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Cutting Edge: Local Recall Responses by Memory T Cells Newly Recruited to Peripheral Nonlymphoid Tissues

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Infection results in the formation of a circulating effector memory T cell population able to enter peripheral tissues either in the steady state or in response to localized infection. As a consequence, recall is thought to result from a phased response first involving those T cells already at the site of infection followed by the infiltration of memory cells from the wider circulation. We have recently reported that tissue-resident T cells can undergo stimulation and proliferation in response to local infection. In this study, we examine the proliferation of memory T cells newly recruited from the circulation. Our results show that although recruitment of circulating memory cells is nonspecific in nature, there is preferential proliferation of specific T cells within infected tissues. Thus, expansion represents a means of local Ag-specific enrichment of T cells recruited from a circulating memory pool of mixed specificities. The Journal of Immunology, 2008, 181: 5837–5841.

Following the cessation of an acute infection, the greatly expanded Ag-specific effector CD8+ T cell population dramatically contracts, leaving a small but numerically significant population of cells that enter the memory T cell pool (1, 2). At least some of these memory cells can enter peripheral tissues (3, 4) and there form the front-line defense against reinfection (5, 6). These cells are effector memory T cells, and they can be found in the wider circulation (4) as well as in all manner of peripheral nonlymphoid tissues (3, 7). As a consequence of this local and disseminated distribution, it has been suggested that the nonlymphoid tissue recall response can be divided into different phases, with an immediate response by those cells already at the site of infection and a later response involving memory T cells recruited from the circulation (8). Although we have recently shown that local T cells are directly stimulated and expanded in situ on reinfection (9), it remained unclear whether this was also true of the recruited memory T cells during this second phase of the peripheral recall response. In the present study we use a transplantation model of HSV-1 reactivation to investigate the activation of memory CD8+ T cells recruited from the circulation. We demonstrate that newly infiltrating memory T cells, like their tissue-residing counterparts, can undergo local proliferation and rapid amplification.

Materials and Methods

Mice

C57BL/6, B6.SJL-PtprcaPep3b/BoyJ (CD45.1), gBT-I × B6.SJL-PtprcaPep3b/BoyJ (gBT-LCD45.1), and OT-1 × B6.SJL-PtprcaPep3b/BoyJ (OT-LCD45.1) were from the Department of Microbiology and Immunology, University of Melbourne (Melbourne, Australia). The gBT-L.CD45.1 TCR-transgenic mice recognize the immunodominant determinant gB998–1005 (where gB is glycoprotein B), and the OT-L.CD45.1 TCR transgenic mice are specific for the OVA epitope OVA257–264.

Viral infections and transplantations and FTY720 treatment

The KOS strain of HSV-1 was used in this study. Mice were infected with 1 × 106 PFU of HSV-1 via flank scarring as previously described (10). Dorsal root ganglia (DRGs) (T8–T12) harvested from naive donor mice or mice infected with HSV-1 >20 days earlier were harvested and grafted beneath the kidney capsule of syngeneic recipient mice (9). The recombinant influenza virus encoding the gB998–1005 epitope within the neuraminidase stalk (Flu-gB) was obtained from Dr. S. Tevetia (Pennsylvania State University College of Medicine, Hershey, PA), and the recombinant influenza virus encoding the OVA257–264 epitope within the neuraminidase stalk (Flu-OVA) (11) was obtained from Dr. P. Doherty (University of Melbourne, Melbourne, Australia). Mice were infected s.c. with 500 PFU of each influenza virus in the hind footpad. Mice were injected i.p. with 2 mg/kg FTY720 (Sapphire Biosciences) commencing at the time of transplantation and continuing every other day for the duration of the experiment.

Generation of memory mice

The method for in vitro activation of CD8+ T cells has previously been described (10). C57BL/6 donor mice received 107 in vitro activated OT-L.CD45.1 and gBT-L.CD45.1 cells via i.v. injection. Alternatively, C57BL/6 donor mice received 1 × 107 naïve gBT-L.CD45.1 and OT-L.CD45.1 lymph node cells via i.v. injection 1 day before s.c. infection with 500 PFU of Flu-gB and Flu-OVA. Animals were allowed to rest for 30 days allowing the conversion of the cells into a memory population.

Flow cytometry, mAbs, BrdU staining

Recovered grafts were digested for 1.5 h at 37°C in collagenase type 3 (Worthington) (3 mg/ml in RPMI 1640 supplemented with 2% FCS). The following Abs from BD Pharmingen were used for flow cytometry: anti-CD8a–allophycocyanin (clone 53–6.7); anti-CD45.1–PE; anti-Vβ8.1,8.2–biotin (clone MR5-2); streptavidin–FITC; and streptavidin–allophycocyanin-Cy7. Pharmingen Spheres blank calibration particles were used to determine cell number. Mice were administered 200 μl of BrdU (6.25 mg/ml in PBS)

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recovered and the number of gBT-I CD8+ T cells. At the indicated time, the graft was regrafted and the number of gBT-I CD8+ T cells (CD45.1+CD8+Vβ8+) and OT-I CD8+ T cells (CD45.1+CD8+Vβ8+) in the graft was determined by flow cytometry. The bars represent the mean ± SEM with three to four mice per group. Data are representative of two independent experiments.

Results and Discussion

Initial Ag nonspecific memory T cell recruitment into infected tissues followed by Ag-specific T cell accumulation

We had previously shown that DRGs harboring latent HSV-1 infection supported local proliferation of resident T cells carried over in a transplantation setting (9). Transplantation itself also resulted in virus reactivation and thus renewed stimulation of the virus-specific T cells within the graft. In these previous experiments, infected ganglia were transplanted into noninfected recipients so that the memory response consisted entirely of the tissue-resident population originating within the graft itself. However, local responses are supposedly phased in nature, with infiltrating T cells taking over from those cells already present in tissues undergoing renewed infection (8). To examine the response of such recruited memory T cells, recipient mice were seeded with in vitro activated HSV-1-specific CD8+ T cells (gBT-I.L-CD45.1+) or irrelevant CD8+ T cells specific for chicken ovalbumin (OT-I.L-CD45.1) 30 days before ganglionic transplantation. These transgenic T cells had converted to a memory pool by the time of transplantation, with the OT-I T cells outnumbering the gBT-I T cells 2.1 in the spleen (data not shown). DRGs latently infected with HSV-1 grafted under the kidney capsule recruited these circulating memory cells within the first 4 days regardless of specificity, with both gBT-I and OT-I T cells present at this time (Fig. 1). This Ag nonspecific recruitment was most likely a consequence of the general inflammation associated with the transplantation. However, gB-specific CD8+ T cells preferentially accumulated thereafter (Fig. 1), consistent with only these cells responding to the reactivating HSV-1. Thus, although initial memory T cell recruitment into inflamed and infected tissues is nonspecific in nature, consistent with what is seen in other systems (12, 13), subsequent accumulation or expansion of these cells is largely Ag-specific.

Ag-specific T cells undergo preferential expansion rather than continuous recruitment from the circulating memory pool

T cell accumulation in Fig. 1 could have resulted from a combination of continuous infiltration and retention of only virus-specific memory T cells or, alternatively, from local Ag-specific T cell expansion. To discriminate between these scenarios, mice containing alleleically marked (CD45.1+) circulating memory OT-I and gBT-I CD8+ T cells, generated by T cell transfers as described above, were infected with HSV-1. On day 6 post-infection (day 0 posttransplantation), the viral infected DRGs, which by this time point were seeded by circulating memory CD8+ T cells (107) were seeded into recipient mice 30 days before flank infection with 106 PFU of HSV-1. On day 4 regardless of specificity, with both gBT-I and OT-I T cells present at this time (Fig. 1). This Ag nonspecific recruitment was most likely a consequence of the general inflammation associated with the transplantation. However, gB-specific CD8+ T cells preferentially accumulated thereafter (Fig. 1), consistent with only these cells responding to the reactivating HSV-1. Thus, although initial memory T cell recruitment into inflamed and infected tissues is nonspecific in nature, consistent with what is seen in other systems (12, 13), subsequent accumulation or expansion of these cells is largely Ag-specific.

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transplantation (day 0) there was a small population of both gBT-I and OT-I CD8+ T cells in the HSV-1-infected ganglia, the former outnumbering the latter. By day 8 posttransplantation the virus-specific gBT-I had increased 10-fold while the OT-I numbers remained unchanged.

The above experiment was repeated using mice infected with recombinant influenza viruses to generate memory T cell populations. To do this, mice were seeded with allelically marked naive gBT-I and OT-I CD8+ T cells before infection with a mixture of recombinant influenza viruses that expressed either the gB epitope (Flu-gB) or the OVA epitope (Flu-OVA) and were left for 30 days to allow the development of memory OT-I and gBT-I CD8+ T cells before coinfection (s.c.) with Flu-gB and Flu-OVA. Mice were left for 30 days before a latent and a naive ganglia graft were transplanted into distinct regions beneath the same kidney capsule. Four days later (day 0) the grafts were re-transplanted beneath the same kidney capsule of a naive recipient. B and C, On day 7 after secondary transplantation the number of gBT-I CD8+ T cells (CD45.1+CD8+gB+) and OT-I CD8+ T cells (CD45.1+CD8+Vb8+) in the latent (B) and naive (C) grafts was determined by flow cytometry. The bars represent the mean ± SEM with eight mice per group pooled from two independent experiments. D and E, Mice were seeded with 5 × 10^4 naive gBT-I.CD45.1+1 day before infection (s.c.) with Flu-gB. Mice were left to rest for 30 days before a latent ganglia graft was transplanted beneath the kidney capsule. Four days later (day 0), the grafts were recovered and re-transplanted beneath the kidney capsule of a naive recipient. Recipient mice were treated with FTY720 until day 8 after secondary transplantation. D, Flow cytometry profiles depicting the mean percentage ± SEM of CD8+ T cell in the blood of animals treated with FTY720 compared with untreated controls. E, The number of gBT-I CD8+ T cells (CD45.1+CD8+) within the latent grafts at days 0 and 8 after secondary transplantation. The bars represent the mean ± SEM with six mice per group pooled from two independent experiments.

**FIGURE 3.** Circulating memory T cell expansion within the peripheral tissue is not due to prior LN stimulation. A. Mice were seeded with 5 × 10^4 naïve gBT-I.CD45.1+ and OT-I.CD45.1+CD8+ T cells 1 day before coinfection (s.c.) with Flu-gB and Flu-OVA. Mice were left for 30 days before a latent and a naïve ganglia graft were transplanted into distinct regions beneath the same kidney capsule. Four days later (day 0) the grafts were re-transplanted beneath the same kidney capsule of a naïve recipient. B and C, On day 7 after secondary transplantation the number of gBT-I.CD8+ T cells (CD45.1+CD8+gB+) and OT-I.CD8+ T cells (CD45.1+CD8+Vb8+) in the latent (B) and naïve (C) grafts was determined by flow cytometry. The bars represent the mean ± SEM with eight mice per group pooled from two independent experiments. D and E, Mice were seeded with 5 × 10^4 naïve gBT-I.CD45.1+1 day before infection (s.c.) with Flu-gB. Mice were left to rest for 30 days before a latent ganglia graft was transplanted beneath the kidney capsule. Four days later (day 0), the grafts were recovered and re-transplanted beneath the kidney capsule of a naïve recipient. Recipient mice were treated with FTY720 until day 8 after secondary transplantation. D, Flow cytometry profiles depicting the mean percentage ± SEM of CD8+ T cell in the blood of animals treated with FTY720 compared with untreated controls. E, The number of gBT-I CD8+ T cells (CD45.1+CD8+) within the latent grafts at days 0 and 8 after secondary transplantation. The bars represent the mean ± SEM with six mice per group pooled from two independent experiments.

**Memory T cell expansion within the peripheral tissue is not due to prior LN stimulation**

The preceding demonstrated that circulating memory T cells infiltrating the ganglia graft could continually expand if severed from the rest of the circulating memory T cell pool. This T cell expansion was not solely due to momentum from prior LN stimulation. To show this, DRGs harvested from naïve and latently infected donor mice were transplanted into distinct regions beneath a single kidney capsule of recipient mice containing memory OT-I and gBT-I T cells (Fig. 3A). Memory CD8+ T cells initially infiltrated in a nonspecific manner due to surgery-induced inflammation. Thus, at the time of harvest (day 0 after re-transplantation) both the infected and naïve ganglia grafts were seeded with relatively equal proportions of OT-I and gBT-I memory T cells (Fig. 3, B and C). These memory T cell-laden grafts were transplanted into secondary naïve recipient mice and 7 days later were recovered and assessed for memory T cell expansion (Fig. 3, B and C). By initially transplanting the naïve and infected ganglia into the same memory animal we ensured that the T cells infiltrating both grafts were subjected to the same external stimulatory cues.

Following disconnection from the circulating memory T cell pool, further expansion of the Ag specific gBT-I CD8+ T cells was only observed within the latently infected graft (Fig. 3B). No further expansion of these memory T cells was detected within the naive graft (Fig. 3C), implying the requirement for local antigenic stimulation to drive this expansion. We repeated the above experiment with latent ganglia alone, but treated the secondary recipients with FTY720. This inhibits lymphocyte egress from LNs, causing profound lymphopenia (Fig. 3D). Memory gBT-I CD8+ T cells could still expand within the latent graft (Fig. 3E), arguing for a LN-independent recall of infiltrating memory T cells.
Local virus-specific proliferation of infiltrating memory T cells

Combined, the above experiments showed that memory T cells could be recruited into nonlymphoid tissues and there apparently undergo an Ag-specific phase of expansion. Reinhardt et al. (14) reported that effector T cells could accumulate in an Ag-specific manner within nonlymphoid tissues in the absence of local proliferation, where such proliferation was measured by BrdU incorporation. We therefore set out to determine whether memory T cells recruited into the reactivating ganglia could indeed incorporate BrdU, consistent with our proposal for local proliferation. Naive or HSV-1 latently infected DRGs were grafted into mice with circulating memory OT-I and gBT-I T cells. On day 7 posttransplantation, mice were administered BrdU i.p. and 1 h later they were sacrificed and the proportion of BrdU-positive memory CD8+ T cells within the ganglia graft, blood, spleen, and kidney draining LN was measured. A short, 1-h BrdU pulse was used in an attempt to largely confine detection to those cells dividing only within the tissue and limit the contribution of any recruited proliferating population to this measurement. Once again both Ag-specific gBT-I and nonspecific OT-I T cells infiltrated the grafted ganglia, thus allowing ready comparison of Ag-specific vs nonspecific infiltrating memory CD8+ T cells (Fig. 4B). Within the grafts, ~8.9% of the virus-specific infiltrating memory CD8+ T cells (gBT-I) were BrdU+ compared with 1.6% of the control OT-I T cells (Fig. 4C). Little gBT-I T cell proliferation was seen in transplanted noninfected (naive graft) DRGs (Fig. 4B), although these tissues also recruited memory T cells. Combined, the results in Fig. 4, B and C, were consistent with proliferation being virus specific, although the higher level of OT-I cell proliferation in grafts vs other tissues suggests that nonspecific inflammatory mediators may make some contribution to cell cycling. In addition, while no other tissues showed the same level of gBT-I BrdU incorporation as the ganglia, the proportion of gBT-I cells incorporating BrdU in the renal draining LN appeared elevated compared with that in the control OT-I T cells, although this difference was found not to be statistically significant. Nonetheless, we do not exclude that this may represent low-level gBT-I proliferation in these lymphoid tissues. Regardless, the strength of BrdU incorporation by the recruited gBT-I cells within the ganglia compared with what is seen in other sites argues that these cells were proliferating directly in the nonlymphoid tissues. This LN-independent recall of memory T cells is consistent with previous studies demonstrating that memory CD8+ T cells can be successfully recalled to mediate allograft rejection (15) or facilitate pathogen control (16) in secondary lymphoid organ-deficient environments.

Overall, the results show that newly recruited memory T cells can undergo local T cell proliferation and expansion in peripheral nonlymphoid tissues, analogous to what we have previously shown for the equivalent resident population (9). Local proliferation of memory T cells makes sense, given the changes that are known to occur in the memory population as a whole. Memory T cells of a given specificity can be replaced by subsequent infection (17–19) and the infiltrating effector memory subsets are lost with time (20, 21), possibly resulting in the slow decrease in those T cells found in nonlymphoid tissues (22). Proliferation at the site of infection of even low numbers of virus-specific infiltrates could therefore compensate for this memory degradation.

Disclosures
The authors have no financial conflict of interest.

References


