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Rapid clearance of herpes simplex virus type 2 by CD8⁺ T cells requires high level expression of effector T cell functions

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Abstract

CD8⁺ T cells are important for resolution of HSV-2 lesions from the female genital epithelium. It is uncertain whether optimal clearance of viruses such as HSV-2 that cause a limited, non-systemic infection solely requires expression of effector functions by infiltrating CD8⁺ T lymphocytes, or if the clearance rate is reflective of the expression level of critical effector functions. To address this, CD8⁺ T cells from normal OT-I mice or OT-I mice deficient in IFN γ (IFN γ ^{-/-}) or the IFN γ receptor (IFN γ R^{-/-}) were activated *in vitro* in the presence of IFN γ or IL-4 to generate a series of effector populations (Tc1 and Tc2-like respectively) that secreted different levels of IFN γ and expressed different levels of HSV-specific cytolytic function. Compared with Tc1 cells, Tc2-like cells produced the type 2 cytokines IL-4 and IL-5, exhibited decreased IFN γ secretion, diminished proliferation *in vitro*, and decreased antigen-specific cytotoxicity *in vivo*. Clearance of an ovalbumin-expressing HSV-2 strain (HSV-2 tk⁻ OVA) by adoptively transferred Tc2-like cells was delayed relative to Tc1 cell recipients. Because donor Tc2-like cells proliferated *in vivo* and infiltrated the infected genital epithelium similar to Tc1 cells, the diminished virus clearance by Tc2-like effector cells correlated with reduced expression of critical effector functions. Together, these results suggest that high level expression of protective T cell functions by effector T cells is necessary for optimal clearance of HSV-2 from the genital epithelium. These results have important implications for vaccines designed to elicit CD8⁺ T cells against viruses such as HSV-2 that infect the genital tract.

Keywords

Interferon- γ ; tumor necrosis factor- α ; CD8⁺ T lymphocyte; female genital tract; cytotoxicity

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1. Introduction

Herpes simplex virus type 2 (HSV-2) is a major cause of sexually transmitted disease, and is the leading cause of ulcerative lesions in the genital tract (Malkin, 2004; Weiss, 2004). As many as 20% of sexually active individuals in the USA are infected with HSV-2 (Xu et al., 2006), but in some developing countries, the seroprevalence reaches nearly 80% (Weiss, 2004).

It is thought that virus-specific CD8⁺ T cells are critical in resolving human HSV-2 lesions and may play a predominant role in limiting the reactivation of latent virus (Cunningham et al., 2006; Koelle et al., 1998). Ulcerative lesion resolution coincides with the arrival of HSV-specific CD8⁺ T cells (Koelle et al., 1998) that express cytolytic activity and secrete IFN γ (Koelle et al., 2001). Results from animal studies have demonstrated that IFN γ secretion by T cells (Milligan and Bernstein, 1995, 1997), in addition to expression of cytolytic effector function (Dobbs et al., 2005), is required for HSV-2 clearance. However, it is not clear whether the rapid clearance of the limited, local HSV-2 infection only requires the expression of these two effector functions or if the rate of clearance is determined by the magnitude of the effector functions expressed by responding T cells. In the current study, we addressed this issue by altering the activation conditions of naïve CD8⁺ T cells to obtain effector T cell populations expressing modified effector functions. Specifically, we produced distinct effector CD8⁺ T cell populations expressing diverse levels of IFN γ secretion and cytolytic function, and compared these cells with conventional Tc1 effector T cells, for their ability to achieve viral clearance. These studies demonstrate that rapid virus clearance is dependent on expression of high levels of relevant effector functions by infiltrating CD8⁺ T cells. These results have significance for the development of vaccines designed to elicit CD8⁺ T cells for protection of the genital epithelium against primary or recurrent HSV infection.

2. Materials and Methods

2.1. Mice

Seven-week-old C57BL/6 (B6), interferon gamma-deficient B6.129S7-Ifng^{tm1Ts/J} (IFN γ ^{-/-}), and interferon gamma receptor-deficient B6.129S7-Ifngr^{tm1Agt/J} (IFN γ R^{-/-}) mice were purchased from The Jackson Laboratories (Bar Harbor, ME, USA). T cell receptor (TCR) transgenic OT-I mice specific for the ovalbumin peptide OVA₂₅₇₋₂₆₄ (Falk et al., 1993) were maintained at the AAALAC-approved University of Texas Medical Branch animal facility and were bred onto an IFN γ ^{-/-} or IFN γ R^{-/-} background (OT-IFN γ ^{-/-} or OT-IFN γ R^{-/-} respectively). All animal work was approved by the UTMB Institutional Animal Use and Care Committee.

2.2. Virus

Working stocks of the ovalbumin (OVA)-expressing, thymidine kinase-deficient (tk⁻) strain HSV-2 tk⁻ OVA (Dobbs et al., 2005) were prepared on Vero monolayers as described previously (Milligan et al., 1998).

2.3. In vitro CD8⁺ T cell activation

OT-I CD8⁺ T cells were isolated using CD8 isolation kits (Miltenyi Biotec, Auburn, CA, USA) and cultured as described previously (Dobbs et al., 2005). Polarization into Tc1 and Tc2-like subsets was accomplished by adding 20 U/mL rIL-2 and 2 ng/mL rIL-12 for Tc1 cells and rIL-2, 200 U/mL rIL-4, and 20 μ g/mL anti-rIFN γ (BD Pharmingen, San Diego, CA, USA) for Tc2-like cells (Cerwenka et al., 1998).

2.4. Adoptive transfer and virus inoculation

B6 mice were irradiated with 650 cGy from a ^{137}Cs source to deplete endogenous T cells, then reconstituted intravenously (i.v.) with 3×10^6 CD8⁺ effector T cells. Mice were treated with 3 mg of medroxyprogesterone acetate (SICOR Pharmaceuticals, Irvine, CA, USA) six days prior to virus inoculation to increase susceptibility of mice to HSV-2 infection (Miller et al., 2000; Linehan et al., 2004). Recipients were challenged intravaginally (ivag) with 5×10^3 PFU of HSV-2 tk⁻ OVA. Vaginal swabs were obtained for quantification of virus by titration on Vero E6 cell monolayers.

2.5. Cytokine quantification

Detection of IL-4, IL-5, IL-10, TNF α , or IFN γ production by CD8⁺ effector T cell populations was accomplished as described previously (Johnson et al., 2010). Briefly, OD₄₉₀ values of culture supernatants were obtained using a VersaMax plate reader (Molecular Devices, Sunnyvale, CA, USA) and compared with the linear portion of the cytokine standard curve. Cytokine concentrations were calculated using SoftMax software (Molecular Devices).

2.6. Intracellular cytokine staining

T cells were re-stimulated with mitomycin C-treated B6 splenocytes pulsed with media as a control, the antigenic peptide OVA₂₅₇₋₂₆₄, or treated with PMA/ionomycin (Sigma) for 2 h followed by 6-h culture with brefeldin A (BD Pharmingen). Cells were blocked with anti-Fc γ RII/III mAb, and stained with anti-CD8 α (APC, BD Pharmingen) then permeabilized using a Cytofix/Cytoperm kit (BD Pharmingen) and stained with fluorochrome-conjugated mAb for IFN γ (PE-Cy7) and IL-2, IL-4, IL-5, IL-10, or TNF α (PE), or granzyme B (PerCP-Cy5.5, BD Pharmingen). Data were acquired on a BD FACSCanto (BD Biosciences, San Jose, CA, USA) and analyzed using FlowJo software (Tree Star, Ashland, OR, USA). No cytokines were detected in cultures of Tc1 or Tc2-like cells in the absence of antigen.

2.7. Proliferation analysis

Naïve OT-I T cells were incubated with 10 μM carboxy-fluorescein succinimyl ester (CFSE, Molecular Probes, Carlsbad, CA, USA) for 10 min at ambient temperature prior to activation into Tc1 or Tc2-like cells. Cells were harvested at 24-h intervals and the CFSE content of CD8⁺ cells was analyzed by flow cytometry. For analysis of adoptively transferred Tc1 and Tc2-like cells, activated donor cells were labeled with CFSE and injected i.v. into irradiated recipient mice. Cells were harvested on days 3, 5, and 7 post HSV-2 tk⁻ OVA inoculation, stained for CD8 and V β 5.1/5.2 expression, and analyzed by flow cytometry.

2.8. In vivo cytotoxic T lymphocyte assay

Activated OT-I Tc1, OT-IFN γ R^{-/-} Tc2, or OT-IFN γ ^{-/-} Tc2 CD8⁺ effector T cells or naïve B6 CD8⁺ T cells (3×10^6) were adoptively transferred into irradiated B6 mice. Mice were inoculated ivag with 5×10^3 PFU HSV-2 tk⁻ OVA virus. On days 4, 6, and 8 after virus inoculation, a mixture of control and OVA peptide-pulsed target cells were prepared as described previously (Johnson et al., 2010) and adoptively transferred i.v. to HSV-infected and naïve mice. After 4 h, splenocytes were analyzed for CFSE content by flow cytometry, and the percentage of specific lysis was calculated as described previously (Johnson et al., 2010).

2.9. Statistics

Data were analyzed by Student's *t*-test or one-way analysis of variance (ANOVA) with the Bonferroni correction for multiple groups, as appropriate, using GraphPad Prism (GraphPad Software, San Diego, CA, USA). *P* values less than 0.05 were considered significant.

3. Results

3.1. Tc2-like CD8⁺ T cells differ in proliferative capacity and cytokine production compared with Tc1 cells

Previous studies demonstrated altered effector function resulting from activation of CD8⁺ T cells in the presence of IL-4 (Cerwenka et al., 1998, 1999; Helmich and Dutton, 2001; Mosmann et al., 1997; Wiley et al., 2001; Woodland and Dutton, 2003). This approach was utilized to prepare populations of CD8⁺ effector T cells with identical antigen specificity, but disparate levels of effector functions. CD8⁺ OT-I T lymphocytes were activated in the presence of recombinant cytokines IL-12 or IL-4 plus anti-IFN γ antibody and the proliferation and cytokine secretion patterns of the resulting activated effector T cells were characterized. Activation of OT-I T cells under IL-4-dominant conditions resulted in a population of cells with both Tc1 and Tc2 properties similar to populations described by others (Cerwenka et al., 1998/1999), which we refer to hereafter as Tc2-like. As shown in Fig. 1A, both Tc1 and Tc2-like cells proliferated in response to OVA₂₅₇₋₂₆₄ peptide antigen; however, by day 4 after stimulation, more than 90% of the OT-I Tc1 cells had proliferated greater than six generations, while only 9% of the OT-I Tc2-like cells exhibited a similar level of expansion. Identical results were obtained with Tc2-like cells generated from naïve CD8⁺ T cells from OT-IFN γ R^{-/-} and OT-IFN γ ^{-/-} mice (data not shown). The same proliferation pattern was obtained by daily quantification of viable Tc1 and Tc2-like cells in activation cultures by trypan blue exclusion (Fig. 1B).

OT-I Tc1 cells predominantly produced IFN γ and/or TNF α following stimulation with antigenic peptide (Fig. 2). By contrast, approximately 14.3% of OT-I Tc2-like cells produced TNF α and IFN γ and a slightly smaller subset of cells produced both IFN γ and the type II cytokines IL-4 or IL-10. Similar cytokine production patterns were observed in Tc2-like OT-IFN γ R^{-/-} T cells. Quantitatively, differentiated OT-I Tc2-like cells produced significantly less IFN γ compared with identical numbers of Tc1 cells (Tc2-like: 199.2 $\pm$ 46.3 ng/ml compared with Tc1: 557.2 $\pm$ 91 ng/ml, *P* < 0.05; Student's *t*-test) following antigen re-stimulation as detected by ELISA. As anticipated, OT-IFN γ ^{-/-} Tc2-like cells did not produce IFN γ , whereas approximately 14% produced TNF α . Approximately 3.5% of these cells produced IL-10. Together, these data indicated both qualitative and quantitative differences in cytokine production between Tc1 cells and the various Tc2-like cell populations, providing effector cell populations exhibiting IFN γ secretion profiles ranging from high (OT-I Tc1), and intermediate (Tc2-like cells derived from OT-I and OT-IFN γ R^{-/-}), to absent (OT-IFN γ ^{-/-} Tc2-like).

3.2. Delayed clearance of HSV-2 tk⁻ OVA by Tc2-like effector T cell recipients

The ability of Tc1 and Tc2-like cells to clear HSV-2 from the genital epithelium was tested using an adoptive transfer model. To test only the ability of the various CD8⁺ effector T cell populations to clear HSV-2 from the vaginal epithelium, *in vitro* activated Tc1 and Tc2-like effector cells were transferred to irradiated B6 mice prior to virus challenge. OT-I Tc1 cell recipients cleared the genital infection by day 10 (Fig. 3). In contrast, naïve CD8⁺ T cells from B6 mice were unable to eliminate HSV from the vaginal epithelium until day 14 and had significantly higher vaginal swab titers on days 6 through 12, compared with Tc1 cell recipients (*P* < 0.001 on days 6 and 8; *P* < 0.01 for days 10 and 12; ANOVA). Tc2-like cell recipients exhibited significantly higher viral titers on days 4 through 10 compared with Tc1

recipients ($P < 0.001$ for days 6 and 8; $P < 0.05$ for days 10 and 12; ANOVA). OT-IFN γ R $^{-/-}$ Tc2-like effector cell recipients exhibited a delayed viral clearance and viral titers were significantly higher than those from the Tc1 counterparts on day 6 ($P < 0.001$; ANOVA). OT-IFN γ R $^{-/-}$ Tc2-like cells, which secreted no IFN γ , were unable to eliminate genital HSV-2 infection in recipient mice through day 14.

Tc1 and Tc2-like cells retained their IFN γ production profiles *in vivo* following virus challenge. Tc1 and Tc2-like cell populations from the experiment described in Fig. 3 were isolated at the termination of the virus clearance study and re-stimulated with OVA peptide for analysis of cytokine production. As shown in Fig. 4, OT-I Tc1, OT-IFN γ R $^{-/-}$ Tc2-like and OT-IFN γ R $^{-/-}$ Tc2-like cells produced IFN γ and TNF α in good agreement with *in vitro* activation results. As shown previously for *in vitro* activated effector cells, OT-IFN γ R $^{-/-}$ Tc2-like cells produced only TNF α and not IFN γ following *in vivo* challenge.

3.3. Reduced *in vivo* cytolytic activity in Tc2-like effector cells

OT-I Tc1 recipients exhibited a high level (83%) of specific lysis of injected OVA₂₅₇₋₂₆₄ peptide-pulsed splenocytes on day 8 after HSV-2 tk $^{-}$ OVA inoculation (Table 1). In contrast, specific lytic activity in OT-IFN γ R $^{-/-}$ Tc2-like cell recipients reached a peak of only 50% on day 8 and was significantly lower than in Tc1 effector cell recipients on all days tested ($P < 0.001$; ANOVA). OT-I Tc2-like cell recipients and OT-IFN γ R $^{-/-}$ Tc2-like cell recipients also expressed significantly reduced specific lysis of OVA-pulsed splenocytes compared with Tc1 recipients on all days tested ($P < 0.001$; ANOVA). In summary, taken together with the results of Figs. 3 and 4, the most rapid clearance of HSV-2 from the genital tract was achieved by effector T cell populations expressing the highest levels of IFN γ production and antigen-specific cytolytic activity.

3.4. Tc1 and Tc2-like cells migrate similarly to the site of HSV infection

Previous findings by others (Cerwenka et al., 1999; Helmich and Dutton, 2001) suggested that Tc2-like cells were unable to home properly to the disease site. To test if a diminished ability of Tc2-like CD8 $^{+}$ T cells to reach the infected genital tract might explain differences in virus clearance between Tc1 and Tc2-like effector cell recipients, CFSE-labeled OT-I Tc1 or Tc2-like effector cells were transferred to irradiated B6 recipients and the level of proliferation and the total numbers of OVA-specific lymphocytes were compared after virus inoculation. Dilution of the CFSE label was pronounced in both Tc1 and Tc2-like cells isolated from secondary lymphoid tissue between days 3 and 7 (Fig. 5A), suggesting that the Tc2-like cells had proliferated in a manner similar to that of the Tc1 cells *in vivo*. There was no significant difference between the total number of Tc1 and Tc2-like cells present in the spleen and draining lymph node on any day tested ($P > 0.05$; Student's *t*-test, Fig. 5B,C). Importantly, these effector cell populations were readily detected in the genital tracts of recipient mice and there was no significant difference in the number of effector cells isolated from the genital tracts of Tc1 and Tc2-like cell recipients on day 7 post HSV-2 tk $^{-}$ OVA challenge ($P > 0.05$; Student's *t*-test, Fig. 5D). These data indicate that the OT-I Tc2-like cells expanded *in vivo* in response to virus-expressed antigen and migrated properly to the genital epithelium site of infection, in a manner similar to OT-I Tc1 cells.

4. Discussion

Differentiation of CD8 $^{+}$ T cells is influenced by the cytokines present early during primary activation (Woodland and Dutton, 2003). We took advantage of this by activating distinct populations of antigen-specific CD8 $^{+}$ T cells with effector responses ranging from OT-I Tc1 cells that had strong proliferation kinetics, secreted large quantities of IFN γ , and were highly cytolytic, to OT-IFN γ R $^{-/-}$ Tc2-like cells that exhibited reduced proliferation *in vitro*,

diminished cytolytic activity *in vivo*, and did not secrete IFN γ . The results of the current studies demonstrated that the ability of CD8⁺ T cells to secrete IFN γ and exhibit cytolytic activity alone is sufficient to achieve HSV-2 virus clearance from the genital tract. However, the overall time to clearance differed greatly depending on the level of these effector functions expressed by responding T cells.

We initially hypothesized that the reduced capability of these Tc2-like cells to clear HSV-2 from the vaginal vault was due to diminished proliferation *in vivo*, resulting in fewer effector cells reaching the site of infection. However, following adoptive transfer, Tc2-like cells proliferated in a manner similar to that of Tc1 cells in secondary lymphoid tissue and were recruited to the infected genital tract in numbers similar to those for Tc1 recipients. The reason for the disparity between the proliferation observed *in vitro* and *in vivo* is not clear. Perhaps critical cellular or molecular signals necessary for proliferation or survival were more readily available *in vivo* or, alternatively, that a sub-population of Tc2-like cells capable of higher proliferation rates was selected *in vivo*. In support of the second possibility, a sub-population of Tc2-like cells was shown to proliferate with kinetics similar to that of Tc1 cells in our *in vitro* studies. It is possible that this population expanded preferentially in recipient animals.

IFN γ has been suggested to play a role in immune cell homing by increasing the expression of adhesion molecules on endothelial cells in the genital tract (Parr and Parr, 2000), and may manipulate the expression of chemokine receptors on T cells (Tokuriki et al., 2002), thereby influencing infiltration of immune cells into the infected genital epithelium. Additionally, previous work suggested that Tc2 cells were unable to home properly after infection (Cerwenka et al., 1999, Helmich and Dutton, 2001). However, we found that Tc1 and Tc2-like cells expanded and accumulated to similar levels in the infected genital epithelium. Thus, the decreased viral clearance by Tc2-like cells did not result from an inability to expand and traffic to infected genitalia.

Recent studies of primary HSV-2 infection have demonstrated that IFN γ acts on parenchymal cells in the genital tract (Bird et al., 2007) to achieve rapid resolution of genital HSV-2 infection. This may reflect synergy between IFN γ and type I interferons to prevent HSV replication and spread between epithelial cells (Mikloska and Cunningham, 2001; Sainz and Halford, 2002). In humans, IFN γ may increase antigen processing and presentation by reversing the ICP47-induced down-regulation of class I MHC (Radosevich et al., 2003). Although not all the critical IFN γ -dependent mechanisms responsible for protection against HSV-2 infection have yet been identified, the results of the current study suggest that locally high concentrations of IFN γ and high levels of HSV-specific cytolytic activity are required for rapid resolution of infection. In the current study, we detected increased intracellular quantities of TNF α in OT-IFN γ R^{-/-} Tc2-like cells. While TNF α has been shown to be important for clearing HSV in some studies (Svensson et al., 2007), the production of TNF α alone by IFN γ ^{-/-} Tc2-like cells was insufficient to fully compensate for the lack of IFN γ and to mediate viral clearance. Co-expression of TNF α and IFN γ has been shown to enhance cytolytic activity (Betts et al., 2006) and it is possible that IFN γ is necessary for antiviral activity of TNF α against HSV-2.

Together, these results suggest that high magnitude expression of protective effector functions by effector T cells is required for rapid clearance of viruses; even those that cause a limited, localized infection, such as HSV-2. Optimal protection against HSV-2 infection may require HSV-specific CD8⁺ T cells in the genital epithelium or sensory neuron/epithelial junction to rapidly limit or prevent virus replication (Milligan et al., 1998; Simmons and Tscharke, 1992). Additionally, CD8⁺ T cells may need to be elicited in the sensory ganglia to protect neurons against infection or modulate HSV reactivation from

latently infected neurons (Khanna, et al., 2003, 2004). Effective management of these stages of HSV infection by high-quality CD8⁺ T cells may ultimately result in decreased virus shedding and, therefore, aid in diminishing the transmission of HSV-2. Therefore, eliciting a CD8⁺ T cell response capable of expressing high-quality effector functions may be required for HSV vaccines designed to provide protection at these tissue sites.

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Abbreviations

APC	antigen-presenting cell
CFSE	carboxy-fluorescein succinimyl ester
CTL	cytotoxic CD8 ⁺ T lymphocyte
HSV-2 tk⁻	thymidine kinase-deficient herpes simplex virus type 2
ivag	intravaginal
ICS	intracellular cytokine staining
MFI	mean fluorescent intensity
OT-IFNγ^{-/-}	IFN γ deficient, OVA ₂₅₇₋₂₆₄ -specific CD8 ⁺ T cells
OT-IFNγR^{-/-}	IFN γ receptor-deficient, OVA ₂₅₇₋₂₆₄ -specific CD8 ⁺ T cells
PFU	plaque-forming unit
TCR	T cell receptor

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Fig. 1.

Antigen-specific proliferation by Tc1 and Tc2-like cells. (A) CFSE-labeled OT-I CD8⁺ T cells were activated under polarizing conditions in culture. On day 4, cells were harvested, stained with fluorochrome-labeled anti-CD8α, and analyzed by flow cytometry. Representative data from one of three experiments are shown. (B) Activation cultures were sampled on the days indicated and viable cell counts were obtained by trypan blue exclusion. Data from two representative experiments are shown.

Fig. 2.

Tc2-like effector cell populations secrete both type 2 and type 1 cytokines. Tc1 or Tc2-like effector cells derived from OT-I, OT-IFN γ R $^{-/-}$, and OT-IFN γ $^{-/-}$ mice were obtained from day 4 activation cultures. Cells were stained for CD8 α and the intracellular cytokines indicated as described in the Materials and Methods. Plots shown are from three representative experiments.

Fig. 3.

Delayed clearance of HSV-2 tk⁻ OVA in recipients of Tc2-like effector cells. Equivalent numbers of OT-I Tc1 cells, OT-I, OT-IFN γ R^{-/-}, or OT-IFN γ ^{-/-} Tc2-like cells or naïve B6 CD8⁺ T cells were adoptively transferred to irradiated B6 mice. Mice were subsequently inoculated ivag with HSV-2 tk⁻ OVA and were swabbed on the days indicated for viral quantification as described in Materials and Methods. Asterisks indicate significant differences between the marked same-day sample and OT-I Tc1 (**, $P < 0.001$; *, $P < 0.05$; one-way ANOVA).

Fig. 4.

In vitro activated Tc1 and Tc2-like effector populations maintain their IFN γ and TNF α secretion profile *in vivo*. CD8⁺ T cells from the experiment described in Fig. 3 were isolated from spleens at the conclusion of the experiment. Spleen cells were stimulated *in vitro* with OVA peptide and stained for the cytokines indicated as described in Materials and Methods. The percentage of each cytokine-producing population is given in the corner of each quadrant. The results of two representative experiments performed are shown.

Fig. 5.

Similar numbers of OT-I Tc1 and Tc2-like cells are detected in secondary lymphoid tissues and the site of HSV infection of effector cell recipient mice. OT-I CD8⁺ T cells were activated in the presence of polarizing cytokines for four days. CFSE-labeled Tc1 or Tc2-like cells were adoptively transferred to irradiated B6 mice, which were subsequently inoculated with HSV-2 tk⁻ OVA. (A) The proliferation of cells isolated from the spleen and iliac lymph nodes on days 3 and 7 following inoculation was assessed by flow cytometric analysis of CFSE staining. (B) On days 3, 5, and 7 single cell suspensions from the spleen and draining lymph nodes were prepared and stained for CD8α and Vβ5.1/5.2 for flow cytometric analysis. The total number of antigen-specific CD8⁺ T cells within the tissues was calculated by multiplying the percentage of Vβ5⁺CD8α⁺ cells by the total number of cells from the tissue. (C) Genital tract lymphocytes were obtained from pooled tissues from five cell recipient mice on day 7 after infection and the number of OT-I cells quantified as described for secondary lymphoid tissues. The data shown are combined from two representative experiments.

Table 1

Reduced antigen-specific in vivo cytolytic activity in Tc2-like cell recipients

		Mean Percentage Specific Lysis (+/- SD)		
	Group	Day 4	Day 6	Day 8
Experiment 1	OT-Tc1	57.3 ± 4.2	68.5 ± 3.6	83.8 ± 2.6
	OT-IFN γ R ^{-/-} Tc2	32.4 ± 4.6 *	48.4 ± 4.5 *	51.3 ± 9.0 *
	Naïve	1.7 ± 1.7 *	0 ± 0 *	0 ± 0 *
Experiment 2	OT-Tc1	75.9 ± 0.8	77.7 ± 0.2	66.1 ± 0.1
	OT-Tc2	37.3 ± 0.2 *	58.4 ± 0.2 *	21.9 ± 0.1 *
	OT-IFN γ R ^{-/-} Tc2	38.8 ± 0.1 *	39.7 ± 0.1 *	37.1 ± 0.1 *

* Indicates significant difference ($P < 0.001$) compared with same-day value of the OT-Tc1 group.