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Cutting Edge: Activation of Virus-Specific CD4 T Cells throughout γ-Herpesvirus Latency

Michael L. Freeman,* Claire E. Burkum,* Kathleen G. Lanzer,* Meghan K. Jensen,* Mushtaq Ahmed,‡ Eric J. Yager,§ Emilio Flaño,§ Gary M. Winslow,† David L. Woodland,* and Marcia A. Blackman*

CD4 T cells are essential for immune control of γ-herpesvirus latency. We previously identified a murine MHC class II-restricted epitope in γ-herpesvirus-68 gp150 (gp150\textsubscript{67-83}-I\textsuperscript{b}) that elicits CD4 T cells that are maintained throughout long-term infection. However, it is unknown whether naive cells can be recruited into the antiviral CD4 T cell pool during latency. In this study, we generate a mouse transgenic for a gp150-specific TCR 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 naive 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 naive cells. The Journal of Immunology, 2011, 187: 000–000.

CD4 T cells play an essential role in the control of persistent infections (1). In human γ-herpesvirus infections, CD4 T cells can be specific for lytic or latent Ags, 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 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.

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 gp150 (gp150\textsubscript{67-83}-I\textsuperscript{A}), a homolog of EBV gp350/220 and KSHV K8.1 (11). In this study, we report the generation of a TCR transgenic (tg) mouse with specificity for the γHV68 gp150\textsubscript{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 (DCs) and B cells to proliferate in an Ag-specific manner, thereby suggesting that naive 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-Pipec/PepCBoy (B6.SJL), Salmonella flagellin peptide (SM1\textsubscript{427-444}-I\textsuperscript{A}) TCR tg (12), Mycobacterium tuberculosis early secreted antigenic target-6 (ESAT\textsubscript{6}-\textsubscript{35}-I\textsuperscript{A}) TCR tg (13), and γHV68 gp150\textsubscript{67-83}-I\textsuperscript{A} TCR tg mice were obtained from the Trudeau Institute animal facility and maintained under specific pathogen-free conditions. Mice were infected intranasally with 400 PFU γHV68 (strain WUMS). All experiments were approved by the Institutional Animal Care and Use Committee of the Trudeau Institute. To generate the gp150\textsubscript{67-83}-I\textsuperscript{A} TCR tg mouse, genes encoding the TCR Va11 and Vb12 chains were cloned from the gp150\textsubscript{67-83}-specific CD4 T cell hybridoma (clone 4211) (9) essentially as described using standard methods (13). Micrinjection of C57BL/6 mouse embryos was performed by the Transgenic Mouse Facility at the University of Vermont (Burlington, VT).

Stimulations of tg CD4 T cells

In vitro stimulations were performed as described (13). For in vivo examination during lytic infection, 4 × 10\textsuperscript{5} cells were injected i.v. into naive congenic mice 24 h before infection. Mediastinal lymph nodes (MLN), spleens, and lungs were examined for number and phenotype of transferred CD4 T cells at various times postinfection (p.i.). For examination during latency, 10\textsuperscript{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 Ag presentation, CD19\textsuperscript{+} B cells or CD11c\textsuperscript{+} DCs were sorted...
from the spleens of mice 4 mo p.i. CD4 T cells were enriched from naive 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 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 tg mouse strain

We previously identified a MHC class II-restricted epitope within γHV68 gp150 (gp150_{67–83-I-Ab}) that stimulates anti-viral CD4 T cells to produce IFN-γ (9, 10). To specifically monitor virus-specific CD4 T cells throughout infection, we generated a TCR tg mouse with specificity for gp150_{67–83} I-Ab.

TCR genes were isolated from a T cell hybridoma that recognizes the gp150_{67–83} epitope (9, 10). The hybridoma expresses a TCR containing the V_{a11} and V_{b12} TCR chains (data not shown). The TCRα and TCRβ genes were cloned onto the human CD2 expression vector (14), and the construct was used to generate germline tg mice. C57BL/6 founder mice were identified based upon their expression of V_{a11} and V_{b12} on the majority of CD4 T cells (Fig. 1 A). To confirm the specificity of the tg CD4 T cells for the gp150_{67–83} I-Ab epitope, we measured the expression of an early activation marker, CD69, in vitro. As shown in Fig. 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-I-A^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 naive CD4 T cells to respond to acute viral infection, we adoptively transferred 4000 gp150 TCR tg cells into congenic mice 1 d 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 d p.i., we could recover tg CD4 T cells from the spleens, MLN, and lung parenchyma (Fig. 2 A). These cells were highly activated, evidenced by expression of CD44 and PD-1, and a lack of CD127 expression in the spleen (Fig. 2 B). Over time, and consistent with the endogenous host CD4 T cells (shown in gray in Fig. 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 tg CD4 T cells during latency

It has been shown previously that CD44^{high} 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 IFN-γ production ∼10-fold (10). These findings suggest that antiviral CD4 T cells are cycling continuously throughout the acute and latent infection, similar to obser-
vations 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 naive cells into the ongoing response (17). To determine whether naive gp150\textsubscript{67–83}I-Ab\textsuperscript{a}-specific CD4 T cells can be activated and contribute to the ongoing immune response during \(\gamma\)HV68 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 h later. When transferred into mice at 1 mo 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 (Fig. 3A, 3B). There was also a clear increase in CD69 expression on gp150 TCR tg CD4 cells transferred into latently infected mice at 4 mo p.i., although the percentage of cells that expressed CD69 was reduced compared with the earlier time point (Fig. 3C). 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 naive virus-specific CD4 T cells.

We next sought to measure whether naive 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 mo previously. We then assessed the donor T cells harvested from spleens or lungs for CD44 expression and CFSE dilution. At 7 d after transfer, we observed an increase in CD44 expression on, and CFSE dilution in, gp150 TCR tg CD4 T cells, but not cotransferred tg CD4 T cells of an irrelevant specificity, confirming that naive virus-specific CD4 T cells could enter the antiviral immune response during latency (Fig. 4A). However, even 14 d after transfer, not all the gp150-specific tg CD4 T cells had proliferated (Fig. 4B), suggesting that naive 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\textsubscript{67–83}I-Ab\textsuperscript{a} epitope expression. Expression of the gp150 protein is associated with late, lytic infection (18) and it is not thought to be expressed during latency; therefore, the gp150\textsubscript{67–83}I-Ab\textsuperscript{a} epitope may only be detectable by CD4 T cells during periodic episodes of virus reactivation. Consistent with other persistent infection models (19, 20), our findings suggest a system in which antiviral T cells generated during acute \(\gamma\)HV68 infection can be supplemented by recruitment of naive cells during viral latency.

**FIGURE 3.** Activation of naive \(\gamma\)HV68 T cells during latency. A, 18 h after transfer of cells into 1 mo 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 three to five mice. B, The percentage of donor cells from \(A\) that are CD69\textsuperscript{+} is shown. C, In a separate experiment, CD45.2\textsuperscript{+} Thy1.2\textsuperscript{+} gp150 TCR tg cells and CD45.2\textsuperscript{+} Thy1.1\textsuperscript{+} SM1 TCR tg cells were cotransferred into CD45.1\textsuperscript{+} Thy1.2\textsuperscript{+} recipient mice 4 mo p.i. Eighteen hours later, spleens, MLN, and lungs were harvested and analyzed by flow cytometry. The percentage of each donor population that is CD69\textsuperscript{+} is shown. All data are representative of at least three experiments.

**FIGURE 4.** Proliferation of naive \(\gamma\)HV68 T 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 \(A\). The percentage of each donor population that exhibited CFSE dilution (CFSE\textsuperscript{narrow}) is shown (\(n = 3\); representative of two experiments).
TCR tg CD4 T cells were stimulated by DCs or B cells from naive 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 naive 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, in which CD4 T cells can prevent lymphoproliferation by recognizing EBV Ags presented by B cells (24).

Given that CD4 T cells serve an important role in immunity to persistent herpesvirus infections, it is essential to dissect the mechanisms of CD4 T cell generation and maintenance to better design antiviral therapies and vaccines. In the studies presented in this work, 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: 1) What are the kinetics of the CD4 T cell response to γHV68?; 2) Can naive CD4 T cells contribute to the ongoing immune response during latency?; and 3) What cell types can present Ags to antiviral CD4 T cells? First, we show that antiviral CD4 T cells specific for a late-lytic viral epitope were primed by 7 d p.i. and maintained for at least 42 d in multiple tissues. Second, we show that naive 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 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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Disclosures
The authors have no financial conflicts of interest.

References

FIGURE 5. Both DCs and B cells from infected mice can stimulate naive CD4 T cells. DCs (top) and B cells (bottom) were sorted out of latently infected B6.SJL mice 4 mo p.i. and cocultured with naive 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 two experiments).


