2009 pandemic H1N1 influenza A virus strains display differential pathogenicity in C57BL/6J but not BALB/c mice

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Influenza A viruses are the causative agents of annual epidemics and occasional pandemics. The pathogenicity of influenza viruses is determined by complex interplay of viral and host factors. While some knowledge exists on viral determinants of pathogenicity, little is known on the host factors involved. Here, we discuss our recent findings on host genetic variations involved in disease outcome. We found that 2009 pandemic H1N1 influenza A virus strains (pH1N1) are low pathogenic in BALB/c but display differential pathogenicities in C57BL/6J mice. In contrast, a highly pathogenic avian influenza A virus (HPAIV) strain of the H5N1 subtype isolated from a fatal human case was more virulent in BALB/c than C57BL/6J mice. As a control, we used a seasonal H1N1 influenza virus that showed marginal pathogenicity in both mouse strains. Thus, differences in pH1N1 virulence become visible in C57BL/6J mice, while intrinsic pH1N1 pathogenicity markers are masked in BALB/c mice. Further, increased pH1N1 virulence correlated with a depressed cytokine response in C57BL/6J mice, while increased H5N1 virulence correlated with an elevated proinflammatory cytokine response in BALB/c mice. These findings indicate that disease severity can be strongly regulated by the host genetic background. Moreover, our findings suggest that differential host determinants contribute to the pathogenesis of pH1N1 and human H5N1 influenza viruses. Further studies are needed to identify the responsible viral factors involved in enhanced pH1N1 virulence in C57BL/6J mice. Also, extensive studies are needed to identify and characterize cellular factors regulating pH1N1 or H5N1 susceptibility in a host-dependent manner. These observations extend our knowledge on influenza virus pathogenicity and highlight the role of host-dependent factors, especially in pH1N1 susceptibility. We propose the C57BL/6J mouse strain as a convenient small animal model to study pH1N1 virulence determinants. Furthermore, the C57BL/6J mouse strain might also represent a suitable model for the assessment of pH1N1 vaccine candidates or the evaluation of antiviral therapies.

Understanding influenza virus pathogenicity is essential for prevention and control of future outbreaks. The pathogenesis of influenza viruses involves a complex interplay of viral and cellular factors. We found that two pH1N1 influenza strains, A/Hamburg/05/09 (hereafter HH05) and A/Hamburg/NY1580/09 (hereafter HH15) display differential virulences in C57BL/6J mice, while BALB/c mice are mostly resistant (Fig. 1). In the following, we focus on the discussion of viral determinants that might contribute to pH1N1 pathogenicity and on the role of host genetic factors involved in disease outcome.

Viral Factors Involved in pH1N1 Influenza Virus Pathogenicity

Major viral factors contributing to influenza virus pathogenicity include the viral hemagglutinin (HA) that triggers the first steps of infection by recognizing appropriate cellular receptors and mediating
The 2009 pH1N1 isolate HH05 is low pathogenic [log mouse lethal dose 50 (MLD50) 5.2 plaque forming units (p.f.u.)] while HH15 is highly virulent (MLD50 3.5 p.f.u.) in C57BL/6J mice. We have sequenced both genomes and found that HH05 differs from HH15 by 12 amino acid substitutions. Seven mutations are localized in the genes encoding the viral ribonucleoprotein complex (PB2, PA and NP). Four mutations are detected in the viral surface glycoproteins, the HA and the neuraminidase (NA). One substitution is also located in the NS1 gene.

Positions differing from the consensus sequence of circulating pH1N1 strains have been reported before in reference 6. Interestingly, mutations in HA S202T (H1 numbering), NA N248D, NP V100I and NS1 I123V were less prevalent in the early pandemic periods (16 April to 15 May 2009) while their prevalence increased during the late stages of the pandemic (15 June to 31 December 2009).

Figure 1. Pathogenicity of seasonal H1N1, pH1N1 (HH05 and HH15 strains) and human H5N1 influenza viruses in BALB/c and C57BL/6J mice. Upright mouse represents non-lethal infection while upside-down mouse represents lethal infection. Mouse lethal dose 50 (MLD50) was determined in plaque forming units (p.f.u.). Cytokine levels were measured in the lungs at 6 d post infection for Th1-type (tumor necrosis factor (TNF-α), interferon (IFN-γ) and macrophage chemo-attractant-protein (MCP-1)) (red) and Th2-type cytokines (interleukin (IL)-4, IL-6 and IL-10) (green). Cytokine levels elevated compared with seasonal H1N1-infected groups are shown in upright and bold letters while downregulated cytokines are indicated in upside-down letters.

Viral pathogenicity determinants of H5N1 influenza have been identified using mostly BALB/c mouse strains. However, these H5N1 virulence markers are not present in circulating pH1N1 influenza strains. Reports confirm that introduction of H5N1 virulence markers do not affect pH1N1 pathogenesis in BALB/c mice. It remains to be seen whether the introduction of HPAIV virulence markers into pH1N1 influenza strains would alter their virulence in C57BL/6J mice. Interestingly heterogeneous virulence of pH1N1 strains has been recently reported in macaques.

Here, we propose the C57BL/6J mouse strain as a suitable and convenient small animal model to identify and characterize novel pH1N1 pathogenicity markers. Viral determinants identified in C57BL/6J mice will need further evaluation in other animal models, such as ferrets, guinea pigs or macaques. This will shed light on the impact of mutations found in virulent pH1N1 strains on mammalian pathogenicity and transmission.
Cellular Factors Involved in pH1N1 Influenza Virus Pathogenicity

It has been shown that host genetic backgrounds substantially contribute to influenza virus susceptibility in mice. However, these studies have concentrated on the DBA/2J mouse strain, which was generally more susceptible to several influenza virus strains (mouse-adapted H1N1, H7N7 and H5N1) compared with the C57BL/6J strain. Moreover, our studies revealed that C57BL/6J mice are particularly important to study differential virulences among pH1N1 influenza virus strains that are not visible in BALB/c mice (Fig. 1).

Level of virus lung titers correlated with the grade of virulence in BALB/c but not C57BL/6J mice, suggesting that lethal outcome in C57BL/6J mice upon influenza virus infection is not only affected by virus replication itself but other factors, such as the host immune response, play a key role.

BALB/c mice are genetically primed to mount a Th2-type (marker cytokines: e.g., IL-4, IL-6 and IL-10) immune response, whereas C57BL/6J mice predominantly induce a Th1-type (marker cytokines: e.g., TNFα, IFNγ and MCP-1) response. Th1-primed cells play a predominant role in cellular immune response, while Th2-primed cells are important for the generation of a humoral immune response. We have analyzed cytokine profiles in the lungs of influenza virus-infected mice. In general, C57BL/6J mice infected with seasonal H1N1, pH1N1 or human H5N1 displayed a significantly lower cytokine response in contrast to BALB/c mice. Increased human H5N1 pathogenicity correlated with elevated levels of proinflammatory cytokines (TNFα, IFNγ, MCP-1 and IL-6). In contrast, enhanced susceptibility to pH1N1 correlated with generally reduced expression of Th1- and Th2-type cytokines in C57BL/6J mice. Cytokines may have beneficial or, in case of an unbalanced response, detrimental effects on viral disease presentation. Indeed “dampening” H5N1-induced hypercytokinemia by using knockout-mouse models was not protective but reduced H5N1 mortality. It is tempting to speculate whether an induction of beneficial cytokines might reduce pH1N1 mortality.

Th2-type cytokines, such as IL-4 and IL-10, were downregulated while proinflammatory cytokines (IFNγ, MCP-1 and IL-6) became more prevalent in pH1N1-infected C57BL/6J compared with BALB/c mice. Remarkably, elevated proinflammatory cytokines and decreased IL-10 levels were also reported in pH1N1 infected humans. It seems that IL-4 and IL-10 cytokines might have some beneficial effect on pH1N1 disease outcome in BALB/c mice. IL-4 is a key cytokine of the Th2-type response antagonizing proinflammatory cytokines. Modulators increasing IL-4 production upon influenza infection in mice have been successfully shown to reduce mortality. Similarly, IL-10 antagonizes Th1-type immune response by suppressing inflammation and cytokine production in the lung. Future studies will reveal whether IL-4 and IL-10 cytokines might have some beneficial impact on pH1N1 pathogenesis.

Another hallmark of lethal influenza virus infection in our studies has been lymphocyte depletion in the corresponding mouse strain. C57BL/6J mice underwent prolonged lymphopenia upon more virulent HH15 infection in contrast to low pathogenic HH05 infection. Accordingly, higher grade of lymphocyte depletion was detected in H5N1-infected BALB/c than C57BL/6J mice. Remarkably, it was reported that lymphocyte count inversely correlated with viral load in pH1N1-infected patients. A reduced leukocyte count has also been detected in the patients who were infected with HH05 and HH15 (unpublished data; HH05: 3.7 Mrd/L; HH15: 3.2 Mrd/L; normal range: 3.8–11.9 Mrd/L). Another distinguishing feature observed in pH1N1-infected animals has been the detection of virus titers in extrapulmonary organs. For example, virus titers were detected in the gut of HH15- but not HH05-infected C57BL/6J mice that likely represented different stages of viremia as no replicating virus was detected. It was reported before that pH1N1 infection courses are often associated with gastrointestinal symptoms in humans. Remarkably, viremia was also reported in patients with pH1N1 requiring intensive care.

Furthermore, studies derived from epidemiologic data also postulated the contribution of host genetic markers to influenza disease outcome. The retinoic acid inducible gene 1 (RIG-I) has been shown to interact with the viral NS1 protein leading to viral evasion from recognition by the host immune system. Very recently, we could show that the cellular importin-α7 (KPNA6) gene is a key regulator of pH1N1 pathogenicity in mice. Mice lacking the importin-α7 gene were resistant to pH1N1 strains but not to an H5N1 strain isolated from humans. This finding further supports our observations that differential host factors contribute to pH1N1 and H5N1 influenza virus disease outcome.

Genotyping of specimens derived from patients with severe pneumonia identified novel single-nucleotide polymorphisms (SNP) on human chromosomes 1 and 17 (FCGR2A and C1QBP). The authors postulated that these factors might potentially play a role in susceptibility to influenza in humans. Several SNPs have been identified in trials comparing safety, immunogenicity and efficacy of influenza virus vaccines in the mannose-binding lectine (MBL) 2 gene and in the TNFα and IL-10 promoter regions. A polymorphism in the IL-10 promoter region at -1,082 (A/G) conferred a significantly lower risk of the development of adverse responses in individuals with the A/A genotype.

In summary, understanding the role of host genetic variations in disease outcome may reveal novel innovative approaches to prevent and control future influenza outbreaks. Furthermore, host genetic markers contributing to disease outcome would be invaluable to define risk groups with predisposition to severe illness. This would allow early intervention strategies and permit more effective distribution of medication, e.g., in the event of a pandemic.

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