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Review

The two faces of heterologous immunity: protection or immunopathology

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ABSTRACT

Immunity to previously encountered viruses can alter responses to unrelated pathogens. This phenomenon, which is known as heterologous immunity, has been well established in animal model systems. Heterologous immunity appears to be relatively common and may be beneficial by boosting protective responses. However, heterologous reactivity can also result in severe immunopathology. The key features that define heterologous immune modulation include alterations in the CD4$^+$ and CD8$^+$ T cell compartments and changes in viral dynamics and disease progression. In this review, we discuss recent advances and the current understanding of antiviral immunity in heterologous infections. The difficulties of studying these complex heterologous infections in humans are discussed, with special reference to the variations in HLA haplotypes and uncertainties about individuals' infection history. Despite these limitations, epidemiological analyses in humans and the data from mouse models of coinfection can be applied toward advancing the design of therapeutics and vaccination strategies. *J. Leukoc. Biol.* 95: 405–416; 2014.

Introduction

The most important consequence of adaptive immunity is the establishment of immunological memory. Memory responses to previously encountered pathogens can alter the immune response and the course of infection of subsequent, unrelated pathogen challenge by a process known as heterologous immunity. This process can lead to a protective immunity or immunopathology [1]. Heterologous immunity appears to be a common phenomenon among closely related pathogens, for example, different strains of influenza or DENV, or among different members of the same virus group, such as hantaviruses, arenaviruses, and flaviviruses [2]. However, it also occurs among unrelated pathogens, including parasites, protozoa, bacteria, and viruses [3]. Infections with distinct pathogens can occur concurrently or sequentially. Infections with different strains of the same pathogen or distinct pathogens are often classified as "coinfections" (when the two infections occur at the same time or within a brief window prior to the establishment of the first strain in the host) or "superinfections" (where a second strain enters after the first strain is well-established). In this review, we mainly discuss the mechanisms of heterologous immunity between unrelated pathogens, with a focus on viruses.

Heterologous immunity differs from classic homologous immunity in several key aspects [4]. Adaptive immunity is believed to be highly specific, meaning that entirely distinct responses can be mounted against different infections. However, the data from animal and human studies have provided convincing evidence that after infection with unrelated pathogens, the host's immune responses to subsequent infections is modulated. For example, in certain circumstances, immunopathologic features are more pronounced in young adults than in children [5, 6], suggesting that prior infections in young adults that have not occurred yet in most children may alter the immune environment in the older patients. Alternatively, it is possible that primary immunization in children programs a distinct type of memory response that is less pathogenic upon challenge. Similarly, considerable interpatient variability in pathology occurs among individuals suffering from certain infections that exist in nature as closely related serotypes (e.g., DENV and PV) [7–9], as well as in those infected with viruses that undergo high rates of mutations in T cell epitope hot spots, including HCV and HIV [7, 9, 10]. Another situation where heterologous immunity is commonly observed is in persistently infected individuals who experience constant, low-level antigenic stimulation that alters their immunity to other pathogens [11].

Multiple immune mechanisms underlie the broad category of heterologous immunity. In this review, we discuss our current understanding of the immune parameters affected by heterologous infection and host/pathogen-rela-

Abbreviations: BCG=bacillus Calmette-Guérin, DENV=Dengue virus, DHF=Dengue hemorrhagic fever, EM=effector memory, HB(C)V=hepatitis B(C) virus, HSV=Herpes simplex virus, IAV=influenza A virus, LCMV=lymphocytic choriomeningitis virus, LM=*Listeria monocytogenes*, NP=nucleoprotein, PD1=programmed cell death 1, PV=papillomavirus, RSV=respiratory syncytial virus, Treg=regulatory T cell, Vβ=variable region β, VV=vaccinia virus

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tionship factors that can explain variable outcomes ranging from resolution with little disease to severe immunopathology. Understanding such issues may help us control and perhaps prevent tissue-damaging viral infections and improve clinical outcome.

HETEROLOGOUS IMMUNITY IN ANIMAL MODELS

Modulation of innate responses

Heterologous immunity can polarize the immune response to incoming pathogens by altering the innate immune environment. Simultaneous coinfections may result in increased viral loads and enhanced immunopathology (unpublished data; **Fig. 1A**). Furthermore, a primary infection or insult results in the maturation of APCs, resulting in enhanced antigen presentation (Fig. 1B). In one study, a modified heat-labile bacterial toxin, administered in mice, altered the microenvironment, such that it improved the immune response to subsequent infection with RSV, influenza virus, or the fungus *Cryptococcus neoformans*. This protective immunity was partially T cell- and B cell-independent [12]. Cytokines produced by activated APCs stimulate T cells to differentiate into polarized subsets (Fig. 1C) and influence the type of immune response that is generated to a second unrelated pathogen. These pathways can also lead to immunopathology (discussed elsewhere in the review). Activation of innate responses by various pathogens increases HIV replication and may lead to faster progression of HIV in coinfected individuals [13]. These examples demonstrate that the suppression or enhancement of immune responses by concurrent infections can have protective or pathogenic effects, depending on the specific features of the individual pathogens and the time interval between the two infections.

The polarizing effect of heterologous infections appears to be driven by the cytokine milieu elicited by the active infection, which may modulate subsequent cellular responses. Coinfections can result in simultaneous Type 1 and Type 2 responses in the same host. In a model of thoracic filarial and foot pad *Leishmania major* coinfection, Type 2 immune responses elicited by filaria were unaffected by *L. major*. However, Type 1 responses against *L. major* were enhanced, and pathology was diminished in coinfected animals [14].

A cascade of inflammatory responses occurs after PRRs are activated by PAMPs. These responses include the secretion of cytokines and chemokines. In this manner, ongoing infections may serve as adjuvants for subsequent infections by inducing costimulatory molecules and receptors that enhance APC function and recruitment. LCMV infection results in activation of Kupffer cells, recruitment of NK cells and T cells, and increased production of TNF-α, IFN-γ, and IFN-α/β, supporting the clearance of HBV in coinfected animals [15]. In response to specific microbial infection, DCs influence the development

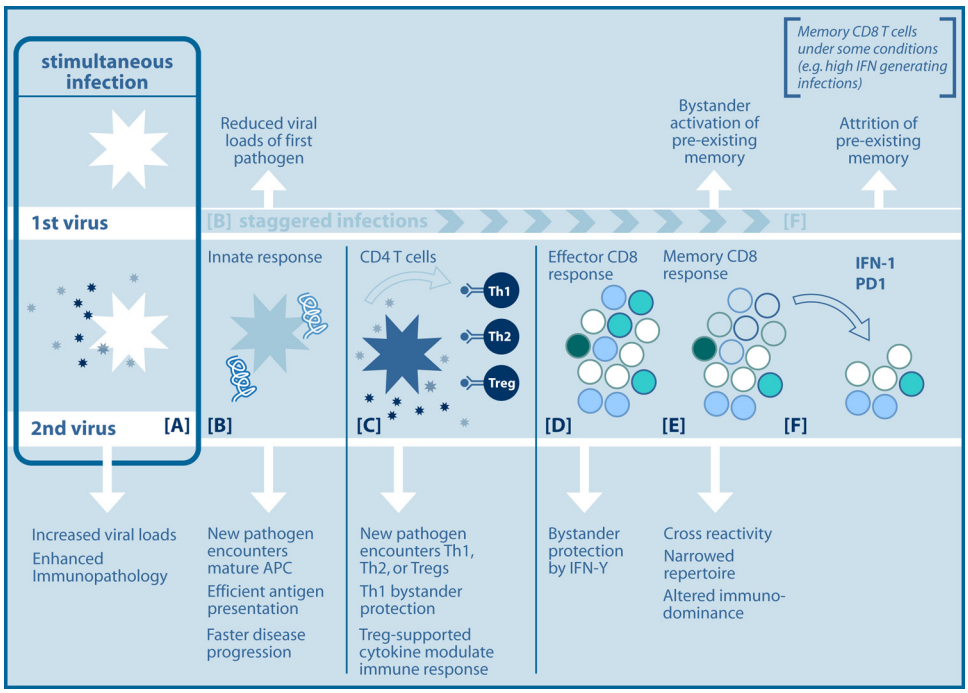

Figure 1. Outcome of infection following heterologous viral infections is dependent on the features of the immune response to a simultaneously or previously encountered pathogen. As an example, for simultaneous or staggered infections with two distinct viruses: (A) dimultaneous infection can enhance immunopathology, potentially as a result of the immune responses to both viruses, reaching peak levels at the same time. (B) Prior infection can result in activation of APC so that a subsequent infection encounters mature APC, resulting in more efficient antigen presentation and faster disease progression and resolution. Also, the new, incoming pathogen creates a strong antiviral state that might result in reduced viral loads of first pathogen. (C) Upon activation, APCs secrete cytokines that result in Th subset differentiation so that a heterologous, incoming pathogen encounters an already polarized immune response. Encounter with a Th1 type of immune response can provide bystander protection against some pathogens or enhanced immunopathology, whereas Tregs can result in suppression of immune responses to incoming pathogen, which may be protective or pathogenic. (D) Subsequent heterologous infection in the presence of an ongoing effector $CD8^+$ T cell response can result in bystander protection from IFN-γ production. (E) When a new virus infects the host with an established memory $CD8^+$ T cell pool, the outcome may be cross-reactivity (can be protective or pathogenic), which can lead to recruitment of a cross-reactive T cell pool, resulting in a narrowed repertoire and altered immunodominance hierarchies. (F) Reciprocally, the incoming pathogen can result in bystander activation or attrition (Type 1 IFN-dependent) of pre-existing memory CD8 T cells. The specific outcome of heterologous viral infection depends on the type and sequence of viruses.

of naive T cells into polarized Th1 cells, Th2 cells, or Tregs [16, 17].

Latent viruses can also confer heterologous protection. Mice latently infected with murine γ herpes virus 68 or murine CMV are resistant to infection with the bacterial pathogens LM and *Yersinia pestis*. This latency-induced protection is not antigen-specific; it involves prolonged production of the antiviral effector cytokine IFN-γ and systemic activation of macrophages [11]. However, prolonged infection with CMV has been correlated with greater susceptibility to heterologous infections in elderly animals and humans [18], possibly through alterations in the naive repertoire to the infecting pathogen.

CD4 T cell responses

The CD4 T cell plays a crucial role in protecting against viral infection and in the development of memory B cells and CD8 T cells. CD4 T cell help for CD8 T cells is important for primary and/or memory responses to some bacterial and viral infections [19]. CD4 T cells can be protective or exacerbate the course of infection under conditions of heterologous immunity. For instance, when CD8 and CD4 subsets from LCMV immune mice were adoptively transferred into naive mice, they resulted in heterologous immunity upon subsequent infections with Pichinde virus or VV [20]. However, the specific functions of heterologous CD4 T cell immunity have not been well-characterized thus far.

$CD4^+$ T cell responses, which were once thought to be pathogen-specific, clearly can protect against diverse challenges. In one study, mice immunized against the BCG strain of *Mycobacterium bovis* (a vaccine against tuberculosis) were also protected against challenge with VV, a poxvirus. This immunity was mediated by memory $CD4^+$ T cells and in particular, by IFN-γ [3]. These observations were interpreted as true cross-reactive heterologous immunity and not bystander activation of noncross-reactive cells by the innate immune system; however, the study did not determine the cross-reactive specificities.

Tregs are an important subset of $CD4^+$ T cells (Fig. 1C). During chronic infections, the outcome may be influenced by the activity of one or more types of Tregs.

Unlike conventional CD4 and CD8 T cells, the majority of expanded Tregs is not antigen-specific after viral infection. Not surprisingly then, although Tregs have not been evaluated extensively in coinfection models, it appears that induction of Tregs by one pathogen can lead to suppression of bystander responses to subsequent pathogen challenge. Tregs modulate pathogenic and protective immune responses to infection in the host and may be important mediators that influence the outcome of coinfections [21, 22]. Tregs also can inhibit inflammatory reactions associated with chronic viral infections [23], and similar responses can limit the magnitude of protective immunity to an acute viral infection or that induced by a vaccine [24, 25].

The activity of Tregs varies, depending on the virus and site of infection. For example, depleting natural Tregs using anti-CD25 prior to infection enhances antiviral responses with virtually no enhanced immunopathology in a footpad model of HSV-1 infection [24]. In contrast, Treg depletion prior to corneal HSV-1 infection results in severe T cell-mediated tissue lesions [26]. These reports indicate that tissues may require different levels of protection from immune-mediated pathology. Other anti-inflammatory mechanisms, such as galectin-9 and T cell Ig domain and mucin domain 3 signaling, can also potentially modulate the virus-specific responses [27] to subsequent infections. For example, following viral infection, the host up-regulates immunoinhibitory receptors on CD8 T cells to limit immunopathology [27]. Thus, subsequent infections at this stage may encounter an immunosuppressive state that may limit the host's ability to clear the second infection but may also protect the host from associated immunopathology [28], although these pathways have been investigted less thoroughly than those of Tregs.

Cross-reactive T cells

T cells are characterized by the presence of heterodimeric receptors. The number of antigenic peptides that can be perceived by the immune system far exceeds that actual number of unique TCRs in an individual at any given time [29]. TCR cross-reactivity (also known as alloreactivity) has been suggested as a primary means of increasing the effective size of the TCR repertoire. Several mechanisms have been proposed for TCR cross-reactivity. First, it may be achieved through changes in the conformation of the flexible loops within the CDR3 region. Second, TCRs may use two very different binding modes to recognize two unrelated ligands. Other proposed mechanisms of TCR cross-reactivity include molecular mimicry [30], and also, the same TCR can recognize different peptide-MHC complexes, resulting in cross-reactivity.

Although cross-reactivity can be caused by amino acid sequence similarity between epitopes, several exceptions to this have been observed. For example, the immunodominant HLA-A*0201-restricted IAV epitope M158-66 and the major EBV-specific epitope BMLF1280-288 demonstrate cross-reactivity to each other but share only 3 of 9 aa. Interestingly, in several studies of heterologous viral infections in C57BL/6 mice, which have two Class I MHC molecules—D^b and K^b—more cross-reactivity is observed with K^b-restricted epitopes than with D^b-restricted epitopes [1]. A likely reason for this is that K^b presents an 8-aa peptide epitope instead of the 9-aa peptide, usually presented by D^b. Shorter peptides are more likely to result in cross-reactivity, with an increased chance for relevant molecular mimicry [1]. Structural studies have shown that K^b molecules have a deep pocket, allowing a smaller portion of the peptide to protrude and be available to engage the TCR, reducing the specificity of those interactions. Thus, variation among the binding features of MHC molecules may also contribute to heterologous immunity.

The presence of cross-reactive cells in infections has immunological consequences, resulting in substantial differences in the responses of a naive host compared with that of an immunologically experienced host [31–33]. Cross-reactivity can narrow an otherwise oligoclonal TCR repertoire, changing the epitope-specific T cell hierarchies and alter immune outcomes [34]. Most likely resulting from a combi-

nation of these mechanisms, cross-reactive CD8 T cells have been detected in multiple heterologous viral infections [35]. As a result of their higher precursor numbers during heterologous challenge, cross-reactive memory cells are induced to expand and activate rapidly [36]. The role of these T cells varies from protective [37, 38] to pathogenic [39] (Fig. 1D and E). The outcome appears to depend, in part, on whether IFN-γ plays a protective or pathogenic role under the specific conditions in which the T cells are activated, as increased IFN-γ production is one of the major consequences of a cross-reactive response [31].

Narrowed TCR repertoire and viral escape

After an infection, a diverse repertoire of T cells is established to a variety of immunodominant epitopes [40] whose TCRs recognize antigens presented by MHC molecules [41]. CD8 T cell responses to a single epitope often use different Vβ families, and even within cells using the same dominant Vβ, many different clonotypes are present [42–44]. A diverse TCR repertoire is important, as it may improve pathogen control and reduce the possibility of immune evasion by T cell escape variants [45, 46]. For example, in LCMV or PV infections of naive mice, the responding CD8 T cells constitute a very diverse repertoire with many distinct clonotypes [47]. When LCMV immune mice are subjected to homologous challenge (LCMV-LCMV), the repertoire of the responding CD8 T cells is largely conserved. However, when LCMV immune mice are challenged with the heterologous PV, the responding repertoire is narrowed significantly compared with that of naive mice infected with PV or the memory LCMV repertoire [47].

Additional examples of the narrowed TCR repertoire after heterologous challenge include secondary infections with HIV [46], HCV [45], and CMV [48]. Similarly, the Vβ TCR repertoire of some IAV epitope-specific memory CD8 T cells was altered after LCMV infection [2, 49]. Although the consequences are clear, we do not understand the mechanisms that drive this narrowing. A narrowed repertoire with a high-affinity clone in some circumstances may be beneficial during the early phase of infection [50], but it may also drive the increased generation of escape variants. Although repertoire narrowing might be expected to occur in most cases of cross-reactivity [47, 51], under conditions in which the baseline repertoire is already narrow, a heterologous challenge may force the recruitment of T cells that are not dominant clones but are expanded by the alternative epitope. Thus, heterologous infections not only have diverse effects on the magnitude of the cellular response but also at the level of the repertoire. These effects include: (1) the narrowing of the repertoire as a result of cross-reactivity, and (2) if the normal naive repertoire is narrow, heterologous infection can result in diversification by recruitment of nondominant clones. As an example of this, in a model with persistent CMV infection, followed by LM challenge, it was shown that persistent viral infection alters the naive CD8 T cell repertoire toward LM to an extent where there was no overlap between the LM responses in naive and persistently infected animals.

Altered T cell immunodominance hierarchies and diminished CD8 T cell responses to superinfecting viruses

Downstream of these effects on TCR repertoire, heterologous infection can alter the hierarchy of CD8-T cell responses. Most infectious challenges in the mouse model result in predictable hierarchies of CD8 T cell responses specific for the different peptide-MHC epitopes derived from the pathogen. These epitope-specific responses that form the immunodominance hierarchy may be dominant, codominant, or subdominant [52].

Cross-reactivity can alter the normal immunodominance hierarchy. In mice, a heterologous PV infection, followed by LCMV infection, results in the otherwise subdominant NP (205–212) epitope becoming dominant. This is most easily explained by cross-reactivity and could be the result of the presence of a higher precursor frequency of NP (205–212)-specific T cells and the fact that memory CD8 T cells respond more vigorously to antigen than do naive T cells. Thus, the original repertoire elicited by one infection can be altered drastically by a new, unrelated pathogen [34]. These examples demonstrate the variability that can result from challenging hosts with distinct immunological histories [53]. However, cross-reactivity by itself may not be the sole factor responsible for altered hierarchies.

Immunodominance results from the interplay of several complex factors, including the efficiency of peptide processing, the affinity of the peptide for the presenting MHC molecule, the overall number of presented peptide-MHC complexes, the availability of a TCR repertoire that recognizes the peptide-MHC complex, and immunodomination, where T cells specific for certain immunodominant epitopes suppress responses to other epitopes [31, 54]. Persistent infections may alter the inflammatory environment sufficiently to affect many of these features, which would result in altered dominance patterns. In latent murine CMV infection, a pronounced alteration in the T cell compartment occurs that is consistent with impaired, naive T cell function. This results in significantly weaker CD8 T cell responses to superinfection with influenza, human herpes virus I and West Nile virus, even 16 months after the original murine CMV infection [55].

Attrition of pre-existing CD8 T cells

Memory CD8 T cells are an important component of protective immunity to viral infections. Following the effector and death phases, a memory CD8 T cell population is established and maintained in the absence of antigen [56]. However, substantial evidence has shown that under some conditions of heterologous challenge, pre-existing immunity erodes, including the number of memory CD8 T cells [57]. Although cross-reactivity can induce a relative increase in the response to a challenge virus, when a secondary infection with strong Type 1 IFN-inducing virus occurs (Fig. 1F), the effect observed is attrition of previously existing memory T cell populations early during infection, in association with the IFN response. In this situation, heterologous immunity compromises the memory response against an antigen encountered previously.

This finding raises the disturbing possibility that vaccines that generate sufficient quantities of memory $CD8^+$ T cells specific for an agent of interest could have catastrophic consequences for the host by displacing memory $CD8^+$ T cells specific for previous infections [58] and vice versa, meaning a new infection could deplete memory cells generated by previous successful vaccination. However, these effects may be limited to specific infection combinations.

With the use of a heterologous prime-boost vaccination strategy [58], with LCMV and VSV, investigators reported contrasting results. In these experiments, the size of the memory CD8 T cell compartment doubled to accommodate the accumulation of newly generated EM $CD8^+$ T cells. Importantly, the numbers of other cell types, such as CD4 T cells, B cells, and naive CD8 T cells, remained unchanged. One difference in these two contrasting reports [57, 58] is that the studies reporting attrition restricted their analyses to secondary lymphoid tissue after primary infections, which result predominantly in the generation of central memory CD8 T cells. Heterologous prime-boost vaccination, on the other hand, preferentially generates EM CD8 T cells that are present within the nonlymphoid compartment [58]. Furthermore, the concept of profound attrition of memory CD8 T cells on exposure to new pathogens may have depended on the ability of those infections to stimulate Type I IFNs, which can induce high levels of apoptosis in memory T cells early [57]. Thus, vaccination strategies that rely on the generation of Type I IFN responses should consider this issue.

Two additional studies provide insights into this question. In a model of LM infection and subsequent vaccination with modified VV Ankara, it was shown that there was only a slight loss of LM-specific memory CD8 T cells (consistent with findings in ref. [58]); however, pre-existing immunity to LM was lost as a result of alterations in the EM population [59]. Another group found that after infection with LM, adoptively transferred transgenic T cells that were not specific for LM antigens were depleted as a result of a lack of cognate antigen recognition [60]. These data support the observations made by Welsh and Selin [57].

The stable survival of memory CD8 T cells in mice can be disrupted by subsequent heterologous viral or bacterial infections in which the number of noncross-reactive memory CD8 T cells decreases via cytokine-dependent mechanisms [61–66]. Broadly, there are two models of attrition: the passive-attrition model, where established memory T cells are replaced by newly formed memory T cells as a result of competition for limited space. In the active-attrition model, pre-existing memory cells undergo direct apoptotic attrition [31, 49, 60]. The IFN-mediated deletion of memory T cells mentioned above is an example of the active-attrition model. Additionally, the inhibitory molecule PD1, which was identified initially as a T cell exhaustion marker [67], also plays a role in the depletion of autoreactive CD8 T cells in mice [68]. It was also shown that PD1 is involved in the attrition of CMV-specific memory CD8 T cells after acute HBV infection (Fig. 1F).

The emerging field of predicting immune outcomes by mathematical modeling has suggested that if several infections occur simultaneously or within close intervals, then a pathogen that might normally be cleared could become persistent and held at low levels by a suboptimal immune response. In this scenario, infections are readily controlled initially, and the total size of the antigen-specific CTL memory pool is relatively small. When the host experiences new infections, viral load increases, as does the total CD8 T cell memory pool. However, viral control is degraded with each subsequent infection, so when the number of allowed infections is surpassed, the efficiency of viral control is lost. Thus, following each new infection, there is a progressive deterioration in the memory CD8 T cell compartment and an eventual failure to control new challenges.

Immunopathology

Whether a series of viral infections results in severe and prolonged lesions or can be resolved with minimal tissue damage depends on numerous factors. Two important contributors are the specific sequence of infections [39] and the route of infection. Consistent with this, when LCMV immune mice are infected with VV, they suffer a severe outcome [69], which neither of the viruses alone could induce [69, 70]. Interestingly, this pathology is not associated directly with viral load.

In naive animals infected with VV, there was robust infiltration of polymorphonuclear leukocytes and mononuclear leukocytes into the interstitium, peribronchial areas, and perivascular areas of the lung. However, VV-infected, LCMV immune mice demonstrate the induction of bronchus-associated lymphoid tissue [37] in the lungs, which is infiltrated with LCMV-specific CD8 T cells. The presence of these activated T cells contributes to the obstruction of the bronchiole (bronchiolitis obliterans). This immunopathology is dependent on a potent Th1 response, as the lesions in the fat and lungs were dependent on the production of IFN-γ [70].

Similarly, LCMV-infected, IAV immune mice have altered immune kinetics compared with that of naive animals, with increased numbers and activation states of CD8 T cells. Under specific infection conditions, IAV-induced memory CD8 and/or CD4 T cells play a role in enhancing lung immunopathology and can even contribute to increased viral titers [71].

In some instances, immunopathology is regulated by factors other than "traditional" immune mediators. For example, influenza infection triggers a generalized stress response that leads to a sustained increase in serum glucocorticoid levels, resulting in a systemic suppression of immune responses. This immunosuppressed state prevents immunopathology in mice coinfected with influenza and LM but at the same time, results in increased bacterial burdens [72]. Alterations in gut microbiota and mucosal immunity by previous viral infections can exacerbate subsequent pathology. For example, underlying CMV infection alters mucosal immunity, which is associated with an increased tendency of colitis to develop in CMV-infected hosts [73].

Autoimmunity

Heterologous viral infections can alter the course of an autoimmune disease. For example, upon LCMV infection, Type 1 diabetes is induced in transgenic mice that express the NP of

LCMV in their pancreatic β cells. Subsequent infection of these animals with PV accelerates the course of autoimmune disease [74].

In another transgenic mouse that expresses the NP of LCMV as self in oligodendrocytes, a first infection with LCMV resulted in the infection of the peripheral tissue but not the CNS. However, after the virus cleared, MHC Class I and II molecules were expressed in the CNS, and a second infection with LCMV resulted in more damaging pathology of the CNS [75]. These findings demonstrate that although sequential viral infections do not initiate the onset of autoimmune disease, they can clearly alter the course of the disease, from a mild to a more severe consequence.

HETEROLOGOUS IMMUNITY IN HUMANS

The role of heterologous immunity during infection is not limited to the murine system. In humans, most infections do not encounter immunologically naive hosts. As it is not clinically feasible to alter a host's history of prior infection, understanding heterologous immune responses could have important therapeutic implications. In humans, heterologous immunity occurs in viral infections, such as IAV, EBV, HCV, and DENV, with the consequences varying from protective immunity to enhanced immunopathology [6, 7, 76–79]. The human body harbors a variety of microflora, including multiple viruses. The immune system shapes the within-host composition of these resident viruses, and the viruses, in turn, impact host immunity and affect human health [80]. In the majority of the well-studied human infections, heterologous immunity leads to enhanced immunopathology. Below, we describe some important clinical studies from the literature.

Immunopathology/cross-reactivity

EBV and IAV. T cells specific for the HLA-A*0201-restricted immune-dominant EBV epitope, BMLF1280, were shown to cross-react with the HLA-A*0201-restricted IAV epitope M158.13 [34]. This cross-reactivity was detectable in some cases of acute mononucleosis; thus, a subset of the IAV epitope M158-specific TCR repertoire that was cross-reactive with the EBV epitope was activated to proliferate. The cross-reactive T cells expanded more rapidly than the EBV-specific, naive T cells following infection. However, the cross-reactive cells that dominate this response have a low affinity for the virus antigen-expressing cells and cannot control the infection adequately. Despite the apparent advantage of a pre-existing immune repertoire, the net result is immunopathology [81]. This effect may underlie the observation that some infections, such as measles, mumps, chickenpox, and EBV, have much more severe consequences in teenagers and young adults than they do in young children.

HCV and IAV. The pathogenesis of HCV is extremely variable in humans, from asymptomatic infection to a highly pathogenic disease progression. When immune control fails, HCV can establish persistent infections [81]. Heterologous immunity has been shown to play a role in this variability [49], as HCV encodes a HLA-A2-restricted epitope, NS31073-1081, which shares 6 of 8 aa with the influenza epitope (NA231 to 239), and T cells from influenza immune individuals with no previous history of HCV infection responded to the HCV epitope in vitro [77]. Many people may be partially immune to HCV as a consequence of this cross-reactivity. In one study, two patients developed rapid necrotizing hepatitis upon HCV infection, and both had a narrow, cross-reactive T cell response between influenza and HCV [7]. These data suggest that the nature of the cross-reactive T cell response may vary among individuals with similar immunological histories and may contribute to their clinical outcome.

DENV. Heterologous immunity is a common phenomenon in DENV infections. Secondary DENV infection causes DHF, which is characterized by sudden vascular permeability generated by cytokines released when T cells attack dengue-infected cells [82]. Several underlying mechanisms have been proposed for DHF and broadly categorized by humoral or cell-mediated effectors. One proposal is that cross-reactive, but non-neutralizing, antibodies opsonize the virus, thereby facilitating its uptake by macrophages, where productive viral replication occurs [82]. Another explanation is that CD8 T cells that are generated following secondary infection have a higher avidity to epitopes present in the DENV serotype encountered previously, suggesting that less-effective, cross-reactive memory CD8 T cells preferentially expanded over T cells with greater avidity to the new serotype that is causing present infection [82]. In this model, these lower avidity cross-reactive T cells may mediate directly a more severe disease outcome, including hemorrhagic fever.

CD8 T cell attrition in humans

The question of whether the development of memory cells to a new infection indeed impairs the pre-existing memory T cell population is of special interest in humans, as they encounter many different antigens during their lifespan. However, as a result of limitations in available reagents and the difficulty in tracking primary infections in humans, very little data addressing this question are available.

In a study of CMV-seronegative renal transplant recipients who experienced a primary CMV infection as a result of receiving a kidney transplant derived from a CMV-seropositive donor, the absolute numbers of pre-existing memory T cells in peripheral blood were not affected by the appearance of $CD8^+$ T cells specific for the new CMV infection. Instead, the $CD8^+$ T cell pool expanded to make space for the newly generated, virus-specific memory cells [83].

During acute EBV infection, pre-existing CMV- and influenza-specific memory $CD8^+$ T cells showed signs of bystander activation. The numbers of CMV- and influenza-specific T cells were comparable before and after acute EBV infection [84]. These data are consistent with the idea that in humans, a robust $CD8^+$ T cell response creates a new memory $CD8^+$ T cell niche without substantially depleting pre-existing memory for heterologous infections. However, studies involving coinfections with CMV and hepatitis virus have produced opposing results: PD1 is important in the attrition of CMV-specific $CD8^+$ T cells during an acute infection of HBV [85].

Similar to observations in the mouse model, some controversy persists about the extent of T cell attrition after heterologous challenge. Focusing on the stability of a single epitope-specific pool, one study reported a half-life of 8–16 years for VV-specific $CD8^+$ T cells [86]. However, VV-specific $CD8^+$ T cells were not detectable in 50% of the subjects who had been vaccinated >20 years earlier, suggesting a significant loss of human memory T cells in a subset of individuals.

Bystander activation

Bystander activation of T cells occurs in the absence of cognate antigen. Several hypotheses have been advanced to explain the underlying mechanisms of this kind of CD8 T cell activation. It is generally believed that most of these cells are activated by nonspecific mechanisms, including cytokine-driven activation. IFN and IL-15 can mediate bystander activation in mice [62, 87, 88]. However, the definitive mechanisms of bystander T cell activation in humans still remain to be determined.

Bystander activation of T cells appears to be a common occurrence during acute infection in humans, and not all activated CD8 T cells observed in peripheral blood during acute viral infection are virus-specific, as shown in HIV [89] and hepatitis B infections [90]. During acute EBV infection, preexisting CMV- and IAV-specific memory CD8 T cells showed signs of bystander activation, including up-regulation of granzyme B [84].

Herpes virus-specific CD8 T cells also display an increased production of the antiviral cytokine IFN-γ during the acute phase of heterologous viral infection. In a clinical study of herpes viruses, such as EBV and CMV, the number of activated virus-specific T cells and total T cells was analyzed in patients with an infection of acute hepatitis B, DENV, influenza, or adenovirus. All acute viral infections triggered the activation and expansion of herpes virus–specific cells, which in turn, contributed to the heterologous, antiviral T cell response. This finding led to the question: what is the biologic significance of herpes virus-specific $CD8^+$ T cell activation during acute heterologous viral infections? One possibility is that the activation/proliferation state of herpes virus-specific $CD8^+$ T cells counteracts the attrition exerted by the expansion of the $CD8^+$ T cells specific to the acute infection [90]. Therefore, the activation of the herpes virus-specific $CD8^+$ T cells might prevent the reactivation of the herpes viruses.

Variability of antiviral responses in humans

Many factors limit the direct translation of results from preclinical immunologic studies into clinical trials. In mouse studies, well-planned experiments are conducted with a particular dose of virus and a defined route of infection. However, in nature, such parameters are variable. Even DNA viruses, which have much less variability than RNA viruses, exhibit significant variation among strains that circulate in human populations, and infection with one strain does not necessarily prevent superinfection with another strain of the same virus, as in CMV infections [91]. Also, very little is known about the influence of the route of infection in humans. Moreover, humans exhibit a greater number of genetic differences than do mice that potentially affect antigen presentation and T cell activation. Whereas humans experience frequent encounters with multiple viruses and other infectious agents, in experimental settings, every effort is used to protect the mice from exposure to microbial agents [92].

Humans are constantly exposed to different viruses; therefore, they most likely possess memory $CD8^+$ T cells that recognize determinants from viruses that have not been encountered previously, particularly those with a large coding capacity. Furthermore, if the T cell memory pool contains T cells that cross-react with an epitope of a newly encountered virus, then those T cells could proliferate and dominate the subsequent response. It may be very difficult to predict whether an epitope is normally immunodominant or if its immunodominance is a result of cross-reactivity with a previously encountered pathogen.

Some studies have suggested that the cross-reactivity between T cells and viral epitopes has less potential than expected. For example, one study involving human subjects failed to detect recognition of any of the >6000 VV-encoded peptides by peripheral blood leukocytes from patients prior to immunization [93]. In another study, PBMCs from six of 10 patients, who were CMV-seronegative, failed to recognize any of the 13,000 CMV-encoded peptides tested [94]. Furthermore, the two seronegative patients showed very limited recognition of HSV-2 peptides [95]. However, these studies were performed using 15-mer peptides, which may not be the most optimal system for stimulating and detecting cross-reactive CD8 T cells that generally recognize shorter peptides. Thus, further research is warranted to establish the extent of cross-recognition in humans.

Some convincing studies have shown that $CD4^+$ T cells can be activated and gain a memory phenotype as a result of cross-reactive antigens. Unlike $CD8^+$ T cells, which are biased toward interactions with endogenously processed antigens, $CD4^+$ T cells tend to interact with external antigens. Therefore, they are thought to be more responsive to environmentally acquired anitgens [96]. Consistent with this hypothesis, HIV-1-reactive T cell clones can respond to several non-HIV-derived microbial peptides [96]. Similarly, while people vaccinated with the influenza vaccine have expansions in influenza HA-specific T cell populations, T cells responsive to a similar peptide sequence from the human commensal bacteria *Finegoldia magna* were stimulated to proliferate. This finding suggests that exposure to one microbe can stimulate immune cells specific for an entirely unrelated microbe, most likely via cross-reactivity among the peptides [97].

IMPLICATIONS OF HETEROLOGOUS IMMUNITY IN THERAPEUTICS AND PREVENTION OF INFECTIONS

How can the knowledge obtained from mouse models of heterologous immunity be used to manage human immunity and immunopathology to heterologous viral infections? It is now clear from mouse models and clinical studies that prior infection or concurrent active coinfections can modify the immune

response to unrelated pathogens. Where possible, this information should be exploited to treat certain infections in humans.

Therapeutics

TNF is required for naive mice to control VV infection. However, with prior LCMV infection, the host does not need TNF to mediate efficient VV clearance. Thus, the use of anti-TNF therapies to treat various diseases (e.g., Crohn's disease and rheumatoid arthritis) may be relatively safe, perhaps, in large part, as a result of heterologous immunity. Similarly, humans are exposed to many pathogens throughout their lives; thus, we have a large, complex pool of memory T cells that have the potential to cross-react with any new pathogen and mediate heterologous, protective immunity [98].

Elevated CMV-specific humoral immunity (an indication of failed virus control) has been correlated with increased mortality of elderly individuals and impaired vaccination responses [99]. Treatment with the antiviral drug valaciclovir, for as long as 12 months in mice with established murine CMV infection, reduced the magnitude of the CMV-specific $CD8^+$ T-lymphocyte response by 80% and reduced the IAV load following challenge [100].

In humans and primate models, narrow oligoclonal responses to infection have been associated with poor prognosis and the generation of epitope-escape viral variants. These results can facilitate clinical decisionmaking by providing the information necessary to select the appropriate treatment.

Transplantation

Heterologous immunity is a major concern in tolerance induction. A transplant recipient's prior infection history can govern his/her responsiveness to alloantigens, and this history is an important factor in the design of novel methods to achieve optimal graft survival, as humans have such large, complex memory T cell populations. Sequential infections with heterologous viruses can increase the frequency of T cells specific for alloantigens, thereby generating a large pool of memory cells that need to be tolerized before engraftment [53]. A critical threshold of memory T cells is needed to promote graft rejection, and $CD8^+$ "central" memory T cells are primarily responsible for this transplantation-associated complication.

Anti-CD25, rapamycin, and blocking mAb against the common γ-chain have all been reported to act synergistically with a costimulation blockade to inhibit allograft rejection [101–103]. However, one possible consequence of these efforts to suppress T cell activation and induce tolerance to the allograft is that antiviral immune responses could be compromised, leading to undesirable consequences for the transplant recipient. Conversely, ongoing viral infections could be a barrier to the successful induction of allograft tolerance. For example, persistent infections, such as HCV, EBV, and CMV, could be a threat to the clinical application of costimulation blockade-based, tolerance-induction regimens. Even more importantly, in the absence of antiviral therapy, such regimens also have the potential to hamper protective antiviral immunity.

Treatment with deoxyspergualin, an inhibitor of NF-κB translocation, and costimulation blockade synergistically impairs memory T cell activation and promotes antigen-specific tolerance of memory [104]. Furthermore, in transplant recipients, the pathogenicity of viruses may be enhanced by concurrent infection with virus. This may be a result of virus–virus interactions or virus–host interactions, resulting from modulation of the host cell functions, production of suppressive cytokines, or secondary effects on host immune responses. Thus, additional therapeutic modalities are needed to address viral infections in transplant recipients [105].

Vaccine design

Heterologous immunity has implications for vaccine design. An important manifestation of heterologous immunity is cross-reactivity. Cross-reactive memory T cells have been shown to correlate directly with disease severity upon heterologous challenge under certain conditions, and the mutating epitopes to present this reactivity improved infection outcomes. Thus, if pathogenic cross-reactive memory T cells are identified, vaccines could be designed with careful elimination of these epitopes [1]. Additionally, if potentially pathogenic epitopes (on one HLA background) are essentially required to be included in a vaccine to achieve optimal protection (possibly on another HLA background), then tolerization to certain peptides to prevent immunopathology in susceptible individuals may be useful [71]. Another possible outcome of cross-reactive memory CD8 T cells is the narrowing of the TCR repertoire. Diagnostically, the repertoire diversity of expanded vaccine-specific clones can be measured to determine whether a narrow cross-reactive repertoire has been selected [47]. Finally, in contrast to the above examples, cross-reactive responses have been shown to be protective in some cases and might hold promise as an effective vaccination strategy [106].

Variations in immune responsiveness occur not only because of individuals' unique histories of previous infections but also because of their unique immune repertoire. Moreover, further research is warranted to understand cross-reactive networks comprehensively in diverse individuals, so that this information can be used in the design of vaccines that do not cause deleterious immune outcomes. Because of T cell cross-reactivity, vaccination is likely to affect unexpectedly immune responses to pathogens unrelated to the vaccine [53, 107].

CONCLUSION

A well-regulated immune response to a plethora of pathogens, expression of an appropriate cytokine and chemokine milieu, and the activation of immune cells with regulatory activity are crucial for the survival of the vertebrate host in the setting of coinfections (Table 1). The phenomenon of heterologous immunity has been shown to occur in mice and humans, and almost all types of immune cells play a role in this process. The general principles of heterologous immunity are currently being clarified, with the ultimate goal of being able to manipulate them to improve infection prevention, therapeutics, and transplantation outcomes. Some current approaches that modulate immune responses in a setting of multiple viral infections in vivo hold promise to manage microbe-induced immu-

TABLE 1. Described Models of Heterologous Infection

Model	Type of interaction	Outcome	Mechanisms	Host: mouse/human
Modified heat-labile bacterial toxin	Divergent/unrelated pathogens	Protective	Respiratory syncytial virus, influenza virus, or the fungus *C. neoformans*	Mouse [12]
Tuberculosis and HIV	Divergent/unrelated pathogens	Faster progression of HIV	Activation of innate responses by previous pathogen increases HIV replication	Mouse [13]
Thoracic filarial and foot pad *L. major*	Divergent/unrelated pathogens	No effect on Type 2 responses elicited by filaria-protective responses to *L. major*	Simultaneous Type 1 and Type 2 responses in the same host; diminished pathology in coinfected animals	Mouse [14]
LCMV and HBV	Divergent/unrelated pathogens	LCMV has protective effect on hepatitis B	Kupffer cells, recruitment of NK cells and T cells, and increased production of TNF-α, IFN-γ, and IFN-α/β	HBV transgenic mice [15]
γ Herpes virus 68/murine CMV and LM/*Y. pestis*	Divergent/unrelated pathogens	Herpes viruses impart protection to bacterial pathogens	Prolonged production of the antiviral effector cytokine IFN-γ and systemic activation of macrophages	Mouse [11]
LCMV and Pichinde virus/VV	LCMV and Pichinde = arenaviruses; LCMV and VV = divergent/ unrelated pathogens	LCMV immune mice = protected from Pichinde and VV challenge	Heterologous CD4 T cell immunity	Mouse [20]
BCG strain of *M. bovis* and VV	Divergent/unrelated pathogens	Protective responses to VV	Heterologous CD4 T cell immunity mediated by IFN-γ	Mouse [3]
HSV-2 and HIV	Divergent/unrelated pathogens	Worsens HIV pathology	Treg-mediated suppression of HIV-specific cytolytic T cell function	Human [21]
LCMV and VV	Divergent/unrelated pathogens	LCMV immune mice protected from VV challenge	Heterologous immunity mediated by memory CD8 T cells	Mouse [37]
Influenza virus and RSV	Divergent/unrelated pathogens	Protective immunity to RSV	Heterologous immunity mediated by memory T cells	Mouse [38]
LCMV and Pichinde virus; secondary infections with HIV, HCV, CMV	LCMV and Pichinde = arenaviruses	Increased generation of escape variants	Narrowed TCR repertoire after heterologous challenge	Mouse [44–47]
Latent murine CMV infection and influenza, human herpes virus I, West Nile virus	Divergent/unrelated pathogens	Enhanced immunopathology	Significantly weaker CD8 T cell responses to superinfecting virus	Mouse [55]
LCMV and influenza	Divergent/unrelated pathogens	Enhanced immunopathology	Increased numbers and activation states of CD8 T cells	Mouse [71]
Influenza and *Listeria*	Divergent/unrelated pathogens	Increased bacterial burdens	Generalized stress response and systemic immune suppression	Mouse [72]
EBV and IAV	Divergent/unrelated pathogens	Immunopathology	Cross-reactivity	Human [81]
HCV and IAV	Divergent/unrelated pathogens	Immunopathology	Cross-reactivity	Human [7, 77]
DENV	Serotype variants	DHF	Cross-reactivity	Human [82]
EBV and CMV	Divergent/unrelated pathogens	Prevent herpes virus reactivation	Bystander activation	Human [84]

nopathology, and we anticipate that this will be a growth area in the field of therapeutics design and development.

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DISCLOSURES

The authors declare no conflict of interest.

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KEY WORDS:
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