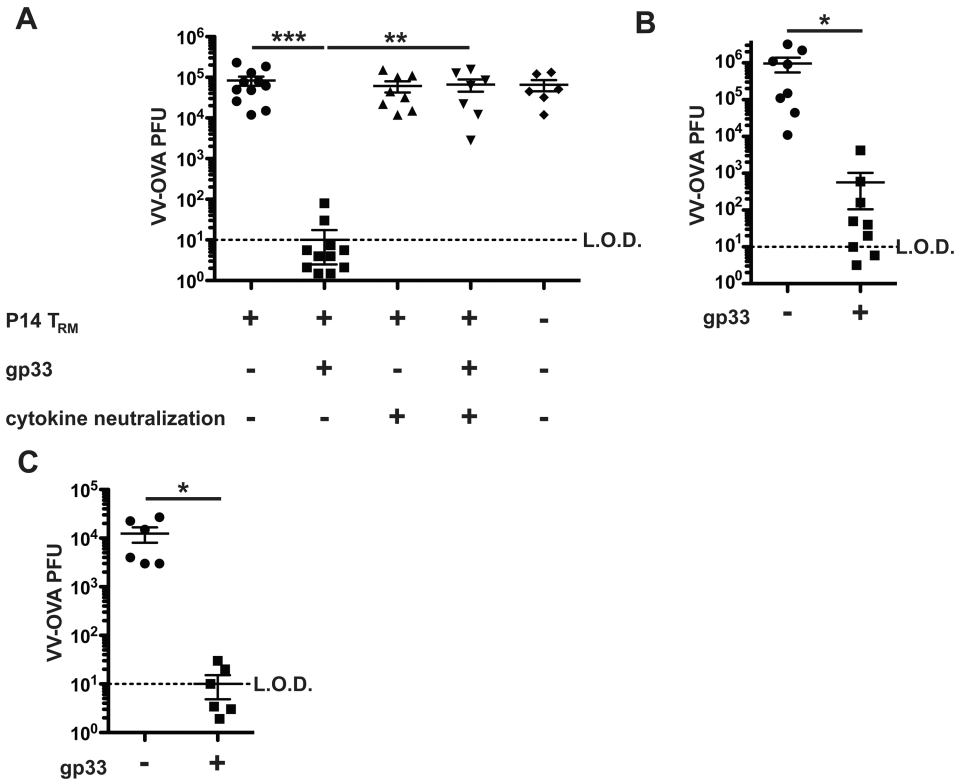

Fig 4. T_{RM} reactivation induces antiviral state

(A) P14 immune chimeras or control naïve mice were challenged t.c. with 4×10^6 PFU of antigenically unrelated VV-OVA in the presence or absence of gp33 reactivating peptide and/or IFNγ, TNFα, and IL-2Rβ blocking antibodies. Two days later, viral titers were evaluated by plaque assay from homogenized FRT. Data pooled from two independent experiments totaling 6-11 mice per group. **(B)** As in A, however gp33 peptide was delivered 12h prior to viral challenge. Data pooled from two independent experiments totaling 8 or 9 mice per group. **(C)** LCMV immune mice (without P14 transfer) were transcervically challenged with 1×10^6 PFU of VV-OVA in the presence or absence of gp33 peptide. Two days after infection, viral titers were evaluated. Data pooled from two independent experiments totaling 6 mice per group. *=p<.05, **=p<.01,***=p<.001, unpaired two-tailed *t*-test, mean±SEM.