Published in final edited form as:
Autoimmunity. 2011 June ; 44(4): 328–347. doi:10.3109/08916934.2011.523277.

Heterologous immunity: immunopathology, autoimmunity and protection during viral infections

Liisa K. Selin[1,*], **Myriam F Wlodarczyk**[1], **Anke R. Kraft**[1], **Siwei Nie**[1], **Laurie L. Kenney**[1], **Roberto Puzone**[3], and **Franco Celada**[2]

[1]Department of Pathology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA, 01655, USA

[2]Department of Rheumatology, Hospital for Joint Diseases, 301 East 17th Street, New York, NY 10003, USA

[3]Department of Clinical Epidemiology, National Institute for Cancer Research, Genoa, Italy

Keywords

heterologous immunity; T cell cross-reactivity; autoimmunity; viruses; immune modeling

INTRODUCTION

There is a strong association between infection-related cell-mediated immunity and autoimmune diseases such as diabetes, multiple sclerosis (MS), rheumatoid arthritis (RA) and lupus erythematosis (SLE)[1]. Infections have also been associated with unusual immunopathologies of unknown origin, such as Wegner granulomatosis, sarcoidosis, colitis, panniculitis, bronchiolitis obliterans and even chronic fatigue syndrome. Despite exhaustive efforts, a definitive link between one particular pathogen and any of one these pathologies has never been found. More often several pathogens have become associated with each of these conditions. For instance multiple sclerosis has been associated with Epstein Barr virus (EBV), measles virus, HHV-6, varicella-zoster virus, and Picornaviruses[2-6]. Panniculitis in the form of erythema nodosum and bronchiolitis obliterans have both been associated with unusual cell-mediated immune responses that occur following non-specified viral or intracellular bacterial infections [7-9]. Erythema nodosum, which has also been associated with Crohn's disease[8], is a very painful condition, where nodules of inflamed subcutaneous fat often on the shins and forearms persist for months. There is no known therapy. Bronchiolitis obliterans is a lethal condition in humans where the bronchioles become occluded with immune cells and fibrinous material, with no known cause or treatment[9].

Chronic fatigue syndrome (CFS) is another unusual multisystem disease which is thought to be associated with immune dysregulation. Over the past two decades millions of patients world wide have suffered from a clinical syndrome of disabling fatigue, myalgias, palpitations and cognitive dysfunction that lasts longer than 6 months. In 50% of cases it develops after a mild viral illness. Cases may appear sporadically or in clusters[10,11]. Many attempts have been made to define the syndrome on the basis of an etiologic agent. These agents have included Epstein-Barr virus[10], Brucella[12], Candida albicans[13], Borrelia burgdorferi, and human herpesvirus-6[14,15]. More recently it has been associated with enteroviruses and xenoretroviruses [16-18]. The general conclusion has been that it is unlikely

[*]Address correspondence to : Liisa K Selin, MD, PhD, Associate Professor, Dept. of Pathology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA, 01655, USA, Phone: 508.856.3039 / Fax: 508.856.0019, Liisa.selin@umassmed.edu.

that the syndrome is caused by a single etiologic agent. The mechanisms mediating CFS are poorly understood, and there are few well designed studies examining its cause. The symptoms of CFS are similar to those experienced during viral infections such as infectious mononucleosis or influenza or in the setting of therapy with cytokines such as interferon or interleukin-2. It has been speculated that some or all the symptoms are reflective of an altered immune response to some pathogen with over production of one or more cytokines. An alternative hypothesis suggests that a number of infectious agents are involved and result in a regulatory imbalance of cytokines and the patient with CFS is unable to reestablish the appropriate balance of cytokines. These theories have been supported by reports of immune deficiency seen associated with CFS[19].

Much effort has been placed on trying to identify both T and B cell auto-antigen responses in autoimmune responses, but often with limited success. It seem that in the last 50 years we have made very slow progress in understanding the pathogenesis of autoimmune diseases and these syndromes of unknown origin, which for the purposes of this review I will call collectively autoimmune syndromes. In this review we would like to suggest a different perspective in trying to understand the mechanisms behind these diseases. Here, we will examine evidence to date arguing that it is not a specific pathogen and the immune response to it which leads to dysregulation of the immune system or to a specific autoantigen response in an autoimmune disease. Instead we would like to suggest that to better understand the pathogenesis of classic autoimmune diseases and these other unusual immunopathologies of unknown origin, which are often considered autoimmune-like syndromes it requires an evaluation of how T cells are regulated and evolve during multiple sequential infections under the influence of heterologous immunity. T cell responses to viral infections are extraordinarily dynamic and highly variable between individuals. The T cell response is impacted by previous infections with putatively unrelated viruses, and subsequent viral infections modulate the memory T cell pool created in response to previously encountered pathogens.[20-23] The concept of "*heterologous anti-viral T cell-mediated immunity*," takes into account the influence that T cell responses to one virus can have on that of another[24,25]. This review will by necessity focus predominantly on CD8 T cell responses, as more is generally known about epitope-specific CD8 than CD4 T cell responses to pathogens. The data presented here indicate that heterologous immunity can alter the outcome of a new infection and determine whether an infection is subclinical or progresses to severe immunopathology and death, even in genetically identical mice. Although superficially it may seem complex, it behooves us to have a better understanding of the general principles of heterologous immunity if we are ever going to understand the mechanisms behind these aberrant immune responses and how best to intervene. In addition to improving our understanding of aberrant immunopathological diseases there are important public health-relevant questions receiving considerable attention by viral immunologists: (1) can one design successful T cell vaccines that will give long term protective immunity without immunopathology, particularly in the elderly, and (2) under what circumstances do virus infections induce severe immunopathology, are there ways to circumvent it, and does this type of immunopathology contribute to the development of autoimmunity? (Figure 1).

Because of the property of viruses to infect cells, peptide cleavage products from many of their encoded proteins get incorporated into nascent class I major histocompatibility complex (MHC) molecules and get presented at the cell surface to CD8 T cells bearing T cell receptors (TCR) specific for the peptide-MHC complex.[26] As a result, viral infections frequently stimulate very potent class I-restricted CD8 T cell responses capable of perforin- or FasL-dependent cytotoxicity, and interferon (IFN)γ and TNF production. Indeed, CD8 T cells are essential regulators of viral infection, playing important roles in the clearance of virus-infected cells and in sometimes causing damaging immunopathology.[27,28] the relative

balances between protective immunity and immunopathology often determine the fate of the virus-infected host (Figure 2).

Dendritic cells (DC) present viral antigen and co-stimulatory signals to T cells [26] causing the release of T cell growth factors (i.e. IL-2) which initiate a programmed expansion that does not require additional exposure to antigen[29-31]. However, the presence of antigen or factors produced from CD4 (T helper) cells may modulate this process[32,33]. After the contraction of this response, some virus-specific T cells are saved into memory, where they undergo steady state homeostatic proliferation[34-37]. During the early phase of most virus infections there is a dramatic reduction in lymphocyte number [38-42], which is associated with a high level of type I interferon (IFN)-dependent apoptosis,[38,42-44] particularly in memory phenotype (CD44+) T cells. Following completion of a viral infection, frequencies of all previous memory cell populations not cross-reactive with the new pathogen decline[22,23]. In general, our studies have shown that an infection with an unrelated virus will induce the formation of new memory cells specific to the second virus and will delete memory cells specific to the previously encountered virus [21]. This is a permanent change that remains for the lifetime of the mouse, though it has never been demonstrated or sufficiently studied in the human. Using the IMMSIM discrete model of a virtual immune system we proposed two models to explain this loss in memory T cell frequency: the *passive attrition* model, whereby old memory cells are lost simply by their competition with newly formed memory cells for survival niches in the immune system after immune response silencing, and the *active attrition* model, whereby there is a directed apoptosis of the pre-existing memory cells [45,46]. Most of our *in vivo* data support the active attrition model [45,47]

The great majority of the T cells responding will be directed against a discrete number of "immunodominant" peptides, which is dependent on a number of factors, including the frequency of available T cells with specificity for the peptide-MHC combination[26,34,48]. This T cell frequency is dependent on thymic selection, but is also a function of whether the host has experienced a T cell expansion to an identical or to a non-homologous cross-reactive epitope. This pattern of immunodominance in a virus infection can thus be greatly altered by previous exposures to other viruses that may encode cross-reactive epitopes[20,21,49]. The specificity of T cells can be very degenerate, and it has been calculated that a single TCR has the potential to react with as many as 10^6 peptide-MHC combinations[50,51]. It is now well-documented that many virus-specific T cells cross-react with epitopes encoded by other viruses and with uninfected targets displaying allogeneic MHC antigens[52-56]. A recent paper has elegantly analyzed the cross-reactive epitopes that we and others have defined and has concluded that seemingly distinct epitopes with low amino acid sequence identity can have biochemical similarity to the point where the authors could predict when two peptides may be cross-reactive, and the authors concluded that T cell cross-reactivity is more common than previously thought.[57]

The IMMSIM discrete model of a virtual immune system predicted that the early active attrition of memory cells may reduce the impact of a cross-reactive T cell response to another pathogen by leveling the playing field, thus allowing for a more diverse immune response[42]. Our computer modeling predicted that without the apoptosis of memory T cells at the early stages of infection, cross-reactive T cells might dominate the response to an infection [42,45]. By selectively reducing the frequency of memory cells at the beginning of an immune response, there actually may develop a more diverse T cell response to a new pathogen, and that diversity may be beneficial to the host for control of the pathogen. We were able to test this prediction *in vivo* in elderly mice, as they undergo less IFN-driven T cell attrition than do young mice[58]. These elderly mice developed higher numbers of cross-reactive T cell responses in both influenza A (IAV)-immune mice challenged with

lymphocytic chorimeningitis virus (LCMV) and the LCMV-immune mice challenged with Pichinde virus (PV)[58].

The hierarchy of T cell responses to immunodominant epitopes in immunologically naive genetically identical mice is very consistent,[59-62] but the amino acid sequences of the TCRs responding to these epitopes differ from mouse to mouse; these are sometimes called "private" specificities[63-66]. In a T cell repertoire, that which is common between individuals, be it TCR Vβ usage or a common amino acid sequence or "motif," is a "public" specificity; that which is different between individuals, such as a CDR3 sequence, is a "private" specificity. Thus, genetically identical hosts such as identical twins have, as a consequence of random DNA recombination events, genetically different immune systems, and this diversity of TCR usage poses a challenge when one considers whether an epitope-specific T cell response may be cross-reactive with another epitope. This is because the expanded clones of virus-specific T cells in different individuals may have different private specificities, such that one individual may have a repertoire cross-reactive between two epitopes, whereas the other individual may not. This may help explain, for instance, why the concordance rate in twins for many autoimmune diseases such as diabetes and multiple sclerosis does not usually exceed 50%. We have shown that private specificity is an integral part of heterologous immunity by demonstrating that genetically identical mice use different cross-reactive T cell responses, resulting in tremendous variability in disease[67-69]. In order to demonstrate private specificity, memory cells from one LCMV-immune mouse were adoptively transferred into three naïve congenic recipients, and it was found that those three mice used similar cross-reactive T cell repertoires upon vaccinia (VV) or PV challenge. However, a different memory donor would generate a completely different cross-reactive response.

The question still remaining is exactly how do heterologous immune responses and, in particular, cross-reactive T cell responses alter the pathogenesis of viral diseases under conditions of sequential infections and persistent infections. There has been a strong association or correlation of cross-reactive T cells playing a role in heterologous immunity. Table 1 summarizes some of the mouse studies in this regard, and Table 2 summarizes human studies which demonstrate that heterologous immunity may be of considerable significance in IAV, EBV, dengue, and HCV viral infections in humans[4,49,70-73]. This review will examine these studies in more detail and also will touch on what role modeling of the immune system with discrete models such as IMMSIM may play in helping us understand these complex issues.

HETEROLOGOUS IMMUNITY AND THE FINE BALANCE BETWEEN PROTECTION AND PATHOLOGY DURING VIRAL INFECTIONS

The term "heterologous immunity" was used when describing differences in protective immunity and immunopathology in C57BL/6 mice immunized with one virus and later challenged with either of five viruses, IAV, LCMV, PV, VV, and murine cytomegalovirus (MCMV)[24,74](Table 1). Only recently has direct evidence been published that heterologous immunity is mediated by cross-reactive T cells, and that cross-reactive T cells may cause dramatic differences in T cell-dependent protection and immunopathology in the fat and lung[69,75](Wlodarczyk,MF & Selin LK unpublished). Heterologous immunity can provide partial protective immunity and, in experimental models, can provide the difference between life and death in the infected individual[24,76,77]. For example, LCMV-immune mice control PV infection, due to the cross-reactivity of T cells specific for the subdominant NP205 epitope[78,79](Chen A, Welsh RM & Selin LK unpublished). LCMV-immune mice also manifest strong protective immunity against infections with the large DNA poxvirus, VV, compared to naïve mice[24]. This heterologous immunity prevented mortality to an otherwise

lethal dose of VV[76,77]. Adoptive transfer studies demonstrated that CD4 and CD8 T cells from LCMV-immune mice were required to transfer protective immunity to naive mice challenged with PV or VV[24]. Selective expansion of LCMV-specific memory CD8 T cells upon VV infection suggested the possibility of cross-reactive CD8 T cell responses between these two viruses [76,78]. In fact, VV-specific CD8 T cell epitopes were identified in mice by searching for sequence similarity to a potentially cross-reactive LCMV epitope [54,80]. These cross-reactive CD8 T cells from LCMV-immune mice mediate protective immunity against VV as demonstrated by adoptive transfer of T cells lines [75].

However, T cells can be mediators not only of protective immunity, but also of substantial immunopathology[27,75,81-85] Classic examples are that of LCMV, where the same clone of T cells responsible for viral clearance can mediate a severe leptomeningitis if the virus is replicating in the brain[27,81]. The pathology that is induced by T cells during an acute infection most likely results from the inflammatory conditions brought about by the presence of high numbers of T cells lysing infected tissues via perforin and FasL, producing pro-inflammatory cytokines including TNF and chemokines which recruit even more cells. An important factor in how rapidly the virus is cleared is the efficiency of the activated T cells. The avidity of the TCR interaction with its ligand is one of the factors which lead to the induction of high potency T cells. Functional studies with altered peptide ligands (APL) show for both CD4 and CD8 T cell clones that high- and low- potency ligands differ in the length of time the TCR interacts with MHC/ligand, often referred to as the “affinity” of the TCR[86-89]. High avidity or agonist TCR interactions for T cell clones have been shown to result in strong signaling and activation of the full functional potential of the cells, including cytokine production, cytotoxicity, and proliferation. Low avidity or weak agonists induce CD4 T cell clones to produce cytokines, but not proliferate, while in CD8 T cell clones they induce all functions, but it requires 10- to 100-fold more ligand. One can easily imagine that a low avidity TCR interaction with a cross-reactive ligand may produce different cytokines, different amounts of cytokines, or be less efficient at killing and proliferating than the high avidity interaction with the original inducing ligand. Recent work using mutations in the $H2K^b$-restricted SIINFEKL epitope of ovalbumin and ovalbumin-specific transgenic T cells indicates that low affinity naïve T cells initially expand with kinetics similar to that of high affinity naive T cells, but leave the lymph node earlier and do not have the sustained expansion of higher affinity T cell clones, which eventually out compete the low affinity clones and dominate the response[90]. Although the exact mechanisms of ligand binding and transmission of this extracellular interaction into a productive intracellular signaling sequence remains incomplete, it has been known for many years that the immunoreceptor tyrosine activation motifs (ITAMs) of the T-cell receptor (TCR):CD3 complex are required for initiation of this signaling cascade. It remains unclear why the TCR:CD3 complex requires 10 ITAMs, while many other ITAM-containing immune receptors, such as Fc receptors (FcRs) and the B cell receptor (BCR), contain far fewer ITAMs. Vignali and his colleagues have recently demonstrated that various parameters of T cell development and activation are influenced by the number, as well as location and type, of ITAMs within the TCR:CD3 complex and hence have proposed that the TCR is capable of ‘scalable signaling’ that facilitates the initiation and orchestration of diverse T-cell functions[91]. Their work would suggest that rather than simply signal initiation, individual ITAMs may also be responsible for the differential recruitment of signaling and regulatory molecules and that this ultimately affects T-cell development, activation, and differentiation.

Not unexpectedly, heterologous immunity is not as protective as homologous immunity, which elicits high avidity T cell and antibody responses against a previously encountered pathogen. Recruitment of a large number of lower avidity cross-reactive memory T cells early in infection by a heterologous virus might be more conducive to amplifying a potent pro-inflammatory response in the presence of on going viral replication, due to the inability

to efficiently clear the virus, thus leading to enhanced immunopathology. Due to the competition between cells that gives rise to immunodominance, low avidity cross-reactive memory cells may also prevent the development of more effective high-avidity T cells responding to the normally immunodominant epitopes. Disease outcome is ultimately based on a fine balance between the number of memory T cells recruited to sites of viral replication, the efficiency of these T cells to clear the virus, and the length of time these T cells are present and amplifying the pro-inflammatory responses before they are able to clear the virus (Figure 2).

Some of the examples that this review will focus on to demonstrate the fine balance that exists within heterologous immunity will include VV infection of LCMV-immune mice, where the price for partial protective T cell immunity is altered immunopathology. After an intraperitoneal inoculation, LCMV-immune mice challenged with VV develop necrosis of visceral fat, termed acute fatty necrosis or panniculitis[24]. This form of panniculitis is analogous to human erythema nodosum and Weber-Christian disease. Also, in a respiratory infection model, reduced mortality of LCMV-immune mice infected with VV is accompanied by altered lung pathology[76]. Their lungs are significantly infiltrated by LCMV-specific T cells, which contributed to obstruction of bronchioles by fibrin and inflammatory cells (bronchiolitis obliterans). As mentioned above, in humans, erythema nodosum and bronchiolitis obliterans are of unknown etiology, but can be seen in some viral and bacterial infections and are also associated with autoimmune diseases[7-9]. Erythema nodosum has been observed after vaccination for smallpox or hepatitis B. The development of bronchiolitis obliterans in lung allografts is associated with transplant rejection[9]. Human T cell cross-reactivity exists between the major immunodominant HLA-A2-restricted epitopes of IAV virus and EBV, and a substantial part of the acute EBV-specific CD8 T cell response during infectious mononucleosis can be mediated by T cells cross-reactive with IAV contributing to the induction of infectious mononucleosis[49]. Other studies have correlated fulminant hepatitis with cross-reactive CD8 T cell responses between IAV and HCV[72,92,93] and have suggested a role for cross-reactive T cells in severe dengue virus infections[71,94]. Heterologous immunity and cross-reactive T cell responses between pathogens may be the basis for greatly dysregulated immune responses and enhanced immunopathology that can contribute to the induction of autoimmune syndromes.

LCMV AND VV

LCMV-immune mice infected with VV, is one of the first mouse models of heterologous immunity to demonstrate significant protective immunity and significant immunopathologies that are very similar to human diseases of unknown origin, falling into to the nebulous category of potentially autoimmune disease processes.

Panniculitis

The type of pathology that these mice develop is dependent on the route of infection. When LCMV-immune mice are infected with VV using the traditional intraperitoneal (i.p.) route some of these mice develop severe panniculitis, in the form of inflammation and necrosis of visceral fat tissue in the presence of a lower virus titer than naïve mice infected with VV[24,95]. This type of abdominal fat pathology is seen in human syndromes of unknown etiology such as Weber-Christian disease or lupus erythematosis, while erythema nodosum, a more benign and more common form of panniculitis involves inflammation of subcutaneous fat tissue [24,95]. Interestingly, this pathology is not directly associated with viral load (Figure 3), and there is tremendous variation between these genetically identical individual mice, with some mice having no pathology and others having very severe pathology that sometimes leads to death[69].

Role of cross-reactive T cells—VV infection activates LCMV-specific memory T cell populations, and we sought to clarify whether and how cross-reactive T cells could be mediating either the protective immunity and/or the pathology [69,75,77,96]. If VV were cross-reactive with an LCMV epitope, then a VV infection of an LCMV-immune mouse should expand LCMV-specific T cells with the cross-reactive specificity [20]. VV sometimes, but not all the time would expand populations of LCMV NP205-specific T cells [76], so we searched the VV proteome for potentially cross-reactive epitopes based on sequence similarity to NP205 and found two, within proteins e7r and a11r, to be recognized by VV-specific T cells [54,75,80]. Both these epitope-specific responses are protective against VV infection [80,97]. Of these two epitopes with sequence similarity to LCMV NP205, only the a11r response turned out to be cross-reactive with LCMV.

About half the time VV infection expands populations of NP205-specific T cells, but sometimes it expands populations of LCMV GP34- or GP118-specific T cells upon adoptive transfers of LCMV-immune splenocyte populations into naïve mice [68]. This variability in responses is not due to random stochastic events, but instead reflects the private specificity of the LCMV-immune T cell repertoire in individual mice. Adoptive transfer into three recipients from the same donor generates the same specificity of outgrowth of LCMV epitope-specific T cells, but recipients from a different donor can stimulate a different specificity. These results suggest that the private specificities of the LCMV memory population dictate which cross-reactive epitope would be recognized. Interestingly, it turned out that T cell responses to the K^b-restricted VV-a11r epitope can cross-react with either LCMV-encoded NP205, GP34, or GP118, all of which are K^b-restricted, though no T cell recognized all epitopes [68,75]. In fact, we found a network of cross-reactive epitopes encoded by VV, LCMV, and PV (Figure 4). Also, even though the e7r-specific T cell population is not cross-reactive with the LCMV-encoded epitopes, a part of the e7r T cell population is cross-reactive with a11r, which engages some T cells specific to each of the three LCMV epitopes or to the PV-encoded NP205 epitope. These experiments indicate that the epitope specificity of a T cell response in genetically identical individuals with the same histories of infection can be influenced by the private specificity of the individual.

What is the evidence that these cross-reactive responses mediate protective immunity or altered immunopathology? A history of an LCMV infection, as well as infections with BCG, PV, IAV, and MCMV, all provide a level of protective immunity to VV, and VV titers at day 3-4 after infection of LCMV-immune mice are generally 1-2 logs lower than those in naïve mice [24,74,76,98]. Adoptive transfer of CD8 and CD4 splenocytes from LCMV-immune mice can provide protective immunity to VV [24], or induce panniculitis [69]. Adoptive transfer of cross-reactive T cell lines generated by stimulating LCMV-immune T cell populations with the VV-encoded and cross-reactive a11r epitope also can mediate protective immunity to VV [75]. Interestingly, the presence of cross-reactivity does not necessarily lead to protective immunity, as some of the VV-a11r-specific cross-reactive lines were not protective against VV. Furthermore, the variation in the presence or level of panniculitis in VV-infected LCMV-immune recipients is a product of the private specificity of the T cells in the LCMV-immune host, as demonstrated by adoptive transfer of LCMV-immune T cells into paired naïve congenic recipients [69]. It still needs to be determined whether there are certain cross-reactive responses that lead to protective immunity versus those that lead to immunopathology.

Potential therapeutics with IFNγ and TNF blockers—*Both* protective immunity and the induction of immunopathology in this system seem to be heavily dependent on IFNγ, as neither protective immunity nor panniculitis occur in mice treated with antibody to IFNγ or in mice lacking IFNγ receptors [24,76]. The panniculitis, but not the protective immunity, was also dependent on TNF, as shown in TNF-deficient mice and by blocking TNF with soluble

TNF receptors (Enbrel) (Nie and Selin, unpublished). In fact, whereas the control of VV infection was partially dependent on TNF in naïve mice, the presence of heterologous protective immunity made resistance to VV less dependent on TNF[77]. Perhaps heterologous immunity may help explain why TNF blockers such as Enbrel and Remicade (anti-TNF antibody) used in the treatment of human autoimmune diseases such as rheumatoid arthritis and colitis are tolerated with relatively few infectious complications[77].

Protection not reciprocal—Heterologous immunity is not necessarily reciprocal, as a history of a VV infection will not confer any protection of mice to LCMV, PV or MCMV [24]. Consistent with this observation, CFSE-labeled LCMV-specific immune cells transferred into mice which then get infected with VV, proliferate substantially (CFSE-loss), but VV-immune cells transferred into mice that are then infected with LCMV, show little to no T cell expansion [78]. The reason for this is unclear, but there are far more potentially cross-reactive NP205-, GP34-, and GP118-specific T cells in the LCMV-immune memory pool than there are A11R-specific cells in the VV-immune memory pool. We also have not resolved all the potential patterns of cross-reactivity between these two viruses, and it may be that one of the many thousand of potential epitopes encoded by the very large VV, which encodes >200 proteins, could engage a sufficient number of memory cells to have a biologically meaningful effect. In contrast, LCMV encodes only four proteins, reducing the likelihood that they could successfully engage the VV-specific memory pool. It is interesting that large viruses like herpes simplex virus (HSV) and CMV encode many proteins that interfere with class 1 antigen presentation[99] and thus perhaps escape the protective effects of heterologous immunity. There remains the possibility that VV-specific memory cells may be qualitatively different than LCMV-specific memory cells. For instance, different cytokines play a role in their T cell maturation, where VV-specific T cells develop in the presence of IL-12, but not IFN1 and LCMV-specific T cells develop under the opposite conditions, high IFN1 and low IL-12[100,101] The long term effect of these cytokines on the responsiveness of these memory cells is uncertain. That being said, VV infection will readily expand populations of VV-specific memory cells, indicating that they are capable of excellent recall responses.

Bronchiolitis Obliterans and Enhanced BALT

Heterologous immunity between LCMV and VV has also been studied in respiratory infections, and the most common route of entry of both viruses in nature is the respiratory route [74,76]. After intranasal (i.n.) infection there was protective immunity and aberrant immunopathology. Acute VV infection of naïve mice was characterized by high virus loads, necrotizing bronchiolitis, acute inflammatory neutrophilic infiltrates, pulmonary edema, and severe respiratory distress of the host. In contrast, in LCMV-immune mice the VV infection was characterized by reduced virus loads, little pulmonary edema, areas of massive chronic lymphocytic infiltration, and enhanced bronchus-associated lymphoid tissue (BALT). As with panniculitis, the severity of the pathology could vary from insignificant to very severe and some mice presented with an unusual pathology, bronchiolitis obliterans, which is an obstruction of the airways with plugs of fibrin and inflammatory cells. This pathology in humans is highly lethal and is thought to be induced by cell-mediated immunity. It is of unknown etiology, but is sometimes seen in humans following viral infections and is strongly associated with lung transplant rejection. Despite these pathologies, the LCMV-immune VV-infected mice experienced less respiratory distress, due to areas of the lung being reasonably intact rather than edematous. In fact, LCMV-immune mice were able to survive doses of VV that were lethal to naïve mice. This is yet another example where models of heterologous immunity can reveal new diseases with pathologies resembling poorly defined autoimmune syndromes in humans.

Another interesting feature of heterologous immunity between LCMV and VV in the respiratory infection model is that there is a profound deviation in virus-induced cytokine production. Acute VV infection of naïve mice is associated with high levels of the proinflammatory cytokine IL-6 and decreased levels of the immune response cytokine IFNγ. Presumably because of the presence of IFNγ-producing cross-reactive memory T cells, the cytokine levels in VV-infected LCMV-immune mice are high in IFNγ and low in IL-6[74,76]. This is consistent with the concept that heterologous immunity is influenced by the cytokine-producing capacity of cross-reactive memory cells which may have the capacity to skew Th1 to Th2 responses during any new infection.

There is always a question of how much of heterologous immunity is mediated exclusively by cross-reactive mechanisms and how much by non-specific bystander mechanisms. Some studies have concluded that memory T cells can be subjected to bystander activation by cytokines independent of TCR signaling and that these non-specifically activated cells may contribute to heterologous immunity[102-105]. IL-12 and IL-18 have been reported to non-specifically stimulate the production of IFNγ by memory T cells[106], and VV infection does induce IL-12 in mice [74]. Thus is the IFNγ-dependent protection against VV entirely a consequence of T cell cross-reactivity or is it in part due to non-specific liberation of IFNγ from memory cells. In our studies, LCMV-specific memory cells need TCR engagement to produce elevated IFNγ levels in the presence of IL-12, but this does not rule out a non-specific effect under certain conditions. It is also possible that up-regulation of host MHC by IFNs and other cytokines provides some low level TCR signaling to memory cells, making them more receptive to activation by cytokines without engagement of viral peptides [107,108]. This area needs more study, but it should be noted that BCG immunization can provide protective immunity to VV by a CD4 T cell-dependent mechanism [98]. Using an *in vivo* IFNγ assay [109], VV infection preferentially induced IFNγ from CD4 T cells in BCG-immune mice, but not from CD8 T cells in LCMV-immune mice [98], although both primary infections resulted in memory phenotype CD4 and CD8 T cell populations in the peritoneal cavity and visceral fat pads. This preferential bias of T cell subtype would argue against non-specific stimulation of cytokines from memory cells under conditions of the natural infection *in vivo* even though the cross-reactive CD4 epitopes have not yet been identified.

Interestingly, to date there are few examples of cross-reactive CD4 T cells in models of heterologous immunity although CD4 T cells, due to their low affinities and longer peptide targets, could be more cross-reactive than CD8 T cells. A problem may lie in the possibility that there may be broad-based CD4 T cell cross-reactivity between the two pathogens, coupled with a rather low proliferative index that would prevent a CD8 T cell-like expansion that can aid in the identification of the cross-reactive epitope. As it stands, this is a system that clearly shows T cell-dependent heterologous immunity between different classes of pathogens which needs further investigation.

LCMV AND PV

LCMV-immune mice produce about 10-fold lower PV titers compared to naïve mice [24,79], and we assume this is due to the cross-reactive T cell response, in part because LCMV-immune splenocytes transferred into naïve mice can partially protect against PV infection. Since LCMV-immune mice are relatively resistant to infections with VV and MCMV, it could be argued that nonspecific activation of memory cells might mediate this protective immunity and that some non-specific mechanisms are at work. However, the NP205 epitope-escape variant[67] of LCMV had reduced ability to protect mice against PV (A. Chen, R.M. Welsh, & LK Selin, unpublished). This correlation of protective immunity and cross-reactivity adds to the argument that protective immunity is not a non-specific phenomenon, but is instead a consequence of MHC-restricted T cell cross-reactivity.

Panniculitis

Although LCMV-immune challenged with PV do not develop significant pathology associated with heterologous immunity we have also observed panniculitis in some mice that are both PV and then LCMV immune and then are rechallenged with PV (A. Chen,R.M. Welsh & LK Selin, unpublished). In this circumstance we would predict that the T cell repertoire to the NP205 cross-reactive response, which varies between mice due to private specificity, would be skewed towards LCMV-specific during the second infection and would not be optimized to protect against PV leading. This might lead to immunopathology.

Role of T cell cross-reactivity—LCMV and PV encode cross-reactive nucleoprotein epitopes at positions 205-212 that have 6 of 8 amino acids in common and differing in amino acids only in their anchoring sites to the K^b MHC [79]. This might suggest that these PV and LCMV epitopes should be similarly recognized by T cells. Most T cells generated in response to one virus will recognize the alternative peptide epitope [79], but with different affinities, as suggested by tetramer binding patterns. The LCMV-induced repertoire is almost exclusively oriented to Vβ16 while the PV-induced repertoire also includes Vβ5-specific T cells, and uses a different CDR3 motif within the Vβ16 T cells [67]. Sequential infections with these viruses could lead to the generation of highly variable and skewed narrow oligoclonal TCR repertoires that differed between hosts, frequently not focused to Vβ16 or Vβ5, reflecting the private specificity of the T cell response. These variations in repertoire reflected private specificity patterns rather than random stochastic patterns produced by a programmed expansion random encounters of cross-reactive T cell clones with its cognate ligand. Transfer of splenocytes from one immune mouse into three recipient mice infected with the heterologous virus generated similar T cell repertoires, but recipients from other donors all generated a different set of repertoires [67]. This indicates that the repertoire available for cross-reactive expansions was established during the first virus infection and was unique for each immune mouse.

Narrowing of TCR repertoire and predictive modeling—Narrowing of the diversity of TCR repertoires for a viral epitope may occur by evolution for the most perfect fit during persistent virus infections, such as those with HIV, HCV, CMV, and EBV, or with repeated antigenic challenges, such as with influenza virus. There are many examples of T cell cross-reactive peptides encoded by different viruses [53,54], and another major cause of TCR repertoire restriction could be due to cross-reactive T cell responses. A rather narrow subset of an epitope-specific T cell memory pool is selectively stimulated to proliferate on exposure to a cross-reactive pathogen [67,110]. On infection with a heterologous virus, these high-frequency, but not-very-diverse set of clones may immunodominate an emerging T cell response from naïve precursors and cause a further restriction of the repertoire, as we have demonstrated in LCMV-immune mice infected with PV [67]. Furthermore, our results using the IMMSIM model, where we incorporated both adoptive transfer and private specificity, would suggest that TCR affinity plays an important role in driving this phenomenon [67].

IAV AND LCMV

A history of an intranasal IAV infection of mice can generate a level of protective immunity against VV, possibly through a similar mechanism as that between LCMV and VV described above. However, a history of IAV infection can lead to enhanced titers of LCMV and MCMV, with altered immunopathology [74]. It has long been known that IAV infection can break down structural barriers to bacterial infection in the lung, and bacterial invasion of the lung is often responsible for the mortality in human influenza patients. Here, however, mice are receiving LCMV and MCMV long after the IAV infection has resolved and when

the lung no longer shows signs of structural damage. This suggests that dysregulated immunity can occur as a consequence of heterologous immunity.

Bronchiolization and Consolidating Mononuclear Pneumonia

Heterologous immunity between IAV and LCMV is quite complex. IAV-immune mice developed enhanced replication of LCMV and enhanced immune pathology in the lung, characterized by massive consolidating mononuclear pneumonia with bronchiolization instead of the mild lymphocytic pneumonitis observed during LCMV infection of naïve mice [74]. Bronchiolization is another novel pathology observed in humans and is of unknown etiology thought perhaps to be associated with lung repair processes[111]. Memory T cell depletion studies indicated that the enhanced LCMV titers seem to be dependent on IAV-specific memory CD4 T cells (perhaps by regulating CD8 T cells), and the enhanced immune pathology is caused by cross-reactive IAV-specific CD8 T cell responses (Wlodarczyk MF & Selin LK, unpublished).

Therapeutics using peptide tolerization, mutant vaccines and IFNγ blockers— Cross-reactive epitopes for IAV and LCMV have been defined in these studies in C57BL/6 mice (Table 1). The importance of these epitopes in these patterns of heterologous immunity was shown by using variants of IAV and LCMV with deletions in these epitopes, and those deletions inhibited the immunopathology associated with the heterologous immunity. In addition, epitope-specific T cells were deleted or functionally inactivated by intravenous infusion of the peptides into mice. This resulted in selective depletion of the epitope-specific memory T cells and decreased lung pathology induced by the heterologous immunity. We were also able to inhibit immunopathology by treating the IAV-immune mice with anti-IFNγ prior to challenge with LCMV. We took 4 different novel approaches that help explain the increased severity in lung pathology during “dysregulated” heterologous immunity, 1. identification of cross-reactive epitopes, 2. correlation of cross-reactive epitope-specific memory responses with disease severity, 3. ablation of inappropriate cross-reactive T cell responses or 4. temporary attenuation of T cell effector cytokines such as IFNγ (Wlodarczyk MF & Selin LK, unpublished). These four approaches suggest for the first time that therapeutic interventions may be possible to circumvent severe immunopathology induced by heterologous immunity[112].

EOSINOPHILIA, IMMUNE DEVIATION AND HETEROLOGOUS IMMUNITY

It seems highly possible that, if memory T cell pools are skewed in a Th1, Th2, or Th17 direction, infection with a cross-reactive pathogen could activate and induce cytokine secretion by some of those T cells, which could then alter the differentiation of the immune response against the pathogen. There have not been studies to unambiguously show this, though there are hints with infection of BALB/c mice with RSV. RSV causes severe respiratory infections in children and adults, and inactive vaccines against RSV in the past have resulted in increased morbidity and mortality in children when the children became naturally exposed to the virus [82]. These children presented with severe lung pathology associated with intense eosinophilia, presumably brought above by a type 2 cytokine response involving IL-5 and other Th2 cell-produced cytokines. This phenomenon can be mimicked in mice immunized with RSV G protein and later challenged with live RSV [113,114]. These mice develop a narrowly focused Vβ14-expressing CD4 Th2 response associated with high levels of IL-5 production and severe eosinophilia. These T cells are directed against a single G-encoded epitope and are rather similar in CDR3 sequences between mice [113]. Priming with RSV G can be done in the context of a VV-G recombinant virus, but if a host first has an infection with IAV, VV-G will no longer prime for a Th2 response [115]. IAV provides a modest level of heterologous immunity to VV, but, importantly, there is a different cytokine milieu in VV-infected IAV-immune mice in

comparison to VV-infected naïve mice [74]. This milieu is deviated in a type 1 cytokine, IFNγ-oriented direction. Thus, it is possible that the IAV infection caused an immune deviation of the VV-G immunization, such that on infection with the third virus, RSV, severe Th2-dependent pathology did not develop. These results would suggest that heterologous immunity could play a role in the development of allergies and asthma following viral infections.

HUMAN EXAMPLES OF HETEROLOGOUS IMMUNITY

In the setting of *human disease* it is more difficult to test whether heterologous immunity can play a role in *protective immunity*. At this time the only direct evidence for this comes from epidemiological vaccine studies in very young children in the third world, where it has been found that BCG or the combined live attenuated measles, mumps and rubella (MMR) vaccine can protect in some unknown manner against mortality from other infectious diseases such as rotavirus-induced diarrhea and pneumonias[116-119]. Interestingly, a subsequent vaccination with the killed combined diphtheria, tetanus and pertussis (DTP) vaccine can reverse these beneficial effects and in fact increase mortality[117,120,121]. We also have data demonstrating that 3 middle-aged EBV sero-negative adults who are constantly being exposed to EBV in their laboratory environment have strong HLA-A2 restricted cross-reactive CD8 T cell responses between IAV $M1_{58}$ and EBV $BMLF1_{280}$, with unique repertoires that are able to produce IFNγ and lyse EBV-infected targets (Watkin et al., unpublished). There are cohorts of sex workers in Kenya which appear to have some form of protective immunity to HIV as long as they continue to work[122,123]. Elderly individuals may also be completely dependent on cross-reactive memory responses when they encounter any new pathogen. The problem with studying the protective effects of heterologous immunity is that reduced sickness would normally go unnoticed, except in a large scale epidemiological study.

Identification of a role of heterologous immunity in the induction of *human immunopathology* is more approachable. CD8 cross-reactive responses have been identified between IAV and HCV or EBV, and these cross-reactive T cells have been associated with the induction of either fulminant hepatitis or infectious mononucleosis, respectively[49,70,75,124]. There are increasing reports of significant side effects to HPV vaccines in young women[125-130], and a new controversy is arising as more and more parents refuse to vaccinate their children for fear of side effects. This even led to a television documentary recently aired on the Public Broadcasting Network, PBS, called the “Vaccine Wars”. In a time of complacency when many childhood infections have almost disappeared due to vaccines, parents are focusing on the side effects of vaccines and demanding demonstrations that giving as many as 6 vaccines in one day and a total of 18 vaccines before 2 years of age is safe. Considering our preliminary studies concerning dramatic changes in T cell responses in mice co-infected with LCMV and PV together compared to singly, where immunodominance hierarchies to the two viruses are completed altered with tremendous variability between individual mice, perhaps the issue should be examined more closely (Kenney L & Selin LK, unpublished). These issues all suggest that it behooves us as viral immunologists to better understand when, in what sequence and how vaccines are given, or we will have ever increasing serious issues with compliance. These issues were recently discussed at a Workshop in Copenhagen on “Nonspecific effects and sex differences in vaccines”, attended by an international group of vaccinologists, where we formed a Consortium to begin to address these types of issues, and a summary of the meeting will be published[117].

Heterologous immunity is, of course, more difficult to study in human systems, but there is evidence that it occurs. Some studies have been done on what has been called “heterotypic”

immunity in the IAV system, where many viral strains and variants are found. T cells cross-reactive between these variants are easy to detect, and an unresolved question is how important these cross-reactive T cells are in immunity or immune pathology associated with human IAV infections [131,132]. Two epidemiological studies have indicated that exposure to one strain of IAV (H1N1) apparently provided some level of protection against another strain of IAV (H2N2) under conditions of very little serological cross-reactivity among the IAV targets of neutralizing antibody[133,134]. Different IAV strains can also encode some extremely conserved epitopes, such as the HLA-A2-restricted M1 epitope, which generates an immunodominant CD8 T cell response [135,136]. Whether that specific response provides any resistance to infection is unclear.

IAV and HCV and Fulminant Hepatitis

A more concrete example of heterologous immunity between unrelated human viruses occurs between IAV and HCV. A T cell response to a defined HCV-encoded HLA-A2-restricted epitope $NS_{31073\text{-}1081}$ was found to stimulate a T cell response in non-HCV-immune individuals and ended up being strongly cross-reactive and sharing 7 of 9 amino acids with an IAV-encoded $NA_{231\text{-}239}$ epitope [92]. In a remarkable study, the breadth of immune responses to the HCV proteome was addressed in a number of HCV-infected patients by ELISPOT analyses of their T cells stimulated against HCV-encoded peptides. Most patients presented with a broadly reactive response with signals seen among a large number of the HCV peptides. Two patients, however, had an extremely focused response against a peptide spanning the HCV and IAV cross-reactive epitopes. These two patients had an unusual presentation for HCV infection, with severe fulminant necrotizing hepatitis [70]. Hence, many of the parameters of heterologous immunity addressed above in mouse models are in play in this study, including a 1. narrowly focused cross-reactive response associated with 2. severe pathology and reflective of 3. private specificity, as all of the patients had likely been exposed to the IAV strains encoding the epitope, but only a subset mounted this immunodominant and narrow response.

IAV and EBV and Infectious Mononucleosis

An unresolved phenomenon of viral infections in humans is that infections with a number of viruses, such as Epstein-Barr (EBV), varicella-zoster virus, measles, or mumps are more severe in young adults than in children[137,138]. Usually there is more immune pathology associated with the young adult infections, and we propose that one reason for that phenomenon may be heterologous immunity. As the memory T cell repertoire of children becomes more diversified due to the acquisition of a sequence of infections, the probability of having memory cells cross-reactive with another pathogen increases, and these may not be the best cells to rapidly clear the virus. Acute infectious mononucleosis (AIM) associated with EBV infections is one of the best examples of such a phenomenon. Children usually have subclinical infections, but teenagers of college age and young adults can have a much more severe and longer lasting infection. The characteristic pathology of AIM is the appearance of “atypical lymphocytes,” which are, in fact, cytotoxic granule-containing activated CD8 T cells responding to EBV-infected B cells and epithelial tissue. There is no evidence that the EBV load is any higher in AIM patients than in the subclinically infected [139]. The major pathological feature of AIM, then, is that of an overzealous CD8 T cell response that is not very effective at controlling the virus. Many HLA-A2+ AIM patients have an increase in the frequency of their IAV-$M1_{58}$ –specific CD8 T cell responses. We have demonstrated human T cell cross-reactivity between the major immunodominant HLA-A2-restricted epitopes of IAV and EBV and find that a substantial part of the acute EBV-specific CD8 T cell response during AIM is mediated by T cells cross-reactive with IAV (Table 2) [49]. We now have data that demonstrates that the

frequency of the IAV $M1_{58}$-specific cells directly correlates with the severity of infectious mononucleosis symptoms (Aslan N & Selin LK, unpublished).

Broad crossreactive T cell repertoire and predictive modeling—We examined cross-reactive responses *in vitro* between two of these cross-reactive epitopes, IAV-M1 and EBV BMLF1, which have little sequence similarity, and found that the cross-reactive repertoire was broad rather than narrow and devoid of highly dominant clonotypes compared to the non-cross-reactive repertoire for each epitope. The breadth of the cross-reactive T cell repertoire may depend on various factors, such as the level of similarity between the epitopes, a hypothesis which was supported by our computer simulation (Clute SC & Selin LK, unpublished). Based on our earlier observations, we hypothesized that if there are a few high frequency memory clones to the initial immunogen with high affinity to the cross-reactive epitope (which may occur when epitopes are more similar *i.e.* "near" cross-reactivity), this type of clone will dominate the TCR repertoire to the cross-reactive epitope and lead to a narrower repertoire. However, if this type of clone does not exist in the memory pool (which may occur when epitopes are more dissimilar *i.e.* "distant" cross-reactivity) the repertoire will actually become broader without any dominant pre-existing cross-reactive memory clones. Cross-reactive TCR repertoires were generated from the two different memory populations to the first antigen. The "near" cross-reacting clonotypes were found in high frequency positions, as their dominance is assured by competition with the development of new clones, thus causing restriction of the repertoire. On the other hand, the "distant" cross-reacting clonotypes were present at low frequencies, and the absence of high frequencies of high affinity clones allow ample space for development of repertoire diversity[124].

Our continued analyses of the CD8 T cell response to EBV in AIM patients and immune controls has revealed a whole network of HLA-A2-restricted cross-reactivities between EBV-encoded and IAV-encoded epitopes[75], much like the K^b-restricted network of cross-reactivities discussed between LCMV-, VV-, and PV- encoded epitopes (Figure 3). Of note is that these cross-reactive T cells were found in some subjects, but not others, likely revealing a strong role for private specificity in this process.

Dengue Viruses and Hemorrhagic Shock Syndrome

Dengue viruses are closely related viruses that are found in four serotypes [140,141]. Other than by neutralization assay, these viruses serologically cross-react and encode cross-reactive T cell epitopes. The most severe manifestation of dengue disease is dengue hemorrhagic fever and shock syndrome, and this most commonly presents when individuals immune to one strain (serotype) of dengue virus become infected with another strain. This has long been thought due to cross-reactive non-neutralizing antibody that binds to viruses without inactivating them and instead enhances the infection of cells like macrophages that bear Fc receptors, in a process known as immune enhancement [141]. However, this may be only part of the mechanism, as extensive T cell cross-reactivity occurs between these viruses (Table 2)[53,142]. One report shows that a substantial part of the T cell response to the second dengue virus infection consisted of CD8 T cells having higher affinity to the previously encountered dengue virus than to the one causing the current infection [71]. Thus, a combination of enhanced viral load due to antibody-dependent immune enhancement plus cross-reactive low affinity T cell responses may help contribute to the severity of the disease.

T REGULATORY CELLS, VIRUSES AND IMMUNOPATHOLOGY

There is another component of the immune system which plays an important role in modulating viral load and immunopathology, regulatory T cells. If regulatory CD4+ T cells (Treg) are generated in the thymus they are referred to as natural Treg cells, but if they

differentiate from naïve T cells into Treg cells in peripheral tissue they are referred to as induced or adaptive Treg cells. Treg cells can suppress the function of many types of immune cells including $CD8^+$ and $CD4^+$ T cells, B cells, DC, NK and NKT cells [143]. Based on factors such as where these cells are generated (thymus or periphery) and which cytokines they release (IL10 and TGFbeta1) Treg cells are divided into different subtypes. The most studied type is the natural Treg cell, $CD4^+CD25^+Foxp3^+$, which make up to 5-10% of the peripheral $CD4^+$ T cell population [144,145] and play an important role in protecting the host against autoimmune diseases like colitis, gastritis and type 1 diabetes [144,146-149]. They have unique features such as expression of Foxp3 ("forkhead/ winged-helix family of transcription factors"), a nuclear transcription factor which plays a critical role in their development and function. They are also highly dependent on exogenous IL-2 for their survival in the periphery [144] and express a variety of accessory molecules including CTLA-4 and GITR which are involved in Treg cell activation, expansion, and suppression. These markers assist in differentiating Tregs from other effector T cells.

Treg cells have been intensively studied in autoimmunity and in the establishment of persistent pathogen infections [145,150-152]. The lack or dysfunction of Treg cells resulted in autoimmune diseases with severe pathology. Increased numbers of Treg cells and a loss of functional virus-specific effector T cells have been reported in persistent, but not in acute virus infection (e.g. HCV, HIV/FV and HSV). Depletion of the suppressive Treg cells during a persistent retroviral infection resulted in enhanced effector T cell function and reduced viral load[153,154]. On the other hand Treg cells can prevent extensive immunopathology during viral infection. Depletion of natural Treg cells, using anti-CD25 prior to infection enhanced anti-viral responses without any evidence of enhanced immunopathology if HSV-1 was injected into the footpad[155]. But, Treg depletion prior to corneal HSV-1 infection resulted in severe T cell-mediated tissue lesions[156]. These results suggest that Treg cells influence disease outcome during viral infection differently depending on the virus and on the site of infection. Do virus-induced Treg cells play any role modulating the balance between viral load and quality of T cell responses during heterologous immunity?

Heterologous immunity between IAV and LCMV is quite complicated. IAV-immune mice developed enhanced replication of LCMV and enhanced immune pathology in the lung, characterized by massive consolidating mononuclear pneumonia with bronchiolization instead of the mild lymphocytic pneumonitis observed during LCMV infection of naïve mice [74]. Memory T cell depletion studies indicated that the enhanced LCMV titers seem to be dependent on IAV-specific memory CD4 T cells (perhaps by regulating CD8 T cells), and the enhanced immune pathology is caused by cross-reactive IAV-specific CD8 T cell responses (Wlodarczyk M and Selin LK, unpublished). In this system T regulatory cells stimulated by IAV infection might be influencing the response to LCMV. Enhanced numbers of FoxP3+ CD25+ Treg phenotype CD4 cells are found in the lung and draining lymph nodes after IAV infection, and depletion of these cells with mAb to CD25 decreases the lung pathology induced by LCMV infection (Kraft A and Selin LK, unpublished).

PRECIPITATION OF AUTOIMMUNITY BY HETEROLOGOUS IMMUNITY

Autoimmune diseases can be initiated by infection of animals with viruses that encode T cell epitopes cross-reactive with self antigens, or infected with recombinant viruses that encode engineered self-epitopes. These have been reviewed elsewhere [4]. For instance, in an HSV-1-induced murine model of autoimmune keratitis T cells specific to an epitope expressed by the UL6 protein of HSV-1 cross-react with a corneal antigen[157]. In the mouse model of MS, both class I and II epitopes in myelin basic protein have been described to induce

autoimmune encephalitis (EAE)[4]. In humans HLA-DR3 predisposes to autoimmune diabetes, targeting the autoantigen glutamic acid decarboxylase. An HLA-DR3-restricted CD4 T cell response has been found to cross-react against epitopes with sequence similarity between an autoantigen and a CMV-encoded antigen[158].

The presence of cross-reactive responses that recognize self and pathogen antigens does not necessarily lead to autoimmunity. There needs to be a precipitating event such as a viral infection. Transgenic mice that express viral proteins as self antigens have been used to study the ability of viral infections to break tolerance[159-162]. LCMV infection induces a transient pancreatitis or encephalitis depending on the model in mice that express LCMV nucleoprotein in the pancreas or brain. A subsequent heterologous infection such as PV or VV, which have a cross-reactive epitope with LCMV, NP205, can induce a second wave of inflammation. In the insulitis model the LCMV infection could break tolerance to NP205, and induce insulitis, but the subsequent PV infection via activation of the cross-reactive T cells pushed the mouse into diabetes[162]. A similar phenomenon may be occurring in the induction of MS as mice infected with VV encoding the CNS protein PLP cleared the virus, but when challenged subsequently with murine CMV they developed CNS white matter lesions[163,164]. In humans, several viruses encode polyarginine sequences that can be recognized by CD4 T cells that have been isolated from the CNS of patients with MS[165]. Thus, heterologous immunity may play an important role in induction of autoimmune diseases such as diabetes or MS. The first virus that encodes a self-like epitope breaks tolerance and initiates an autoimmune process. The subsequent second virus amplifies the cross-reactive response pushing the self reactive T cell response above a certain threshold in the presence of a strong inflammatory response, shifting a controlled response into an overt autoimmune disease.

CONCLUSIONS

Heterologous immunity may be a common event in human infections and help explain the variability in disease outcome from asymptomatic to death in different individuals exposed to the same pathogens. It may occur (1) with diseases such as EBV-associated mononucleosis, where immunopathological features are more pronounced in young adults than in children[49,124]; (2) with diseases associated with marked variations in pathogenesis, such as dengue and HCV[70,94,166]; (3) with viruses that exist as related serotypes (e.g. dengue-, entero-, papillomaviruses (HPV)) or quasi-species with variations in T cell epitopes (e.g. HIV, HCV)[70,71,166]; and (4) with persistent infections, where constant stimulation by antigens may alter immunity to other pathogens[167]. Heterologous immunity may also be a factor in unusual responses to vaccination, particularly with vaccines designed to induce strong T cell responses in adults, who have highly developed memory pools, and in young children who frequently get multicomponent vaccines. Human vaccinations can sometimes lead to erythema nodosum, a form of panniculitis (inflammation of fat tissue), which we see as acute fatty necrosis (AFN) of abdominal fat pads under experimental conditions of heterologous immunity in mice [25,168]. The recent adenovirus vector-based vaccine for HIV is another example, where vaccinees with higher initial antibody titers to adenovirus responded poorly with less protective immunity to HIV, and it has been suggested that heterologous immunity may have affected this outcome[169]. Peptide vaccines for HCV currently being constructed include an epitope cross-reactive between HCV and IAV, and there is no telling how this will affect the immune response after live virus challenge. Data are now showing complications in some individuals vaccinated with HPV including panniculitis, where there are many closely related strains[125-130,170,171]. Many parents are now refusing to vaccinate their children due to perceived severe pathological side effects of vaccines. Some of the best anti-viral vaccines are with attenuated viruses, which can stimulate CD8 T cell responses[172], but insights on heterologous immunity are necessary for

the intelligent design of effective modern vaccines without unwanted side effects. We contend that heterologous immunity is the norm rather than the exception in human viral infections, and that manifestations of heterologous immunity may vary with the virus sequence and the host. We would predict that there are general principles of heterologous immunity which can be elucidated and ultimately manipulated. A better understanding of heterologous immunity and how it influences viral immunopathogenesis could lead to potential therapeutic interventions to circumvent severe disease that may contribute to the development of autoimmune syndromes and also to the intelligent design of vaccines.

Acknowledgments

This research was supported by US National Institute of Health (NIH) grants AI-49320, AI-42845, AI-054455, AI17672, AI46629, AI46578, AI49320, AR35506 DR-32520, an immunology training grant 5 T32 AI-07349-16 and DFG fellowship CO310/1-1. The contents of this publication are solely the responsibility of the authors and do not represent the official view of the NIH.

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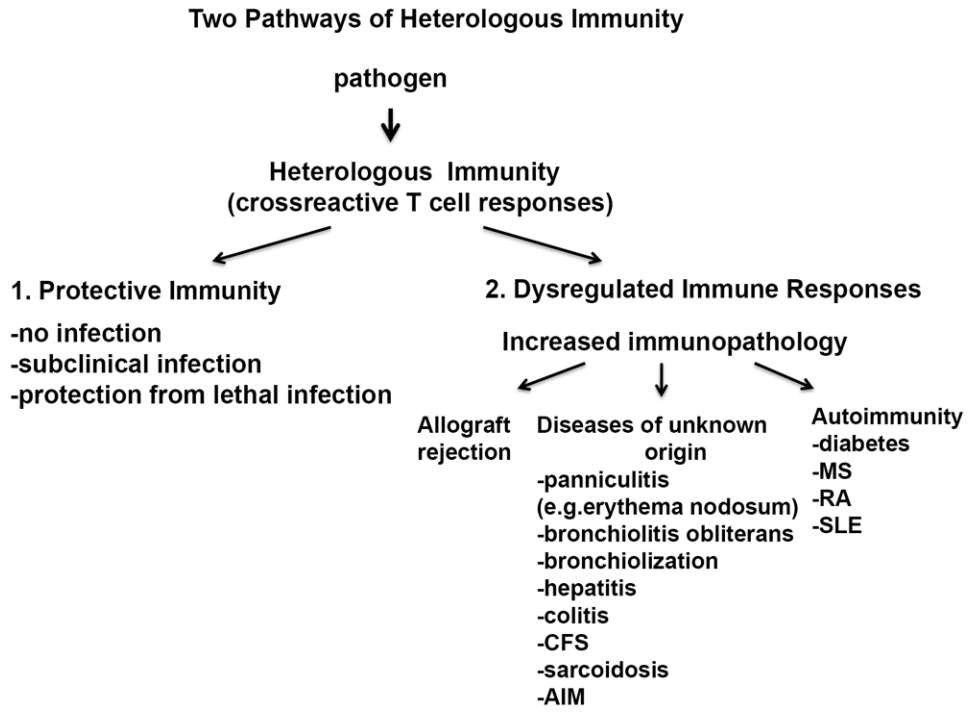

Figure 1. Two alternative pathways for disease outcome during heterologous infections

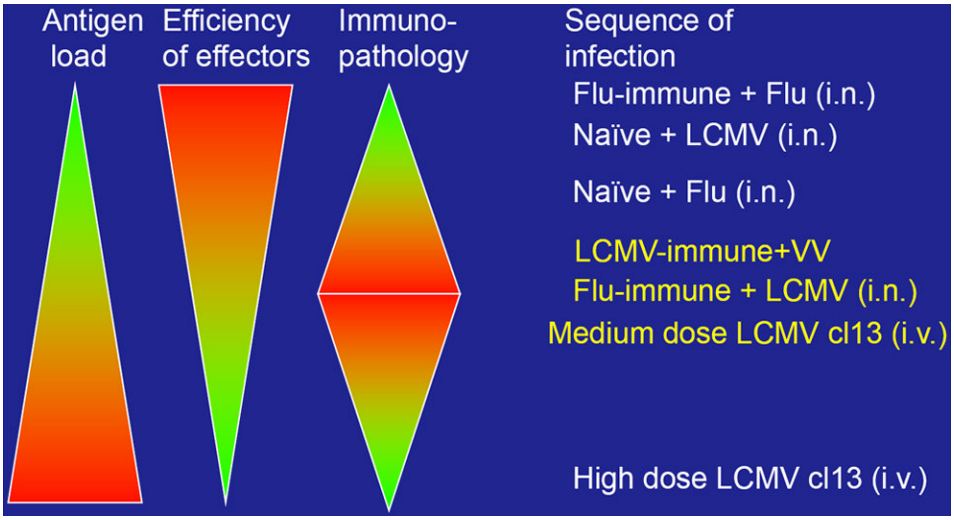

Figure 2. Disease outcome during a viral infection is dependent on a balance between antigen load and efficiency of effector function

The increase in pathology observed during heterologous immunity may be due to dysregulation of the tight balance between the antigen load during the infection and the efficiency of the effector T cells to clear the infection. Efficient memory T and B cell responses clear virus rapidly without inducing immunopathology, as in a secondary infection of Flu-immune or LCMV-immune mice. During a primary Flu, LCMV or PV response virus is cleared more slowly and can lead to some immunopathology. If the efficiency of the T cell response is diminished, by low affinity cross-reactive memory T cells, the virus is cleared slower giving time for recruitment of many memory effector T cells, which can induce immunopathology and collateral damage by producing IFNγ, TNFα, and FasL, as seen in LCMV-immune mice challenge with VV. If the viral load becomes extremely high very rapidly it can result in clonal exhaustion of the T cell response and little immunopathology, as is seen in high dose LCMV clone13 infection.

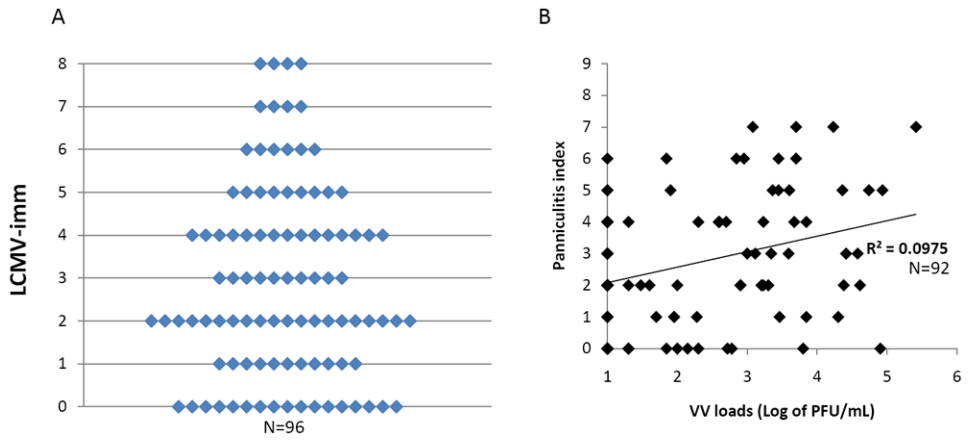

Figure 3. Independent of VV load, variable levels of panniculitis were found in genetically identical LCMV-immune mice during VV

LCMV-immune (*A* and *B*) mice were challenged with VV i.p. At day 6 of infection, the levels of panniculitis were recorded, and VV loads in fat-pads were assayed. *A*. Level of panniculitis varied between individual mice after VV challenge. No correlation between VV loads and levels of panniculitis were found in either LCMV-immune (*B*) mice (adapted from Nie et al. 2010.)

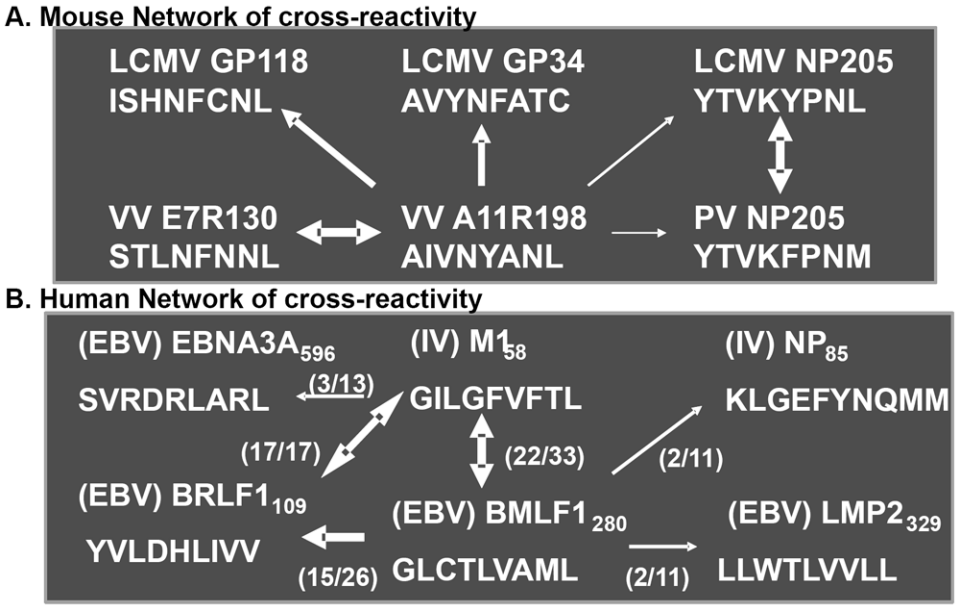

Figure 4. Summary of networks of cross-reactive patterns of in vitro and *in vivo* generated T cells stained with tetramers or tested in ICS assays in a large number of studies

A.) represents $H2K^b$-restricted responses in B6 mice. B.) shows HLA-A2-restricted responses in humans. Numbers in parentheses represents number of positive responses per individual (adapted from Cornberg et al. 2010).

Table 1

Sequence of Heterologous Virus Infections Influences Viral Clearance, Histopathology and cross-reactivity between viruses in mouse models.

Mouse				
Model	**Virus titer**	**Pathology**	**Cross-reactivity**	**Sequence similarity**
LCMV-immune + VV *versus* acute VV i.n. (ref. 75,76)	↓	• Dramatically decreased edema • Increased chronic mononuclear (lymphocytic) response • Decreased acute inflammatory (PMN) response • Early very prominent BALT (lymphocytic) • Bronchiolitis obliterans	VV a11r$_{198}$ A I V **N** Y A **N L** LCMV GP$_{118}$ I S H **N** F C **N L** VV a11r$_{198}$ **A** I V **N** Y **A** N L LCMV GP$_{34}$ **A** V Y **N** F **A** T C	3/8 aa 40% 3/8 aa 40%
LCMV-immune + VV *versus* acute VV i.p. (ref. 24,69,75)	↓	• Acute fatty necrosis	VV a11r$_{198}$ A I **V** N **Y** A **N L** LCMV NP$_{205}$ Y T **V** K **Y** P **N L**	4/8 aa 50%
MCMV-immune + VV *versus* acute VV i.n. (ref. 74)	↓	• Decreased edema • Increased chronic mononuclear (lymphocytic) response • Decreased acute inflammatory (PMN) response • Moderate BALT • Endothelial activation	Not done	
Flu-immune + VV *versus* acute VV i.n. (ref. 74)	↓	• Decreased edema • Increased chronic mononuclear (lymphocytic) response • Decreased acute inflammatory (PMN) response • Mild BALT • Mild consolidation	Not done	
LCMV-immune + PV *versus* acute PV i.p. (ref. 21, 24, Chen A. et al.)	↓	• Acute fatty necrosis	LCMV NP$_{205}$ **Y T V K** Y **P N** L PV NP$_{205}$ **Y T V K** F **P N** M	6/8 aa 75%
Flu-immune + LCMV *versus* acute LCMV i.n. (ref, 74, Wlodarczyk et al.)	↑	• Increased mononuclear infiltrates • Increased bronchiolization • increased BALT • Consolidation with mononuclear cells	IAV PA$_{224}$ **S** S L **E N** F R A **Y** V LCMV GP$_{276}$ **S** G V **E N** P G G **Y** C L IAV PB1$_{-703}$ S S **Y** R R P V G I LCMV GP$_{34}$ A V **Y** N F A T C	4/10 aa 40% 1/9 aa 10%

Mouse				
Model	**Virus titer**	**Pathology**	**Cross-reactivity**	**Sequence similarity**
Flu-immune + MCMV *versus* acute MCMV i.n. (ref. 74)		• Increased bronchiolization • increased BALT • Massive consolidation with lymphocyte and mononuclear cells	Not done	

Table 2

Sequence of Heterologous Virus Infections Influences Viral Clearance, Histopathology and cross-reactivity between viruses in humans.

Human				
Model	**Virus titer**	**Pathology**	**Cross-reactivity**	**Sequence similarity**
Flu-immune + EBV (ref. 49)	Not done	• infectious mononucleosis (IM)	Flu $M1_{58-66}$ **G** I L G F **V** F T L	3/9 aa
			EBV $BMLF1_{280}$ **G** L C T L **V** A M **L**	30%
			Flu $M1_{58-66}$ G I **L** G F V F T **L**	1/9 aa
			EBV $BRLF1_{109}$ Y V **L** D H L I V V	10%
Flu-immune + HCV (ref 70,92)	Not done	• Severe liver pathology during HCV infection	HCV $NS3_{1073}$ **C** I **N** **G** V **C** W **T** **V**	6/9 aa
			Flu NA_{231} **C** V **N** **G** S **C** F **T** **V**	70%
Coronavirus + HPV (ref. 56)	Not done	• Patient with cervical cancer present with ineffective T-cell responses	HPV 16E7 Y **M** **L** **D** L **Q** **P** **E** T	6/9 aa
			$NS2_{52-60}$ T **M** **L** **D** I **Q** **P** **E** D	70%
Different dengue serotypes (ref. 53,142,166)	↑	• increase dengue fever (DF) • severe dengue hemorrhagic fever (DHF) with thrombocytopenia, plasma leakage, bleeding and shock	High variety in cross-reactivity between $NS4b_{111-119}$, $NS4b_{181-189}$, $NS4a_{56-64}$ and $E_{211-219}$ within the four serotypes. With sequence similarity between 3/9 (30%) to 8/9 (90%) depending on the serotype.	
Flu-immune + HIV (ref 55)	Not done	Not done	HIV-1 p17 Gag_{77-85} S L Y N T I A V **L**	1/9 aa
			Flu $M1_{58-66}$ G I L G F V F T **L**	10%