Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Magee-Womens Research Institute and Foundation

2. **Reporting Period (start and end date of grant award period):** 01/01/09 – 12/31/09

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Cheryl Richards, MBA

4. **Grant Contact Person's Telephone Number:** (412) 641-8932

5. **Grant ME Number or SAP Number:** 4100047639

6. **Project Number and Title of Research Project:** Project 3 - Immune responses to herpes simplex virus type 2 and *Chlamydia muridarum* in a murine model of co-infection

7. **Start and End Date of Research Project:** 03/09/09 – 12/31/09

8. **Name of Principal Investigator for the Research Project:** Thomas L. Cherpes, MD

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ 63,416.77

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Cherpes	Principal Investigator	25%	$29,958.98

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____X___ No_____

If yes, please indicate the source and amount of other funds:

National Institutes of Health K23 AI064396 ($12,000)

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Tregs: sculpting a balance between protection and pathology during viral infection	☒NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	11/09	$1,920,809	$ (pending)
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans: We will await funding decision of NIH regarding R01 submission detailed in Section 11(A).

12. Future of Research Project. What are the future plans for this research project?

Our results demonstrate IL-17-mediated inflammatory responses contribute to the genital pathology and viral encephalopathy observed upon murine vaginal HSV-2 infection, and that cells displaying a regulatory T cell phenotype ($CD4^+CD25^+$) diminish these immunopathological processes without compromising viral clearance. We plan to further delineate mechanisms by which IL-17-mediated inflammatory responses contribute to tissue injury and the mechanisms by which regulatory T cells (Tregs) prevent collateral tissue damage and viral spread to the central nervous system (CNS). The ultimate objective of our studies is to inform the development of therapeutic strategies that prevent invasive human pathogens from eliciting immunopathologic tissue damage.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female	3			
Unknown				
Total	**3**	**0**	**0**	**0**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	3			
Unknown				
Total	**3**	**0**	**0**	**0**

	Undergraduate	Masters	Pre-doc	Post-doc
White	3			
Black				
Asian				
Other				
Unknown				
Total	**3**	**0**	**0**	**0**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No____X_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Goals and objectives of the project as stated in strategic plan:

The intent of this research is the production of a murine model that allows the virus to progress from the point of primary infection in the vagina and establish a non-lethal, latent (dormant) central nervous system infection. The benefits of this model include: better understanding of the immune responses that provide optimal control of primary HSV-2 vaginal infection, more comprehensive delineation of the immune mechanisms used by the host to prevent HSV-2 reactivation from latency, and improved knowledge of local interactions between HSV-2 and Chlamydia, two of the most common sexually transmitted diseases.

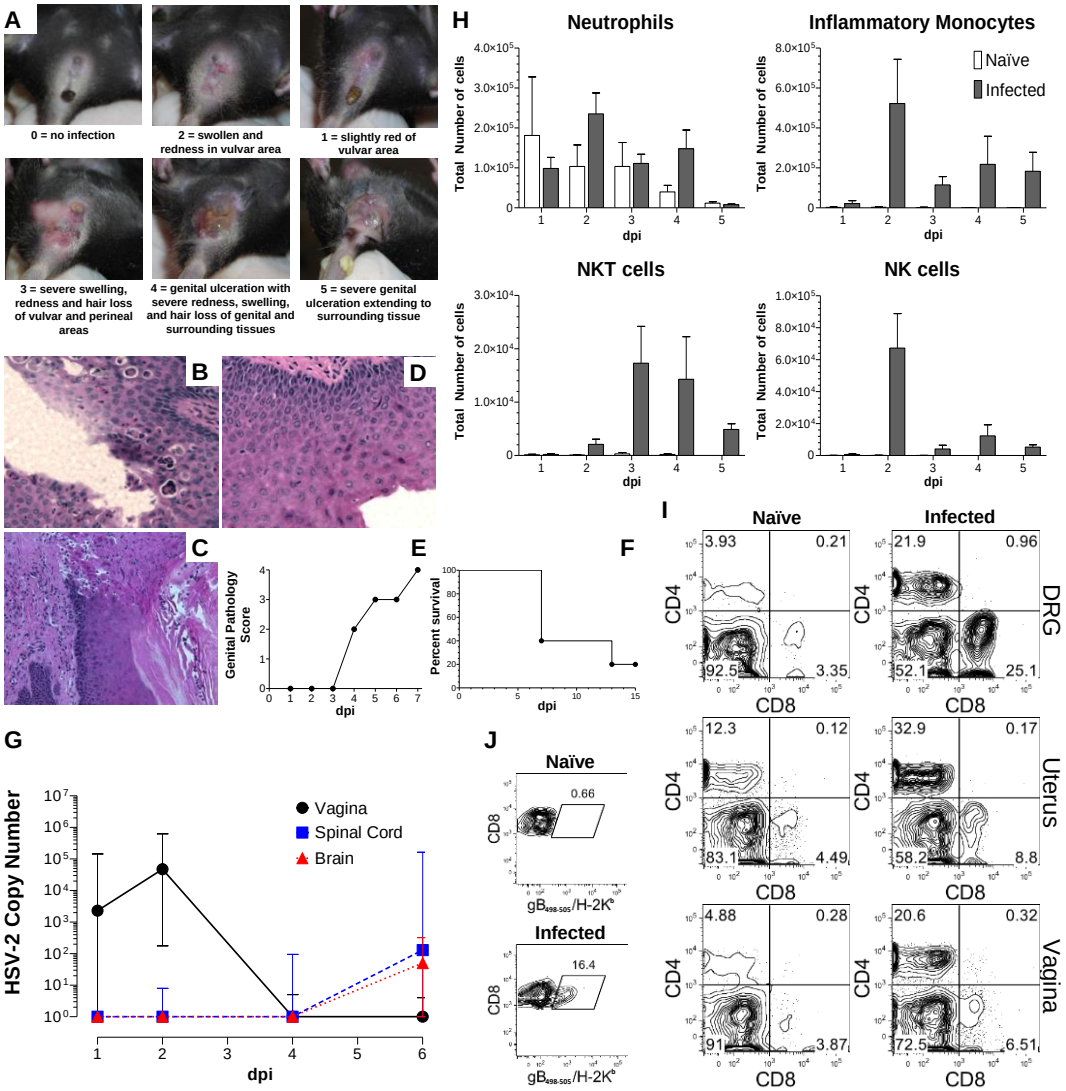
Specific aims of the project as stated in strategic plan:
Specific Aim #1: We hypothesize that the adoptive transfer of CD4 cells from acutely infected donor mice to newly infected recipients will limit viral replication, limit viral induced pathology, and decrease mortality compared to infected controls
Specific Aim #2: We hypothesize that regulatory T cells will be the specific CD4 positive donor cells responsible for rescue of mice vaginally infected with HSV-2
Specific Aim #3: Using our model of vaginal HSV-2 infection to investigate effects of antecedent HSV-2 infection on subsequent genital tract infection with *Chlamydia muridarum*, we hypothesize co-infection with result in increased upper genital tract pathology compared to those mice vaginally infected with HSV-2 or *C. muridarum* alone.

Achievement of research goals/objectives/aims:
This progress report will detail findings demonstrating that adoptive transfer of $C4^+$ T cells from acutely infected donors was able to rescue mice vaginally infected with HSV-2 (completion of Specific Aim #1). It will also detail findings suggesting that regulatory $CD4^+$ T cells from infected donors were the cell type responsible for this rescue (completion of Specific Aim #2). Finally, this progress report will provide data demonstrating that our hypothesized association between co-infection and increased genital tract pathology appears to be incorrect (completion of Specific Aim #3).

Detailed results of the project:

1. Vaginal HSV-2 infection induced an IL-17 mediated inflammatory response that was associated with collateral host damage: Inbred 6-10 week old C57Bl/6 female mice purchased from Jackson Laboratories (Bar Harbor, ME) were used in all of the following studies. To increase susceptibility to infection, all mice were injected subcutaneously with 1.0 mg progesterone 3 days prior to infection. Mice were anesthetized with a parenteral anesthetic and infected intravaginally with 10 µl of RPMI containing 10^5 PFU of wild-type HSV-2 333. Under the continued influence of anesthetic, mice were placed in ventral recumbency for at least 45 min, a maneuver designed to ensure infectivity of the inoculum. Genital pathology induced by vaginal HSV-2 infection was scored on a 5-point scale as photographically illustrated in Figure 1A. As shown in Figures 2B and C, vaginal HSV-2 infection caused an inflammatory infiltrate associated with significant tissue damage. Infected mice demonstrated progressive genital pathology and high mortality rates (Figures 1E, F, and G). To continuously monitor host inflammatory response, we obtained cells from daily cervicovaginal lavages (CVL) by pipetting 30 µl of phosphate-buffered saline (PBS) in and out of the vagina several times. Obtained cells were stained for flow cytometric analyses. We observed an early infiltrate of neutrophils ($CD11b^+Ly\text{-}6C^+Ly\text{-}6G^+F4/80^-$), inflammatory monocytes ($CD11b^+Ly\text{-}6C^+Ly\text{-}6G^-F4/80^{-/low}$), as well as NK ($CD3^-NK1.1^+MHC\text{-}II^-$) and NKT ($CD3^+NK1.1^+MHC\text{-}II^-$) cells (Figure 1H), but no lymphocytes were detected. To study the lymphocyte infiltrate, we sacrificed mice at day 6 post-infection (dpi 6), harvested vagina, uterus and regional dorsal root ganglia (DRG), and collagenase digested the tissues into single cell suspensions, and observed an intense infiltration of $CD4^+$ and $CD8^+$ T cells in the collected tissues (Figure 1I). We further characterized the $CD8^+$ T cell population by determined that a small percentage of cells were capable of recognizing the $gB_{498\text{-}505}/H\text{-}2K^b$ tetramer (indicating the HSV epitope gB is not an immunodominant protein during HSV-2 infection) (Figure 1J).

Figure 1. Vaginal HSV-2 infection causes tissue damage associated with recruitment of inflammatory cells. A, Genital tract pathology scoring system. B-D, Vaginal inflammatory infiltrate 7 dpi predominated by PMN and microabscess formation (B) and disruption of mucosal microarchitecture (C) as compared to control mice (D). (H&E sections viewed with oil immersion). E-F, Mice infected vaginally with HSV-2 develop progressive genital pathology (E) and display high mortality (F) (n=13). G, Lethality and progressive disease is associated with viral invasion of the central nervous system. Data shown is median ± interquartile range (n=31). H, Neutrophils, Inflammatory monocytes, NK and NKT cells are recruited early in the course of infection. Data shown is mean ±SD (n=7 in infected group, n=3 in naïve group). I, $CD4^+$ and $CD8^+$ T cells infiltrate vagina, uterus and DRG late in the course of infection. Dot plots representative from one mouse each representative of experimental groups (n=3). J, A small percentage of CD8+ T cells infiltrating the vagina recognize the $gB_{498-505}$/H-$2K^b$ tetramer (n=3).



2. Initial immune response to vaginal HSV-2 infection is characterized by an intense inflammatory response and early production of IL-17: We next investigated changes in the vaginal tissue induced by the inflammatory response triggered by HSV-2. Vaginal tissues from mice vaginally infected with HSV-2 were obtained daily until dpi 7. Tissues were mechanically homogenized in preparation for RNA extraction and quantitative real-time PCR analyses and for cytokine/chemokine detection using multiplex technology. As shown in Figures 2A and B, an intense inflammatory response was elicited by infection. Interestingly, we detected, in both CVL and vaginal tissue, a previously unreported production of IL-17 early in the course of the vaginal infection (Figures 2C and D). To identify cells producing IL-17, we performed 6 h *in vivo* brefeldin A (BFA) treatments of mice 4 dpi. Tissues were harvested and rendered into single cell suspensions. We identified $CD4^+$ and $CD8^+$ T cells as the main IL-17 producers (Figure 2E); data confirmed by ex vivo stimulation with splenic antigen presenting cells (APCs) loaded with inactivated HSV-2 lysate (Figure2F). We also observed lower levels IL-17 expression in $CD45^+CD3^-CD11b^+$ cells, (Figure 2E).

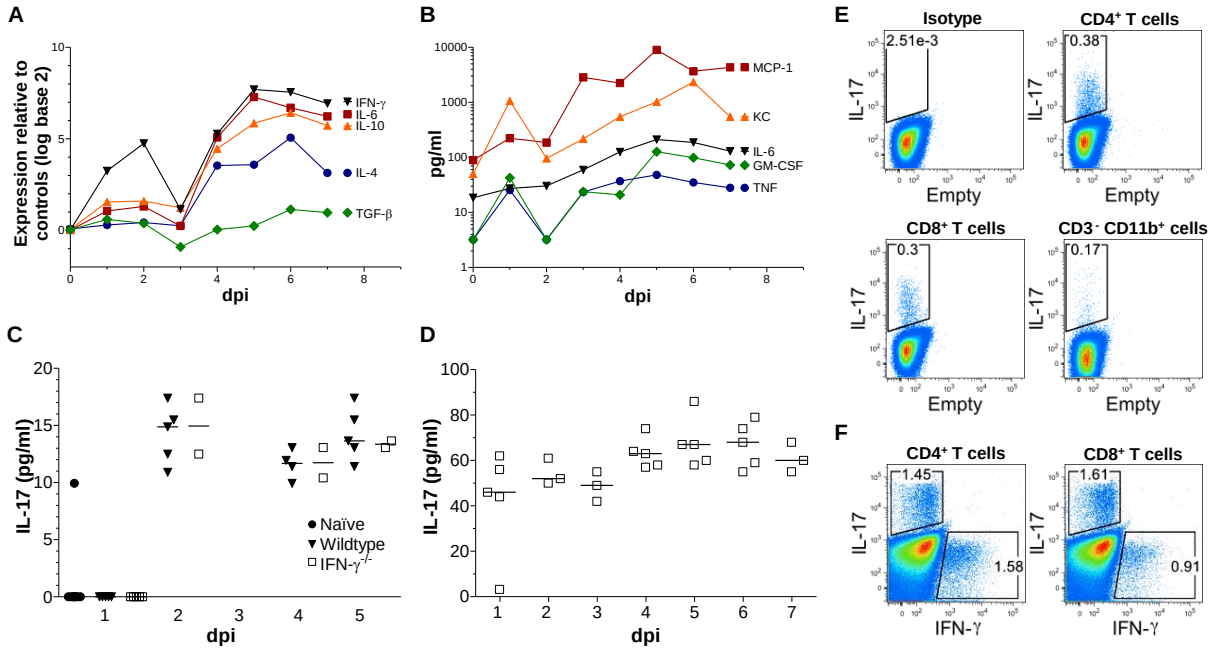


Figure 2. Vaginal HSV-2 infection induced a robust inflammatory response characterized by IL-17 production. HSV-2 induced high expression of classical inflammatory cytokines as shown by mRNA expression (**A**) and protein production (**B**). IL-17, not normally detected in the CVL of naïve mice, was readily demonstrated upon HSV-2 infection in CVL (**C**) and vaginal tissue (**D**). Using BFA *in vivo* treatments we identified cell types producing IL-17. As shown in **E**, CD4+ and CD8+ T cells expressed high IL-17 levels, as did, to a lesser degree, $CD45^+CD3^-CD11b^+$ cells. To confirm these results, we performed ex vivo stimulation of $CD4^+$ and $CD8^+$ T cells using splenic antigen presenting cells loaded with inactivated HSV-2 lysate. As shown in **F**, at dpi 6 $CD4^+$ and $CD8^+$ T cells present in the DLN produced high levels of IL-17 or IFN-γ, in response to antigen specific stimulation.

3. IL-17 mediated inflammatory response is associated with tissue damage and inability to control viral replication: As IL-17 has been implicated with the immunopathologic damage caused by some pathogens, we next investigated if neutralization of IL-17 (using a monoclonal antibody (M210) would prevent tissue damage and mortality. Mice were infected as described and CVL collected daily for the first 14 dpi (CVL were stored at -70° C for posterior detection of replicating virus by plaque assay to determine the infection status of each experimental animal; of note, we found at least one positive plaque assay from each mouse on $\geq$ 1 days sampled). As demonstrated in Figures 3A and B, inhibition of IL-17 prevented genital pathology and increased survival. Even though we have not yet tested viral replication in these αIL-17 treated animals, we speculate that blockade of IL-17 induced a response capable of preventing invasion of CNS by HSV-2 as viral replication in these tissues is known to precede encephalopathy and death. Furthermore, αIL-17 treatment was associated with a decrease in the neutrophilic infiltrate (Figure 3C), while increasing the number of inflammatory monocytes and NK cells recruited into the vaginal tissue (Figures 3 C and D) as shown by examination of CVL taken dpi 2. It was interesting that naïve vaginas contained a high numbers of neutrophils, identified as $CD11b^{+}$Ly-$6C^{+}$Ly-$6G^{+}F4/80^{-}$. Although IL-17 is a key element in our defense mechanisms against a variety of pathogens via neutrophil recruitment which limit pathogen replication, in our model it seems that IL-17 elicited immunopathologic immune responses that were also incapable of controlling viral dissemination into the CNS.

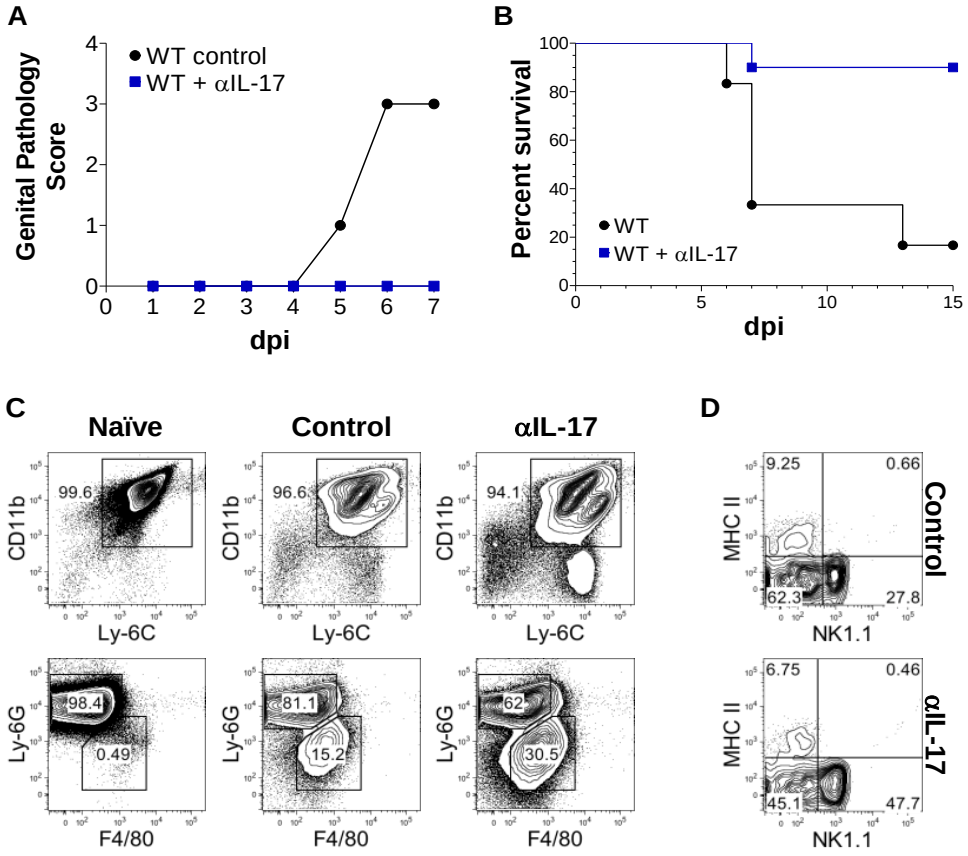


Figure 3. Vaginal HSV-2 infection induced IL-17 mediated inflammation associated with tissue damage and viral CNS spread. Wildtype mice receiving αIL-17 showed less genital pathology (**A**) and increased survival (**B**) after HSV-2 infection compared to controls receiving rat IgG**.** Representative experiment of three performed with similar results. Genital pathology scores shown are medians, $p<0.0001$ by two-way ANOVA. Survival curves, $p<0.05$ by Log-rank test. (n=10 in control group, n=10 in αIL-17 treatment group). CVL were also obtained from both groups dpi 2 for flow cytometric analyses. We observed an increase in inflammatory monocytes and a decrease in the neutrophilic infiltrate (**C**), accompanied by increases in the number of NK cells present (**D**). Representative contour plots are shown (n=3 in each group).

4. Transfer of $CD4^+$ splenocytes from HSV-2 infected donor mice decreased genital pathology and decreased mortality associated with vaginal HSV-2 infection: In preliminary investigation, we observed that mice receiving an intravenous transfer of splenocytes from HSV-2 infected donors were considerable less likely to develop genital tract pathology and more likely to survive vaginal HSV-2 infection (data not shown). As it has been shown that $CD4^+$ T cells play a role in disease prevention in mice vaccinated with sub-lethal doses of HSV-2 or with replication deficient virus strains, we next investigated if similar increases in survival of HSV-2 infected mice would be achieved with the transfer of $CD4^+$ T cells rather than bulk splenocytes. Spleens were harvested 7 dpi, and $CD4^+$ T cells were selected using the magnetic activated cell sorting (MACS) $CD4^+$ T cell Isolation Kit from Miltenyi Biotec. $5x10^6$ $CD4^+$ splenocytes were transferred intravenously into individual mice that had been treated with 1.0 mg of progesterone 3 days prior to infection (other mice from this same progesterone treated cohort received no transfer of splenocytes). Furthermore, to determine if the beneficial effects on the development of genital pathology produced by transfer of $CD4^+$ splenocytes from infected donors was the result of $CD4^+$ T cell effector functions mediated by IFN-γ production, a group of mice receiving donor splenocytes from infected HSV-2 IFN- $\gamma^{-/-}$ mice was included in the experiment. All donor mice were then vaginally infected with $1x10^5$ PFU of wild-type HSV-2 333. CVL were obtained as described for detection of replicating virus or analyzed using rt-PCR to determine HSV-2 copy number. As demonstrated in Figure 4A, mice that received transfer of $CD4^+$ T cells from infected donors concomitant with vaginal infection demonstrated decreased genital pathology and a decreased rate of mortality compared to mice that did not receive transfer of these cells. Moreover, mice receiving IFN-$\gamma^{-/-}$ donors demonstrated similar decreases in genital pathology and mortality compared to control mice. These results strongly suggested that the mechanism underlying the improved outcomes observed with transfer of $CD4^+$ T cells from ocularly infected donors is not dependent of the release of IFN-γ from transferred splenocytes. Of note, we also explored the possibility that transferred $CD8^+$ T donor cells might produce similar findings to those seen after transfer of $CD4^+$. However, we observed no apparent contributions from $CD8^+$ T cells for suppression of immunopathology or increased survival as was observed after $CD4^+$ T cell transfer (data not shown).

5. Transfer of $Foxp3^+$ Tregs from HSV-2 infected wildtype mice decreases genital pathology and decreases the mortality associated with vaginal HSV-2 infection while preventing viral spread into CNS: Because IFN-γ production from $CD4^+$ splenocytes was not necessary to confer protection against vaginal HSV-2 infection, we considered the possibility that the adoptive transfer of regulatory T cells (Tregs) could be contributing to the decrease in genital pathology and mortality observed after vaginal infection. Spleens from wildtype donors ocularly infected with HSV-2 were harvested 7 dpi, and splenocytes were selected using the MACS $CD4^+CD25^+$ Regulatory T Cell Isolation Kit. Either $2x10^5$ $CD4^+CD25^+$ or $2x10^5$ $CD4^+CD25^-$ splenocytes were transferred intravenously into progesterone treated mice (control mice from this same cohort received no adoptive transfer of splenocytes). All mice were vaginally infected with $1x10^5$ PFU of wild-type HSV-2 333, and observed daily for the presence of genital pathology or death. Mice that received transfer of $CD4^+CD25^+$ splenocytes demonstrated less genital pathology and were more likely to survive infection long-term than controls or mice that received $CD4^+CD25^-$ splenocytes (Figure 4B). Interestingly, transfer of $CD4^+CD25^-$ cells conferred some protection, compared to control mice, against development of genital pathology and death, so it was possible that these findings were due to the inclusion of small numbers of

Foxp3^{+} Tregs in the CD4^{+}CD25^{-} transfer population. To test this hypothesis, we repeated the experiment using Foxp3^{+GFP} mice as donors, to be able to isolate only Foxp3^{+} cells from splenocytes of infected donors using flow cytometric cell sorting. As shown in Figure 4C, 7.5x10^{4} CD4^{+}Foxp3^{+GFP} donor cells were capable of preventing tissue damage and mortality, while 7.5x10^{4} CD4^{+}Foxp3^{-} cells did not change the course of the infection seen in untransferred controls. Because transfer of CD4^{+}CD25^{+} T cells was associated with more favorable, we next determined if this same intervention altered the kinetics of viral clearance from the lower genital tract. Using the same experimental set-up described immediately above we determined, using rt-PCR, the HSV-2 burden in vaginal lavage and spinal cord specimens between controls and mice receiving 2x10^{5} CD4^{+}CD25^{+} splenocytes concomitant with vaginal HSV-2 infection. Surprisingly, as displayed in Figure 4D, despite significant decreases in genital pathology, no detectable effects on viral burden were observed between mice receiving transfer of CD4^{+}CD25^{+} splenocytes and controls. On the other hand, spinal cord invasion by HSV-2 was almost completely prevented by transfer. These results indicate that Foxp3^{+} Tregs from HSV-2 infected donors modulated the response to HSV-2 in a way that prevented tissue damage while enhancing responses capable of controlling invasive viral replication.

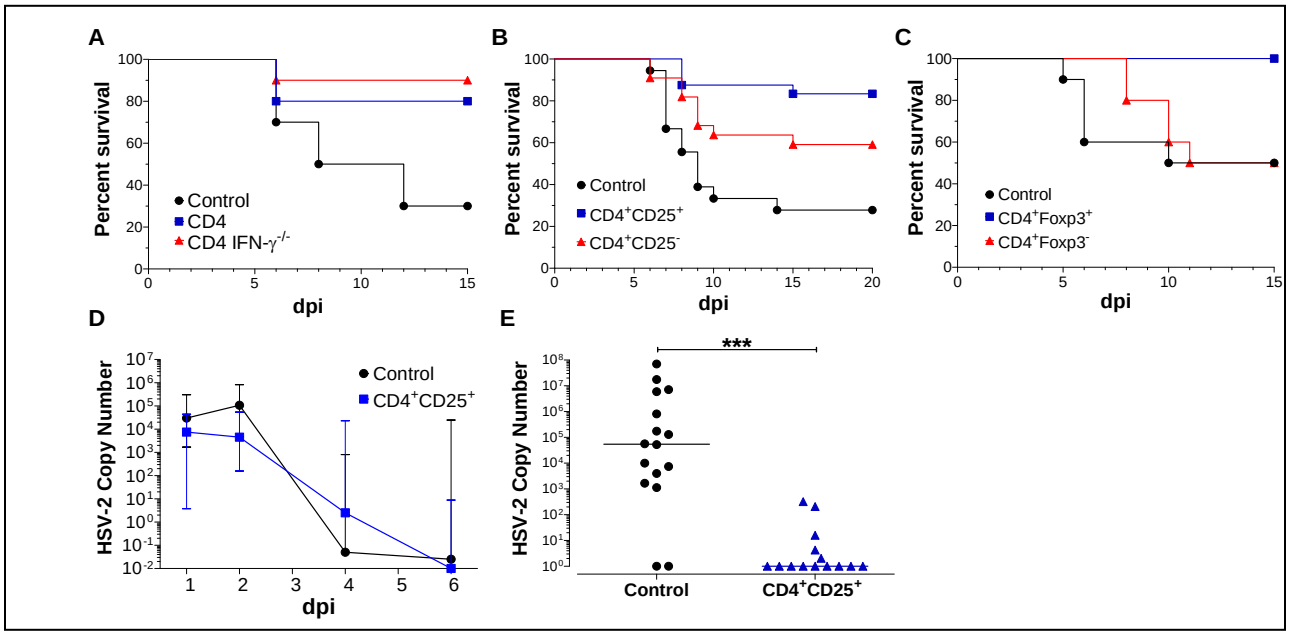


Figure 4. **A**, Transfer of 5x10^{6} CD4^{+} wildtype and IFN-γ$^{-/-}$ splenocytes from infected donors to naïve recipients was associated with reduced pathology and increased survival after vaginal HSV-2 infection. Survival curves, $p<0.05$ by Log-rank test (n=10 in control group, n=8 in CD4+ group, n=7 in IFN-γ$^{-/-}$ CD4^{+} group). **B**, Transfer of 2x10^{5} CD4^{+}CD25^{+} splenocytes from ocularly infected mice to naïve recipients was associated with decreased genital pathology and with increased survival after vaginal HSV-2 infection. Data pooled from 3 experiments; survival curves, $p<0.01$ by Log-rank test (n=21 in each group). **C**, Transfer of 7.5x10^{4} CD4^{+}Foxp3^{+GFP} splenocytes from infected mice to naïve recipients was associated with decreased genital pathology and with increased survival after vaginal HSV-2 infection. Survival curves, $p<0.05$ by Log-rank test (n=7 in control group, n= 6 in Foxp3^{+} group and n=7 in Foxp3^{-} group). **D**, Similar viral clearance kinetics in the lower genital tract of mice transferred with HSV- induced CD4^{+}CD25^{+} Tregs. HSV-2 copy numbers shown are median and their interquartile range, p=

0.1520 by two-way ANOVA. (n=14 in control group, n=6 in $CD4^+CD25^+$ group). **E**, Transfer of HSV- induced $CD4^+CD25^+$ Tregs prevents viral invasion of the spinal cord. HSV-2 copy numbers shown, line indicates mean, p= 0.01 by one-tailed t student test.

6. Summary of Specific Aims #1 and #2: Treg responses can both preserve host tissue by limiting immunopathologic damage elicited by anti-pathogen immune responses and facilitate pathogen persistence by subverting protective host responses. Understanding the mechanisms of Treg activation and migration, the nature of the antigens they recognize, and mechanisms by which they regulate host immune responses to viral infection are the logical next steps for the sculpting of novel therapeutic approaches that create acceptable balances between pathology and protection during infection. A number of clinically important infections, such as influenza and the hemorrhagic viruses, are caused by invasive pathogens that often elicit devastating immunopathologic host responses. Many of these infections lack suitable experimental models and require high level biocontainment laboratories for their investigation. HSV-2 has evolved nearly in parallel with the human immune system, and, as such, is well adapted for survival in its host. Possibly because it did not evolve as a murine pathogen, vaginal HSV-2 infections of mice are not characterized, as they are in humans, by the establishment of life-long latency. Instead, murine vaginal HSV-2 infection behaves as an aggressive invader, eliciting exuberant inflammatory responses that produce extensive collateral host damage. The preliminary data we have collected suggests that vaginal HSV-2 infection of mice will prove to be an ideal model for in vivo exploration of IL-17-mediated inflammatory responses and the mechanisms by which adaptive Tregs prevent development of IL-17-mediated immunopathology. Our model may be of particular utility for future investigation of the host immune responses that achieve a homeostatic balance between protection and pathology as HSV-2 can be used in lower level biocontainment facilities than those required for many other invasive viral pathogens. These data demonstrate transfer of a $CD4^+CD25^+$ splenocyte population, the majority of which were $Foxp3^+$, from infected to naïve mice at the time of HSV-2 vaginal infection suppresses the development of genital pathology and prevented dissemination of virus to the spinal cord and brain. Therefore, elucidation of Treg-regulated immune responses that prevent tissue damage and permit viral clearance may also inform the design of therapeutic approaches against other clinically relevant invasive pathogens.

7. Antecedent HSV-2 genital infection did not affect course of lower genital tract infection with *C. muridarum*: For 3 consecutive days, female C57Bl/6 mice who had previously been vaginally infected with $1x10^5$ PFU of wild-type HSV-2 (35-40 dpi) and naïve female C57Bl/6 mice were intravaginally infected with $1x10^5$ inclusion forming units (IFU) of *C. muridarum*. Additional age-matched control groups included both naïve mice and mice previously infected with HSV-2 (35-40 dpi) that were not challenged with vaginal chlamydiae infection. Analysis of endocervical swabs collected every fourth day after infection demonstrated that the chlamydial infection peaked 4 dpi and resolved by 29 dpi in both naïve mice and mice with antecedent HSV-2 infection challenged vaginally with *C. muridarum*, strongly suggesting that antecedent HSV-2 infection had no effect on the course of lower genital infection with *C. muridarum* (p = 0.875) (Fig. 5).

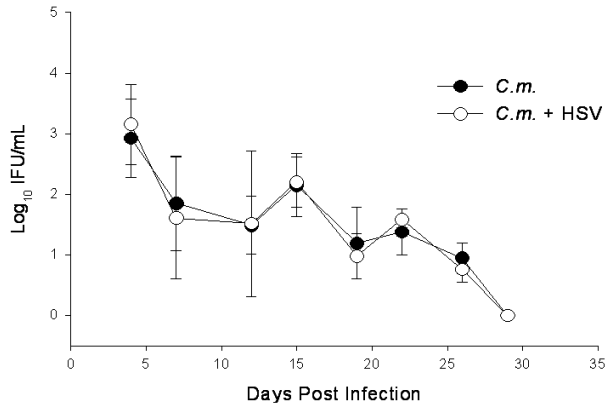
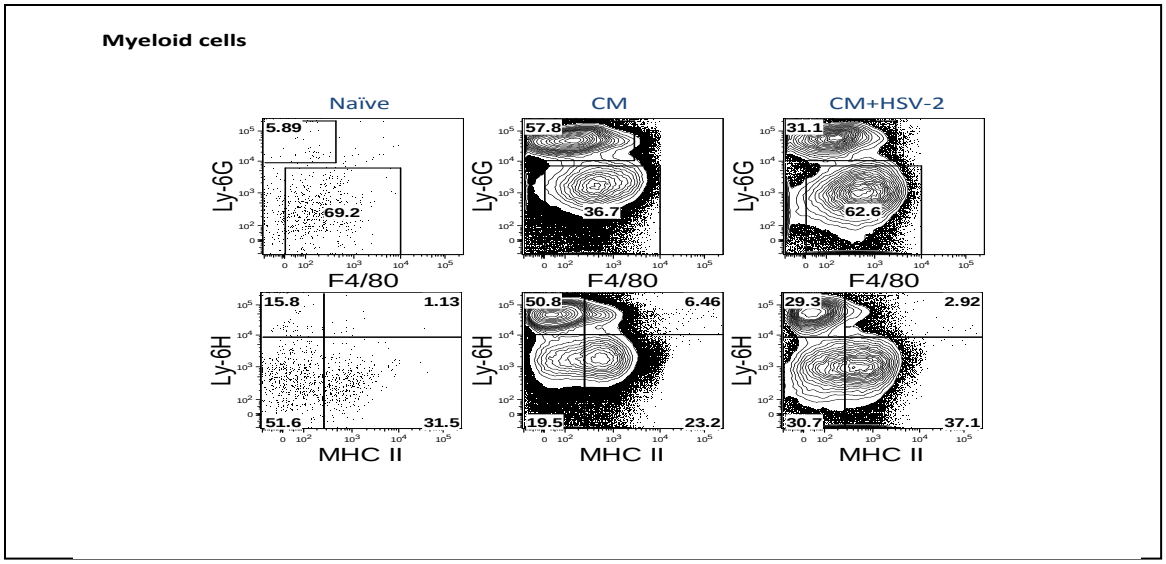


Figure 5. Antecedent HSV-2 infection does not affect the course of lower genital tract infection with *C. muridarum.* Data shown are mean ± SD of log transformed IFU/ml for mice positive on that day (N = 9 in *C. muridarum*; N = 4 in *C.muridarum* & HSV-2).

8. Antecedent HSV-2 genital infection did not affect upper genital tract inflammatory response elicited by infection with *C. muridarum*: Mice that were either naïve, latently infected with HSV-2, or co-infected with HSV-2 and *C. muridarum* were sacrificed 9 dpi, and upper genital tract tissues were processed by mechanical dissociation into single cell suspensions for flow cytometric analyses. Upper genital tract tissues from other similarly treated mice were histologically examined for evidence of oviduct pathology, but no differences were observed between groups (data not shown). Similarly, no significant differences regarding inflammatory infiltrate were seen between co-infected mice or those infected with HSV-2 (alone) (Figure 6). After completion of this set of experiments during the summer of 2009, it appeared that co-infection did not increase upper genital tract pathology as originally hypothesized, and a decision was made to focus on the objective outlined in Specific Aim #1 and Specific Aim #2 of this proposal.

A



B

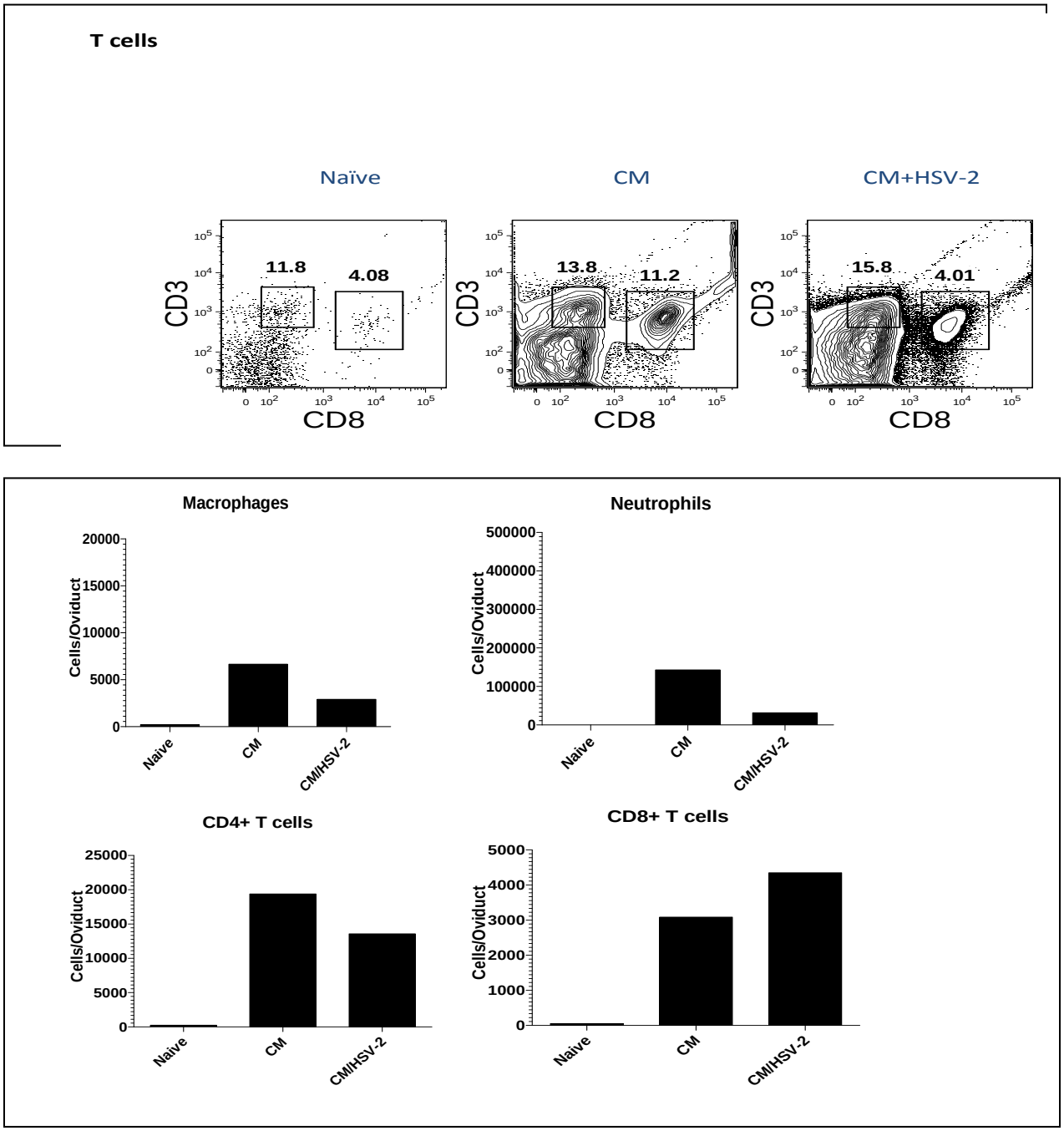


Figure 6. Representative flow cytometric analysis and histograms comparing upper genital tract inflammatory infiltrate among naïve mice, mice latently infected with HSV-2, and mice infected with both HSV-2 and *C. muridarum*. No statistically significant differences were observed between groups.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American

_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X___No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer

Project 3 – Zhang – Publication 2 – Lung Cancer
If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

We plan to submit a peer-reviewed publication regarding our findings this summer.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.
None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.
None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

The principal investigator received his medical degree from Wake Forest University, and completed American Board of Internal Medicine Research Pathway training at West Virginia University (internal medicine) and the University of Pittsburgh (infectious disease). He is a member of the American Society of Microbiology and the American Association of Immunologists. The principal investigator's research interests include herpes virology and the immunology of infectious diseases, and he has previously conducted bench, translational, and clinical research.

Selected Peer-reviewed publications

1. Cherpes TL, Meyn LA, Hillier SL. Cunnilingus and vaginal intercourse are risk factors for herpes simplex virus type 1 acquisition in women. Sex Transm Dis 2005; 32: 84-9.
2. Cherpes TL, Melan M, Kant J, Cosentino LA, Meyn LA, Hillier SL. Genital tract shedding of herpes simplex virus type 2 in women: effects of hormonal contraception, bacterial vaginosis, and vaginal Group B Streptococcus colonization. Clin Infect Dis 2005; 40:1422-8.
3. Cherpes TL, Wiesenfeld H, Melan MA, Kant JA, Cosentino SL, Meyn LA, Hillier SL. The associations between pelvic inflammatory Disease, Trichomonas vaginalis infection, and positive herpes simplex virus type 2 serology. Sex Transm Dis 2006; 33: 747-52.
4. Divito S, Cherpes TL, Hendricks RL. A triple entente: virus, neurons, and CD8+ T cells maintain HSV-1 latency. Immunol Res 2006; 36: 119-26.
5. Cherpes TL, Hillier SL, Meyn LA, Busch JL, Krohn MA. A delicate balance: risk factors for acquisition of bacterial vaginosis include sexual activity, absence of hydrogen peroxide producing lactobacilli, black race, and positive herpes simplex virus type 2 serology. Sex Transm Dis 2008; 35: 78-83.
6. Cherpes TL, Marrazzo JM, Cosentino LA, Meyn LA, Murray PJ, Hillier SL. Hormonal contraceptive use modulates the local inflammatory response to bacterial vaginosis. Sex Transm Inf 2008; 84: 57-61.
7. Cherpes TL, Busch JL, Sheridan BS, Harvey SA, Hendricks RL. Medroxyprogesterone acetate inhibits CD8+ T cell viral specific effector function and induces herpes simplex virus type 1 reactivation. J Immunol 2008; 181:969-75.
8. Leyland B, Kennedy MR, Wimberly YH, Levine BJ, Cherpes TL. Serologic detection of herpes simplex virus type 2 antibodies among pregnant women using point-of-care test from Focus Diagnostics. J Clin Virol 2009; 44:125-8.
9. Sheridan BS, Cherpes TL, Urban J, Kalinski P, Hendricks RL. Reevaluating the CD8 T-cell response to herpes simplex virus type 1: involvement of CD8 T cells reactive to subdominant epitopes. J Virol 2009; 83:2237-45.

10. Vicetti Miguel RD, Sheridan BS, Harvey SA, Schreiner RS, Hendricks RL, Cherpes TL. 17-β estradiol promotion of herpes simplex virus type 1 reactivation is estrogen-receptor dependent. J Virol (in press).
11. Frank GM, Lepisto AJ, Freeman ML, Sheridan BS, Cherpes TL, Hendricks RL. Early $CD4^+$ T cell help prevents partial $CD8^+$ T cell exhaustion and promotes maintenance of HSV-1 latency. J Immunol (in press).
12. Vicetti Miguel RD, Cherpes TL, McKenna KC. Impaired Tumoricidal Activity of Intratumoral $F4/80^+$ Macrophages Contributes to the Failure of $CD8^+$ to Control Ocular Tumor Development. J Immunol (in press).

Research support

<u>On-going Research Support</u>
HSV-2 Shedding: Role of hormonal contraception and BV (K23AI064396)
Principal Investigator: Thomas L. Cherpes
Agency: NIH/NIAID, Period: 5.1.2005 – 4.30.2010, (6 calendar months)

UPMC Collaborative Research Grant to Study Sexually Transmitted Infections (U19A1084024)
Principal Investigator: Toni Darville
Agency: NIH/NIAID, Period: 9.30.2009 – 08.31.2014, (6 calendar months)

<u>Completed Research Support</u>
AIDS and Molecular Microbiology and Epidemiology Training Program
Principal Investigator: John W. Mellors
Agency: NIH/NIAID, Type: T32, Period: 7.1.2002 – 4.30.2005

Evaluation of the HerpeSelect® Express Rapid Test among a cohort of pregnant women
Principal Investigator: Thomas L. Cherpes
Agency: Focus Technologies, Type: Industry sponsored, Period: 11.1.2006 – 3.1.2007
Description: Evaluation of a point-of-care HSV-2 serology assay in pregnant women

Multicenter, randomized, double-blind study to compare efficacy and safety of patient-initiated Famciclovir 1d 1000 mg BID to 3d Valacyclovir 500 mg BID in immunocompetent adults with recurrent genital herpes
Principal Investigator: Thomas L. Cherpes
Agency: Novartis Pharmaceuticals
Type: Industry sponsored, Period: 8.1.2006 - 9.30.2007
Description: Treatment trial of valacyclovir versus famciclovir for recurrent genital herpes

A randomized, multicenter, double-blind study to compare the efficacy of single-day treatment (1000 mg BID) with famciclovir compared to that of placebo in patient-initiated episodic treatment of recurrent genital herpes in immunocompetent black patients
Principal Investigator: Thomas L. Cherpes
Agency: Novartis Pharmaceuticals
Type: Industry sponsored, Period 10.1.2007 – 4.1.2008

Description: Treatment trial of famciclovir versus placebo for recurrent genital herpes in a cohort of African-American study participants

Immune responses to herpes simplex virus type 2 and chlamydia muridarum in a murine model of co-infection
Principal Investigator: Magee Womens Research Institute
Agency: Pennsylvania Department of Health
Period 3.1.2009 – 12.31.2009