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MINI-REVIEW

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Influenza Pathogenesis in Genetically Defined Resistant and Susceptible Murine Strains

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The murine infection model is a cornerstone for influenza virus research and includes aspects such as disease pathogenesis, immunobiology, and vaccine and antiviral drug development. One compelling feature of the murine model is the availability of inbred mouse strains, each with a unique genetic makeup and potential for variable responses to influenza infection. Using highly controlled infection studies, the response to influenza virus infection is classified on a spectrum from susceptible to resistant, reflecting severe morbidity and high mortality, to limited or no morbidity and no mortality. Although there have been a variety of studies establishing disparate pathogenesis amongst various murine strains, thus far, there is no consensus regarding the determinants of the outcome of infection. The goal of this review is to explore and discuss the differences in pathogenesis, as well as the innate and adaptive immune responses to influenza infection that have been described in susceptible and resistant mouse strains. Understanding how host genetics influences the response to influenza infection provides valuable insight into the variable responses seen in vaccine or drug efficacy studies, as well as indicates possible mechanisms contributing to increased disease severity in humans infected with influenza virus with no known risk factors.

INTRODUCTION

Influenza A virus is a seasonal pathogen and a major public health concern. Each year, seasonal influenza virus infection causes between 200,000 and 500,000 deaths globally, predominantly in the very young, elderly, and individuals with identified risk factors [1,2]. Efficacious vaccines are available; however, overall effectiveness varies from year to year and was most recently estimated at 48 percent for the 2016-17 influenza season [3]. Approximately 25 percent of primary influenza viral pneumonia cases occur in individuals without known risk factors such as obesity, hypertension, asthma, and heart disease [2,4]. This suggests that other features may be contributing to disease severity. Genetic factors that contribute to host susceptibility and disease pathogenesis is an area of extreme interest for research; however, human studies, while appealing, are difficult to complete and results are complicated by lifelong exposures to influenza, vaccination, and other variables. Studies of influenza disease severity in genetically defined inbred mouse strains provide a controlled and compelling approach to elucidate how minor genetic differences can influence influenza infection and disease.

VARIATION IN INFECTION AND PATHOGENESIS

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†Abbreviations: mTECs, murine tracheal epithelial cells; MPO, myeloperoxidase; QTL, quantitative trait locus; IPA, ingenuity pathway analysis; SNPs, single nucleotide polymorphisms; mLN, mediastinal lymph node.

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The mouse is an established model for influenza virus infection and has been used to study influenza disease pathogenesis, as well as vaccine safety and efficacy. Several studies have shown that disease pathogenesis in mice varies from susceptible (DBA/2) to resistant (C57BL/6 and BALB/c), and is dependent on the mouse strain used. This variation in susceptibility has been studied across a wide variety of influenza subtypes and species of origin, including human H1N1 and H3N2, avian H5N1 and H7N7, and swine H1 and H3 strains [5-12]. Variation in murine susceptibility is not unique to influenza and has been demonstrated to occur in a variety of infectious diseases including bacteria (*Mycobacterium bovis* and *tuberculosis*, *Orientia tsutsugmushi*, *Chlamydia trachomatis*, *Leishmania donovani*, and *Salmonella typhimurium*), parasites (*Plasmodium chabaudi* and *Toxoplasma gondii*), fungi (*Candida albicans*), and even with toxins (lethal factor produced by *Bacillus anthracis* [13-21].

For influenza virus infection, increased disease severity seen in DBA/2 mice compared to resistant strains has been illustrated by greater weight loss, reduced survival time, and enhanced pathogenesis [5,7,10,22]. The increased pathogenesis in DBA/2 has been described through increased lung weight by five days post-infection, pronounced lung pathology characterized by increased consolidation and necrosis, and increased blockage of airways (cellular debris and infiltrates) by two days post-infection as compared to resistant C57BL/6 mice [5,23]. Furthermore, increased percentages of granulocytes and decreased percentages of lymphocytes from total white blood cell counts have been shown to correlate with severity of disease [24]. Finally, increased disease severity in DBA/2 mice has also been associated with higher lung viral load as early as 12 to 24 hours post-infection and greater viral spread into the alveolar regions of the lung compared to resistant mouse strains [5-8,11]. While these phenotypes have been ascribed to susceptible mouse strains, the mechanisms of increased disease are still in question.

Several potential mechanisms for the significant differences in disease and viral load between resistant and susceptible mouse strains have been proposed. One hypothesis is that a difference in sialic acid receptor expression within the respiratory tract supports increased infection in susceptible mouse strains. Human-origin influenza A viruses preferentially bind α-2,6 linked sialic acids, avian influenza viruses preferentially bind α-2,3 linked sialic acids, whereas swine-origin influenza viruses may bind either, all of which is dependent on the hemagglutinin [25,26]. A study by Pica et al. compared the virulence of a panel of influenza viruses, including human, avian, and swine origin influenza A viruses, mouse-adapted influenza A viruses, and influenza B viruses in DBA/2 and C57BL/6 mice. While their study consistently demonstrated increased disease severity in the DBA/2 mice, with pathogenicity correlating with lung virus titers, they found no correlation between disease severity and sialic acid binding specificity of the viruses [11]. Although the presence of specific sialic acids was not assessed in the DBA/2 and C57BL/6 mice, the lack of difference between human and avian viruses suggests that differential receptor expression was not responsible for the increased disease severity in DBA/2 mice [11]. Earlier research utilizing an *ex vivo* primary differentiated cell culture approach demonstrated that murine tracheal epithelial cells (mTECs†) derived from C57BL/6 mice predominantly express the avian α-2,3-linked sialic acid receptor [27]. Subsequent analysis of the lung airways also showed only α-2,3-linked sialic acids on the ciliated epithelial cells, which were preferentially infected upon *in vivo* infection with mouse-adapted H1N1 influenza virus. In addition, a human-origin H1N1 failed to infect the mTEC cell cultures or C57BL/6 mice [27]. Casanova et al. demonstrated small, albeit significantly increased expression of the α-2,3 linked sialic acids but no difference in α-2,6 linked sialic acid expression on alveolar macrophages and mTECs derived from DBA/2 mice compared to cells derived from C57BL/6 mice [23]. In this study, infection of the DBA/2 mTECs with a mouse-adapted H1N1 virus also resulted in increased virus titers compared to the C57BL/6 mTECs, which was abrogated with neuraminidase treatment, suggesting that differences in receptor expression may partially explain the difference in viral load during early influenza infection [23]. While this may in part explain the increased pathology and severity of disease in DBA/2 mice, the receptor specificities of the mouse-adapted viruses used in these studies are unclear. Moreover, increased α-2,3 linked sialic acid expression would not account for differences in disease severity seen with human and swine influenza viruses having α-2,6 linked sialic acid specificities. Other mechanisms of increased disease severity in the DBA/2 mice must play a role, i.e. the host innate immune response.

VARIATION IN THE IMMUNE RESPONSE POST INFECTION

Innate Immune Response

The innate immune response to influenza infection in susceptible mouse strains has been categorized as hyper-inflammatory [9]. Studies show increased infiltration of neutrophils and macrophages to the lung by two and six days post-infection in DBA/2 compared to C57BL/6 mice [5,23]. Furthermore, alveolar macrophages derived from DBA/2 mice were shown to have greater phagocytic activity, compared to C57BL/6 derived macrophages. In the same study, myeloperoxidase (MPO) activity, a

marker for neutrophil activity in the lung, was increased in DBA/2 mice compared to C57BL/6 mice by day 4 post-infection. In addition, despite infecting C57BL/6 mice with an almost 100 times greater inoculum, MPO activity did not increase [23]. In contrast, a previous study infected C57BL/6 with 100 times greater inoculum resulting in CCL2 and TNFα production increasing to similar concentrations as DBA/2 mice given the lower inoculum dose [22]. Together these data suggest that the hyper-inflammatory immune response in DBA/2 mice is only partially due to viral load, and host genetics regulating the innate immune response contributes as well. Several studies agree that in addition to greater infiltration of neutrophils and macrophages, greater production of pro-inflammatory chemokines and cytokines contribute to the hyper-inflammatory response seen in the influenza susceptible DBA/2 strain. Pro-inflammatory cytokines including TNFα, IL-6, and IFNγ have been demonstrated to be produced to greater levels in the lungs of DBA/2 mice anywhere from 1 to 7 days post-infection. Furthermore, these studies have also shown increased chemokine production, including MCP-1, KC, MIP2, IP-10, and G-CSF early during infection in the lungs of DBA/2 mice compared to C57BL/6, although akin to the cytokine responses, the exact kinetics is unclear (Table 1) [5-7,22,23,28]. Importantly, a variety of inoculums and influenza strains including reassortants of highly pathogenic avian H5N1 (HK213) and mouse-adapted H3N2 (X31) and H1N1 strains (swine and PR8) were used across these studies, which could contribute to the differences in kinetics and discrepancy in IL-1β production. Separate studies have compared influenza infection in A/J mice, another susceptible strain to C57BL/6 mice. In this susceptible versus resistant comparison, several proinflammatory cytokines and chemokines were increased in the susceptible A/J mice, including IL-6, TNFα, and IL-10, as well as interferon β (IFNβ) and GM-CSF [29]. Importantly, an early study by Szretter et al. demonstrated a role for TNFα in morbidity during influenza virus infection [30], supporting the potential impact of early increased levels of TNFα production in the susceptible DBA/2 and A/J mouse strains. While a variety of studies support the role of early inflammatory cytokine responses, additional studies to refine the kinetics of specific cytokine and chemokine production are needed to clarify their contribution to susceptibility to infection and relate these studies to human disease.

Adaptive Immune Response

The adaptive immune response has also been studied among resistant and susceptible mouse strains. Historically, many of the studies focusing on the T cell response to influenza infection in the murine model used the more resistant C57BL/6 and BALB/c strains. In these strains, $CD8^+$ T cells begin to expand in the mediastinal lymph node (mLN) 3 to 5 days post-infection but are not detectable in the lung until at least day 5, peaking between days 9 to 11 days post-infection, and contracting over the following week [31-34]. Studies analyzing the $CD4^+$ T cell response to influenza virus infection demonstrate similar kinetics, albeit a reduced magnitude of response [35,36]. Interestingly, a more recent study assessing $CD4^+$ T cell responses to H1N1 influenza virus infection in resistant and susceptible mouse strains (BALB/c and A/J mice, respectively) demonstrated both strain elicited robust $CD4^+$ T cell responses in the mLN and spleen. The precise epitopes differed as the BALB/c and A/J mice have distinct MHC haplotypes ($I\text{-}A^d$, $I\text{-}E^d$ and $I\text{-}A^k$, $I\text{-}E^k$, respectively) [37]. However, while the A/J mice were more susceptible to primary infection, prior exposure to influenza (i.e. sub-lethal infection) elicited comparable immune memory and protection from subsequent lethal challenge, indicating immune memory is sufficient to protect even highly susceptible mouse strains from enhanced disease [37]. Finally, while some mouse strains have been described as Th1 or Th2 biased, being predisposed to pro-inflammatory or anti-inflammatory adaptive immune responses, respectively [38], these descriptions do not translate to resistance or susceptibility. C56BL/6 and BALB/c mice are categorized as Th1- and Th2- biased, respectively, but are both resistant to influenza virus infection. In contrast, the susceptible DBA/2 strain is categorized as an intermediate phenotype between Th1 and Th2 [38]. Ultimately, while there are differences in cellular adaptive immune responses in susceptible and resistant mouse strains, the proposed hyper-inflammatory response and higher viral load occur within the first few days of influenza infection, prior to development of the primary T cell response. Thus, differences in the T cell response are unlikely to contribute to the increased disease pathogenesis described in susceptible mouse strains.

Humoral immunity has also been considered as a possible contributing factor to differences in influenza pathogenesis in resistant versus susceptible mouse strains. In addition to assessing the potential contribution of influenza virus receptor expression, Pica et al. assessed the antibody response to sub-lethal infection with influenza, measuring IgA, IgM, IgG1, IgG2a, IgG2b, and IgG3 isotypes. It was established that there was no difference in antibody responses between DBA/2 and C57BL/6 mice [11]. Serum complement proteins have also been considered. DBA/2 mice have a two base-pair deletion rendering them deficient in the fifth complement protein (C5), whereas both C57BL/6 and BALB/c strains are C5 sufficient [39], suggesting a potential mechanism for disease susceptibility. However, in a recent study, Casanova et al. administered compliment-sufficient serum by infu-

Table 1. Cytokines and chemokines increased in DBA/2 relative to C57BL/6 mice in response to influenza virus infection. The virus strain used in each study is indicated.

	Days Post Infection						
	1	2	3	4	5	6	7
			CYTOKINES				
G-CSF (CSF3)	-	-	-	-	X31	X31	X31
	-	PR8	PR8	PR8	-	-	-
	-	-	HK213	-	-	-	-
IFNα	-	HK213#	HK213	-	-	-	-
	swH1N1*	swH1N1*	-	-	-	-	-
IFNβ	-	-	HK213	-	-	-	-
	swH1N1*	-	-	-	-	-	-
IFNγ	-	-	-	-	X31	X31	X31
	-	-	-	-	-	swH1N1	swH1N1
IL-1α	-	PR8	PR8	-	-	-	-
IL-1β	-	-	-	-	X31*	X31*	X31*
	-	-	swH1N1	swH1N1	swH1N1	-	-
IL-5	-	PR8	PR8	PR8	-	-	-
IL-6	X31	-	X31	-	X31	X31	X31
	-	-	swH1N1	swH1N1	swH1N1	swH1N1	swH1N1
	-	PR8	PR8	PR8	-	-	-
IL-12	-	-	-	PR8	-	-	-
TNFα	-	-	X31	-	X31	X31	X31
	-	HK213#	HK213	-	-	-	-
	-	-	-	swH1N1	swH1N1	swH1N1	swH1N1
			CHEMOKINES				
IP-10	X31	-	X31	-	X31	X31	X31
	-	PR8	PR8	PR8	-	-	-
KC	X31	-	X31	-	X31	X31	X31
	-	PR8	PR8	PR8	-	-	-
	-	-	swH1N1	swH1N1	swH1N1	swH1N1	swH1N1
MCP-1 (CCL2)	X31	-	X31	-	X31	X31	X31
	-	PR8	PR8	PR8	-	-	-
	-	HK213#	-	-	-	-	-
	-	-	-	swH1N1	swH1N1	swH1N1	swH1N1
MIG	-	PR8	PR8	PR8	-	-	-
MIP1α	X31	-	X31	-	X31	X31	X31
	-	PR8	PR8	PR8	-	-	-
	-	-	-	-	-	swH1N1	swH1N1
MIP2 (CXCL2)	-	-	HK213	-	-	-	-
	-	PR8	PR8	PR8	-	-	-
	swH1N1	-	swH1N1	swH1N1	swH1N1	swH1N1	-
RANTES	-	PR8	PR8	PR8	-	-	-

Table 1 cont'd.
X31 – A/Aichi/2/68 (H3N2) x31 (6:2 reassortment with PR8) [7,59]
PR8 – mouse-adapted A/Puerto Rico/8/34 (H1N1) [5]
HK213# – reverse genetics A/Hong Kong/213/2003 (PB1 segment A/Chicken/Hong Kong/Y0562/2002) (H5N1) [28]
HK213 – reverse genetics A/Hong Kong/213/2003 (PB1 segment A/Chicken/Hong Kong/Y0562/2002) (H5N1) [6,22]
swH1N1 – mouse-adapted A/Swine/Iowa/4/1976 (H1N1) [23]
-No data available or data available lacks statistical significance
*C57BL/6 > DBA/2

sion to DBA/2 mice prior to influenza infection with no effect on survival or body weight [23]. Together this data suggest that the humoral response does not contribute to the differences in influenza pathogenesis between DBA/2 and C57BL/6 mice; however, further studies are needed to dismiss the role of humoral immunity.

DIFFERENTIALLY REGULATED GENES POST-INFECTION

One major benefit of using the mouse model is the availability of a variety of inbred strains, each with their own unique genetic profile. Although it has been established that influenza pathogenesis in the mouse model does not completely correlate with human disease, there has been some debate whether the genetic expression profile in response to infection closely mimics human infection. One study utilizing C57BL/6 mice determined poor genomic correlation between the murine model and human response to a variety of inflammatory stressors [40]; however, weaknesses in the approaches and overly broad conclusions reduce the concerns raised by the report [41]. In contrast, a second study utilizing both C57BL/6 and BALB/c mice demonstrated significant correlation between the mouse and human genetic response to inflammatory conditions [42]. Furthermore, a recent study found that the collaborative cross founder strains including C57BL/6J, 129S1/Svlmj, CAST/EiJ, and PWK/PhJ resulted in gene signature profiles that closely mimicked the human response to influenza A virus [43]. The *Mx1* gene in most inbred murine strains (including BALB/c, C57BL/6, A/J, and DBA/2), has a large deletion or nonsense mutation, resulting in a loss of function. In humans, the *Mx* gene is fully functional and capable of conferring resistance to influenza infection [44-47]. The importance of interferon induced Mx resistance has been shown both *in vitro* and *in vivo*, by use of genetic crosses with A2G mice (an inbred mouse strain with an intact *Mx1* gene) [46,48,49]. A more recent study demonstrated even the reduced expression of the *Mx1* gene in human monocytes and macrophages is correlated with increased expression of influenza genes while *Mx1* sufficient mice are more resistant to influenza infection than their wild type counterparts [50]. Although Mx protiens confer protection against influenza infection, early and rapid replication of the virus can overcome this antiviral response [51]. Moreover, while the data are compelling, *Mx1* is only one gene out of many that contribute to the pathogenesis outcome of influenza infection in mice and in humans.

Studies have used a variety of methods and programs including quantitative trait locus (QTL), gene chip array, genome-wide linkage analysis, ingenuity pathway analysis (IPA), and gene ontology to connect the influenza pathogenesis phenotype to specific genes and molecular pathways that differ in resistant and susceptible murine strains. Using gene ontology, one study compared DBA/2 and C57BL/6 mice 1 to 4 days post influenza infection and established DBA/2 had an overall greater number of genes upregulated. Moreover, the genes upregulated in DBA/2 were associated with the immune response whereas the genes upregulated in C57BL/6 were generally associated with cell cycle and cell division. In addition, of the interferon and interferon related genes, only *IFNβ1* and *IFNγ* were upregulated in DBA/2, but not C57BL/6, while among chemokines only *CXC11*, associated with lung inflammation, was remarkably increased in DBA/2 mice. With the use of IPA, various genes found to be upregulated in DBA/2 and not C57BL/6 upon influenza infection, were associated with eicosanoid signaling, apoptosis, and coagulation [9]. Another study by Boon et al. used principal component analysis comparing DBA/2 and BALB/c mice. Genes upregulated in BALB/c mice 7 days post-infection were associated with T and B cell function, including cell adhesion molecules, and antigen processing and presentation. In contrast, susceptible strains, including DBA/2 mice, continued to upregulate cytokines. It was also noted that susceptible strains expressed similar proinflammatory cytokines as resistant strains, but to a considerably greater extent [22]. These studies highlight that differential gene expression may drive aspects of the susceptible versus resistant phenotype, but the magnitude of the host response may also mediate resistance or susceptibility.

Several studies have concentrated on using gene loci to focus on a smaller cohort of genes that could be associated with susceptibility or resistance to influenza virus infection in mice. An early study by Boon et al. used QTL mapping of a panel of recombinant inbred mouse lines to identify several loci on chromosomes 2, 7, and 17 associated with the resistant phenotype. Furthermore, they identified 30 candidate genes including *Trim12*, *Trim34*, *Plekhb1*, *Prkrir*, *Trpc20*, *Med1*, and *Hc* [6]. Additional testing on the role of *Hc* (hemolytic complement, identified on chromosome 2) to validate the QTL

analysis compared influenza infection in *Hc* competent and *Hc* knockout mice. Boon et al. found a dose-dependent resistant phenotype in *Hc* intact mice compared to *Hc* knockout mice [6]. Subsequent studies by Boivin et al. suggest *Hc*-related susceptibility may be dominant in female mice, highlighting the complexities of resistance and susceptibility studies and the potential for sexual dimorphism [29]. Loci on chromosome 5, 16, 17-1, 17-2, and 19 have also been associated with resistance to influenza-mediated disease, leading to approximately 30 candidate genes including *Sik1*, *Eif2ak1*, *Itgb6*, *Ifih1*, *Robo1*, *Nrip1*, and *LST1*, some of which regulate innate immune pathways [52]. Further studies on the role of *LST1* (leukocyte specific transcript 1) in influenza infection demonstrated increased weight loss and a slight increase in mortality in *LST1* knockout mice compared to the C57BL/6 parental strain. Interestingly, DBA/2 mice have a deletion in the *LST1* gene; however, there was no difference in the histopathology or immune cell infiltrates found in the lungs of wild type C57BL/6 compared to $LST1^{(-/-)}$ mice, suggesting that while *LST1* may contribute to the susceptibility seen in DBA/2 mice, there are additional factors contributing to the phenotype [53]. A single locus on chromosome 6 has recently been associated with greater inflammation as demonstrated by increased production of both TNFα and IFNα within 48 hours of infection. Comparing this locus in a variety of resistant and susceptible murine strains, genes *Sam9l*, *Slc25a13*, and *Ica1* all contained single nucleotide polymorphisms (SNPs). In this locus, the gene *Col28a1* contained an in-frame deletion as well. However, there was no significant difference in cytokine production, morbidity, or mortality resulting from influenza infection between a *Slc25a13* knockout and its parental strain [28]. Much of the QTL analysis has been done using C57BL/6 and DBA/2 strains or a variety of BXD crosses (recombinant inbred strains derived from a cross of C57BL/6J ($H\text{-}2^b$) and DBA/2J ($H\text{-}2^d$)). However, a recent study utilized the collaborative cross inbred mouse panel [54] to assess influenza susceptibility and resistance against a diverse genetic background representative of the human population. This study found several loci contributing to the disease phenotype, including a novel allele of *Mx1*, identified in the well-described influenza resistance locus on chromosome 16. Moreover, a novel locus on chromosome 7 potentially associated with weight loss was identified and includes candidate genes *Nox4* and *Il16*. Additionally, a locus on chromosome 1 associated with changes in pulmonary edema and a locus on chromosome 15, potentially associated with differences in neutrophil infiltrates in the airway were discovered [55]. These findings suggest that the host pathways that drive increased inflammation and subsequent increases in morbidity and mortality are regulated by a complex network of genes and gene products having overlapping and sometimes competitive effects. Thus, connecting candidate genes found by transcriptional analysis and the innate immune pathways regulated by those genes is the next step in discerning this complicated web of interactions that can ultimately result in the difference between susceptibility and resistance to influenza infection.

CONCLUSIONS AND FUTURE PERSPECTIVES

The immune response to influenza infection in the murine model can be categorized by strain on a scale from susceptible to resistant based on morbidity and mortality. Substantial weight loss, a high lung viral load, and a robust proinflammatory response characterize strains that are susceptible to influenza infection. The pro-inflammatory response includes increased neutrophil and macrophage recruitment and increased production of cytokines and chemokines within a few days of influenza virus infection. Thus, disease in susceptible strains reflects the acute and excessive pro-inflammatory, antiviral response. However, in some studies, a high virus load in the lung, seen within 48 hours of infection, may contribute to the increased inflammatory response seen in susceptible mouse strains. While several groups have postulated mechanisms for the increased viral load early during infection, there is still no definitive answer. Furthermore, there is still debate whether a higher viral load elicits a proinflammatory response in resistant strains similar to that found in susceptible strains. Other host factors are almost certainly playing a role. Elucidating what host factors contribute to susceptibility to influenza infection in the murine model, may reveal possible factors that modulate the immune response in humans.

This review has focused on the murine model; however, there are other established animal models of influenza virus infection. Ferrets are a well-established model for influenza infection and considered superior to mice by some researchers as they are susceptible to infection with most human influenza strains without prior adaptation and the symptoms mimic human disease. Importantly, ferrets can be used for both pathogenesis and transmission studies, whereas mice do not readily transmit influenza virus. However, there are few immunologic reagents available for the ferret and the genetics are as yet poorly defined, limiting mechanistic studies in this model. Other animals used in influenza research include hamsters, cotton rats, guinea pigs, swine, and non-human primates (reviewed in detail by Bouvier and Lowen [56]). Each of these animal models has specific benefits and drawbacks, but only the mouse model has the array of genetically defined strains and transgenic or knock out mice and robust tools for analyzing the host response to infection critical for dissection of determinants of influenza susceptibility.

The mouse model is a widely accepted animal model for influenza virus infection and particularly useful for interrogation of the immune response to infection. A variety of disease endpoints are commonly used, including weight loss, survival, lung virus titer, lung weight, and histopathology [56]. However, these endpoints may not fully measure acute respiratory distress syndrome (ARDS) in humans [57], which is associated with viral pneumonia, the primary complication of influenza infection in humans [58]. Many of the studies addressing susceptibility to influenza infection do not directly consider ARDS, which needs to be considered for translation to human disease.

The comparison of host responses to influenza virus infection in resistant (C57Bl/6) and susceptible (DBA/2) mice has established a useful model system for interrogating host determinants of disease. Consideration of endpoints more relevant to human disease (i.e. ARDS) when defining susceptibility and resistance will only strengthen this model. Future studies should continue to develop the network of host immune pathways involved in the response to influenza virus infection. Identification of determinants of susceptibility or resistance to influenza-associated disease is critical for risk assessment as well as development of effective treatments for individuals with severe influenza disease.

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REFERENCES

1. Organization WH. Influenza (seasonal) fact sheet N211. 2014.
2. Centers for Disease C, Prevention. Prevention and control of seasonal influenza with vaccines. Recommendations of the Advisory Committee on Immunization Practices--United States, 2013-2014. MMWR Recomm Rep. 2013;62(RR-07):1-43.
3. Flannery B, Chung JR, Thaker SN, Monto AS, Martin ET, Belongia EA, et al. Interim Estimates of 2016-17 Seasonal Influenza Vaccine Effectiveness - United States, February 2017. MMWR Morb Mortal Wkly Rep. 2017;66(6):167-71.
4. Barker WH, Mullooly JP. Impact of epidemic type A influenza in a defined adult population. Am J Epidemiol. 1980;112(6):798-811.
5. Srivastava B, Blazejewska P, Hessmann M, Bruder D, Geffers R, Mauel S, et al. Host genetic background strongly influences the response to influenza a virus infections. PLoS One. 2009;4(3):e4857.
6. Boon ACM, deBeauchamp J, Hollmann A, Luke J, Kotb M, Rowe S, et al. Host genetic variation affects resistance to infection with a highly pathogenic H5N1 influenza A virus in mice. Journal of virology. 2009;83:10417-26.
7. Trammell RA, Liberati TA, Toth LA. Host genetic background and the innate inflammatory response of lung to influenza virus. Microbes Infect. 2012;14(1):50-8.
8. Blazejewska P, Koscinski L, Viegas N, Anhlan D, Ludwig S, Schughart K. Pathogenicity of different PR8 influenza A virus variants in mice is determined by both viral and host factors. Virology. 2011;412(1):36-45.
9. Alberts R, Srivastava B, Wu H, Viegas N, Geffers R, Klawonn F, et al. Gene expression changes in the host response between resistant and susceptible inbred mouse strains after influenza A infection. Microbes and infection / Institut Pasteur. 2010;12:309-18.
10. Zaraket H, Bridges OA, Russell CJ. The pH of activation of the hemagglutinin protein regulates H5N1 influenza virus replication and pathogenesis in mice. J Virol. 2013;87(9):4826-34.
11. Pica N, Iyer A, Ramos I, Bouvier NM, Fernandez-Sesma A, Garcia-Sastre A, et al. The DBA.2 mouse is susceptible to disease following infection with a broad, but limited, range of influenza A and B viruses. J Virol. 2011;85(23):12825-9.
12. Otte A, Gabriel G. 2009 pandemic H1N1 influenza A virus strains display differential pathogenicity in C57BL/6J but not BALB/c mice. Virulence. 2011;2(6):563-6.
13. Shaw MH, Boyartchuk V, Wong S, Karaghiosoff M, Ragimbeau J, Pellegrini S, et al. A natural mutation in the Tyk2 pseudokinase domain underlies altered susceptibility of B10.Q/J mice to infection and autoimmunity. Proc Natl Acad Sci U S A. 2003;100(20):11594-9.
14. O'Brien AD, Rosenstreich DL, Taylor BA. Control of natural resistance to Salmonella typhimurium and Leishmania donovani in mice by closely linked but distinct genetic loci. Nature. 1980;287(5781):440-2.
15. Skamene E, Gros P, Forget A, Kongshavn PA, St Charles C, Taylor BA. Genetic regulation of resistance to intracellular pathogens. Nature. 1982;297(5866):506-9.
16. Watters JW, Dietrich WF. Genetic, physical, and transcript map of the Ltxs1 region of mouse chromosome 11. Genomics. 2001;73(2):223-31.
17. Medina E, North RJ. Resistance ranking of some common inbred mouse strains to Mycobacterium tuberculosis and relationship to major histocompatibility complex haplotype and Nramp1 genotype. Immunology. 1998;93(2):270-4.
18. Tuite A, Mullick A, Gros P. Genetic analysis of innate immunity in resistance to Candida albicans. Genes Immun. 2004;5(7):576-87.
19. Fortin A, Stevenson MM, Gros P. Complex genetic control of susceptibility to malaria in mice. Genes Immun. 2002;3(4):177-86.
20. Groves MG, Osterman JV. Host defenses in experimental scrub typhus: genetics of natural resistance to infection. Infect Immun. 1978;19(2):583-8.
21. Bernstein-Hanley I, Balsara ZR, Ulmer W, Coers J, Starnbach MN, Dietrich WF. Genetic analysis of susceptibility to Chlamydia trachomatis in mouse. Genes Immun. 2006;7(2):122-9.
22. Boon AC, Finkelstein D, Zheng M, Liao G, Allard J, Klumpp K, et al. H5N1 influenza virus pathogenesis in genetically diverse mice is mediated at the level of viral load. MBio. 2011;2(5).

23. Casanova T, Van de Paar E, Desmecht D, Garigliany MM. Hyporeactivity of Alveolar Macrophages and Higher Respiratory Cell Permissivity Characterize DBA/2J Mice Infected by Influenza A Virus. J Interferon Cytokine Res. 2015;35(10):808-20.
24. Dengler L, Kuhn N, Shin DL, Hatesuer B, Schughart K, Wilk E. Cellular changes in blood indicate severe respiratory disease during influenza infections in mice. PLoS One. 2014;9(7):e103149.
25. Gambaryan AS, Karasin AI, Tuzikov AB, Chinarev AA, Pazynina GV, Bovin NV, et al. Receptor-binding properties of swine influenza viruses isolated and propagated in MDCK cells. Virus Res. 2005;114(1-2):15-22.
26. Rogers GN, Paulson JC. Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin. Virology. 1983;127(2):361-73.
27. Ibricevic A, Pekosz A, Walter MJ, Newby C, Battaile JT, Brown EG, et al. Influenza virus receptor specificity and cell tropism in mouse and human airway epithelial cells. Journal of virology. 2006;80:7469-80.
28. Boon AC, Williams RW, Sinasac DS, Webby RJ. A novel genetic locus linked to pro-inflammatory cytokines after virulent H5N1 virus infection in mice. BMC Genomics. 2014;15:1017.
29. Boivin GA, Pothlichet J, Skamene E, Brown EG, Loredo-Osti JC, Sladek R, et al. Mapping of clinical and expression quantitative trait loci in a sex-dependent effect of host susceptibility to mouse-adapted influenza H3N2/HK/1/68. J Immunol. 2012;188(8):3949-60.
30. Szretter KJ, Gangappa S, Lu X, Smith C, Shieh WJ, Zaki SR, et al. Role of host cytokine responses in the pathogenesis of avian H5N1 influenza viruses in mice. J Virol. 2007;81(6):2736-44.
31. Tripp RA, Sarawar SR, Doherty PC. Characteristics of the influenza virus-specific CD8+ T cell response in mice homozygous for disruption of the H-2lAb gene. J Immunol. 1995;155(6):2955-9.
32. Flynn KJ, Belz GT, Altman JD, Ahmed R, Woodland DL, Doherty PC. Virus-specific CD8+ T cells in primary and secondary influenza pneumonia. Immunity. 1998;8(6):683-91.
33. Lawrence CW, Braciale TJ. Activation, differentiation, and migration of naive virus-specific CD8+ T cells during pulmonary influenza virus infection. J Immunol. 2004;173(2):1209-18.
34. Lawrence CW, Ream RM, Braciale TJ. Frequency, specificity, and sites of expansion of CD8+ T cells during primary pulmonary influenza virus infection. J Immunol. 2005;174(9):5332-40.
35. Eichelberger M, Allan W, Zijlstra M, Jaenisch R, Doherty PC. Clearance of influenza virus respiratory infection in mice lacking class I major histocompatibility complex-restricted CD8+ T cells. J Exp Med. 1991;174(4):875-80.
36. Doherty PC, Topham DJ, Tripp RA, Cardin RD, Brooks JW, Stevenson PG. Effector CD4+ and CD8+ T-cell mechanisms in the control of respiratory virus infections. Immunol Rev. 1997;159:105-17.
37. Alam S, Sant AJ. Infection with seasonal influenza virus elicits CD4 T cells specific for genetically conserved epitopes that can be rapidly mobilized for protective immunity to pandemic H1N1 influenza virus. J Virol. 2011;85(24):13310-21.
38. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol. 2000;164(12):6166-73.
39. Wetsel RA, Fleischer DT, Haviland DL. Deficiency of the murine fifth complement component (C5). A 2-base pair gene deletion in a 5'-exon. J Biol Chem. 1990;265(5):2435-40.
40. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A. 2013;110(9):3507-12.
41. Cauwels A, Vandendriessche B, Brouckaert P. Of mice, men, and inflammation. Proceedings of the National Academy of Sciences. 2013;110(34):E3150.
42. Takao K, Miyakawa T. Genomic responses in mouse models greatly mimic human inflammatory diseases. Proc Natl Acad Sci U S A. 2015;112(4):1167-72.
43. Elbahesh H, Schughart K. Genetically diverse CC-founder mouse strains replicate the human influenza gene expression signature. Sci Rep. 2016;6:26437.
44. Lindenmann J. Inheritance of Resistance to Influenza Virus in Mice. Proc Soc Exp Biol Med. 1964;116:506-9.
45. Lindenmann J. Resistance of mice to mouse-adapted influenza A virus. Virology. 1962;16:203-4.
46. Staeheli P, Grob R, Meier E, Sutcliffe JG, Haller O. Influenza virus-susceptible mice carry Mx genes with a large deletion or a nonsense mutation. Mol Cell Biol. 1988;8(10):4518-23.
47. Haller O, Kochs G. Interferon-induced mx proteins: dynamin-like GTPases with antiviral activity. Traffic. 2002;3(10):710-7.
48. Haller O, Arnheiter H, Gresser I, Lindenmann J. Virus-specific interferon action. Protection of newborn Mx carriers against lethal infection with influenza virus. J Exp Med. 1981;154(1):199-203.
49. Staeheli P, Haller O, Boll W, Lindenmann J, Weissmann C. Mx protein: constitutive expression in 3T3 cells transformed with cloned Mx cDNA confers selective resistance to influenza virus. Cell. 1986;44(1):147-58.
50. Pillai PS, Molony RD, Martinod K, Dong H, Pang IK, Tal MC, et al. Mx1 reveals innate pathways to antiviral resistance and lethal influenza disease. Science. 2016;352(6284):463-6.
51. Grimm D, Staeheli P, Hufbauer M, Koerner I, Martinez-Sobrido L, Solorzano A, et al. Replication fitness determines high virulence of influenza A virus in mice carrying functional Mx1 resistance gene. Proc Natl Acad Sci U S A. 2007;104(16):6806-11.
52. Nedelko T, Kollmus H, Klawonn F, Spijker S, Lu L, Hessman M, et al. Distinct gene loci control the host response to influenza H1N1 virus infection in a time-dependent manner. BMC Genomics. 2012;13:411.
53. Leist SR, Kollmus H, Hatesuer B, Lambertz RL, Schughart K. Lst1 deficiency has a minor impact on course and outcome of the host response to influenza A H1N1 infections in mice. Virol J. 2016;13:17.
54. Churchill GA, Airey DC, Allayee H, Angel JM, Attie AD,

Beatty J, et al. The Collaborative Cross, a community resource for the genetic analysis of complex traits. Nat Genet. 2004;36(11):1133-7.
55. Ferris MT, Aylor DL, Bottomly D, Whitmore AC, Aicher LD, Bell TA, et al. Modeling host genetic regulation of influenza pathogenesis in the collaborative cross. PLoS Pathog. 2013;9(2):e1003196.
56. Bouvier NM, Lowen AC. Animal Models for Influenza Virus Pathogenesis and Transmission. Viruses. 2010;2(8):1530-63.
57. Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, et al. An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Biol. 2011;44(5):725-38.
58. Short KR, Kroeze EJ, Fouchier RA, Kuiken T. Pathogenesis of influenza-induced acute respiratory distress syndrome. Lancet Infect Dis. 2014;14(1):57-69.
59. Toth LA, Rehg JE, Webster RG. Strain differences in sleep and other pathophysiological sequelae of influenza virus infection in naive and immunized mice. J Neuroimmunol. 1995;58(1):89-99.