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Activation of virus-specific CD4 T cells throughout gammaherpesvirus latency[1]

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Abstract

CD4 T cells are essential for immune control of gamma-(γ)-herpesvirus latency. We previously identified a murine MHC class II-restricted epitope in γHV68 glycoprotein 150 ($gp150_{67–83}IA^b$) that elicits CD4 T cells that are maintained throughout long-term infection. However, it is unknown if naïve cells can be recruited into the antiviral CD4 T cell pool during latency. Here we generate a mouse transgenic for a gp150-specific T cell receptor and show epitope-specific activation of transgenic CD4 T cells during acute and latent infections. Furthermore, although only dendritic cells can stimulate virus-specific CD8 T cells during latency, we show that both dendritic cells and B cells stimulate transgenic CD4 T cells. These studies demonstrate that naïve CD4 T cells specific for a viral glycoprotein can be stimulated throughout infection, even during quiescent latency, suggesting that CD4 T cell memory is maintained in part by the continual recruitment of naïve cells.

Introduction

CD4 T cells play an essential role in the control of persistent infections (1). In human gamma (γ)-herpesvirus infections, CD4 T cells can be specific for lytic or latent antigens, and disruption of CD4 T cell surveillance can lead to recrudescence and development of cancer (2–4). Virus reactivations and malignancies associated with the human γ-herpesviruses Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) are frequently found in HIV-infected patients with CD4 T cell deficiency (5). CD4 T cells are currently being targeted for therapies and vaccines for EBV-associated cancers (6, 7). However, how antiviral CD4 T cells are activated and maintained throughout persistent γ-herpesvirus infection is unclear.

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In this study we analyze the activation and proliferation of virus-specific CD4 T cells using a tractable small animal model of γ-herpesvirus infection and immunity – infecting mice with murine γ-herpesvirus-68 (γHV68), a γ2-herpesvirus related to EBV and KSHV (8). Throughout γHV68 infection, antiviral CD4 T cells are maintained at high frequencies and have a high turnover rate (9, 10). The best characterized CD4 T cell epitope in C57BL/6 mice is derived from γHV68 glycoprotein 150 ($gp150_{67–83}IA^b$), a homolog of EBV gp350/220 and KSHV K8.1 (11). Here we report the generation of a T cell receptor (TCR) transgenic (tg) mouse with specificity for the γHV68 $gp150_{67–83}$ epitope. Using this mouse, we demonstrate that gp150-specific CD4 T cells are activated and expand after γHV68 infection, traffic to multiple tissues, and can be stimulated during latency by both dendritic cells and B cells to proliferate in an antigen-specific manner, thereby suggesting that naïve CD4 T cells can be recruited into the ongoing immune response during quiescent latency.

Materials and Methods

Mice and viral infections

C57BL/6, B6.SJL-*Ptprc*^{a}*Pepc*^{b}/Boy (B6.SJL), *Salmonella* flagellin peptide ($SM1_{427–441}IA^b$) TCR tg (12), *Mycobacterium tuberculosis* early secreted antigenic target-6 ($ESAT6_{1–20}IA^b$) TCR tg (13), and γHV68 $gp150_{67–83}IA^b$ TCR tg mice were obtained from the Trudeau Institute animal facility and maintained under specific-pathogen free conditions. Mice were infected intranasally (i.n.) with 400 PFU of γHV68 (strain WUMS). All experiments were approved by the Institutional Animal Care and Use Committee of the Trudeau Institute. To generate the $gp150_{67–83}IA^b$ TCR tg mouse, genes encoding the TCR Vα11 and Vβ12 chains were cloned from the $gp150_{67–83}$-specific CD4 T cell hybridoma (clone 4211) (9) essentially as described using standard methods (13). Microinjection of C57BL/6 mouse embryos was performed by the Transgenic Mouse Facility at the University of Vermont (Burlington, VT).

Stimulations of transgenic CD4 T cells

In vitro stimulations were performed as described (13). For *in vivo* examination during lytic infection, 4×10^3 cells were injected intravenously (i.v.) into naïve congenic mice 24 hours before infection. Mediastinal lymph nodes (MLN), spleens, and lungs were examined for number and phenotype of transferred CD4 T cells at various times p.i. For examination during latency, 10^6 cells of each type were injected i.v. into congenic mice at various times p.i. Tissues were examined for phenotype of transferred CD4 T cells. To assess *in vitro* antigen presentation, $CD19^+$ B cells or $CD11c^+$ dendritic cells (DCs) were sorted from the spleens of mice 4 months p.i. CD4 T cells were enriched from naïve gp150 TCR tg mice by negative enrichment and labeled with CFSE (Molecular Probes). CD4 T cells were combined with B cells or DCs at varying effector to target (E:T) ratios. On day 3 of culture, cells were collected and analyzed by flow cytometry.

Results and Discussion

Generation of a gp150-specific TCR transgenic mouse strain

We previously identified an MHC class II-restricted epitope within γHV68 gp150 ($gp150_{67–83}IA^b$) that stimulates antiviral CD4 T cells to produce IFNγ (9, 10). To specifically monitor virus-specific CD4 T cells throughout infection, we generated a TCR tg mouse with specificity for $gp150_{67–83}IA^b$.

TCR genes were isolated from a T cell hybridoma that recognizes the $gp150_{67–83}$ epitope (9, 10). The hybidoma expresses a TCR containing the Vα11 and Vβ12 TCR chains (data not shown). The TCRα and TCRβ genes were cloned into the human CD2 expression vector

(14), and the construct was used to generate germ-line tg mice. C57BL/6 founder mice were identified based upon their expression of Vα11 and Vβ12 on the majority of CD4 T cells (Figure 1*A*). To confirm the specificity of the tg CD4 T cells for the $gp150_{67–83}IA^b$ epitope, we measured the expression of an early activation marker, CD69, *in vitro*. As shown in Figure 1*B*, TCR tg CD4 T cells were activated in the presence of cognate peptide, but not an irrelevant control peptide (influenza $NP_{311–325}IA^b$), demonstrating that they exhibited the intended specificity.

Activation of gp150-specific tg CD4 T cells during acute γHV68 infection

To analyze the ability of naïve CD4 T cells to respond to acute viral infection, we adoptively transferred 4000 gp150 TCR tg cells into congenic mice 1 day prior to γHV68 infection. Importantly, transferring 4000 tg CD4 T cells had no protective effect on either the initial lytic infection in the lung or the establishment of latency in the spleen (data not shown). As early as 7 days p.i., we could recover tg CD4 T cells from the spleens, MLN, and lung parenchyma (Figure 2*A*). These cells were highly activated, evidenced by expression of CD44 and PD-1, and a lack of CD127 expression in the spleen (Figure 2*B*). Over time, and consistent with the endogenous host CD4 T cells (shown in gray in Figure 2*B*), PD-1 levels decreased and CD127 expression increased on the donor tg cells. Unlike γHV68-specific CD8 T cells (15), we did not observe sustained expression of KLRG-1 on the tg CD4 T cells.

Stimulation of gp150-specific transgenic CD4 T cells during latency

It has been shown previously that $CD44^{hi}$ CD4 T cells proliferate during γHV68 latency, as measured by incorporation of BrdU, and that treatment of latently-infected mice with a cell-cycle specific cytotoxic drug reduces $gp150_{67–83}$-specific IFNγ production approximately 10-fold (10). These findings suggest that antiviral CD4 T cells are cycling continuously throughout the acute and latent infection, similar to observations with virus-specific CD8 T cells (16). The CD4 T cell response can be maintained either by proliferation of a self-renewing population or by the continual recruitment of naïve cells into the ongoing response (17). To determine whether naïve $gp150_{67–83}IA^b$-specific CD4 T cells can be activated and contribute to the ongoing immune response during γHV68 latency, we transferred CD4 T cells from uninfected gp150 TCR tg mice into latently-infected congenic mice and assessed their activation by measuring CD69 expression 18 hours later. When transferred into mice at 1 month p.i., an early stage of latency, we observed a robust increase in CD69 expression on gp150 TCR tg CD4 T cells, but not on cells from non-tg littermate control mice (Figure 3*A*,*B*). There was also a clear increase in CD69 expression on gp150 TCR tg CD4 cells transferred into latently-infected mice at 4 months p.i., although the percent of cells that expressed CD69 was reduced compared to the earlier time point (Figure 3*C*). Notably, at neither time did we observe activation of tg CD4 T cells recovered from the lungs of infected mice, suggesting the stimulatory environment in the lung is insufficient to activate naïve virus-specific CD4 T cells.

We next sought to measure whether naïve CD4 T cells activated during latency went on to proliferate. CFSE-labeled TCR tg CD4 T cells were transferred into B6.SJL mice that had been infected 2 months previously. We then assessed the donor T cells harvested from spleens or lungs for CD44 expression and CFSE dilution. At 7 days after transfer, we observed an increase in CD44 expression on, and CFSE dilution in, gp150 TCR tg CD4 T cells, but not co-transferred tg CD4 T cells of an irrelevant specificity, confirming that naïve virus-specific CD4 T cells could enter the antiviral immune response during latency (Figure 4*A*). However, even 14 days after transfer, not all the gp150-specific tg CD4 T cells had proliferated (Figure 4*B*), suggesting that naïve CD4 T cell recruitment during latency may be inefficient or occur infrequently. The apparent low-level recruitment may also be a

consequence of the timing of $gp150_{67–83}IA^b$ epitope expression – expression of the gp150 protein is associated with late, lytic infection (18) and it is not thought to be expressed during latency, so therefore, the $gp150_{67–83}IA^b$ epitope may only be detectable by CD4 T cells during periodic episodes of virus reactivation. Consistent other persistent infection models (19, 20), our findings suggest a system in which antiviral T cells generated during acute γHV68 infection can be supplemented by recruitment of naïve cells during viral latency.

Both DCs and B cells from infected mice can stimulate naïve gp150-specific CD4 T cells

During γHV68 infection, it has been shown that CD8 T cells specific for a model lytic antigen and MHC class I-restricted γHV68-specific hybridomas are activated only by DCs (21, 22), even though B cells represent the major reservoir of latent virus (23). However, both DCs and B cells express MHC class II molecules, raising the possibility that both cell types could present antigens to CD4 T cells. To test this hypothesis, we sorted DCs and B cells from latently-infected mice and analyzed their ability to stimulate naïve gp150 TCR tg CD4 T cells. At an E:T ratio of 0.1, we observed a substantial increase of CD69 expression (Figure 5*A*) and CFSE dilution (Figure 5*B*) of the tg cells after stimulation by either DCs or B cells *in vitro*. We did not observe appreciable CD69 expression or proliferation (as measured by CFSE dilution) when naïve gp150 TCR tg CD4 T cells were stimulated by DCs or B cells from naïve mice (data not shown). Although the source of the antigenic signal *in vivo* is unclear, we conclusively show that both DCs and B cells isolated from the spleens of infected animals each retain the capacity to stimulate naïve CD4 T cells. This finding is in contrast to stimulation via MHC class I, which is limited to signals from the DC compartment (21, 22), and is consistent with data from EBV infections, where CD4 T cells can prevent lymphoproliferation by recognizing EBV antigens presented by B cells (24).

Given that CD4 T cells serve an important role in immunity to persistent γ-herpesvirus infections, it is essential to dissect the mechanisms of CD4 T cell generation and maintenance in order to better design antiviral therapies and vaccines. In the studies presented here, we describe the generation of a new CD4 TCR tg mouse that allowed us to answer three fundamental questions about CD4 T cell immunity during γHV68 infection – what are the kinetics of the CD4 T cell response to γHV68, can naïve CD4 T cells contribute to the ongoing immune response during latency, and what cell types can present antigens to antiviral CD4 T cells? First, we show that antiviral CD4 T cells specific for a late-lytic viral epitope were primed by 7 days p.i. and maintained for at least 42 days in multiple tissues. Second, we show that naïve antiviral CD4 T cells were continually recruited into the immune response in a tissue-specific manner during latent infection *in vivo*. Third, we demonstrate that both DCs and B cells from latently-infected mice stimulated antiviral CD4 T cells *in vitro*. These findings enhance our understanding of T cell immunity during γ-herpesvirus infections, providing key insight into the mechanisms by which the antiviral CD4 T cell response is activated and maintained during long-term infection.

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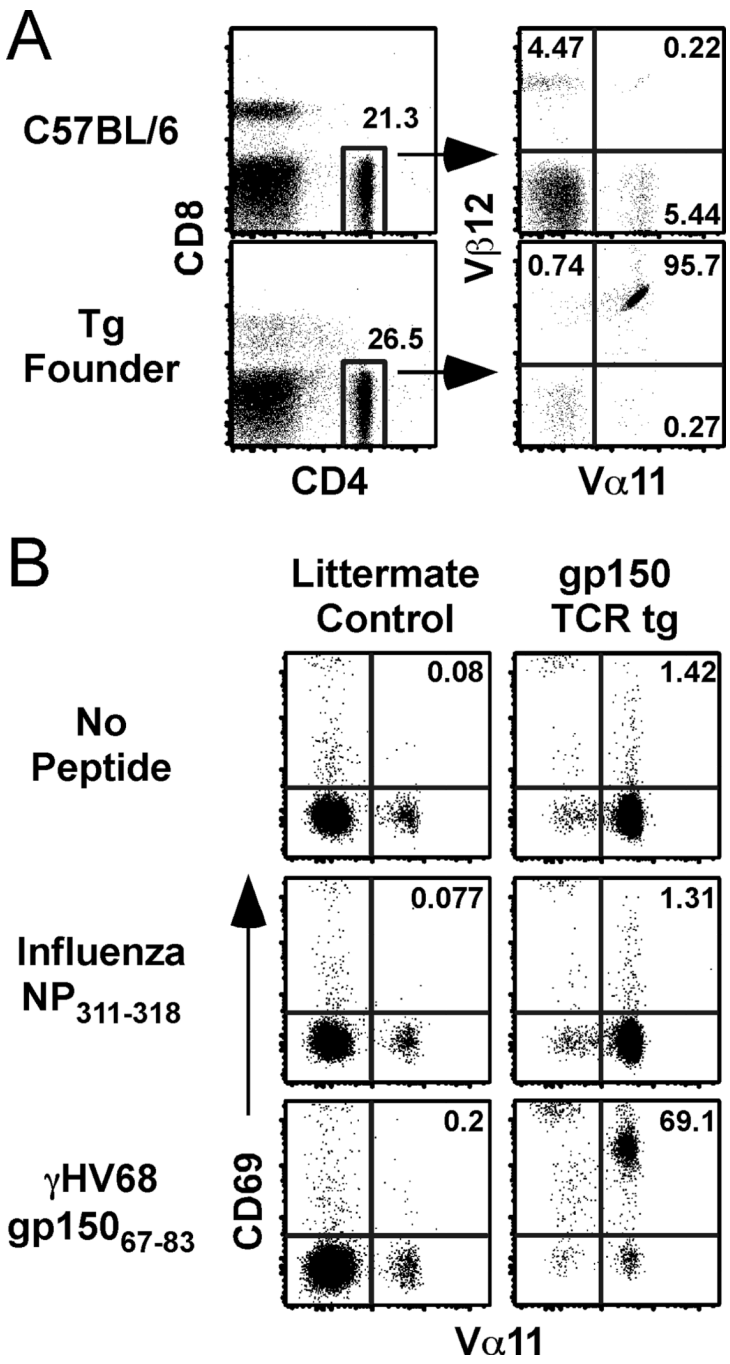

Figure 1.

Generation of a $gp150_{67–83}$/I-A^b-specific transgenic mouse. *A*, Splenocytes from a C57BL/6 littermate or the transgenic founder mouse were analyzed for expression of Vα11 and Vβ12. *B*, Splenocytes from transgenic or littlemate control mice were incubated for 24 h with congenic APCs plus influenza $NP_{311–325}$, γHV68 $gp150_{67–83}$, or no peptide control. The dot plots are gated on CD4 T cells and show Vα11 and CD69 expression after stimulation.

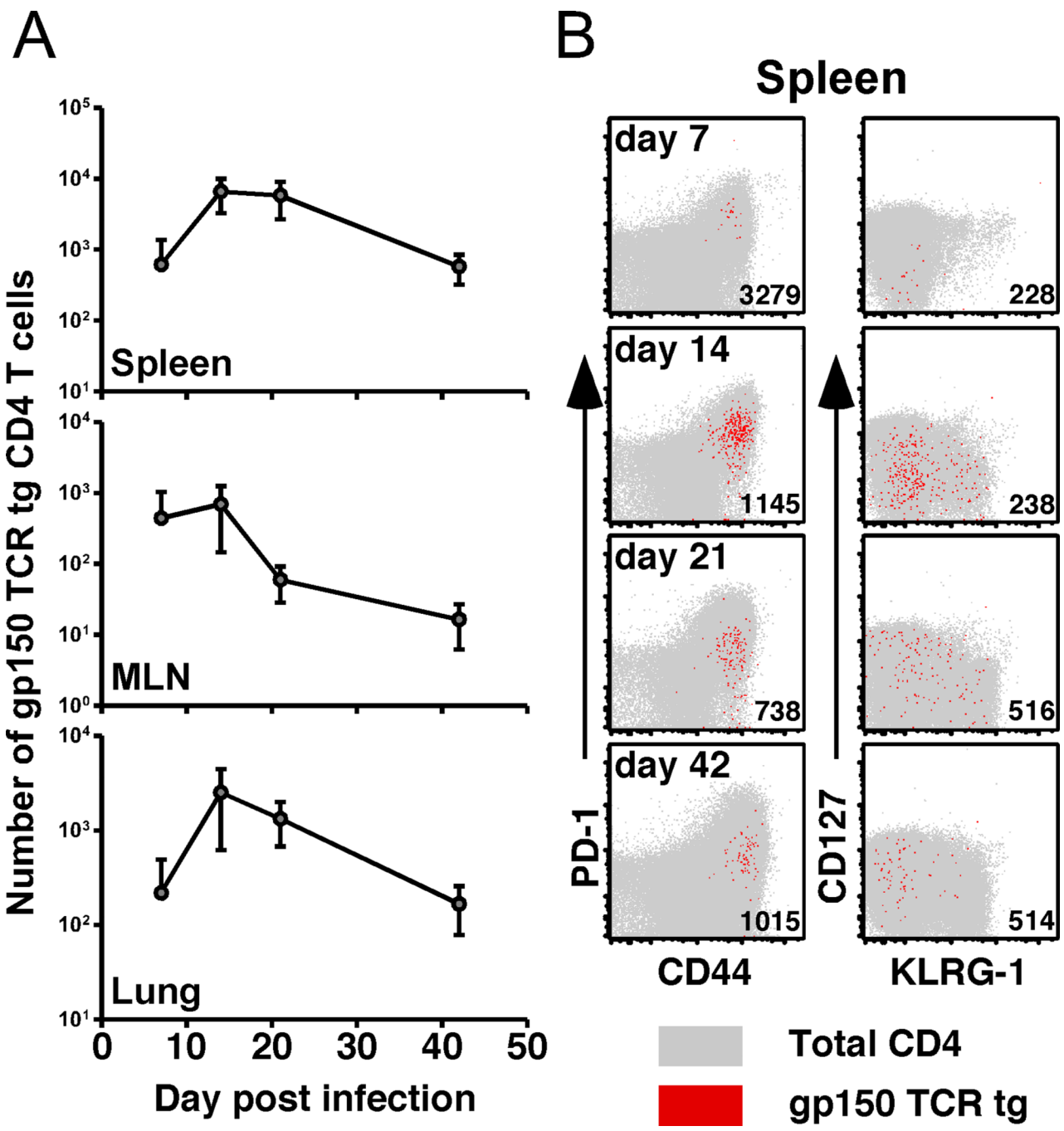

Figure 2.

CD4 T cell response kinetics after intranasal γHV68 infection. *A*, Number of gp150-specific TCR tg CD4 T cells recovered from spleens, MLN, and lungs at the indicated times (n=4–8/time point). *B*, Spleens were harvested at the indicated times. CD45.2$^+$ gp150 TCR tg CD4 T cells from multiple mice are shown in red; the endogenous host CD45.1$^+$ CD4 T cell population from a representative mouse is shown in gray. Numbers in the plots show average MFI for PD-1 (left column) or CD127 expression (right column) in the tg cells (n=4–8/time point, data are representative of 2 experiments).

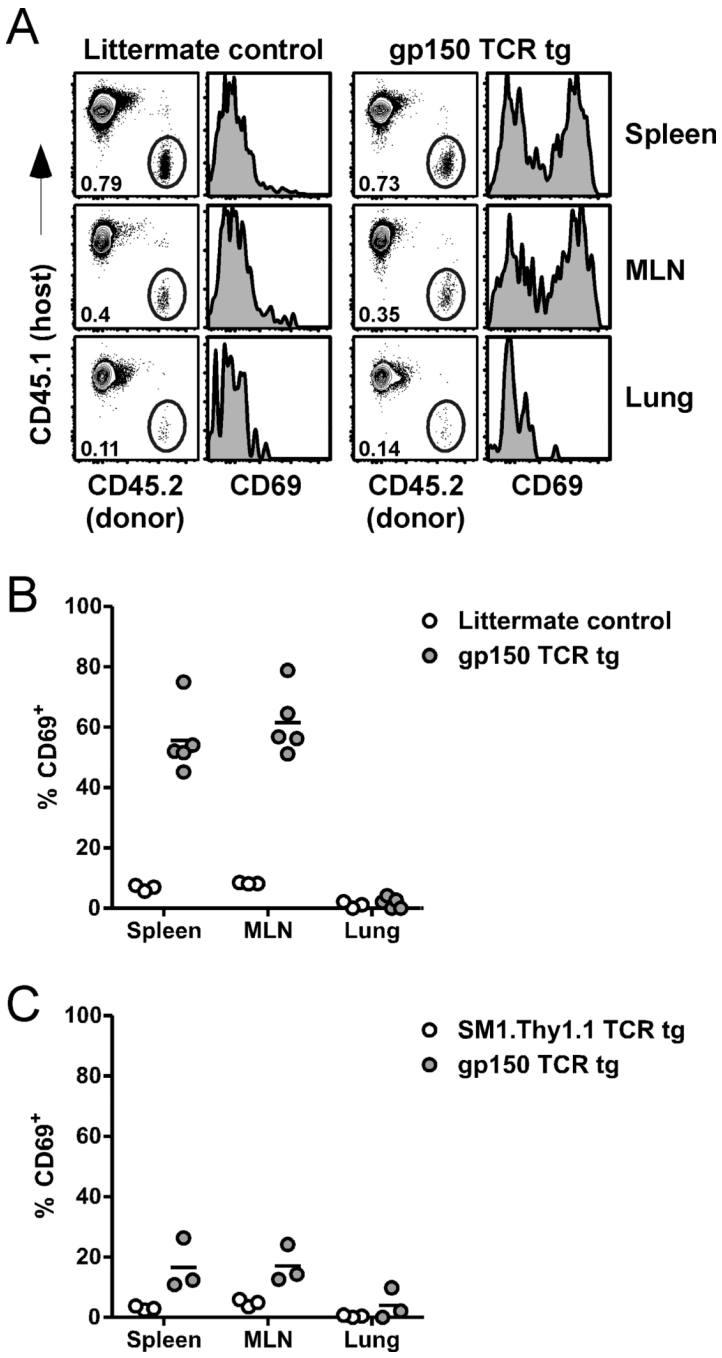

Figure 3.

Activation of naïve transgenic cells during latency. *A*, 18 h after transfer of cells into 1 month p.i. mice, spleens, MLN, and lungs from recipient mice were harvested. Dot plots show the percentage of CD4 T cells that are donor-derived. Histograms show the level of CD69 expression on the donor-derived cells. Data are representative of 3–5 mice. *B*, The percent of donor cells from panel *A* that are $CD69^+$ is shown. *C*, In a separate experiment, $CD45.2^+$ $Thy1.2^+$ gp150 TCR tg cells and $CD45.2^+$ $Thy1.1^+$ SM1 TCR tg cells were co-transferred into $CD45.1^+$ $Thy1.2^+$ recipient mice 4 month p.i. 18 h later, spleens, MLN, and lungs were harvested and analyzed by flow cytometry. The percent of each donor population that is $CD69^+$ is shown. All data are representative of at least 3 experiments.

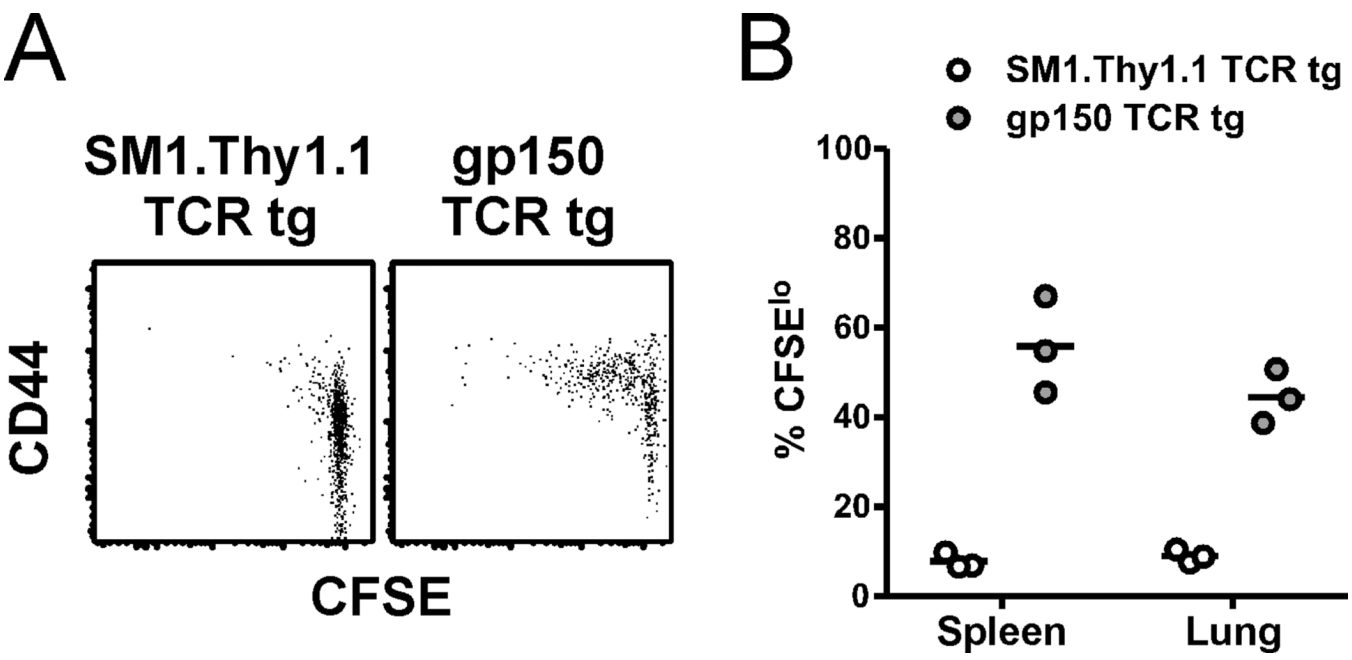

Figure 4.

Proliferation of naïve transgenic cells during latency. *A*, 7 d after transfer, spleens from recipient mice were harvested. Dot plots show the CFSE levels and CD44 expression on the indicated donor populations. *B*, 14 d after transfer, spleens and lungs were harvested and stained as in panel *A*. The percent of each donor population that exhibited CFSE dilution ($CFSE^{lo}$) is shown (n=3, representative of 2 experiments).

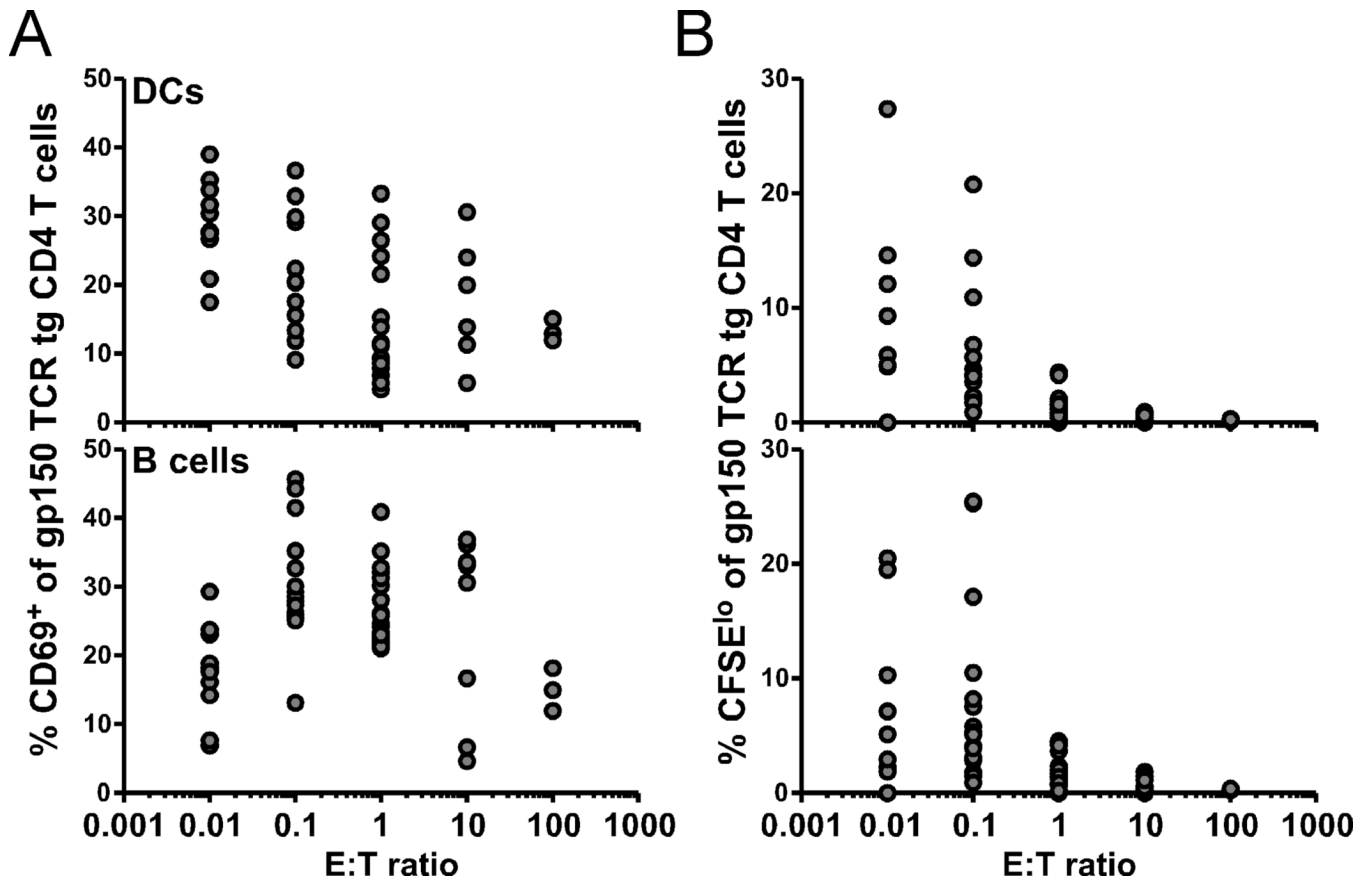

Figure 5.

Both DCs and B cells from infected mice can stimulate naïve CD4 T cells. DCs (top) and B cells (bottom) were sorted out of latently-infected B6.SJL mice 4 months p.i. and co-cultured with naïve CFSE-labeled gp150 TCR tg CD4 T cells at indicated E:T ratios. After 3 d, cultures were analyzed for CD69 expression (*A*) and dilution of CFSE (*B*) (n=3–10, representative of 2 experiments).