Supplemental materials

Detailed discussion of 25 overlapping genes (Table 2)

Zhou et al [1] combined an siRNA screen with expression analysis in the human lung epithelial cell line A549 after infection with PR8 virus. They identified 300 genes as significantly up-regulated and subsequently performed an siRNA screen for these genes. This screen detected 52 genes as regulators of viral replication, including 40 genes that were not reported previously. We found 25 genes that overlapped with the 52 genes identified by [1].

One of these genes, Fmr1, was investigated by others in detail and its relevance for viral ribonucleoprotein assembly could be demonstrated in vitro and in vivo [1].

From the 25 overlapping genes, six genes (Stat1, B2m, Lgals3bp, Dusp5, Nfkbia, Il15ra) were also identified as host factors involved in influenza virus replication by Shapira and colleagues [2]. They used human bronchial epithelial cells for transcriptional profiling and combined the data with results from a yeast two-hybrid approach where ten major viral proteins of PR8 were tested against 12,000 human proteins. Furthermore, genes acting downstream of RIG-I binding to viral RNA like Irf7, Irf9, Stat1 and NF-kB were also found amongst the genes that overlapped with the list from Zhou et al. [1]. STAT1, IRF9 and STAT2 form a heterotrimer, known as the ISGF3 complex that mediates induction of interferon stimulated genes. Another candidate with reported antiviral activity, ZC3HAV1 (zinc finger CCCH type, antiviral 1) correlated with survival of chicken infected with H5N1 variants [3]. Plscr1 is induced by interferon and is involved in amplifying the interferon response [4]. It is a TLR9-interacting protein that plays an important role in pDCs type 1 IFN responses by regulating TLR9 trafficking to the endosomal compartment [5]. We speculate that genes like Cxcl2, Lcn2, Il15ra, LgalS3BP, Casp1, Cd274, Tnfaip2 and Atf3 (a negative regulatory transcription factor in TLR pathways) reflect the pathogen induced activation of macrophages. The detrimental role of Cd274 (programmed death ligand 1) in influenza infection was demonstrated recently [6-8]. The knockout of caspase 1 (Casp1), a key mediator of inflammatory processes revealed a higher susceptibility to influenza [9, 10]. The importance of the transcription factor NF-kB for viral replication has also been shown [11, 12]. Recently this pathway was assessed as potential therapeutic target [13].
Detailed discussion of correlating genes (Table 3)

Since viral and host transcripts can be followed in the same individual, we were able to correlate changes in host gene expression with changes in the level of virus gene expression. We studied host gene expression in C57BL/6J lungs and viral transcripts including both the period of increasing viral load (day 1 to day 5 p.i.) as well as the period of decrease in viral load (day 8 to 14 p.i.). We found 182 host genes that were positively or negatively correlated with influenza gene expression in infected C57BL/6J mice.

The role in immune response processes of many of the positively correlated genes is already known. For example, it has been shown that Flotillin 1 (Flot1) plays an important role during neutrophil recruitment and migration [14] whereasOrm1 inhibits neutrophil migration to the focus of infection after polymicrobial sepsis [15]. Porcine Ifit3 is highly induced by IFN-α/β and swine influenza virus (SIV) and is able to inhibit SIV replication as well as to enhance IFN-β production [16]. Moreover increased expression of Ifit3 has been revealed during lymphocytic choriomeningitis virus (LCMV) and West Nile virus (WNV) infection in the murine central nervous system (CNS). Thus, a dominant role in the host response to different viruses in the CNS was suggested [17].

Other genes interfere with pathways known to play important roles in the immune response after influenza A virus infection: Tnip2 which is required for optimal activation of the Erk signaling pathway and the inhibition of NfκB activation [18-21]. It has been shown recently that the macrophage stimulating 1 receptor (Mst1r) plays an important role in controlling cytokine secretion in inflammatory bowel disease (IBD) [22], negatively regulates TNFα production in alveolar macrophages [23] and its signaling promotes cell survival [24]. Additionally, the tyrosine kinase of this receptor plays a regulatory role in the immune response during acute lung injury [25]. The function of several of these genes has already been validated in knock-out mouse models: Ccr12 deficient mice exhibit alteration in trafficking of antigen-loaded lung dendritic cells and the authors suggest that this receptor might control excessive airway inflammatory responses [26]. In Serpind1 KO mice an increased susceptibility to Pseudomonas aeruginosa was observed and a new role of Serpind1 as a factor of host defense in innate immunity was concluded [27].

Among the negatively correlated genes, interleukin-7 (Il7) represents a critical cytokine for the induction of T follicular helper (Tfh) cells [28]. The negative correlation
may account for the fact that the immune response can have beneficial as well as detrimental effects to the host. Genes that are highly expressed and are negatively correlated with flu counts are: *Ift140, Tmem106c, Zmat3, Phkdh1* and *Cyb5rl.*

Several genes that overlap with previously identified siRNA genes, such as *Dusp5, B2m, Pnpt1, Areg, Fam46a, Ppp1r15a, Tnfaip2,* have not yet been associated with the host response to influenza virus infections. Similarly, for several genes that were positively correlated with virus genome expression and expressed at high levels in the lung after influenza infection, no biological function has been described yet: *Tdrd7, Dyn11h1, CD177, I830012016Rik* and *D14Ertd668e.* These genes are promising candidates for further investigations.

**References**


pandemic influenza A(H1N1) virus impairs the human T cell response. 


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