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Comparison of Immunoprotection of Simultaneous to Individual Vaccinations in a Murine Model

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Laurie Kenney

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APPROVED:

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Liisa Selin, Ph.D.
Pathology
Umass Medical Center
Major Advisor

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David Adams, Ph.D.
Biology and Biotechnology
WPI Project Advisor

ABSTRACT

In response to a viral antigen, the immune system produces epitope-specific T-cells. The majority become cytotoxic T-cells that clear the virus, while a small population remains in memory form to protect against future infections. To determine if multiple, simultaneous infections compromise this protection, mice were immunized with either a single or combination of viruses, then immuno-protection levels were analyzed after a single virus rechallenge by viral titer, viral epitope frequency, and animal weight loss. The results indicate that when immunized against the rechallenge virus, either alone or with another virus, mice receive sufficient protection at day 5. Weight analysis, however, shows that the protection may be dependent upon the viruses used for vaccinations and rechallenge infections.

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Upon first entering the lab, I worked closely with Frances Saccoccio. As a 2003 WPI alumnus, her advice helped guide me through the beginning of my project. After her departure for graduate school, Michael Brehm became my main mentor in the lab. As a newly appointed instructor at the University of Massachusetts Medical School, he had extensive experience with all the laboratory techniques and always took extra time to answer my continuous questions. I would like to extend my sincerest thanks to him for his help.

I would also like to extend my appreciation to Professor David Adams as my MQP advisor. His help to initiate my project at Umass during the summer and continual advice allowed me to get the most out of this opportunity. His recommendations with my writing helped me shape my final report.

BACKGROUND

To understand how simultaneous and individual vaccinations affect the immune responses of mice, a solid understanding of the T-cell adaptive immune system is required. Specifically, development and activation of memory CD8 T-cells by viral peptides is the main determinant of the protection provided by the vaccinations. To further explain the results of this MQP, the infection pathways of two viruses, Lymphocytic Choriomeningitis Virus (LCMV) and Pichinde Virus (PV) will also be explained.

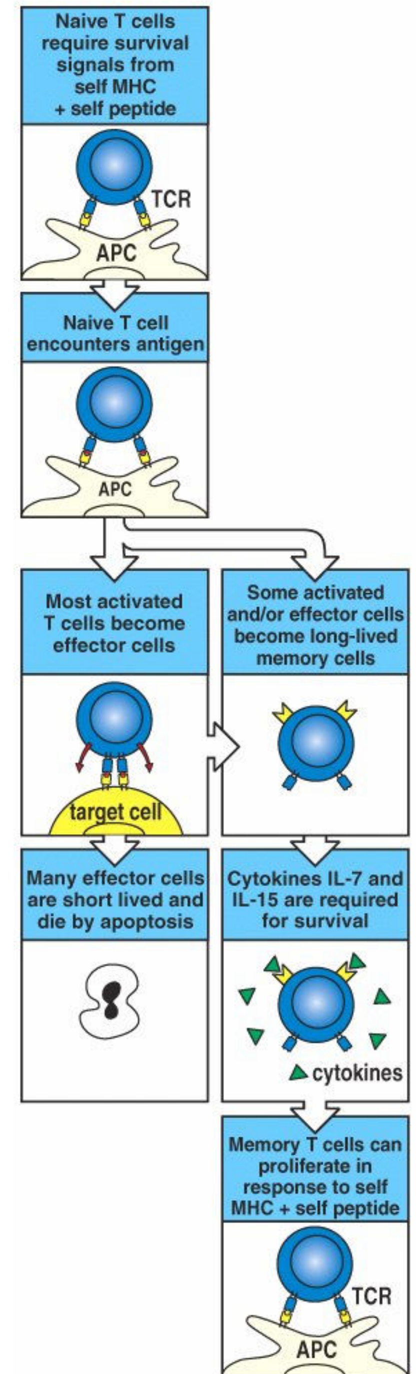


Figure 1. Development of CD8 T-Cells. Figure from Janeway, 2005-Fig. 10.34).

T-Cells

Upon encountering a foreign antigen, naïve T-cells differentiate and proliferate into cytoxic effector T-cells (Figure 1). These effector T-cells are cytokine-producing cells that act to clear the foreign antigen from the body. Once the foreign antigen is cleared, these cells are no longer needed, and decrease in number by apoptosis. Some of these cells, however, remain in the periphery of the body in memory form (Kaech and Ahmed, 2001). Memory T-cells require less interaction with antigen to become re-activated, as compared to naïve cells,

and are thus able to quickly provide protection against future infections, or to clear existing persistent infections (Selin and Welsh, 1997).

The memory T-cells exist in a repertoire made up of a set number of cells. This limitation forces a preferential hierarchy of epitope specific memory T-cells. Upon each new infection the frequencies of these cells changes. As an evolving environment, the memory T-cells that are cross-reactive with a second pathogen are conserved, while non-cross-reactive T-cells are lost (Selin and Welsh, 2004).

Degeneracy of T-Cell Recognition

Upon initial viral infection, the virus enters the host's cells and can follow several different pathways depending on the virus, environmental factors, and host cell type. The mostly common pathway is when a virus utilizes the organelles of a host cell for replication. During this process, some of the produced viral proteins are broken down and presented on the surface of the cell by MHC class I molecules (Figure 2). This presentation then identifies the cell as infected with non-self antigen; normal, self-proteins of the cell also undergo this same presentation process, but T-cells active against self-antigens are eliminated (Janeway, 2005).

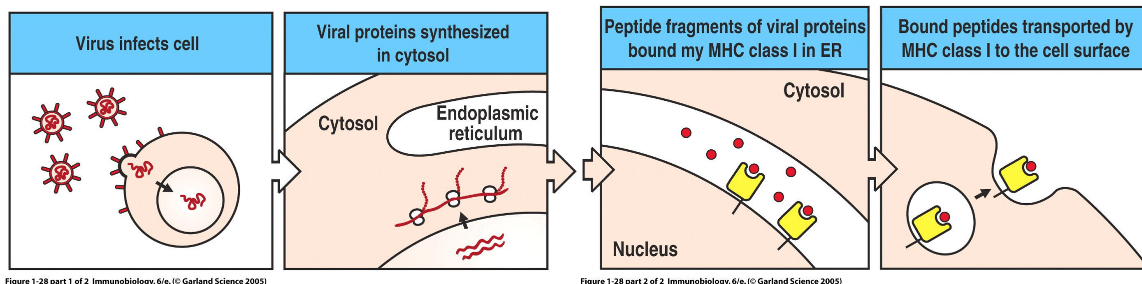


Figure 2. MHC Presentation of Viral Peptides (Janeway, 2005- Fig. 1.28).

These small presented proteins are recognized by antigen presenting cells (APC's), such as dendritic cells, whose main function is to activate the naïve T-cells that have the appropriate T-cell receptor. T-cell receptor (TCR) recognition of viral antigen is limited to 8-10 amino acid length peptides. These peptides or epitopes are presented in MHC class I molecules on the surface of infected cells or APC's (Falk et al, 1991) (Figure 3).

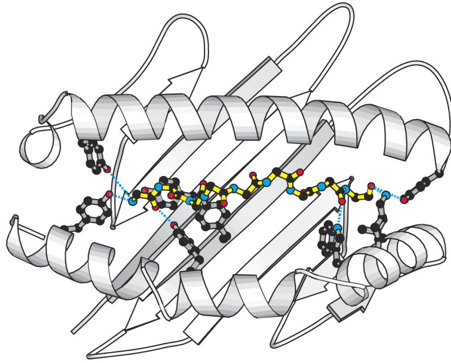


Figure 3. Structure of the binding site of a MHC class I molecule for foreign proteins (Janeway, 2005- Fig. 3.23).

The interactions of TCR's with epitopes, located in a MHC class I molecules, occur through the side chains of only a few amino acids, therefore changes in the remaining viral amino acid sequence would not have a great impact on the recognition (Bjorkman, 1997).

Cross-reactivity

Cross-reactive T-cells have the ability to recognize multiple epitopes. For example, in some cases of Chlamydia infection, antibodies induced against Chlamydial surface proteins also cross-react with heart muscle proteins to induce inflammation. In this process, TCRs recognize antigen that they were not originally primed against, but kill the cells in a similar manner. There are multiple explanations for this occurrence (Figure 4). Molecular mimicry is a mechanism in which one protein induces an immune response against a similar protein. Another mechanism, known as alternative recognition, suggests

that antigens may bind to different places on the TCR (Daniel et al, 1998). T-cells may also have two different TCRs, which have highly variable recognition abilities (Alam and Gascoigne, 1998).

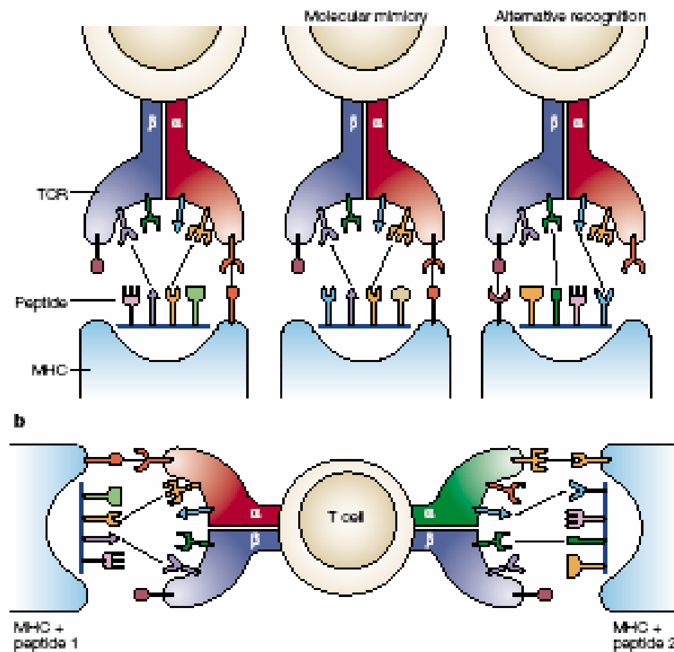


Figure 4. Possible Mechanisms of T-cell cross-reactivity. Interactions of TCRs with peptides in MHC class I molecules.
Top- left- Peptide interaction with an appropriate TCR.
Top-middle-Same TCR of left interacting with a second peptide through the same amino acids (molecular mimicry).
Top-right- Same TCR interacting with a third peptide through different amino acid side chains. (alternative recognition)
Bottom- Two different TCRs on a single T-cell allowing it to interact with multiple peptides.
(Welsh and Selin, 2002)

Cross-reactivity has been found to occur commonly between related pathogens, but can also occur between unrelated ones. Calculations predict that a single TCR has the ability to recognize 10^6 different 9-amino acid epitopes (Mason, 1998). When taken together, the highly evolving T-cell memory pool and the cross reactivity of TCRs makes the immune system capable for protecting against a wide range of pathogens. In theory, after multiple viral challenges the T-cell memory pool will be composed of highly effective cross-reactive T-cells, which would provide at least partial protection against the majority of pathogens.

Epitope Hierarchy

Another influence on the frequency of memory T-cells is the variety of viral specific epitopes. Each virus has multiple epitopes to which the immune system responds. These resulting epitope specific T-cells will persist in memory for the life of the subject. Experiments have shown that in naïve mice infected with a single virus, the epitope hierarchy is very predictable (Chen et al, 2000). The LCMV virus for example produces GP33, NP396 and GP276 immunodomiant epitopes, which have the strongest responses appearing in the greatest number during both acute and memory phases. There are also several other subdominant or weaker epitopes such as GP92, GP118, and NP205. The Pichinde virus has far fewer epitopes, two are immunodominat (NP38 and NP122), and one subdominant (NP205) (Table 1).

Virus	Epitopes	
	Dominant	Subdominant
LCMV	GP 33 NP 396 GP 276	GP 92 GP 118 NP 205*
PV	NP 38 NP 122	NP 205*

Table 1. Viral Epitopes for LCMV and PV.
* cross-reactive epitopes between LCMV and PV

The nomenclature for the epitopes has to do with the type of protein and its location in the genome. As seen in Table 1 the two types of epitopes are NP and GP. NP is a nucleocapsid protein, which often coats the viral genome, while GP is a glycoprotein often found at the viral surface. The number that follows is the nucleotide at which the protein starts.

The dominance of an epitope is affected by several factors. The ability of infected cells and APCs to process and present the epitope affects its dominance. The affinity of the viral peptide to both the MHC molecule and the TCR also affects the rate at which the

naïve T-cells are primed to this epitope (Yewdell and Bennink, 1999). The faster the epitope specific T-cells are activated the greater the frequency of them.

The LCMV epitope NP205 is cross-reactive with PV NP205. These two proteins share 6 out of 8 amino acids at this locus. The normally subdominant NP205 epitope can become dominant when these viruses are given in sequence (i.e. an LCMV-immune mouse rechallenged with PV, or a PV-immune mouse rechallenged with LCMV). This cross-reactivity can produce immunoprotection that can save a mouse from a lethal dose of the second virus (Brehm et al, 2002).

Immunopathology

Along with partial protection, immunopathology can also develop in response to the proliferation of cross-reactive T-cells (Doherty and Zinkernagel, 1974). At the peak of an infection, cytotoxic T-cells lyse infected cells and produce inflammatory cytokines. When the virus is cleared quickly by memory T-cells, there is limited cell lysis and minimal immunopathology. If the memory T-cells formed from the first viral infection are only slightly cross-reactive with the new second virus, there will only be slight protection, and thus slower viral clearance. Also due to the competition of cells within the immune system, this cross-reactive response, although weak, may inhibit a better response produced from naïve T-cells (Hemmer et al, 1998).

This heterologous immunity produced by cross-reactive T-cells provides a new explanation of the different responses between individuals exposed to the same virus. An individual's immune system is a pool of memory cells that reflects that individual's previous infections. Immunity is most effective when the memory T-cell repertoire is

composed of cross-reactive T-cells that have the ability to quickly clear a viral infection. Each individual has a unique pool of memory cells, which have an unpredictable protection level.

Cytokines and Surface Receptors

As cells differentiate the receptors on their surface and the cytokines they release change. The two main surface receptors that were analyzed in this MQP were CD8 and CD44. CD8 is a protein marker on the cell surface of CD8 cytotoxic T-cells, as opposed to CD4 helper T-cells. CD4 helper T-cells are responsible for recognizing MHC class II molecules presentation of epitopes as compared to CD8 cytotoxic T-cells that recognize the MHC class I molecules presentation of viral epitopes. CD44 is a cell adhesion molecule that is present on the surface of naïve T-cells, but only at low levels. After stimulation, this receptor increases in frequency and remains on the surface of memory cells to help with cell-to-cell interactions.

The two cytokines that were used in this MQP were interferon gamma (IFN-gamma) and tumor necrosis factor alpha (TNF-alpha). The majority of interferons are involved with the innate immune response to viral infections. IFN-gamma, however, is released by T-cells in response to stimulation. TNF-alpha plays a similar role as IFN-gamma. In mice that are depleted of these two cytokines death will ensue a few days after a viral infection (Janeway, 2005).

Murine Arenaviruses Used in This Project

The two murine viruses used in this MQP were LCMV (Lymphocytic Choriomeningitis Virus) and PV (Pichinde Virus) which are both members of the Arenaviridae family. This family has been found to form chronic infections in rodents across Europe, Africa and the Americas. LCMV is an Old World arenavirus while PV is a member of the New World arenavirus group. However, the relationship between the two viruses is very limited as shown by their minimal cross-reactivity (NP205 has been found to be highly conserved between all Old and New World arenaviruses) (Buchmeir, 2001). Neither of these viruses are cytolytic. The majority of the damage is produced by the immune systems of the mice.

LCMV (Figure 5 & 6) was the first arenavirus to be isolated in 1933. It is understandably, one of the most widely understood viruses in murine models. As this virus invades the cells of mice, there is a balance between protection and damage with the proliferation of T-cells (Buchmeir, 2001).

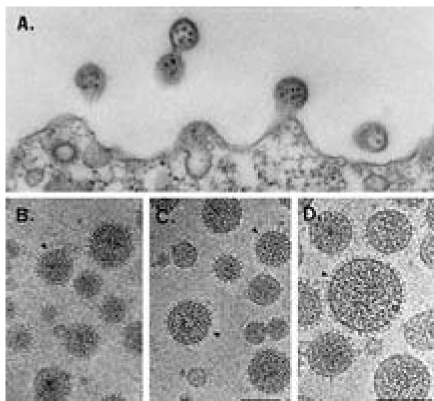


Figure 5. Electron Microscopy of LCMV. A. virons budding from infected BHK-21 cells. B-D. Purified LCMV virons with surface glycoproteins visible. Bar indicates 100nm.

Micrography from studies by M.B.A. Oldstone, Peter W. Lambert, and Michael Buchmeier.

http://www.scripps.edu/newsandviews/e_20021028/print-oldstone.html

Pichinde virus (Figure 6) is not as aggressive as LCMV, and is given at much higher doses (an increase of ~2.5 logs) to get a response that is easily detectable. It also produces much fewer epitopes compared to LCMV.

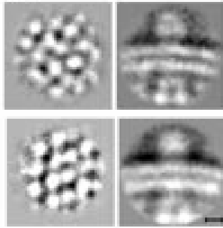


Fig. 6. Image Analysis of Pichinde and LCMV Arenaviruses. The arenavirus glycoprotein complexes are present in 2-dimensional, orthorhombic arrays on the surface of the virion. Images of New World Pichinde (top row) and Old World lymphocytic choriomeningitis virus (bottom row) are shown. Scale bar = 50 Å. http://www.scripps.edu/news/sr/sr2004/np04buchmeier.html

Two strains of LCMV were used in this MQP: Armstrong and Clone 13 (cl 13). These two strains differ in only two amino acids, but cl 13 is found to cause a much stronger infection (Ahemd et al, 1984). LCMV Armstrong was used to immunize all of the mice, which received LCMV. LCMV cl 13 was used as the secondary, rechallenge virus because it is a more aggressive strain known to cause persistent infections at the given dose. To add to the evidence of immunopathology of viral infections, mice given a high dose of LCMV cl 13 (10X higher than dose used in this MQP), which induces clonal exhaustion, will live longer and show less weight loss than mice given a persistent infection dose (dose used in this MQP) (Cornberg, unpublished).

Another element of LCMV that makes it more complicated than PV is the induction of a neutralizing antibody. This antibody is produced by the subject to help combat the infection. When this antibody is transferred into a mouse, it is found to protect against a lethal dose of LCMV (Webster and Kirk, 1974).

PROJECT PURPOSE

Memory T-cells that exist in individuals perviously infected with a virus (or who have received a vaccination for a virus) will provide immunoprotection to future infections with the same virus or other pathogens presenting similar proteins. When these memory cells are only partially cross-reactive with the secondary infection, immunopathology may also develop.

The Selin Lab has done several experiments looking at the evolution of the memory T-cell repertoire in mice after several infections with a variety of viruses. These experiments show changes in viral epitope hierarchies are unpredictable in a sense that cross-reactive epitopes are not known until the two given viruses are used to infect mice in a series of experiments, which examine the protection and T-cell responses. This protection has also been found to be dependent on the order of the infections. If virus A was given to a virus B-immune mouse the protection by cross-reactive T-cells may be different than if virus B was given to a virus A-immune mouse.

This MQP focuses on a slightly different aspect of viral immunoprotection. It assesses the protection to a single virus, by the memory T-cells acquired from either simultaneous or individual vaccinations. It examines the ability and limits of the immune system to produce T-cells when exposed to multiple viruses at the same time. The extent of the defense provided by these activated memory T-cells is analyzed in terms of viral titer, viral epitope frequency, and animal weight change. Another strong influence on the applications of simultaneous vaccinations is the possibility of immunopathology due to T-cell proliferation.

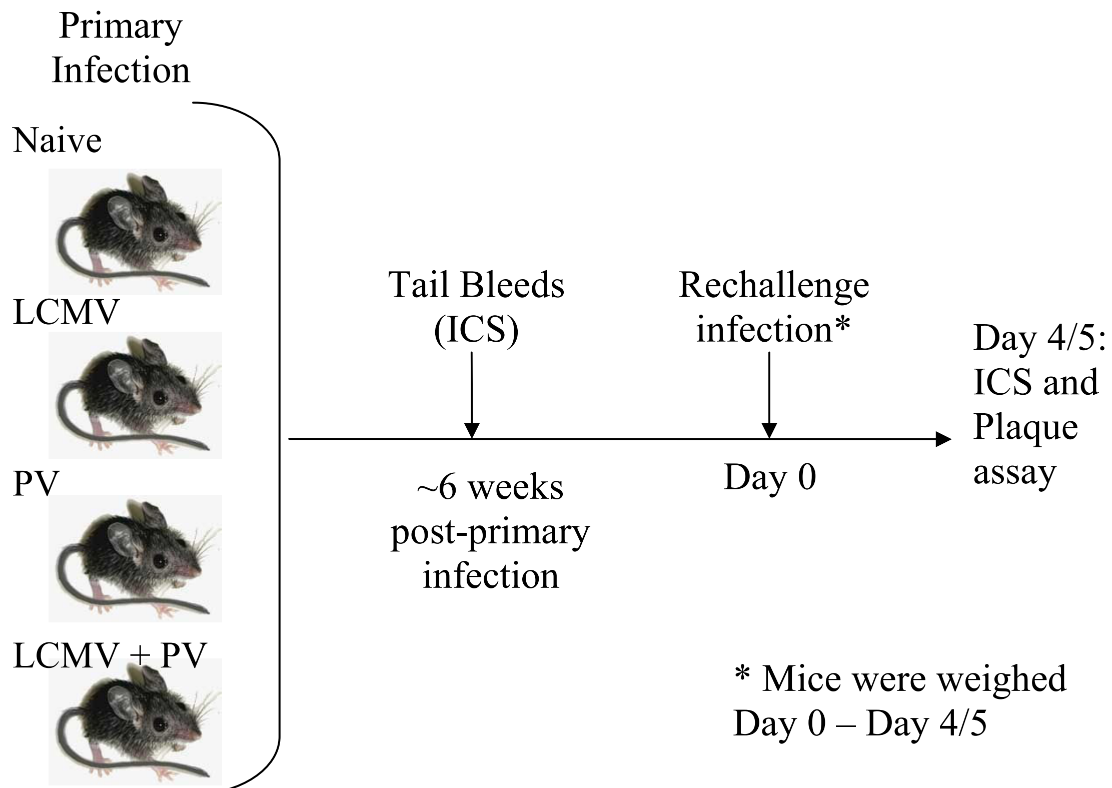
The conclusions from the MQP could be applied to the administration of vaccines to humans. If mice can be effectively immunized with two viruses simultaneously then perhaps humans can be also. This also raises questions to how many vaccines can be given simultaneously and the development of immunopathology.

MATERIALS AND METHODS

Experimental Design

Mice were given a primary infection of one (LCMV or PV), both (LCMV + PV) or none (Naïve) of the viruses. Mice were allowed to develop immune responses to the primary infections. At approximately 6 weeks post-primary infection their memory T-cell responses were analyzed by an intracellular cytokine assay using blood taken from the tails of the mice (Figure 7).

Figure 7. Experiment Outline



Mice were then rechallenged with a secondary infection of either LCMV or PV. At four or five days post-rechallenge (two out of the three PV rechallenge experiments were harvested at day 4 post rechallenge) the mice were harvested using ½ spleen for another intracellular cytokine assay while the remaining ½ spleen, liver, and fatpads were taken for a plaque assay to determine viral titer. For each virus (LCMV and PV) three experiments were performed. Within each experiment there were four groups (naïve, LCMV, PV, and LCMV + PV). Each group contained five mice (Figure 7).

IP/IV Injections

The mice used for these experiments were all male C57BL/6J at least 6 weeks of age. Mice were given LCMV Armstrong IP at $5X10^4$ pfu per mouse and PV at $2x10^7$ pfu per mouse for primary infections. Mice were allowed to clear the viruses (6 weeks) and then rechallenged. Secondary infections were LCMV cl 13 at $2x10^5$ pfu per mouse and PV at the same dosage as the primary infection. For IP (intraperitoneal) injections, mice were given 100 µl of virus. Cl 13 was diluted into Hanks basic salt solution and each mouse received 200 µl IV (intravenous) into the tail vein.

Plaque Assays

Plaque assays were used to determine viral titer in various tissues. Fatpads, livers, and ½ spleens were ground, triple aliquoted at 300 µl per tube and frozen at -80˚C. Vero cells were used at 60-70% confluency at 1.5 X10^5 cells per well in 6 well plates. For fatpads and spleens 1:10 serial dilutions were made from 0-5. Livers were extended to six dilutions due to the high enzymatic activity of this organ. 100 µl of sample for each dilution was added to the well and allowed to incubate at 37˚C for 1.5 hours (rocked at 45

min). Plates were overlayed with 4 ml of 1:1 ratio of EMEM and 1% agarose. Neutral red stain was used for both viruses. PV plates were stained on day 3 and read at days 4 and 5. LCMV was stained on day 4 and read at days 5 and 6.

Intracellular Cytokine Assays (ICS)

Intracellular cytokine assays were used to determine viral epitope hierarchy and cytokine production. In this MQP, intracellular cytokine assays were preformed with both blood samples and ½ spleens.

Six weeks after the primary infection approximately 400ul of blood was taken from the tails of each mouse, except the naïve groups. Blood cells were lysed and resuspended in RPMI media. Due to the low frequency of lymphocytes in blood only five stimulations were done for each mouse: negative control, GP33, LCMV-NP205, NP396 and NP38. Cells were stained for CD44 (FitC), CD8 (PerCP Cy 5.5), IFN-gamma (APC), and TNF-alpha (PE).

The remaining half of the spleen from the day 4/5 post-rechallenge harvest was ground, cells were counted with tryphan blue, and placed in a 96 well plate at $2x10^7$ cells per well. Naïve ½ spleens were pooled in each experiment, but all others were kept separate. For each mouse the splenocytes were stimulated with: no stim (negative control), anti-CD3 (positive control), GP33, GP92, GP118, LCMV-NP205, GP276, NP396, NP38, NP122, and PV-NP205. Splenocytes were allowed to incubate with peptides for 5 hours at 37˚C. This allows for the production of cytokines from the stimulated T-cells. Cells were then stained for surface receptors: CD44 (FitC) and CD8 (PerCP Cy5.5) and for cytokines: TNF-alpha (PE) and IFN-gamma (PE Cy7).

For both cell types samples were then read by the FACS machine and analyzed in FloJo. Cells were selected by a series of gating: live cells, CD8+ and then analyzed by IFN-gamma vs. TNF-alpha for total IFN-gamma production.

Weight Analysis

Mice were weighed from day 0 to day 4/5 post rechallenge infection at approximately the same time each day. Analysis was determined by percentage of weight change.

RESULTS

This MQP compares the immunoprotection provided by either simultaneous or individual vaccinations in response to a rechallenge infection of a single virus. The protection from the vaccines was determined by pre-rechallenge memory T-cell frequencies and viral titer, weight change analysis, and post-rechallenge T-cell frequencies. There is one set of results presented for each of these areas on each of the experiments (Figure 8).

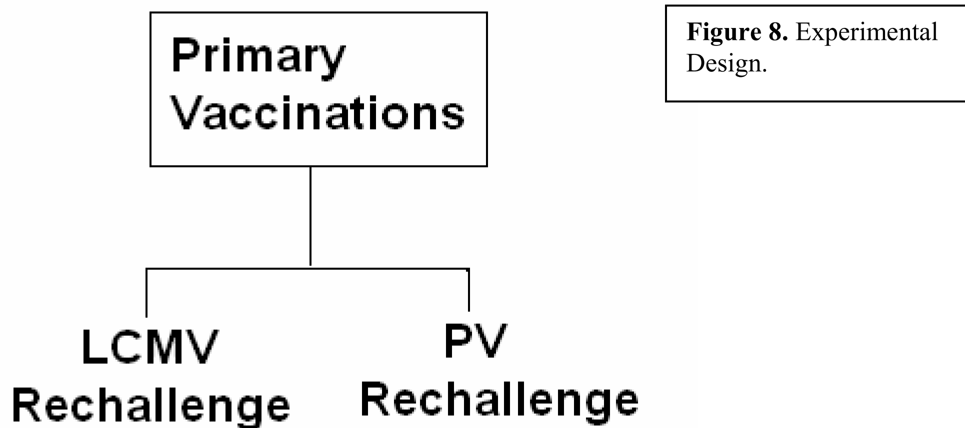


Figure 8. Experimental Design.

Pre-rechallenge Memory T-cell Frequencies

In order to determine the frequencies and hierarchies of the memory T-cells for the mice in each group (Naïve, LCMV-immune, PV-immune, LCMV + PV-immune) before the rechallenge infections, intracellular cytokine assays were performed on T-cells isolated from blood samples (Figure 9). From these results, it was determined that all groups of mice in both experiments had protection against the virus to which they were immunized.

Since both of these rechallenge groups received the same vaccinations they should have similar memory T-cell profiles. LCMV-immune mice had high levels of

GP33 and NP396 specific CD8 T-cells as expected due to their immunodominance with a low frequency of the subdominant NP205 specific CD8 T-cells. The PV-immune mice showed dominant levels of the NP38 specific response in both experiments.

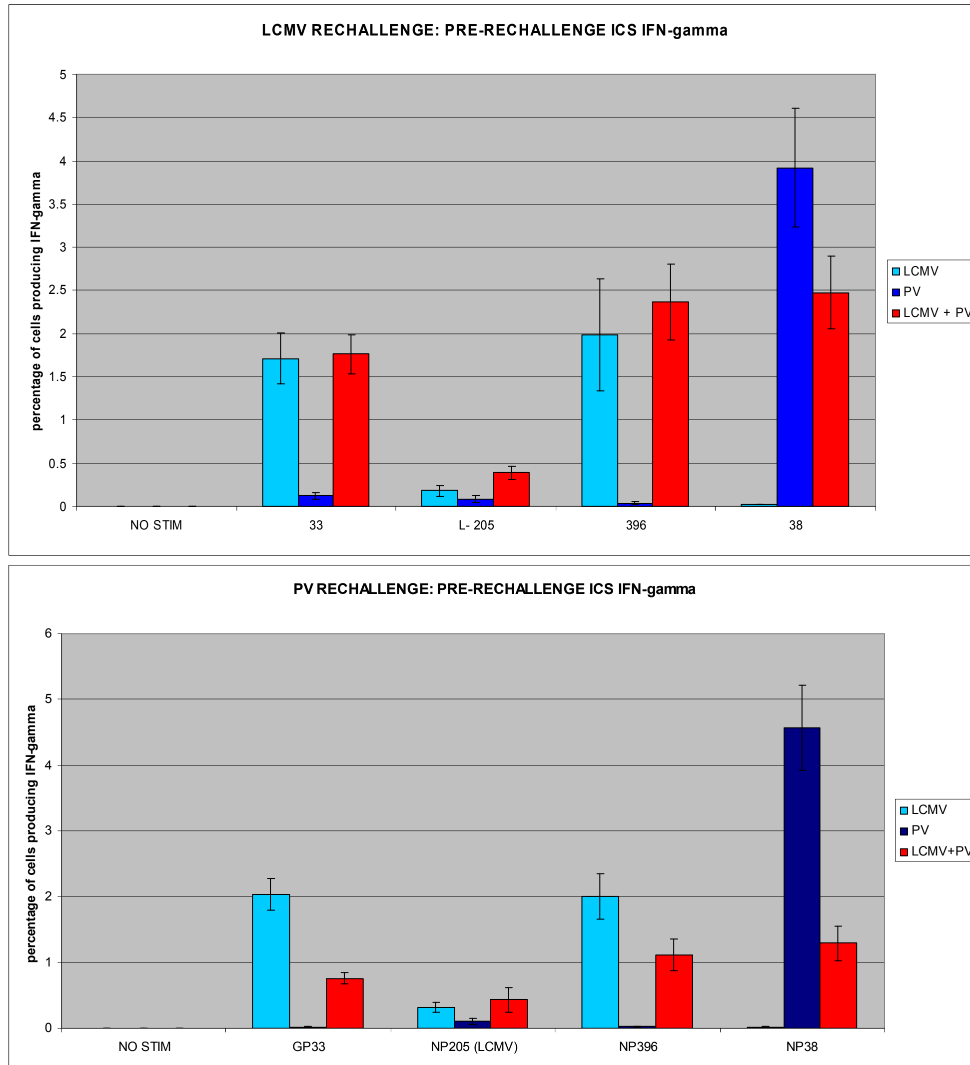


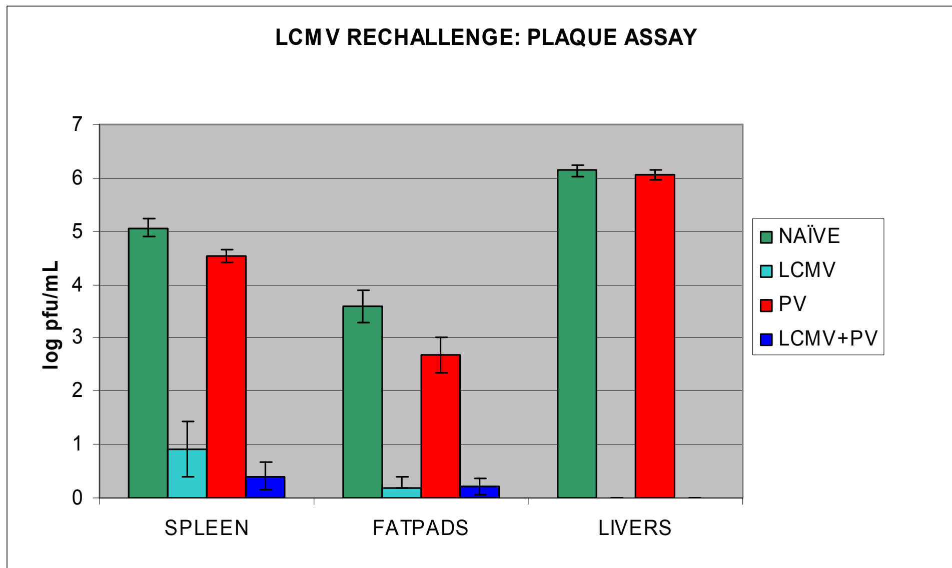
Figure 9. Percentages of cells producing IFN-gamma (memory cells) for LCMV and PV specific epitopes. Mice rechallenged with LCMV (top) and PV (bottom). Y-axis shows Percentage of cells producing IFN-gamma. Data presented as a mean of 10 mice +/- SEM.

Individually, LCMV + PV-immune mice had high levels of variability and prioritized the dominant epitopes for either of the viruses. This variability between individual mice was also seen in the differences between the two experiments. The responses were much lower in the LCMV + PV-immune group of the PV rechallenge as

compared to the LCMV rechallenge. This could potentially affect the immunoprotection that these mice developed.

Viral Titers

The viral titers of the mice were determined on day 4/5 post-rechallenge by plaque assay of spleens, livers, and fatpads (Figure 10).



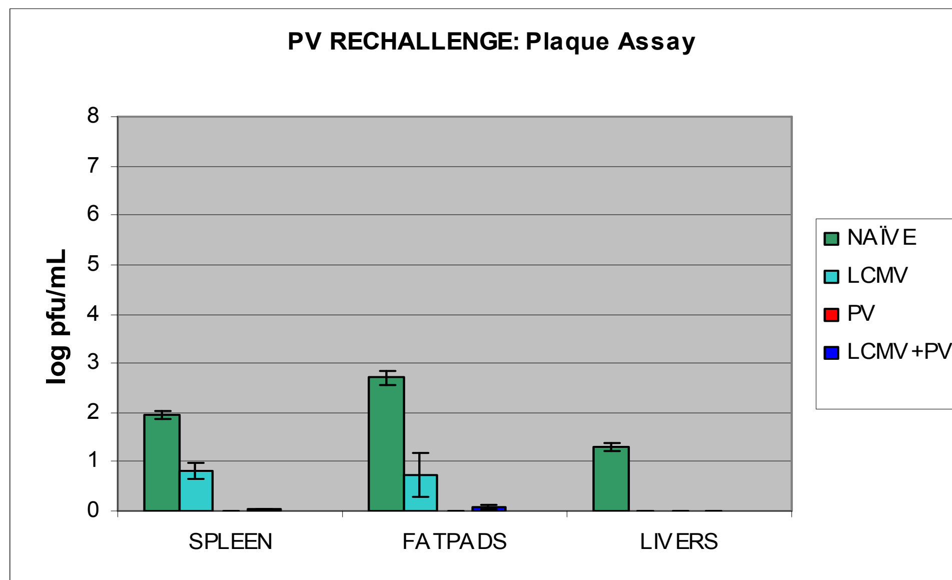


Figure 10. Viral Titers determined by plaque assay of ½ spleens, fatpads, and livers. Mice rechallenged by LCMV (top) and PV (bottom). Data is presented as a mean of 10 mice +/- SEM.

The different viral titers are indicators of both immunoprotection, but also the time course of the virus. Naïve mice rechallenged with LCMV cl 13 will usually die at day 12 post-infection while by day 8 of a PV infection the virus has been cleared. This was depicted in both of these experiments by the large difference in viral titers. The naïve mice of the PV rechallenge experiment had an approximate 99% inhibition compared to the naïve mice of the LCMV rechallenge experiment.

In the LCMV rechallenge experiment, (Figure 10 upper panel) PV-immune and naïve mice had much higher viral titers than LCMV-immune or LCMV + PV-immune mice. PV-immune mice had a slight decrease in viral titer in spleen and fatpads as compared to the naïve mice. This may be due to the cross-reactive T-cell response. The LCMV-immune and LCMV+PV-immune groups were expected to have immunoprotection to the virus due to their memory T-cells specific for this virus. This was proven by the similar pattern of low titers of LCMV in all these organs at day 5 post-rechallenge (<1 log pfu/ml).

When rechallenged with PV (Figure 10 lower panel) viral titers are significantly lower is all groups. Naïve mice showed the highest concentrations of virus in fatpads, which is characteristic of this virus. The cross-reactive T-cell responses between LCMV and PV provided partial protection for the LCMV-immune group, which showed over 90% inhibition compared to naïve animals. PV-immune mice had undetectable virus while LCMV + PV-immune mice had slight amounts virus detectable in only a few individuals. Upon statistical analysis, however, this difference is not significant (p=0.1).

Weight Loss

The percentage of weight change in the mice was calculated after the rechallenge infection (Figure 11). Weight change in mice infected with these viruses is comparable to symptoms that develop in humans and other large mammals.

Similar to the viral titers, the weight change curves for the two experiments are slightly different due to the differences between the viruses. When rechallenged with LCMV cl 13 (Figure 11 upper panel) the naïve and PV-immune mice lost the most weight the fastest after infection. After day 3, however, the PV-immune mice rebounded while the naïve mice continued to lose weight. This rebounding effect could be due to the cross-reactive T-cell responses between the two viruses and correlates with the viral titer results. LCMV-immune and LCMV + PV-immune mice lost far less weight and both had regained their weight by day 5. LCMV-immune mice, however, dropped ~2% by day 1 post rechallenge while the LCMV + PV-immune mice took longer to lose weight and only lost ~1%. These findings indicate that even though these two groups had similar weights and viral titers at day 5, LCMV + PV-immune mice has less severe symptoms.

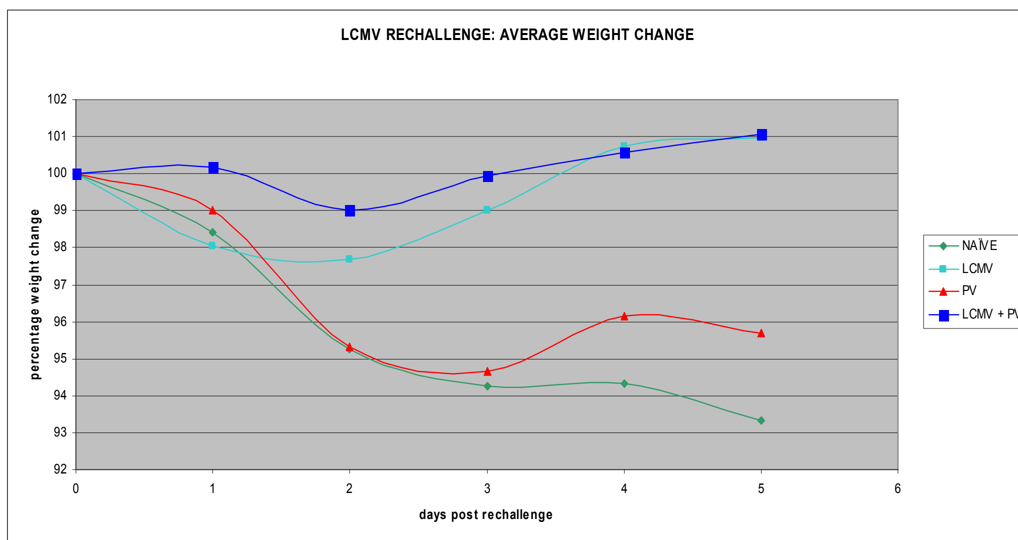
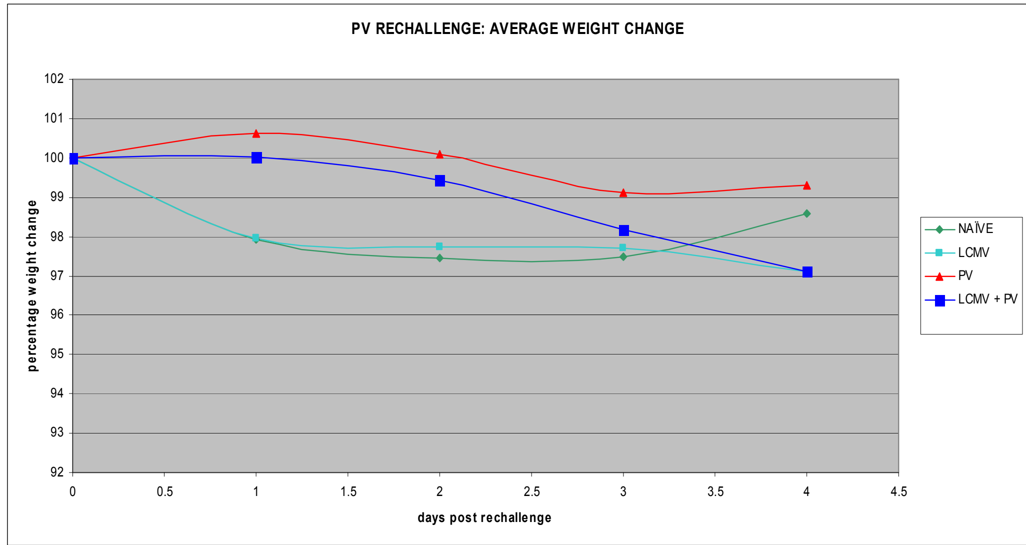


Figure 11. Percentage weight loss of mice rechallenged with LCMV (top) and PV (bottom). Data is presented as a mean of 10 mice.



The weight results from the PV rechallenge experiment (Figure 11 lower panel) provided a different conclusion. The naïve and LCMV-immune mice showed a similar dramatic loss of weight soon after rechallenge. Naïve mice, however, rebounded ~1% at day 4 while the LCMV-immune remained low. These weight losses showed little correlation with the viral titers. The weight curves for the PV-immune and LCMV + PV-immune mice showed a similar trend until day 4. Both groups took longer to lose the weight than LCMV-immune or naïve mice. This showed immunoprotection to the rechallenge. At day 4, LCMV + PV-immune mice continued to drop to their weight levels similar to LCMV-immune mice while PV-immune mice rebounded. The LCMV immunity of the LCMV and LCMV + PV-immune groups seems to be the downfall of these mice. Immunopathology, in the form of increased weight loss, was mediated by the immune response. It is possible that the cross-reactive T-cell responses in LCMV-immune mice are being actively recruited to sites of infection resulting in symptoms, but are not very efficient at clearing the virus.

Post-Rechallenge Memory T-cell Frequencies

After the rechallenge infections, a second intracellular cytokine assay was performed on the spleens from the individual animals (naïve pooled) (Figure 12). Changes in viral epitope hierarchy from before rechallenge were identified.

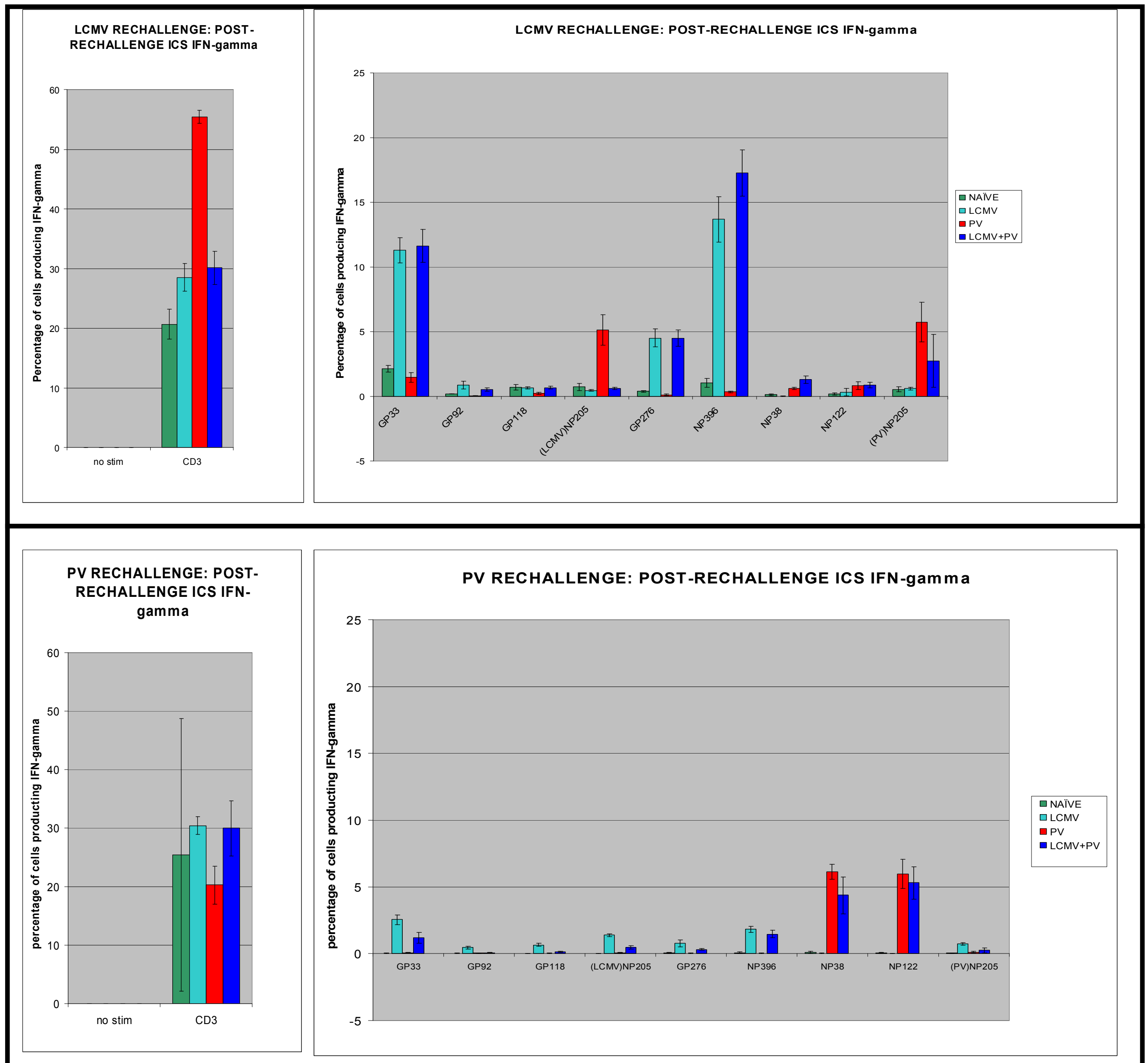


Figure 12. Percentages of cells producing IFN-gamma for LCMV and PV specific epitopes (right) and positive and negative controls (left).
Mice rechallenged with LCMV (top) and PV (bottom).
Data presented as a mean of 10 mice +/- SEM.

The intracellular cytokine assay (ICS) showed the specific viral epitopes used by the immune system of the mouse to respond to the virus. When vaccinated mice had memory cells that were either cross-reactive or specific for the rechallenge virus, their frequency in the mouse increased due to their efficiency in clearing the virus.

In the LCMV rechallenge experiment (Figure 12 upper panel), the naïve mice had low total IFN-γ levels induced by all specific viral epitopes, with the exception of GP33 (left side of the figure). This may be a developing response, but day 5 may be too early to see the full proliferation of the T-cells. PV-immune mice show significantly high levels of NP205 (both LCMV and PV). These are cross-reactive memory T-cells that are being activated and used to clear the virus. Like the naïve mice, the PV-immune mice are developing a GP33 response showing that the cross-reactivity is not enough to combat LCMV cl 13. The LCMV-immune mice show normal levels of all dominant LCMV epitopes (GP33, GP276 and NP396). The LCMV + PV-immune mice have LCMV epitope frequencies comparable to the LCMV-immune mice. Thus, the dominant LCMV epitopes are activated as the immune system attempts to clear the virus.

When rechallenged with PV (Figure 12 lower panel) there much lower levels of T-cell proliferation at day 4 as compared to the LCMV rechallenge experiment at day 5. LCMV-immune mice have low frequencies of viral specific memory T-cells, but show only a slight increase in NP205 cross-reactive epitope. From previous experiments, there was a greater expected increase in NP205 specific memory T-cells, however, a greater proliferation may be seen at later time points. PV-immune mice showed a significant increase in the PV epitopes, NP38 and NP122. When mice are vaccinated against both LCMV + PV the PV specific epitopes proliferate in response to a PV rechallenge. T-cells

specific for the LCMV epitopes were detected at low, memory-like frequencies in the maintained hierarchy.

Applying these results to human vaccine administration would show that multiple vaccines can be given to an individual at a single time and they will develop sufficient protection to future infections. As seen with the differences between LCMV and PV there may be cases in which immunopathology will develop due to low efficiency of cross-reactive memory T-cells. Here lies a major difference between murine models and humans that makes definitive conclusions much harder: humans are constantly being exposed to pathogens while experimental mice are kept in sterile conditions. There is no way to predict the memory T-cells of a specific individual in order to determine if any cross-reactivity will occur. Due to the high potential for TCR recognition cross-reactivity is expected to exists. In this MQP cross-reactivity occurred and the results showed that protection was produced; vaccination will therefore still have an effect in the case of unpredictable cross-reactivity.

DISCUSSION

Due to the presence of viral specific memory T-cells in mice that received either simultaneous or individual vaccines, they had sufficient immunoprotection at day 4/5 to clear a post-rechallenge infection. With similar T-cell frequencies and viral titers in singly and multiply infected mice at this time point, it seems as though the effect of receiving multiple vaccines at the same time is minimal. Evidence from the weight change analysis, however, leaves several questions about the efficiency at which the virus was cleared in the simultaneously vaccinated mice compared to the individually vaccinated mice. To access these questions further experiments should be performed at earlier and later time points.

LCMV Rechallenge

This experimental group tested the rechallenge of the mice with a stronger, more lethal virus, LCMV cl 13. Weight analysis showed that early after rechallenge the simultaneously immunized mice maintained their weight more than any other group (Figure 11). The correlation between T-cell proliferation and weight change would suggest that the T-cells that these mice produced a high affinity for LCMV and cleared the virus well, or that the LCMV neutralizing antibody was assisting the clearing of virus. By day 5, the T-cell frequencies of the LCMV-immune and LCMV + PV-immune mice were equal so determining if any specific epitopes were used to clear virus early in infection is not possible with these results. Experiments using earlier time points, however, would provide this information.

PV Rechallenge

The results for this experiment vary greatly from the LCMV rechallenge experiment. LCMV and PV, however, are only distantly related so their infection time courses are understandably different. The pre-rechallenge T-cell frequencies of the PV rechallenge experiment were slightly lower than expected from the results of the LCMV rechallenge experiment. This could be due to several factors. All of the mice for both experiments had the same date of birth and were immunized on the same day, but were bled or rechallenged on the different days. This may have introduced unknown variables, however, replication and duplication were performed in both cases. This may also just be an another indicator of the wide variability in the LCMV+PV-immune memory cell repertoires.

The weight loss for this experiment was similar to that of the LCMV experiment in the fact that the two groups that were not immunized against the rechallenge virus lost the most weight the fastest, but by day 4 there were different results. The two groups that had LCMV memory T-cells had the lowest weights and did not show any weight regain (Figure 11). In terms of viral titer, the naïve mice had the highest titer levels followed by LCMV-immune mice approximately 90% inhibition (Figure 10). Of the few LCMV + PV-immune mice that had not cleared the virus by day 4, there were very low viral titers showing that they were indeed clearing the virus even though they were losing weight. The weight change in LCMV-immune and LCMV + PV-immune mice can be explained by the stimulation of NP205 cross-reactive memory CD8 T-cells that have little efficiency in clearing virus (Figure 12).

In conclusion the results show a trend of sufficient immunoprotection in all mice that have received a vaccination to the rechallenge virus, be it individually or in conjunction with a second vaccination. Weight analysis identified distinct differences in the rate of weight change, or T-cell proliferation, in the different groups soon after the second infection in both experiments. These differences may prove to show different immunopathological developments from these viruses.

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