Trends in Immunology

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Review

Making Mouse Models That Reflect Human Immune Responses

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Humans are infected with a variety of acute and chronic pathogens over the course of their lives, and pathogen-driven selection has shaped the immune system of humans. The same is likely true for mice. However, laboratory mice we use for most biomedical studies are bred in ultra-hygienic environments, and are kept free of specific pathogens. We review recent studies that indicate that pathogen infections are important for the basal level of activation and the function of the immune system. Consideration of these environmental exposures of both humans and mice can potentially improve mouse models of human disease.

Trends

A significant contributor to variation in immune responses is bystander infection.

Specific pathogen-free mouse husbandry affects the basal state of the mouse immune system and changes mouse immune responses.

Bystander infections in mice may increase correlation between mouse models and human immune responses.

Bystander Infections in Mouse Models

Recent work suggests that specific pathogen-free (SPF) husbandry has broad and unexpected effects on the immune system of mice [1,2]. As their name indicates, SPF mice are free of specific pathogens. The list of organisms tested varies from facility-to-facility and room-to-room, but the organisms usually include both disease-causing pathogens and opportunistic and commensal organisms that do not cause disease in healthy mice. Mice raised in SPF conditions represent the benchmark for studies of the immune system. However, SPF mice may have immune systems that are immature compared with wild rodents or mice infected with specific pathogens [1,2]. In particular, this raises the concern that the immune system of SPF mice is less representative of that of adult humans. This advocates for an expansion of our definition of the normal microflora in mice to include a broader range of bacteria, viruses, parasites, and fungi. It also indicates that as a research community we need to redefine what we consider to be the 'normal' or baseline immune response in mice. Finally, we must consider the history of their microbial exposure to recapitulate human disease phenotypes and immune responses in mice.

Human and mouse physiology is influenced by the microbiome. Wide ranges of disease states are associated with microbiome changes. For example, nutrition and obesity, hematopoiesis, inflammatory bowel disease, cancer, and rheumatoid arthritis are all associated with microbiome alterations [3,4]. In all cases, these studies focus on the changes in the gut microflora, illustrating the profound effect intestinal bacteria and viruses have on a diverse set of disease phenotypes. However, it is important to note that the microbiota can refer to all host-associated microorganisms in multiple tissues, not just the gut. Moreover, some members of the microbiota are potentially pathogenic under predisposing conditions, and thus classified as pathobionts [5].

Less well studied are the effects of the pathogenic or opportunistic elements of the microbiome, including bacteria, viruses, parasites, and fungi, on immune responses to other pathogens. Recent work is beginning to uncover the significant influences of bystander infections on

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immune responses [6]. The metagenome, or the sum of host and microbial genes, drive phenotypes in the host [7]. We now appreciate that human and mouse physiology is not solely determined by genotype. However, we still need further investigation into the effects of bystander infections on disease states to define mechanisms.

In this review, we summarize recent work that indicates that bystander infections change mouse immune responses, and drive some of the observed differences between disease models in mice and humans. We explore the hypothesis that some mouse models of human diseases could be improved through understanding the contribution of bystander infections to immune cell function and immune responses.

Mice As Model Organisms

The mouse model is the cornerstone of biomedical research. The use of genetically inbred strains reduces variability. Researchers can easily manipulate the mouse genome, especially with the implementation of clustered regularly interspaced short palindromic repeats (CRISPR) technology. Mice are easy to breed and economical to house in animal facilities. Through the use of SPF husbandry, microbial exposures can be controlled, further reducing variability. Experimentation in mice has promoted an explosion of knowledge in immunology and other biomedical fields.

Despite the significant advances we have made in understanding the immune system from mouse studies, there is increasing concern over the utility of mouse models. Some emphasize the numerous differences between mouse and human immune systems, including differences in the balance of leukocyte subsets, Toll-like receptors, antibody subsets, and defensins [8]. Another major concern is that many therapeutics that have shown efficacy in mice have not translated into treatments for human diseases such as cancer and autoimmunity [9,10]. Genomic comparisons of mice and humans revealed significant overlap in transcriptional programs, but also expose noteworthy differences [11–14]. It has also been suggested that there is an overreliance on mice as a model system, and that there should be more emphasis on human immunology [15]. However, mice are still the foundation of basic research, and offer too many advantages to discard. The concerns raised emphasize the need for a better understanding of all the elements that drive variation between mice and humans. More research on factors that contribute to the basal state of the immune system in mice and humans is critical to understanding interspecies and interindividual variation.

Infection History and Variation in Immune Responses

In humans, interindividual variation in immune responses can be driven by nonheritable influences. In a study of monozygotic twins, it was found that nonheritable factors contribute significantly to variation in cell populations, cytokine responses, and serum proteins between individuals [16]. Moreover, genetically identical twins diverge in immune measurements with age, suggesting that environmental factors drive variation. Within this data set, human cytomegalovirus seropositivity was analyzed, and among the twin pairs that were discordant for human cytomegalovirus status, there was increased variation between the individuals in the parameters measured. While this is just one example of a potential environmental variable, it implicates microbial exposure as a long-term modulator of immune responses.

Because infectious diseases are one of the most important causes of mortality in the human population, it is likely that polymorphisms associated with the immune response to infection are under selective pressure. MHC genes, innate immune response genes, and interleukin (IL) genes exhibit evidence of natural selection [17]. Moreover, growing evidence suggests that certain loci associated with inflammatory bowel disease, and probably other human diseases, were targets of pathogen-driven selection [18]. The extent and mechanisms by which common

variants in genes interact with the environment to contribute to disease risk remain incompletely understood. Recent work indicates that many single nucleotide polymorphisms in human dendritic cells and monocytes only display functional variability upon stimulation [19,20]. This implies that critical genes may be missed in analyses when they are only examined at baseline without infectious exposure.

The evolution of the immune system was largely shaped through interactions with pathogens. While recent studies have focused on disease-susceptibility loci that were driven by balancing selection, it is likely that the functional outcome of a gene requires exposure to pathogens. In this case, even humanized mice carrying human genes, cells, tissues, or organs may still fail to recapitulate human disease. However, a combination of particular genotypes and housing conditions, including bystander infections, could allow the development of a model that closely mimics the phenotype observed in humans.

Evidence That Bystander Infection Changes Immune Response

Infection Modulates Genetics

Genetic ablation of a gene in mice does not always lead to concordance in phenotype compared with genetic deficiency in humans [21–24]. In many cases, immunodeficient mice are more susceptible to a wide range of infections, whereas genetically deficient humans are susceptible to a smaller subset of pathogens. The question is whether this is actually due to disparate functions of these genes in mice and humans, or whether it is due to differences in nonheritable factors between the two species.

A recent example illustrates that an environmental factor changes genetic immunodeficiency in mice. Genetic deficiency in mice of the *Hoil-1* gene (*Rbck1*) led to greater susceptibility to *Listeria monocytogenes* challenge, in addition to other pathogens [25]. However, humans with mutations in *Hoil-1* have varying amounts of hyperinflammation and immunodeficiency. Interestingly, *Hoil-1*$^{-/-}$ mice were protected from lethal *Listeria* challenge by chronic γ-herpesvirus infection. γ-Herpesvirus infection promoted a hyperinflammatory state in these mice, similar to what is seen in humans with deficiency in *Hoil1*. These data indicate that a particular component of the virome, the viral component of the microbiome [26], modulates a genetic immunodeficiency. Moreover, it implies that the difference between the phenotype in mice and humans may reflect a difference in the virome between barrier-raised mice and humans, and suggests that modifying the pathogen exposure of mice enhances agreement between mouse and human phenotypes.

In line with these observations, the contribution of a gene to disease phenotypes sometimes requires particular environmental factors that may be lost in mouse barrier facilities. Mice harboring a mutation in *Atg16l1*, a Crohn's disease-susceptibility gene, displayed Paneth cell abnormalities similar to Crohn's disease patients when they were raised in a conventional barrier facility [27]. However, *Atg16l1*-mutant mice embryonically rederived into an SPF facility did not have Paneth cell abnormalities. Infection of the mice in the enhanced barrier facility with murine norovirus, an intestinal pathogen, restored the Paneth cell abnormalities [28]. These data indicate that mutant mice raised in clean barrier facilities may not display the same phenotype as humans with similar mutations, due to a lack of pathogens. It also suggests that highly prevalent disease-susceptibility alleles in the human population may be linked to infrequent disease phenotypes through infection.

Disease phenotypes are also driven by complex interplays between bystander infections and the commensal microbiota. The bacterial sensor Nod2 is associated with Crohn's disease in humans [29], and mice deficient in *Nod2* displayed small intestinal abnormalities [30]. These intestinal abnormalities were dependent on expansion of *Bacteroides vulgatus* in the intestinal

microbiota. Importantly, infection of *Nod2*$^{-/-}$ mice with the helminth parasites, *Trichuris muris* or *Heligmosomoides polygyrus*, protected mice from intestinal abnormalities and prevented the colonization of *Bacteroides* species [31]. Parasite infection promoted the colonization with Clostridiales, which were protective in mice against small intestinal abnormalities. Notably, the pathogenic nature of Bacteroidales was only found in *Nod2*$^{-/-}$ hosts, indicating that a particular blend of genetic and bacterial factors combines to lead to inflammatory disease. Interestingly, helminth-positive humans with higher egg burden also had expansion of Clostridiales and reduced Bacteroidales. Given the association between helminth infections and reduced inflammatory bowel disease, this is notable. Only under certain genetic conditions is the pathogenic nature of a commensal bacteria revealed. Moreover, helminth infections tip the balance in favor of the host by promoting outgrowth of Clostridiales in the intestines, leading to protection from disease in a normally genetically susceptible background. These data suggest that disease phenotype requires not only genetic deficiency, but also perturbed microbiota. It also suggests that to understand the complex nature of disease phenotypes in humans, and to account for variability between human populations in disease susceptibility, we will have to consider the differences in pathogen exposure between diverse populations.

Bystander Infections Alter Basal Immune Activation

Work in specific experimental systems is beginning to address the mechanisms underlying bystander infection-driven changes on immune responses. Much of the earlier work, reviewed elsewhere [6], was mostly correlative. However, recent work implicates both innate and adaptive mechanisms for bystander infection-mediated changes in immune responses.

Bystander infections alter the cytokine environment in the host, which has consequences for subsequent immune responses. One or more herpesviruses chronically infect virtually all humans, and are constituents of the virome [26,32]. Importantly, herpesvirus latency and reactivation change the host immune response to other pathogens [33–35]. Herpesvirus latent infection induces a chronic, low-level production of interferon-γ, tumor necrosis factor-α, and interferon-β, increased activation of macrophages, and arming of natural killer cells [33,36–38]. These cytokines and activated cells may be important for herpesvirus-mediated cross-protection against bacterial challenge. They may also be important for effects observed on bystander T cells in herpesvirus-infected mice that were challenged with an unrelated virus [39]. Interestingly, helminth infection of a herpesvirus-infected animal led to production of IL-4 and IL-13, which subsequently induced herpesvirus reactivation from macrophages [40], demonstrating that new pathogens can influence the status of pre-existing infections. In addition to IL-4/IL-13, there are other cytokines that regulate viral gene expression of γ-herpesvirus [36,41,42], indicating that this mechanism of sensing host immune signals may be utilized by herpesviruses and other pathogens. This example illustrates that a component of the virome alters the host immune system, and that infection with multiple pathogens can create a complex cytokine milieu with the potential to alter immune responses to unrelated stimuli.

Co-infection alters the inflammatory environment, leading to changes in T-cell responses to secondary challenges. Intestinal bacteria influence the generation of T-cell subsets by stimulating innate immune sensors and producing fatty acids [3,4]. Recent work suggests that chronic viral and parasitic infections also change the T-cell responses to unrelated antigens. Differentiation of unrelated memory T cells is altered by chronic viral, protozoan, and helminth infections [43]. Moreover, activation and proliferation of virus-specific CD8$^+$ T cells are impaired in mice infected with an intestinal helminth [44]. While it was previously shown that certain intestinal helminths alter the microbiota [45–48], importantly, Osborne *et al.* [44] demonstrated that the effects of helminth infection were independent of changes to the gut microbiota. The authors determined that Ym1, a chitinase-like molecule produced by alternatively activated macrophages, impairs virus-specific T-cell responses. Significantly, these findings and the

findings described earlier for helminth–herpesvirus co-infection both implicate macrophages as important cells in innate immunomodulation of virus–helminth co-infection [40,44]. Macrophages may play a key role in sensing different types of infections, and modulating T-cell responses to unrelated antigens through cytokine production.

It was recently proposed that some acute bacterial infections produce an 'immunologic scar' that persists long after the bacterial infection is resolved [49]. Infection of mice with the gastrointestinal pathogen, *Yersinia pseudotuberculosis*, provoked local immunological damage in the gut by inducing remodeling of the mesentery, increased lymphatic leakage in mesenteric adipose tissue, and reduced migratory dendritic cell accumulation in lymph nodes. Interestingly, antibiotic treatment of mice partially restored mucosal immunity, suggesting that *Y. pseudotuberculosis* infection promoted changes in the microbiota that sustain tissue damage after the pathogen was cleared [49]. Another group found that acute infection of Toll-like receptor 1-deficient mice with *Yersinia enterocolitica* drove alterations in microbiota composition that persisted after *Yersinia* clearance [50]. This group also observed increased inflammation in tissue months after pathogen clearance. Together, these studies have implications for how we explain the association of inflammatory bowel disease or other autoimmune diseases with acute infections, and they indicate that in a susceptible genetic background, acute infections promote permanent changes in the microbiota leading to chronic diseases.

These examples highlight the complex interactions between the host immune system and pathogens, and the diverse mechanisms that govern these interactions. In some cases, bystander infections alter the T-cell response to viral pathogens, and thus change the course of infection. In other cases, infection damages lymphatic architecture and increases inflammation in mesenteric adipose tissue. Infections alter macrophage and dendritic cell phenotypes and organization, which subsequently impairs secondary immune responses to unrelated antigens [44,49,51]. There are examples where gut microbiota changes are critical for the effects of bystander infections, and other examples where those changes are not essential for the effects observed [44,49,50]. Future work will be needed to identify paradigms of bystander infection-mediated changes to immune responses. The effects will likely depend on the type of pathogen, and will comprise both innate and adaptive arms of the immune response.

Does Pathogen Exposure Humanize the Mouse Immune Response?

The data described thus far indicate that altered microbiome, as well as infection with chronic and acute pathogens, change immune responses to unrelated pathogens. Now the question is whether these changes are relevant to the comparison between mice and humans. Do laboratory mice that are infected with a diverse set of pathogens have immune responses more similar to human immune responses (Figure 1)? Two recent studies provide evidence that the answer is yes.

The first study compared the immune response of laboratory mice from an SPF facility with the immune responses of mice caught in the wild or mice purchased from a pet store [2]. While laboratory mice lack differentiated memory T cells, feral and pet store mice have many more differentiated memory T cells in lymphoid and nonlymphoid tissue, similar to adult humans. In addition, the T-cell phenotype in pet store mice could be transferred to barrier-raised laboratory mice through cohousing, suggesting that a transmissible agent affects the differentiation state of T cells. Approximately 20% of the cohoused mice died during the first 2 months of cohousing. Serological testing of pet store and cohoused laboratory mice revealed immune responses to a number of pathogens, suggesting that the T-cell phenotype changes and mortality could be due to pathogen exposure. The authors compared gene expression data of peripheral blood mononuclear cells (PBMCs) from pet store and laboratory mice with human cord PBMCs and adult PBMCs [52]. They found that the gene signature of pet store mice

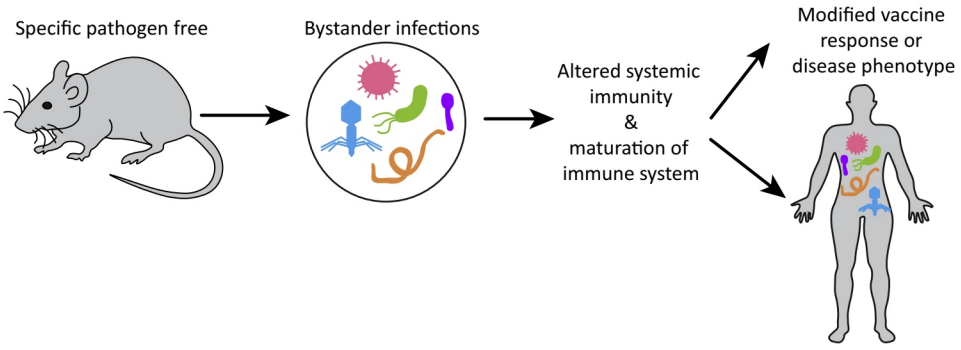

Figure 1. Bystander Infections Alter Systemic Immunity and Mature the Immune System. Specific pathogen-free mice infected with a combination of pathogens or cohoused with pet store mice have altered basal immune activation that changes vaccine response or resistance to pathogen challenge. This alteration in systemic immunity matures the immune responses and enhances correlation between the mouse and adult human immune systems.

PBMCs was similar to that observed in adult human PBMCs. While there was an overlap in both innate and adaptive immune pathways between pet store and adult humans, the most noteworthy overlap was in pathways related to interferon response. There was also a significant overlap in gene signatures between the laboratory mice with neonatal PBMCs, with significant enrichment of a naïve lymphocyte signature in both groups. The pet store mice and laboratory mice cohoused with pet store mice had reduced bacterial and parasite burden when challenged with *L. monocytogenes* and *Plasmodium berghei*, respectively, and increased effector $CD8^+$ T-cell response to lymphocytic choriomeningitis virus challenge [2]. These data suggest that mice with diverse environmental exposures have more mature immune responses, similar to human adults, whereas laboratory mice have immature or neonate-like immune systems. These data also imply that transmissible pathogen(s), rather than age alone, may influence the maturity of the immune system.

Another study directly tested whether pathogen exposure changes the basal state of the immune system. Barrier-raised mice were sequentially infected with three chronic pathogens and one acute pathogen (co-infected), and were compared with age-matched mice that did not receive any pathogen infections (mock infected) [1]. Gene expression of PBMCs identified markedly different expression profiles in the previously co-infected versus mock-infected mice. The co-infected PBMCs were enriched in interferon response and effector and memory lymphocyte pathways, while the mock-infected PBMCs were enriched in naïve lymphocyte pathways. When the co-infected and mock mice were challenged with the vaccine strain of yellow fever virus (YFV-17D), the co-infected mice made fewer antibodies to yellow fever virus, indicating that altered baseline immune activation changed response to vaccination. Comparison of the gene expression data with data from human cord PBMCs and adult PBMCs [52] revealed that pathways related to type I interferon response in the co-infected gene signature were enriched in the maternal adult blood gene signature. By contrast, the naïve lymphocyte signature in mock-infected PBMCs was enriched in the human cord blood [1]. These data indicate that diverse microbial exposure alters both innate and adaptive immune pathways, and possibly matures immune responses. Moreover, comparison of the co-infected and mock gene signatures with the pet store and laboratory signatures from the Beura *et al.* [1] study identified significant overlap between the co-infected mice and the pet store mice. Taken together, these data suggest that infecting laboratory mice with multiple pathogens recapitulates the microbial exposure and immune response found in pet store mice.

These studies suggest that age alone does not define maturity of the immune system. Both studies indicate that laboratory mice have immune systems more similar to neonatal humans, with lower innate immune activation and more naïve lymphocytes. By contrast, mice with diverse microbial exposure have enhanced interferon and effector/memory lymphocyte signatures that are more similar to adult humans. Further studies will be required to fully define what 'mature' and 'immature' is for the immune system, and whether it is a continuum or discrete state. However, both studies implicate innate and adaptive immune changes as being important. These studies have implications for how we think about mouse husbandry in laboratories and modeling human disease.

What Is the Normal Microflora of a Mouse?

With the expansion of mouse research there has been a push to make mouse facilities cleaner. We have eliminated many of the pathogens commonly found in laboratory animals and wild rodents. Through sentinel monitoring, mouse rooms are tested routinely for a variety of viral, bacterial, and parasitic pathogens. The pathogens that are routinely monitored depend on the facility and the room. A subset of these pathogens is summarized in Table 1. The impetus behind this is a desire to eliminate variables that confound results. Importantly, many of the immunodeficient strains generated are susceptible to the eliminated pathogens; therefore, eliminating them enhances survival of immunodeficient mice.

There is significant anecdotal and experimental evidence that particular mouse pathogens that 'contaminate' mouse research colonies alter disease phenotypes (Table 1). In some cases, phenotypes disappear when colonies are positive for particular pathogens. An example of this is the nonobese diabetic mouse model for type I diabetes. When these mice are bred in SPF conditions the incidence of diabetes is high, whereas when mice are bred in conventional facilities the incidence is low or absent [53,54]. Moreover, infection of nonobese diabetic mice with individual viruses, bacteria, and parasites reduced the incidence of diabetes [54]. By contrast, researchers at various institutions have noticed that phenotypes are altered when mouse colonies are rederived into cleaner facilities [28]. However, it is notable that many of these 'contaminating' pathogens are actually normal pathogens that are present in wild mice [55,56]. In many cases, immunocompetent mice have subclinical infections without significant pathology. These pathogens likely represent components of the mouse microbiome, and may influence many aspects of mouse physiology in important ways.

There is experimental evidence that these eliminated pathogens have profound effects on the immune system. Pinworms, for example, are monitored in mouse colonies, because they strongly alter mouse immune systems. Experimental infections with pinworms led to increased lymphoma development in nude mice, enhanced autoimmune disease in neonatal mice given self-peptide, and altered allergic phenotype of mice given oral antigens [57–59]. Enterotropic strains of murine hepatitis virus cause asymptomatic infections in most immunocompetent mice. However, infected mice can have increased epithelial lysis and atrophy of villi in the small intestines and colon [60]. Murine hepatitis virus infections also promoted thymic variations, changed T-cell proliferation and cytokine production, altered disease in autoimmune models, and increased resistance to *Salmonella typhimurium* [61–69]. Murine cytomegalovirus (MCMV) is a beta-herpesvirus commonly found in wild mice, but has been eliminated from many laboratory mouse colonies [55,56]. Mice with latent MCMV infection were protected from lethal doses of the *L. monocytogenes* and *Yersinia pestis* [33]. Moreover, latent MCMV infection led to a decrease in the naïve T-cell pool, an increase in effector memory $CD8^+$ T cells, and a decrease in $CD8^+$ T-cell response to influenza virus, herpes simplex virus, and West Nile virus challenge [70–72]. Together, these data suggest that this persistent virus commonly found in wild mice changes immune responses to unrelated pathogens.

Table 1. Examples of Organisms Excluded from Mouse Colonies and Their Impact on the Mouse Immune System

Monitored organisms	Impact on mouse experiments	Refs
Viruses		
Lactic dehydrogenase elevating virus	Reduced the development of autoimmune diseases in susceptible mice	[82–84]
	Impaired host resistance to bacterial infection	[85,86]
	Suppressed protective immune responses against nematode infection	[87]
Lymphocytic choriomeningitis virus	Impaired control of *Leishmania major* infection	[88]
	Chronic infection-induced splenic atrophy leading to decreased vaccine response	[89]
	Enhanced susceptibility to bacterial infection	[90–92]
	Reduced immune response to secondary viral infection	[93–95]
	Enhanced tumor susceptibility in immunocompetent mice	[96,97]
Mouse cytomegalovirus	Improved control of subsequent retrovirus infection	[98]
	Immune aging and impaired CD8 response to virus and bacterial superinfection	[70–72]
	Altered host resistance to bacterial/fungal infections	[33,99–102]
	Attenuated disease course of murine multiple sclerosis	[103]
	Induced autoimmune diseases including myocarditis and Sjögren's syndrome in susceptible mice	[104–110]
	Induced pulmonary fibrosis in mouse model of sepsis	[111]
Mouse hepatitis virus	Increased resistance to *Salmonella typhimurium* infection	[66]
	Changed incidence of autoimmune diseases	[65,67–69]
	Altered thymus and T cells	[61–64]
Mouse parvoviruses	Inhibited tumorigenesis induced by oncogenic viruses and chemical carcinogens	[112–114]
	Altered hematopoiesis	[115,116]
Bacteria		
Helicobacter sp.[a]	Impaired oral tolerance	[117]
	Enhanced gallstone formation	[118]
	Enhanced colitis	[119–121]
	Increased hepatitis and hepatic tumors	[122,123]
Parasites		
Pinworms	Enhanced Th2 responses and autoimmune disease in neonatal mice	[58]
	Induced lymphoma in athymic mice	[57]
	Exacerbated allergic reaction to ovalbumin	[59]
Helminths and other roundworms	Altered antiviral immunity	[40,44,124–126]
	Altered bacterial clearance and inflammation	[127–131]
	Altered intestinal microbiota diversity	[47,132,133]
	Decreased protective efficacy of vaccines	[134–137]
	Protected mice from autoimmune inflammation and disease	Reviewed in [138,139]

[a]Only monitored and excluded from the highest-level barrier facilities.

A recent study identified a novel protist in mouse intestines that induced gut inflammation without tissue damage [73]. This parasite was found in multiple animal facilities, but was absent from mice procured from Jackson Laboratories. Significantly, colonization with this protist protected mice from *S. typhimurium* infection. However, colonization exacerbated inflammation in a colitis model, and increased tumor burden in a colonic tumor model [73]. This work highlights the often-underappreciated role eukaryotic microbes, termed the eukaryome [74], play in the microbiome and host physiology. It also demonstrates that there are probably many species of viruses, bacteria, and parasites that contribute to heath and disease we have yet to identify in humans and mice.

Perspective

If we accept that humans and mice have some level of ongoing infection from a variety of pathogens and commensals, then should we change the way we model disease in mice? We think the answer to this is yes. We described just a few of the examples of mouse 'pathogens' that regularly infect wild mice. Given the prevalence of many of these organisms in wild mice, and the fact that they often do not cause disease in immunocompetent mice, these mouse 'pathogens' may be more accurately classified as 'pathobionts'. Moreover, they likely represent a major selective force in the evolution of the mouse immune system, much like infectious diseases in humans. To be clear, we do not propose the end of SPF husbandry or barrier facilities. However, it is evident that the basal state of the immune system is changed by chronic and acute infections, and so far we have limited understanding about how these changes influence immune responses to secondary challenges. To obtain a better grasp of this, we need more research into the mechanisms underlying these phenomena, both prior to and after secondary challenge.

To determine the effects of bystander infections on the immune system, disease models and therapeutics should be assessed after controlled reintroduction of pathogens into laboratory mice (Figure 2). Primary screens are still needed in SPF mice, where pathogen exposure and microbiome alterations are monitored and controlled. Appropriate reporting and documentation of all experimental conditions are essential for reproducibility across institutions and laboratories [7]. After the primary screen, a secondary screen can be performed where SPF mice are made 'dirty' by exposure to bystander pathogens prior to testing the disease model, vaccine, or therapeutic. The choice of bystander infections is still open for debate.

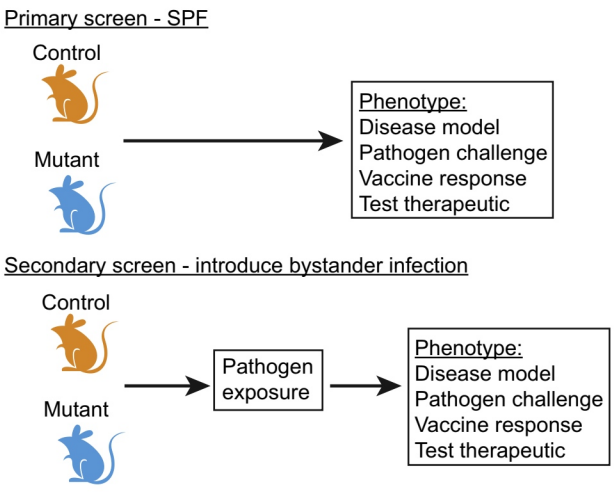

Figure 2. Proposed Experimental Design to Test the Effect of Bystander Infection on Disease Models in Mice. Primary screens for the effect of mutations in mice should be performed in similar conditions currently used, with mice bred in specific pathogen-free (SPF) facilities that are tested free from defined pathogens. Following this primary screen, additional secondary screens are needed. As part of a secondary screen, SPF mice should be exposed to pathogens prior to modeling diseases or testing new treatments. Altered systemic microbiota can be achieved through reintroduction of specific pathogens, moving mice to conventional facilities, or cohousing mice with pet store mice.

Cohousing mice with pet store mice is one option. However, given the variable nature of pet store mice, this may contribute to reproducibility issues, even if serology for a set of pathogens is reported. In addition, laboratory mice cohoused with pet store mice may have increased mortality upon exposure to pet store mice, thus necessitating larger cohorts of mice [2]. Another option is to define a group of pathogens for the secondary screen. This may include chronic viruses, such as herpesviruses, bacteria, fungi, and/or parasites. Further work is needed to define a reasonable set(s) of pathogens. These experiments may be best achieved through collaboration with other labs with experience in polymicrobial infections.

Given the long-standing correlation between infection and autoimmunity, mouse models of autoimmunity should be tested with and without bystander infections. For example, herpesviruses represent an important component of the systemic microbiome with a putative role in the genes-plus-environment etiology of multiple autoimmune diseases [75–77]. The mouse model of gammaherpesvirus infection has been shown to exacerbate experimental autoimmune encephalomyelitis, resulting in disease that more closely resembles human multiple sclerosis [78,79]. Herpesvirus infection in young children versus adolescents may have different consequences for development of multiple sclerosis [76]. This suggests that timing of infection is also an important variable, and that mouse models will be important for defining mechanism. Moreover, herpesviruses are just one example of a virus that may have a role in modifying autoimmune phenotypes. Many other pathogens, including helminths, bacteria, and other viruses, may modify autoimmune disease in positive and negative ways [53].

Environmental factors, including the presence of bystander infection and inflammation, may be important modifiers of tumor progression that we currently fail to model in mice. More basic research is needed to understand these putative effects. The low rate of translation of findings in animal models to clinical trials for cancer therapeutics challenges us to assess the limitations of animal models [9].

Concluding Remarks

If we consider the multifarious infection history of humans and compare it with the infection history of laboratory mice, it is clear that the ultra-hygienic environment of experimental mice does not recapitulate the environmental exposures of humans. Work from multiple groups now indicates that infection history changes the mouse immune system, and alters the way in which it responds to challenges. Moreover, increasing a mouse's infection exposure may enhance correlation between mouse and adult human immune responses. This approach combined with new efforts to overcome species-specific difference by humanizing mice with genes and cells from humans could lead to improved models for human disease [80,81] (see Outstanding Questions).

Outstanding Questions

- What are the mechanisms by which bystander viral, parasitic, and bacterial infections change response to vaccine?
- Do bystander infections differentially affect certain types of immune responses?
- What are the roles of bystander infections in tumor progression, autoimmunity, and immunometabolism?
- Can we define a combination of pathogens that enhances correlation between mouse and human immune responses?
- How does the effect of bystander infection change with age and genetic background?
- What are the effects of bystander infection on the intestinal microbiome and vice versa?
- How do other environmental variables, such as diet, sex, age, temperature, and stress, influence immune responses in mice?

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References

1. Reese, T.A. *et al.* (2016) Sequential infection with common pathogens promotes human-like immune gene expression and altered vaccine response. *Cell Host Microbe* 19, 713–719
2. Beura, L.K. *et al.* (2016) Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature* 532, 512–516
3. Hooper, L.V. *et al.* (2012) Interactions between the microbiota and the immune system. *Science* 336, 1268–1273
4. Honda, K. and Littman, D.R. (2016) The microbiota in adaptive immune homeostasis and disease. *Nature* 535, 75–84
5. Chow, J. *et al.* (2011) Pathobionts of the gastrointestinal microbiota and inflammatory disease. *Curr. Opin. Immunol.* 23, 473–480
6. Stelekati, E. and Wherry, E.J. (2012) Chronic bystander infections and immunity to unrelated antigens. *Cell Host Microbe* 12, 458–469

7. Stappenbeck, T.S. and Virgin, H.W. (2016) Accounting for reciprocal host-microbiome interactions in experimental science. *Nature* 534, 191–199
8. Mestas, J. and Hughes, C.C.W. (2004) Of mice and not men: differences between mouse and human immunology. *J. Immunol.* 172, 2731–2738
9. Mak, I.W. *et al.* (2014) Lost in translation: animal models and clinical trials in cancer treatment. *Am. J. Transl. Res.* 6, 114–118
10. von Herrath, M.G. and Nepom, G.T. (2005) Lost in translation: barriers to implementing clinical immunotherapeutics for autoimmunity. *J. Exp. Med.* 202, 1159–1162
11. Seok, J. *et al.* (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc. Natl. Acad. Sci. U. S. A.* 110, 3507–3512
12. Takao, K. and Miyakawa, T. (2015) Genomic responses in mouse models greatly mimic human inflammatory diseases. *Proc. Natl. Acad. Sci. U. S. A.* 112, 1167–1172
13. Liao, B.-Y. and Zhang, J. (2008) Null mutations in human and mouse orthologs frequently result in different phenotypes. *Proc. Natl. Acad. Sci. U. S. A.* 105, 6987–6992
14. Shay, T. *et al.* (2013) Conservation and divergence in the transcriptional programs of the human and mouse immune systems. *Proc. Natl. Acad. Sci. U. S. A.* 110, 2946–2951
15. Davis, M.M. (2008) A prescription for human immunology. *Immunity* 29, 835–838
16. Brodin, P. *et al.* (2015) Variation in the human immune system is largely driven by non-heritable influences. *Cell* 160, 37–47
17. Fumagalli, M. and Sironi, M. (2014) Human genome variability, natural selection and infectious diseases. *Curr. Opin. Immunol.* 30, 9–16
18. Jostins, L. *et al.* (2012) Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 491, 119–124
19. Lee, M.N. *et al.* (2014) Common genetic variants modulate pathogen-sensing responses in human dendritic cells. *Science* 343, 1246980
20. Fairfax, B.P. *et al.* (2014) Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. *Science* 343, 1246949
21. Bernuth von, H. *et al.* (2008) Pyogenic bacterial infections in humans with MyD88 deficiency. *Science* 321, 691–696
22. Cohen, A.C. *et al.* (2006) Cutting edge: decreased accumulation and regulatory function of $CD4^+$ CD25(high) T cells in human STAT5b deficiency. *J. Immunol.* 177, 2770–2774
23. von Bernuth, H. *et al.* (2012) Experimental and natural infections in MyD88- and IRAK-4-deficient mice and humans. *Eur. J. Immunol.* 42, 3126–3135
24. Cypowyj, S. *et al.* (2012) Immunity to infection in IL-17-deficient mice and humans. *Eur. J. Immunol.* 42, 2246–2254
25. MacDuff, D.A. *et al.* (2015) Phenotypic complementation of genetic immunodeficiency by chronic herpesvirus infection. *Elife* 4, e04494
26. Virgin, H.W. (2014) The virome in mammalian physiology and disease. *Cell* 157, 142–150
27. Cadwell, K. *et al.* (2008) A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* 456, 259–263
28. Cadwell, K. *et al.* (2010) Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. *Cell* 141, 1135–1145
29. Cleynen, I. *et al.* (2016) Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. *Lancet* 387, 156–167
30. Ramanan, D. *et al.* (2014) Bacterial sensor Nod2 prevents inflammation of the small intestine by restricting the expansion of the commensal *Bacteroides vulgatus*. *Immunity* 41, 311–324
31. Ramanan, D. *et al.* (2016) Helminth infection promotes colonization resistance via type 2 immunity. *Science* 352, 608–612
32. Virgin, H.W. *et al.* (2009) Redefining chronic viral infection. *Cell* 138, 30–50
33. Barton, E.S. *et al.* (2007) Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* 447, 326–329
34. Nguyen, Y. *et al.* (2008) Gammaherpesvirus modulation of mouse adenovirus type 1 pathogenesis. *Virology* 380, 182–190
35. Saito, F. *et al.* (2013) MHV68 latency modulates the host immune response to influenza A virus. *Inflammation* 36, 1295–1303
36. Barton, E.S. *et al.* (2005) Alpha/beta interferons regulate murine gammaherpesvirus latent gene expression and reactivation from latency. *J. Virol.* 79, 14149–14160
37. White, D.W. *et al.* (2010) Latent herpesvirus infection arms NK cells. *Blood* 115, 4377–4383
38. Reese, T.A. (2016) Coinfections: another variable in the herpesvirus latency-reactivation dynamic. *J. Virol.* 90, 5534–5537
39. Barton, E.S. *et al.* (2014) Gammaherpesvirus latency differentially impacts the generation of primary versus secondary memory $CD8^+$ T cells during subsequent infection. *J. Virol.* 88, 12740–12751
40. Reese, T.A. *et al.* (2014) Helminth infection reactivates latent γ-herpesvirus via cytokine competition at a viral promoter. *Science* 345, 573–577
41. Goodwin, M.M. *et al.* (2010) Murine gammaherpesvirus 68 has evolved gamma interferon and stat1-repressible promoters for the lytic switch gene 50. *J. Virol.* 84, 3711–3717
42. Mandal, P. *et al.* (2011) A gammaherpesvirus cooperates with interferon-alpha/beta-induced IRF2 to halt viral replication, control reactivation, and minimize host lethality. *PLoS Pathog.* 7, e1002371
43. Stelekati, E. *et al.* (2014) Bystander chronic infection negatively impacts development of $CD8^+$ T cell memory. *Immunity* 40, 801–813
44. Osborne, L.C. *et al.* (2014) Virus-helminth coinfection reveals a microbiota-independent mechanism of immunomodulation. *Science* 345, 578–582
45. Broadhurst, M.J. *et al.* (2012) Therapeutic helminth infection of macaques with idiopathic chronic diarrhea alters the inflammatory signature and mucosal microbiota of the colon. *PLoS Pathog.* 8, e1003000
46. Rausch, S. *et al.* (2013) Small intestinal nematode infection of mice is associated with increased enterobacterial loads alongside the intestinal tract. *PLoS One* 8, e74026
47. Walk, S.T. *et al.* (2010) Alteration of the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus*. *Inflamm. Bowel Dis.* 16, 1841–1849
48. Li, R.W. *et al.* (2012) Alterations in the porcine colon microbiota induced by the gastrointestinal nematode *Trichuris suis*. *Infect. Immun.* 80, 2150–2157
49. Fonseca, D.M. *et al.* (2015) Microbiota-dependent sequelae of acute infection compromise tissue-specific immunity. *Cell* 163, 354–366
50. Kamdar, K. *et al.* (2016) Genetic and metabolic signals during acute enteric bacterial infection alter the microbiota and drive progression to chronic inflammatory disease. *Cell Host Microbe* 19, 21–31
51. Gaya, M. *et al.* (2015) Host response. Inflammation-induced disruption of SCS macrophages impairs B cell responses to secondary infection. *Science* 347, 667–672
52. Votavova, H. *et al.* (2011) Transcriptome alterations in maternal and fetal cells induced by tobacco smoke. *Placenta* 32, 763–770
53. Okada, H. *et al.* (2010) The "hygiene hypothesis" for autoimmune and allergic diseases: an update. *Clin. Exp. Immunol.* 160, 1–9
54. Bach, J.-F. (2002) The effect of infections on susceptibility to autoimmune and allergic diseases. *N. Engl. J. Med.* 347, 911–920
55. Smith, A.L. *et al.* (1993) A serologic survey for viruses and *Mycoplasma pulmonis* among wild house mice (*Mus domesticus*) in southeastern Australia. *J. Wildl. Dis.* 29, 219–229
56. Parker, S.E. *et al.* (2009) Infectious diseases in wild mice (*Mus musculus*) collected on and around the University of Pennsylvania (Philadelphia) Campus. *Comp. Med.* 59, 424–430

57. Beattie, G. *et al.* (1980) Induction of lymphoma in athymic mice: a model for study of the human disease. *Proc. Natl. Acad. Sci. U. S. A.* 77, 4971–4974
58. Agersborg, S.S. *et al.* (2001) Intestinal parasitism terminates self tolerance and enhances neonatal induction of autoimmune disease and memory. *Eur. J. Immunol.* 31, 851–859
59. Michels, C. *et al.* (2006) Infection with *Syphacia obvelata* (pinworm) induces protective Th2 immune responses and influences ovalbumin-induced allergic reactions. *Infect. Immun.* 74, 5926–5932
60. Baker, D.G. (1998) Natural pathogens of laboratory mice, rats, and rabbits and their effects on research. *Clin. Microbiol. Rev.* 11, 231–266
61. Lee, S.K. *et al.* (1994) Apoptotic changes in the thymus of mice infected with mouse hepatitis virus, MHV-2. *J. Vet. Med. Sci.* 56, 879–882
62. de Souza, M.S. *et al.* (1991) Infection of BALB/cByJ mice with the JHM strain of mouse hepatitis virus alters *in vitro* splenic T cell proliferation and cytokine production. *Lab. Anim. Sci.* 41, 99–105
63. Kyuwa, S. *et al.* (1996) Characterization of T cells expanded *in vivo* during primary mouse hepatitis virus infection in mice. *J. Vet. Med. Sci.* 58, 431–437
64. Cray, C. *et al.* (1993) *In vitro* and long-term *in vivo* immune dysfunction after infection of BALB/c mice with mouse hepatitis virus strain A59. *Lab. Anim. Sci.* 43, 169–174
65. Wilberz, S. *et al.* (1991) Persistent MHV (mouse hepatitis virus) infection reduces the incidence of diabetes mellitus in non-obese diabetic mice. *Diabetologia* 34, 2–5
66. Fallon, M.T. *et al.* (1991) Mouse hepatitis virus strain UAB infection enhances resistance to *Salmonella typhimurium* in mice by inducing suppression of bacterial growth. *Infect. Immun.* 59, 852–856
67. Musaji, A. (2004) Exacerbation of autoantibody-mediated thrombocytopenic purpura by infection with mouse viruses. *Blood* 104, 2102–2106
68. Musaji, A. *et al.* (2005) Enhancement of autoantibody pathogenicity by viral infections in mouse models of anemia and thrombocytopenia. *Autoimmun. Rev.* 4, 247–252
69. Aparicio, J.L. *et al.* (2011) Autoimmune hepatitis-like disease in C57BL/6 mice infected with mouse hepatitis virus A59. *Int. Immunopharmacol.* 11, 1591–1598
70. Cicin-Sain, L. *et al.* (2012) Cytomegalovirus infection impairs immune responses and accentuates T-cell pool changes observed in mice with aging. *PLoS Pathog.* 8, e1002849
71. Smithey, M.J. *et al.* (2012) Lifelong persistent viral infection alters the naive T cell pool, impairing CD8 T cell immunity in late life. *J. Immunol.* 189, 5356–5366
72. Mekker, A. *et al.* (2012) Immune senescence: relative contributions of age and cytomegalovirus infection. *PLoS Pathog.* 8, e1002850
73. Chudnovskiy, A. *et al.* (2016) Host-protozoan interactions protect from mucosal infections through activation of the inflammasome. *Cell* 167, 444–456 e14
74. Lukeš, J. *et al.* (2015) Are human intestinal eukaryotes beneficial or commensals? *PLoS Pathog.* 11, e1005039
75. Tselis, A. (2012) Epstein-Barr virus cause of multiple sclerosis. *Curr. Opin. Rheumatol.* 24, 424–428
76. Ascherio, A. and Munger, K.L. (2007) Environmental risk factors for multiple sclerosis. Part I: the role of infection. *Ann. Neurol.* 61, 288–299
77. Draborg, A.H. *et al.* (2013) Epstein-Barr virus in systemic autoimmune diseases. *Clin. Dev. Immunol.* 2013, 535738–535739
78. Casiraghi, C. *et al.* (2012) Gammaherpesvirus latency accentuates EAE pathogenesis: relevance to Epstein-Barr virus and multiple sclerosis. *PLoS Pathog.* 8, e1002715
79. Casiraghi, C. *et al.* (2015) Latent virus infection upregulates CD40 expression facilitating enhanced autoimmunity in a model of multiple sclerosis. *Sci. Rep.* 5, 13995
80. Rongvaux, A. *et al.* (2013) Human hemato-lymphoid system mice: current use and future potential for medicine. *Annu. Rev. Immunol.* 31, 635–674
81. Brehm, M.A. *et al.* (2014) Generation of improved humanized mouse models for human infectious diseases. *J. Immunol. Methods* 410, 3–17
82. Hayashi, T. *et al.* (2001) Reduction of serum interferon (IFN)-γ concentration and lupus development in NZBxNZWF(1)mice by lactic dehydrogenase virus infection. *J. Comp. Pathol.* 125, 285–291
83. Hayashi, T. *et al.* (1993) Suppression of development of antinuclear antibody and glomerulonephritis in NZB x NZWF1 mice by persistent infection with lactic dehydrogenase virus: possible involvement of superoxide anion as a progressive effector. *Int. J. Exp. Pathol.* 74, 553–560
84. Inada, T. and Mims, C.A. (1986) Infection of mice with lactic dehydrogenase virus prevents development of experimental allergic encephalomyelitis. *J. Neuroimmunol.* 11, 53–56
85. Isakov, N. and Segal, S. (1983) A tumor-associated lactic dehydrogenase virus suppresses the host resistance to infection with *Listeria monocytogenes*. *Immunobiology* 164, 402–416
86. Bonventre, P.F. *et al.* (1980) Impaired resistance to bacterial infection after tumor implant is traced to lactic dehydrogenase virus. *Infect. Immun.* 30, 316–319
87. Morimoto, M. *et al.* (1998) Lactic dehydrogenase virus infection enhances parasite egg production and inhibits eosinophil and mast cell responses in mice infected with the nematode *Nippostrongylus brasiliensis*. *Immunology* 93, 540–545
88. Crosby, E.J. *et al.* (2015) Lymphocytic choriomeningitis virus expands a population of $NKG2D^{+}CD8^{+}$ T cells that exacerbates disease in mice coinfected with *Leishmania major*. *J. Immunol.* 195, 3301–3310
89. Mbanwi, A.N. *et al.* (2017) Irreversible splenic atrophy following chronic LCMV infection is associated with compromised immunity in mice. *Eur. J. Immunol.* 47, 94–106
90. Navarini, A.A. *et al.* (2006) Increased susceptibility to bacterial superinfection as a consequence of innate antiviral responses. *Proc. Natl. Acad. Sci. U. S. A.* 103, 15535–15539
91. Merches, K. *et al.* (2015) Virus-induced type I interferon deteriorates control of systemic *Pseudomonas aeruginosa* infection. *Cell. Physiol. Biochem.* 36, 2379–2392
92. Gumenscheimer, M. (2006) Stage of primary infection with lymphocytic choriomeningitis virus determines predisposition or resistance of mice to secondary bacterial infections. *Med. Microbiol. Immunol.* 196, 79–88
93. Honke, N. *et al.* (2016) Immunoactivation induced by chronic viral infection inhibits viral replication and drives immunosuppression through sustained IFN-I responses. *Eur. J. Immunol.* 46, 372–380
94. Lee, L.N. *et al.* (2009) Multiple mechanisms contribute to impairment of type 1 interferon production during chronic lymphocytic choriomeningitis virus infection of mice. *J. Immunol.* 182, 7178–7189
95. Diet, N.H. and Libíková, H. (1979) Selective resistance to togaviral superinfection in mice with tolerant lymphocytic choriomeningitis virus infection. *Acta Virol.* 23, 385–392
96. Güttler, F. *et al.* (1975) Transient impaired cell-mediated tumor immunity after acute infection with lymphocytic choriomeningitis virus. *Scand. J. Immunol.* 4, 327–336
97. Kohler, M. *et al.* (1990) Enhanced tumor susceptibility of immunocompetent mice infected with lymphocytic choriomeningitis virus. *Cancer Immunol. Immunother.* 32, 117–124
98. Francois, S. *et al.* (2013) NK cells improve control of friend virus infection in mice persistently infected with murine cytomegalovirus. *Retrovirology* 10, 1–1
99. Leung, W.C. and Hashimoto, K. (1986) Modification of susceptibility to *Klebsiella pneumoniae* during murine cytomegalovirus infection. *Microbiol. Immunol.* 30, 761–776
100. Bale, J.F. *et al.* (1982) Enhanced susceptibility of mice infected with murine cytomegalovirus to intranasal challenge with *Escherichia coli*: pathogenesis and altered inflammatory response. *J. Infect. Dis.* 145, 525–531
101. Hamilton, J.R. and Overall, J.C. (1978) Synergistic infection with murine cytomegalovirus and *Pseudomonas aeruginosa* in mice. *J. Infect. Dis.* 137, 775–782

102. Hraiech, S. *et al.* (2016) Cytomegalovirus reactivation enhances the virulence of *Staphylococcus aureus* pneumonia in a mouse model. *Clin. Microbiol. Infect.* 23, 38–45

103. Pirko, I. *et al.* (2012) CMV infection attenuates the disease course in a murine model of multiple sclerosis. *PLoS One* 7, e32767

104. Ohyama, Y. *et al.* (2006) Severe focal sialadenitis and dacryoadenitis in NZM2328 mice induced by MCMV: a novel model for human Sjogren's syndrome. *J. Immunol.* 177, 7391–7397

105. Fleck, M. *et al.* (1998) Murine cytomegalovirus induces a Sjögren's syndrome-like disease in C57Bl/6-lpr/lpr mice. *Arthritis Rheum.* 41, 2175–2184

106. Fairweather, D. *et al.* (1998) Wild isolates of murine cytomegalovirus induce myocarditis and antibodies that cross-react with virus and cardiac myosin. *Immunology* 94, 263–270

107. O'Donoghue, H.L. (1990) Autoantibodies to cardiac myosin in mouse cytomegalovirus myocarditis. *Immunology* 71, 20–28

108. Lawson, C.M. *et al.* (1992) Mouse cytomegalovirus infection induces antibodies which cross-react with virus and cardiac myosin: a model for the study of molecular mimicry in the pathogenesis of viral myocarditis. *Immunology* 75, 513–519

109. Tsunoda, I. *et al.* (2007) Sequential polymicrobial infections lead to CNS inflammatory disease: possible involvement of bystander activation in heterologous immunity. *J. Neuroimmunol.* 188, 22–33

110. Onyeagocha, C. *et al.* (2009) Latent cytomegalovirus infection exacerbates experimental colitis. *Am. J. Pathol.* 175, 2034–2042

111. Cook, C.H. *et al.* (2006) Pulmonary cytomegalovirus reactivation causes pathology in immunocompetent mice. *Crit. Care Med.* 34, 842–849

112. Marchini, A. *et al.* (2015) Oncolytic parvoviruses: from basic virology to clinical applications. *Virol. J.* 12, 6

113. Wetzel, K. *et al.* (2007) MCP-3 (CCL7) delivered by parvovirus MVMp reduces tumorigenicity of mouse melanoma cells through activation of T lymphocytes and NK cells. *Int. J. Cancer* 120, 1364–1371

114. Rommelaere, J. and Cornelis, J.J. (1991) Antineoplastic activity of parvoviruses. *J. Virol. Methods* 33, 233–251

115. Segovia, J.C. *et al.* (1995) Myeloid depression follows infection of susceptible newborn mice with the parvovirus minute virus of mice (strain i). *J. Virol.* 69, 3229–3232

116. Segovia, J.C. *et al.* (2003) Parvovirus infection suppresses long-term repopulating hematopoietic stem cells. *J. Virol.* 77, 8495–8503

117. Matysiak-Budnik, T. (2003) Gastric *Helicobacter* infection inhibits development of oral tolerance to food antigens in mice. *Infect. Immun.* 71, 5219–5224

118. Maurer, K.J. *et al.* (2005) Identification of cholelithogenic enterohepatic *Helicobacter* species and their role in murine cholesterol gallstone formation. *Gastroenterology* 128, 1023–1033

119. McBee, M.E. *et al.* (2008) Modulation of acute diarrheal illness by persistent bacterial infection. *Infect. Immun.* 76, 4851–4858

120. Ward, J.M. *et al.* (1996) Inflammatory large bowel disease in immunodeficient mice naturally infected with *Helicobacter hepaticus*. *Lab. Anim. Sci.* 46, 15–20

121. Kullberg, M.C. *et al.* (1998) Helicobacter hepaticus triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12- and gamma interferon-dependent mechanism. *Infect. Immun.* 66, 5157–5166

122. Ward, J.M. *et al.* (1994) Chronic active hepatitis and associated liver tumors in mice caused by a persistent bacterial infection with a novel Helicobacter species. *J. Natl. Cancer Inst.* 86, 1222–1227

123. Ward, J.M. *et al.* (1994) Chronic active hepatitis in mice caused by *Helicobacter hepaticus*. *Am. J. Pathol.* 145, 959–968

124. Actor, J.K. *et al.* (1993) Helminth infection results in decreased virus-specific $CD8^+$ cytotoxic T-cell and Th1 cytokine responses as well as delayed virus clearance. *Proc. Natl. Acad. Sci. U. S. A.* 90, 948–952

125. Furze, R.C. *et al.* (2006) Amelioration of influenza-induced pathology in mice by coinfection with *Trichinella spiralis*. *Infect. Immun.* 74, 1924–1932

126. Scheer, S. *et al.* (2014) *S. mansoni* bolsters anti-viral immunity in the murine respiratory tract. *PLoS One* 9, e112469

127. Hsieh, Y.J. *et al.* (2014) Helminth-induced interleukin-4 abrogates invariant natural killer T cell activation-associated clearance of bacterial infection. *Infect. Immun.* 82, 2087–2097

128. Su, L. *et al.* (2014) Coinfection with an intestinal helminth impairs host innate immunity against *Salmonella enterica* serovar *Typhimurium* and exacerbates intestinal inflammation in mice. *Infect. Immun.* 82, 3855–3866

129. Chen, C.-C. *et al.* (2005) Concurrent infection with an intestinal helminth parasite impairs host resistance to enteric *Citrobacter rodentium* and enhances *Citrobacter*-induced colitis in mice. *Infect. Immun.* 73, 5468–5481

130. Elias, D. *et al.* (2005) Low dose chronic *Schistosoma mansoni* infection increases susceptibility to *Mycobacterium bovis* BCG infection in mice. *Clin. Exp. Immunol.* 139, 398–404

131. Potian, J.A. *et al.* (2011) Preexisting helminth infection induces inhibition of innate pulmonary anti-tuberculosis defense by engaging the IL-4 receptor pathway. *J. Exp. Med.* 208, 1863–1874

132. Zaiss, M.M. *et al.* (2015) The intestinal microbiota contributes to the ability of helminths to modulate allergic inflammation. *Immunity* 43, 998–1010

133. Lee, S.C. *et al.* (2014) Helminth colonization is associated with increased diversity of the gut microbiota. *PLoS Negl. Trop. Dis.* 8, e2880

134. Da'Dara, A.A. *et al.* (2006) Helminth infection suppresses T-cell immune response to HIV-DNA-based vaccine in mice. *Vaccine* 24, 5211–5219

135. Bobat, S. *et al.* (2014) Natural and vaccine-mediated immunity to *Salmonella typhimurium* is impaired by the helminth *Nippostrongylus brasiliensis*. *PLoS Negl. Trop. Dis.* 8, e3341

136. Su, Z. *et al.* (2006) Reduced protective efficacy of a blood-stage malaria vaccine by concurrent nematode infection. *Infect. Immun.* 74, 2138–2144

137. Urban, J.F. *et al.* (2007) Infection with parasitic nematodes confounds vaccination efficacy. *Vet. Parasitol.* 148, 14–20

138. Maizels, R.M. and McSorley, H.J. (2016) Regulation of the host immune system by helminth parasites. *J. Allergy Clin. Immunol.* 138, 666–675

139. Weinstock, J.V. and Elliott, D.E. (2014) Helminth infections decrease host susceptibility to immune-mediated diseases. *J. Immunol.* 193, 3239–3247