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Original article

Gene expression changes in the host response between resistant and susceptible inbred mouse strains after influenza A infection

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Abstract

Inbred mouse strains exhibit differences in susceptibility to influenza A infections. However, the molecular mechanisms underlying these differences are unknown. Therefore, we infected a highly susceptible mouse strain (DBA/2J) and a resistant strain (C57BL/6J) with influenza A H1N1 (PR8) and performed genome-wide expression analysis. We found genes expressed in lung epithelium that were specifically down-regulated in DBA/2J mice, whereas a cluster of genes on chromosome 3 was only down-regulated in C57BL/6J. In both mouse strains, chemokines, cytokines and interferon-response genes were up-regulated, indicating that the main innate immune defense pathways were activated. However, many immune response genes were up-regulated in DBA/2J much stronger than in C57BL/6J, and several immune response genes were exclusively regulated in DBA/2J. Thus, susceptible DBA/2J mice showed a hyper-inflammatory response. This response is similar to infections with highly pathogenic influenza virus and may serve as a paradigm for a hyper-inflammatory host response to influenza A virus. © 2010 Elsevier Masson SAS. All rights reserved.

Keywords: Inbred mouse strains; Influenza A virus; Gene expression microarray

1. Introduction

Influenza A virus is a major threat for human health. During the 1918 “Spanish flu” pandemic, approximately 50 million people died world-wide [1], whereas the 1957 and 1968 pandemics caused about 1 million deaths world-wide [2]. The seasonal yearly epidemic is responsible for about 1 million deaths per year world-wide [3]. A new subtype of influenza, H5N1, commonly known as “avian influenza” or “bird flu”,

infects humans that are in close contact to birds and cause a severe pneumonia that is fatal in 50% of infected individuals [4,5]. Furthermore, a recent outbreak of “swine flu” alarmed the world for a new pandemic threat [6,7].

Whereas the genetic changes of the virus have been studied in great detail, very little is known about genetic factors in the human host that contribute to resistance or susceptibility to influenza A infections, because it is very difficult to study such factors in humans. Here, the mouse represents an ideal model system to analyze viral virulence factors and genetic host factors contributing to disease development and outcome [8–10]. In a previous study [11], we showed that various mouse strains show differences in weight loss, viral load and cytokine/chemokine kinetics after infection with influenza A virus, subtypes H1N1 (PR8) and H7N7 (SC35M). The highly

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susceptible DBA/2J mice died within the first 6–8 days after infection. These observations demonstrate the importance of genetic factors to the host response and provide a basis to identify susceptibility and resistance loci in the mouse model system.

Here, we infected one resistant and one susceptible mouse strain with influenza A/H1N1 (PR8) virus and performed comparative microarray gene expression profiling. Our results showed that the susceptible strain, DBA/2J, activated all pathways that are relevant to innate immune defense. However, many genes related to inflammatory responses were expressed to a much higher level than in the resistant C57BL/6J mouse strain indicating a detrimental over-reaction of the immune system in DBA/2J mice.

2. Materials and methods

2.1. Virus, mouse strains and infections

The mouse-adapted influenza strain A/Puerto Rico/8/34 (H1N1; PR8) was propagated in the chorio-allantoic cavity of 10-day-old embryonated hen eggs for 48 h at 37 °C. Inbred mouse strains C57BL/6J and DBA/2J were obtained from Janvier, France. Mice were anesthetized with a mixture of 1 ml Ketamin (50 mg/ml, Invesa Arzneimittel GmbH, Freiburg), 0.5 ml Rompun (2% Xylazine hydrochloride, Bayer HealthCare, Leverkusen), and 8.5 ml of 0.9% sterile NaCl (Delta-Select GmbH, Dreieich) and administered intra-peritoneally based on body weight (10 ml/kg body weight). Infections were performed intra-nasally with influenza PR8 at a dose of 2×10^3 FFU in a total volume of 20 µl sterile PBS. The experiment was repeated in three rounds. In each of the rounds, 3 C57BL/6J and 3 DBA/2J mice were sacrificed per time point (days 1–4 after infection, and mock-infected control samples with only PBS at day 2) and individual lungs were extracted and stored in RNAlater solution (Qiagen) at –80 °C. Mice were maintained under specific pathogen free conditions and according to the German animal welfare law. All experiments were approved by an external committee according to the German regulations on animal welfare.

2.2. Extraction of RNA, semi-quantitative, and real-time PCR

Total RNA was extracted from individual lungs using the RNeasy Midi kit (Qiagen, Hilden, Germany). The quality and quantity of the RNA was checked using Agilent 2100 bio-analyzer. For microarray analysis, the three individual samples per mouse strain, time point and round were pooled. Thus, for each strain and time point three replicates were available and each replicate was representing a pool from three individual mice. For RT-PCR, 500 ng RNA was digested with DNase I, followed by cDNA synthesis with random hexamer primers (Invitrogen) by using Bioscript™ (Bioline GmbH, Germany) following manufacturer's instructions. For semi-quantitative PCR, cDNA of the chosen genes was amplified with specific primers (Supplementary Table 1) using Taq polymerase

(Genecraft, Germany) and analyzed by electrophoresis. Real-time PCR was performed with the DNA Master SYBR Green I kit (Roche, Mannheim, Germany) using a LightCycler 480 apparatus (Multiwell Plate 384, Roche). The housekeeping gene β -actin was used for normalization.

2.3. Microarray analysis

Gene expression profiling was performed using Affymetrix GeneChip Mouse Genome 430 2.0 Arrays. Samples were amplified according to the recommended protocols by the manufacturer (Affymetrix, Santa Clara, CA, USA). 10 µg of each biotinylated cRNA preparation was fragmented and placed in a hybridization cocktail. Samples were hybridized for 16 h. After hybridization, GeneChips were washed, stained with SA-PE, and read using an Affymetrix GeneChip fluidic station and scanner.

2.4. Data analysis

Affymetrix CEL files were preprocessed using the RMA method [12,13]. Regulated genes were identified as those having at least a two-fold change in expression and being differentially expressed at a false discovery rate <0.05 using Rank Products [14]. Principal component analysis was performed in Statistica 7.0. The R package GOstats [15] was used to test gene lists for over-representation of GO terms. Pathway analysis was performed with Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com).

3. Results and discussion

3.1. A higher number of genes is activated in the susceptible mouse strain

We previously described strong differences to infections with influenza A virus (PR8, H1N1) in several inbred mouse strains [11]. DBA/2J mice are highly susceptible and die within a few days after infection at low infection doses. However, C57BL/6J mice are able to clear the virus and survive. To get further insight into the molecular mechanisms of the different responses in the two mouse strains, we performed genome-wide expression analysis of infected mice from both strains on days 1–4 after infection.

We first did a global analysis of gene expression changes in infected versus control mice. Genes were classified as “regulated” if they exhibited a two-fold or higher change in signal and were differentially expressed at a false discovery rate of 5%. Using this definition, 1.2% of all genes on the array were up-regulated and 0.2% were down-regulated in C57BL/6J mice, whereas 2.2% and 1.2% were up- and down-regulated in DBA/2J, respectively.

Principal component analysis of all regulated genes confirmed that the expression changes are true biological variations and are not caused by variations in experimental conditions (data not shown).

Subsequently, we separated the regulated genes into four classes. “Public C57BL/6J genes” are genes that were regulated in C57BL/6J, irrespectively of their behavior in DBA/2J, whereas “private C57BL/6J genes” are genes that were regulated only in C57BL/6J and not in DBA/2J. Similar classes were created for DBA/2J. We found 25, 430, 637 and 684 public C57BL/6J genes on days 1–4 after infection, respectively. In contrast, 91, 932, 1516 and 1460 public DBA/2J genes were found on days 1–4. Similar amounts of regulated genes after infection with influenza A virus have been obtained in other studies (Cameron et al. [16] ca. 2300 genes; Rosseau et al. [17] ca. 1300 genes). The private genes showed strong differences between both strains. On days 1–4, 9, 26, 114 and 153 genes, respectively, were exclusively regulated in C57BL/6J, whereas 75, 528, 993 and 929 genes, respectively, were only regulated in DBA/2J (see also [Supplementary Fig. 1](#)).

Thus, many more genes were regulated in infected DBA/2J mice compared to C57BL/6J mice. As described earlier [11], the cytokine/chemokine response was much stronger in DBA/2J than in C57BL/6J mice. Our gene expression analysis confirms these earlier observations and supports the hypothesis that DBA/2J mice do not suffer from a global lack of host response.

Ding et al. [18] infected BALB/cByJ and C57BL/6J mice with influenza virus A/HKX31 (H3N2) and compared gene expression in lung and basal forebrain. They identified 361 genes that were regulated after infection in the lungs of BALB/cByJ mice and 16 genes in C57BL/6J mice. They compared their BALB/cByJ results with data obtained by Kash et al. [8] who infected BALB/c mice with the highly pathogenic 1918 influenza A virus, and found 51 overlapping genes. 43 of them were also regulated in DBA/2J mice in our data, and 29 in C57BL/6J. When we compared our data with the results from Kash et al. we found an overlap of 324 out of 1516 publicly regulated genes on day 3 in DBA/2J and an overlap of 232 out of 637 publicly regulated genes on day 3 in C57BL/6J mice ([Supplementary Table 2](#)). These observations indicate that the host response in highly susceptible DBA/2J mice to a low pathogenic influenza virus is similar to the response in a resistant mouse strain to a highly pathogenic virus observed.

3.2. GO annotation of regulated genes reveals immune response genes and differences between mouse strains

To determine whether specific functional groups of genes were regulated after infection, we tested all genes regulated at day 3 for over-representation of Gene Ontology (GO) terms. For both mouse strains, the public up-regulated genes showed a strong over-representation of GO terms relating to immune responses ([Supplementary Table 3](#)). In the down-regulated genes mainly GO terms related to development, differentiation and morphogenesis were found.

Differences between strains became apparent when comparing the private up-regulated genes. In DBA/2J we mainly found immune response terms whereas for C57BL/6J we found cell cycle and cell division terms ([Supplementary](#)

[Table 3](#)). The over-representation of immune response genes is in good agreement with the stronger activation of chemokines and cytokines in DBA/2J mice [11]. The up-regulation of cell-cycle regulated genes in the C57BL/6J private genes indicates that tissue repair has been initiated. Alternatively, the expression of these genes could be related to the division of immune cells infiltrating the lung. These genes are not activated in the susceptible strain where virus replication and tissue damage is continuing [11]. Up-regulation of genes related to cell growth and proliferation has also been observed in ferrets infected with the less virulent H3N2 virus, whereas this group of genes was down-regulated in ferrets infected with the highly pathogenic H5N1 virus [16]. From these and our observations, we speculate that the up-regulation of cell growth and proliferation genes is related to tissue repair and/or division of immune cells, whereas severe disease is indicated by absence or down-regulation of these genes.

3.3. Innate immune response genes

We then studied different functional groups of genes relating to the host innate immune defense against pathogens. Of nine interferon regulatory genes (*Irf*) monitored by our arrays, five were regulated in both mouse strains ([Supplementary Fig. 2](#)). Amongst them, the *Irf7* gene showed strongly increased expression in both strains. Of 14 interferon and interferon receptor genes, only *Ifnb1* and *Ifng* were up-regulated in DBA/2J but not in C57BL/6J mice.

The *Irf* gene family is regulated by Tlrs and other molecular sensors of infection and they play an important role in the establishment of a protective host interferon response [19]. In particular, *Irf7* is essential for the induction of interferon α and β [20]. *Irf7* mutant mice infected with influenza A virus exhibit an impaired interferon induction and are more susceptible to influenza infection [20].

Of the 46 interferon-induced genes, 70% were regulated upon infection. In general, the genes were up-regulated in both strains, but mostly they were up-regulated stronger in DBA/2J ([Fig. 1A](#)). A similar observation was made in ferrets infected with high or low pathogenic influenza virus. Interferon-induced genes were activated in both groups to a similar extent [16]. Furthermore, Zou et al. [21] described the activation of genes in interferon-treated macrophages of wild type and *Ubp43* mutant mice. Of the 749 genes described in their analysis for the *Ubp43* mutant after treatment, 235 were also regulated in our data set. Of the 77 genes activated in wild type mice in their study, 53 overlap with our set of regulated genes. The up-regulation of interferon-induced genes in DBA/2J mice showed that the interferon pathway functions normally in the highly susceptible mouse strain. The stronger response in DBA/2J mice may simply reflect the higher viral loads present in the lungs of mice from this strain after infection [11].

Of the 88 chemokine and cytokine activity genes on our arrays, 36% were regulated ([Fig. 1B](#)). Whereas some genes were up-regulated at a similar level in DBA/2J and C57BL/6J mice, most genes were stronger up-regulated in DBA/2J. *Cxcl11* showed the most striking difference. In addition, *Il6*

Fig. 1. Heatmaps of regulated immune response genes. Absolute $\log_2$ expression values are given for the control samples (C) and differences compared to the control samples, on $\log_2$ scale, are given for day 1 until day 4 (d1–d4). (A) interferon stimulated genes. (B) chemokine and cytokine activity. (C) chemokine receptor activity, cytokine binding and cytokine receptor activity.

gene	C57BL/6J					DBA/2J					expression GNF	lung epithelium embryo
	C	d1	d2	d3	d4	C	d1	d2	d3	d4		
Chia	10.7	0.0	-0.4	-0.6	-0.5	10.5	-0.4	-1.2	-1.6	-2.0	salivary gland, stomach	no data
Lgi3	10.5	0.1	-0.3	-0.5	-0.6	10.0	-0.2	-1.0	-1.5	-2.2	lung, brain	good signal
Scd1	10.7	0.5	0.1	-0.4	-0.4	10.7	-0.2	-1.3	-1.7	-2.1	liver, lung, other organs	possibly weak signal
Hc	12.4	0.1	-0.1	-0.4	-0.4	10.9	-0.8	-1.2	-2.1	-2.4	lung, liver	no data
Scgb3a1	12.6	0.3	0.1	-0.4	-0.4	12.6	-0.3	-1.1	-1.7	-2.7	lung	good signal
Cpm	10.6	-0.2	-0.8	-0.8	-0.8	10.3	-0.6	-1.7	-2.3	-2.6	lung	good signal
Aox3	11.0	0.2	-0.5	-0.9	-0.8	9.5	-0.9	-1.5	-2.2	-2.5	liver, lung, lacrimal gland	possibly weak signal
Ang	11.4	0.2	-0.3	-0.3	-0.3	7.8	-1.4	-2.3	-1.8	-2.2	liver (very low in lung)	possibly weak signal
Plunc	12.5	0.3	0.0	-0.8	-1.0	11.7	-0.5	-2.1	-3.2	-4.7	lung, heart	good signal
Clca3	9.2	-0.7	-0.3	-0.3	0.1	9.6	-2.8	-4.1	-4.7	-5.0	large intestine	possibly weak signal

Fig. 2. Heatmaps of genes that were strongly down-regulated in DBA/2J. Previously described expression in lung (GNF, BioGPS) or lung epithelium of the developing embryo (Eurexpress) is indicated.

and *Cxcl10* were already very strongly induced in DBA/2J mice at day 1, and *Il1a* and *Ccl20* were up-regulated in DBA/2J mice from day 2. Of the 15 genes that were up-regulated at least 8-fold in our data set, 12 genes were also up-regulated stronger in 1918 virus infected mice compared to mice infected with seasonal H1N1 virus [8]. Thus, only specific sub-sets of chemokine/cytokine genes and their receptors were activated in influenza A infected mice. In most cases, the kinetics of activation were similar for both mouse strains, but almost always higher expression levels were observed in DBA/2J compared to C57BL/6J.

Chemokine and cytokine gene expression has been described in several animal model systems. In ferrets infected with the highly pathogenic influenza virus H5N1, *Cxcl10* was strongly up-regulated compared to infections with low pathogenic virus [16]. *Cxcl9*, *Cxcl10* and *Cxcl11* bind to the same receptor *Cxcr3* which is expressed on circulating blood B, T and natural killer cells and is up-regulated on activated T-cells [22]. Inhibition of the *Cxcr3* pathway with an antagonist resulted in longer survival, reduced weight loss, improved lung function and activity scores in ferrets infected with H5N1 virus but did not affect viral load [16]. Up-regulation of *Cxcl9* and *Cxcl10* is part of a lung inflammation response common to different pathogens and exposures [23]. The strong up-regulation of *Cxcl11* observed here may thus be specific for influenza infections of a highly susceptible host.

Of the 56 chemokine/cytokine receptors, 32% were regulated in our data set. Amongst them, the *Ccr5* gene showed up-regulation of almost 8-fold in both strains, whereas *Ccr1*, *Csf3r*, *Il1r2* and *Ccr2* were up-regulated more than 4-fold only in DBA/2J mice (Fig. 1C).

Infected respiratory epithelial cells and alveolar macrophages are thought to represent the main cell populations for the early release of *Ccl2*, *Ccl5*, *Ccl3*, *Ccl4*, *Cxcl8* and *Cxcl10* [24–29]. A strong induction of chemokines and cytokines was also observed in mice infected with the high pathogenic 1918 virus [8,9] and the H5N1 virus [30,31] and were associated with unfavorable disease outcome [4,32]. Particularly, the expression of *Ccl2* by alveolar cells, the activation of its receptor on macrophages and their subsequent recruitment to the lung has been shown to cause detrimental immune-

pathology [33,34]. *Ccr2* mutant mice showed less severe lung leakage and increased survival after influenza A infections [34]. On the other hand, mouse mutants deficient in the *Ccr5* receptor which is also expressed on macrophages, showed increased lethality from influenza A infections [35]. This susceptibility can be rescued in a double mutant deficient in both the *Ccr5* and the *Cxcr3* genes [36], whereas the deletion of *Ccl5* or *Cxcr3* alone did not appear to affect viral load [29]. Recently, Aldridge et al. [37] reported that excessive recruitment of a subset of dendritic cells, tipDCs, was responsible for the high chemokine expression and associated mortality in mice infected with highly pathogenic influenza virus. We are currently analyzing cellular infiltrates to test the possible contribution of different immune cells to the high susceptibility in DBA/2J mice.

3.4. Differential regulation of genes and gene families between the two strains

Investigation of genes that showed the largest differences between the two mouse strains revealed ten genes that were strongly down-regulated in DBA/2J mice after infection but changed only slightly in infected C57BL/6J mice (Fig. 2). Except for *Ang* and *Clca3*, all these genes were reported previously to be expressed in normal lungs (<http://biogps.gnf.org/?referer=symatlas#goto=welcome>). So far, only the *Chia* gene was described as being down-regulated in infected lungs of BALB/c mice [18]. During an influenza infection, massive cell death in the infected lung epithelium is observed [11]. Therefore, the strong ‘down-regulation’ of these genes in DBA/2J mice may simply reflect a reduction in the number of differentiated respiratory epithelial cells. In this case, the expression of these genes may serve as a molecular marker for severe epithelial damage in the lung during the course of an influenza infection, and possibly other severe respiratory diseases. Alternatively, down-regulation of these genes may be a direct result of viral infection, e.g. suppression by viral virulence factors.

By plotting regulated genes along the genome, we identified several regulated gene clusters (data not shown). In particular, one cluster on chromosome 3 was identified in which several members of the *Lce* gene family were down-

Fig. 3. A cluster of *Lce* genes on chromosome 3, down-regulated only in C57BL/6J. (A) heatmap of the regulated genes. (B) chromosomal region of the gene cluster.

regulated only in C57BL/6J mice (Fig. 3). After influenza infection, we observed up-regulation of the *Sprr1a* in DBA/2J and specific down-regulation of *Lce1a2*, *Lce1c*, *Lce1d*, *Lce1f*, *Lce3b*, *Lce3f*, and *Sprr3* genes in C57BL/6J mice. Expression of the *Sprr* and *Lce* genes is associated with epidermal terminal differentiation [38] and psoriasis [39]. *Lce* genes are organized in three sub-clusters which exhibit common regulation of gene expression [40]. In the mouse, genes from all groups are also expressed in stomach, umbilical cord and cornea (<http://biogps.gnf.org/?refer=symatlas#goto=welcome>). We thus hypothesize that down-regulation of the *Sprr3* gene and *Lce* genes may be associated with regeneration of the respiratory epithelium, a process observed only in C57BL/6J infected mice.

3.5. Specific pathways in susceptible versus resistant mouse strains

We then used Ingenuity Pathway Analysis (Ingenuity® Systems, www.ingenuity.com) to further identify pathways involved in the host response to influenza A infections and found three pathways that were only apparent in DBA/2J infected mice: ‘Eicosanoid Signaling’, ‘Apoptosis Signaling’ and ‘Coagulation System’. The ‘Eicosanoid Signaling’ pathway suggests the involvement of prostaglandins in the strong inflammatory response observed in DBA/2J mice. As shown in Fig. 4, three genes exhibited a stronger activation in DBA/2J compared to C57BL/6J infected mice: *Fpr2* (*Fpr-rs2*), *Ptger4*, and *Pla2g7*.

Fig. 4. Heatmaps of genes of the Eicosanoid Signaling pathway.

Fig. 5. Quantitative RT-PCR results for five genes in C57BL/6J (B6) and DBA/2J (D2) control samples and on day 3 after infection.

Fpr2 represents a low affinity receptor for N-formyl-methionyl peptides, which are powerful neutrophil chemotactic factors resulting in the activation of neutrophils (www.genecards.org). *Ptger4* encodes the prostaglandin E2 receptor which can activate T-cell factor signaling (www.genecards.org). Both genes may thus be responsible for the enhanced inflammatory response observed in infected DBA/2J mice. *Pla2g7* represents a secreted enzyme catalyzing the degradation of platelet-activating factor, PAF. PAF is a pro-inflammatory phospholipid mediator causing the production of cytokines and eicosanoids via activation of G protein-coupled PAF receptor [41]. A higher activity of PAF-acetylhydrolase in mouse lungs co-infected with influenza and *Streptococcus pneumoniae* suggests that inactivation of PAF may contribute to mortality and enhanced lung pathology [42]. Also, hyperinduction of *Cox2* was described in human macrophages and in lung tissue of humans infected with the highly virulent H5N1 virus [43]. Cox2 inhibitors could reduce the hyperinduction of chemokines

[43]. Furthermore, Mazur et al. [44] reported that application of acetylsalicylic acid (ASA), another Cox2 inhibitor, reduced the morbidity and mortality in infected C57BL/6J mice.

Therefore, we studied if the application of ASA may rescue DBA/2J infected mice. However, no effect on the rate of survival or weight loss was observed in DBA/2J mice after intra-venous injection of ASA (Supplementary Document 1). But it should be noted that Mazur et al. [44] treated mice by applying the drug directly into the lung via a nebulizer connected to an intra-tracheal tube or by using an intraperitoneal injection followed by giving the drug in the drinking water. In our treatment regime, mice were treated by i.v. injections. The latter may have resulted in a less effective dose in the lungs and thus explain the difference to Mazur et al. [44]. Alternatively, DBA/2J mice may exhibit a different or more deleterious adverse response compared to C57BL/6 mice which were used by Mazur et al. [44], and that this response cannot be rescued by ASA.

3.6. Validation of microarray data

For several genes we verified the expression changes observed in the microarrays by semi-quantitative PCR and real-time PCR analysis. Of 42 genes analyzed, 32 genes showed identical trends in the semi-quantitative PCR assay (Supplementary Table 4). For five genes, the changes in expression were only detected in DBA/2J mice but not in C57BL/6J. For five other genes, the semi-quantitative results did not match with the array results. Thus, for most cases, the semi-quantitative PCR results were in good agreement with the results from the array experiments. We further analyzed five genes by real-time PCR (Fig. 5). The results were in good agreement with the measurements from the arrays, when comparing non-infected versus infected C57BL/6J and DBA/2J mice at day 3. Thus, most of the expression changes from the arrays could be reproduced.

4. Discussion

During the course of an infection molecular signatures of the pathogen are recognized by host receptors (TLRs and NLRs) which activate specific signaling pathways leading to the subsequent activation of transcription factors and the expression and synthesis of chemokines, cytokines, interferons and interferon-response genes [45–48]. Our array analysis showed that in highly susceptible DBA/2J mice the expression of many chemokines, cytokines and interferon-response genes was activated upon infection. We thus conclude that DBA/2J mice do not have a general defect in pathogen recognition and subsequent activation of the TLR, NLR and interferon-response pathways. However, DBA/2J mice showed a much stronger expression of genes associated with inflammatory responses, *e.g.* chemokine and cytokines, their receptors and prostaglandin-pathway genes than resistant C57BL/6J mice. These observations suggest that the susceptible DBA/2J mouse strain mounts a very strong immune response which may be detrimental to the host and together with the high viral load [11] causes the death of infected mice. Similar conclusions were drawn from gene expression studies with different viruses and in other species [8,9,16–18,30,31,42,49,50]. Thus, the comparison of a susceptible and resistant mouse strain, as described here, may serve as a paradigm for the molecular responses to infections in mammals with highly pathogenic influenza A viruses and help to better understand the molecular mechanisms leading to severe disease and death.

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Appendix. Supplementary data

The supplementary data associated with this article can be found, in the online version at [doi:10.1016/j.micinf.2010.01.008](https://doi.org/10.1016/j.micinf.2010.01.008)

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