Antiviral Gene Expression

Subjects: Biology

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Influenza A virus (IAV) is a respiratory virus that alone or in combination with secondary bacterial pathogens can contribute to the development of acute pneumonia in persons >65 years of age.

antiviral   H3N2   H1N1   influenza

1. Introduction

Historically, influenza has been recognized as one of the leading causes of respiratory tract infection and contributes to seasonal epidemics. Due to antigenic variation and interspecies transmission, influenza remains a threat to global health.

Production of type I interferons (IFN) represents one of the key lines of defense against influenza. Host innate immune antiviral signaling early in response to influenza is essential to inhibit early viral replication and guide the initiation of the adaptive immune response. Infection with influenza results in the production of pathogen-associated molecular patterns (PAMPs), which are recognized by pattern recognition receptors (PRR). Several PRRs, such as Toll like receptors (TLR), NOD-like receptors (NLR), and retinoic acid inducible gene-I (RIG-I) like receptors (RLR) play a key role in the activation of the antiviral immune response to influenza. TLR 3 and 7 recognize double stranded (dsRNA) and single stranded (ssRNA) RNA, respectively and can initiate TIR domain-containing adaptor inducing IFN-β (TRIF) or myeloid differentiation factor-88 (MyD88) dependent antiviral signaling [1][2][3][4][5][6].

2. Impact of Chronological Aging on Host Responses to Mouse-Adapted H1N1 and H3N2 Strains of Influenza

2.1. Morbidity and Histological Changes in the Young and Aged Murine Adult Lung

To understand if there were age-associated differences in the host response to influenza, we infected young (3 months) or aged-adult (18–20 months) mice with mouse-adapted strains of influenza (strain: A/Puerto Rico/8/1934, PR8, H1N1) or (strain: A/Aichi/2/1968, HKx31, H3N2). In response to both strains of influenza, significantly sustained loss of weight was observed in aged adult mice when compared to young (**Figure 1**A,B). When compared to young, there was also a significant increase in viral titer present in aged lung in response to either H1N1 (**Figure 1**C) or H3N2 (**Figure 1**D). Histological examination of lung tissue illustrated increased cellular infiltration, with a notable increase in inflammation and intra-alveolar edema detected in aged adult lung at day 7 of

infection (**Figure 1**E). In response to H1N1, there was increased cellular recruitment to aged adult murine lung, with detectable damage to the alveolar capillary barrier (**Figure 1**E). Interestingly, when compared to lung tissue collected from aged H1N1 infected mice, there was less inflammation and inflammatory damage in aged H3N2 infected lung detected at day 7 post infection (**Figure 1**E).
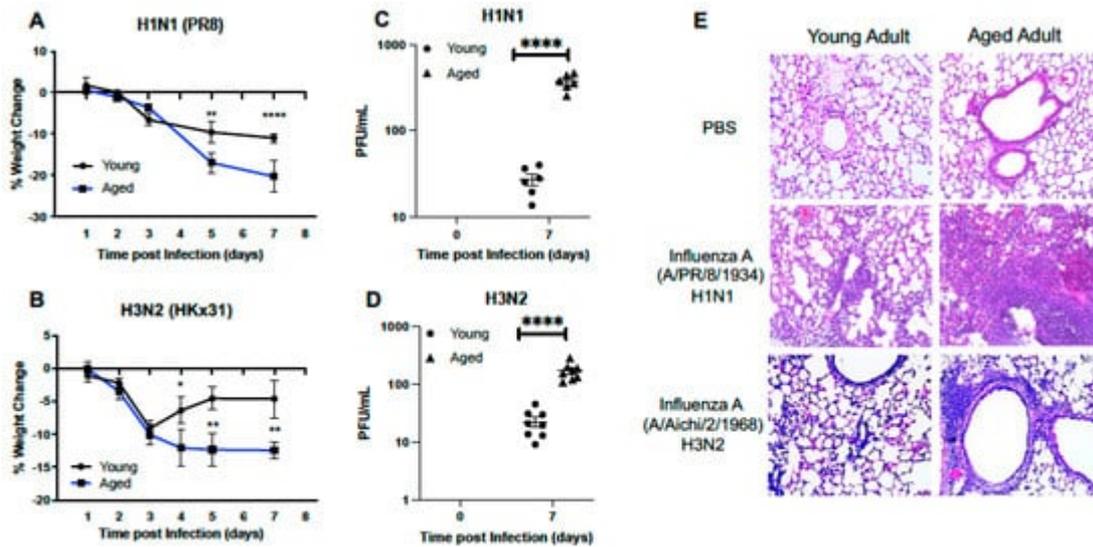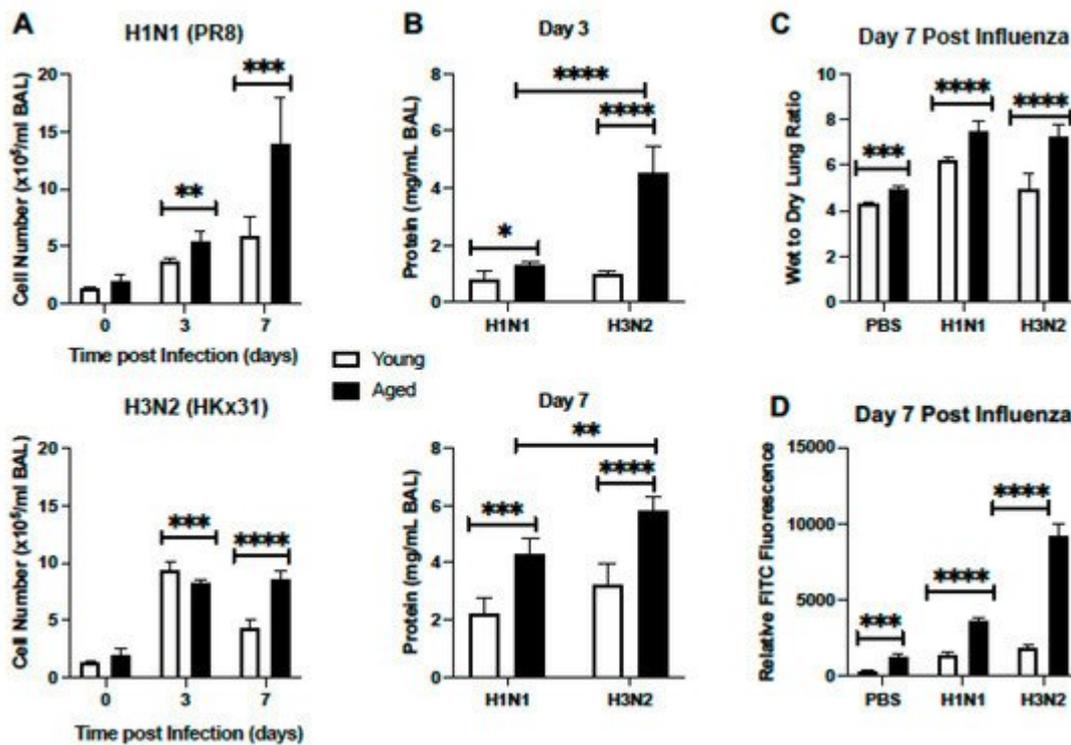

Figure 1. Morbidity and Histological Changes in the Young and Aged Murine Adult Lung. Young (3 months) and aged (18–20 months) adult mice were intranasally instilled with 12.5 PFU of influenza (strain: A/Puerto Rico/8/1934, PR8, H1N1) or (strain: A/Aichi/2/1968, HKx31, H3N2). Weight measurements were taken for young and aged mice at select time points post (**A**) H1N1 or (**B**) H3N2 infection. Viral titer in BAL was quantified and PFU/mL for (**C**) H1N1 and (**D**) H3N2. (**E**) Lung tissue was collected at day 7 post infection and H&E staining was performed to assess inflammation and cellular recruitment to lung. For H1N1 and H3N2 samples, each tissue section (20X magnification) shown represents a different mouse. Student's *t*-test: * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$. Similar results were obtained from at least three independent experiments, with $N = 10$ per group. Data are expressed as the mean ± SD.

2.2. Cellular Infiltration and Lung Injury Is Increased in Aged Murine Lung in Response to Influenza

Based on these histological findings, we next examined cell numbers present in bronchoalveolar lavage (BAL) fluid isolated from young and aged adult lung at select time points post H1N1 or H3N2 infection. In response to H1N1, there was a significant increase in cells present in BAL detected at day 3 post infection (**Figure 2**A). Cell number continued to increase in aged lung, with significantly heightened quantities detected at day 7 post infection (**Figure 2**A). We next evaluated cellular numbers present in young and aged lung in response to H3N2 infection. At day 3 post infection, there was a marked increase in cellular recruitment, with significantly higher numbers of cells quantified in young lung when compared to aged (**Figure 2**A). By day 7 post infection, cell numbers remained significantly elevated in aged H3N2 infected lung (**Figure 2**A). We next examined protein levels in young and aged adult BAL samples collected from mice at day 3 and 7 post H1N1 or H3N2 infection. At day 3 post infection there was a significant increase in protein detected in aged adult lung in response to influenza, with heightened levels

detected in response to H3N2 (**Figure 2**B). Similarly, at day 7 post influenza, there was significantly increased protein concentrations present in aged lung, with notably higher levels quantified in H3N2 lung (**Figure 2**B). We next investigated the amount of lung water accumulation in young and aged adult lung in response to H1N1 or H3N2 infection. At day 7 post H1N1 or H3N2 infection there was a significant increase in water accumulation in aged adult lung, as illustrated by increased wet to dry lung ratio (**Figure 2**C). To examine potential changes in alveolar epithelial and endothelial permeability in young and aged murine lung in response to H1N1 or H3N2, we quantified relative fluorescence levels in plasma post lung instillation with FITC. In response to influenza, there was a marked increase in permeability, as illustrated by increased FITC fluorescence in aged influenza infected lung, with significantly higher levels detected in response to H3N2 (**Figure 2**D).

Figure 2. Cellular Infiltration and Lung Injury is Increased in Aged Murine Lung in Response to Influenza. Young (3 months) and aged (18–20 months) adult mice were intranasally instilled with 12.5 PFU of influenza (strain: A/Puerto Rico/8/1934, PR8, H1N1) or (strain: A/Aichi/2/1968, HKx31, H3N2). (**A**,**B**) At select time points post infection BAL was collected from mice and (**A**) cell number or (**B**) protein concentration was quantified. (**C**) Lung tissue was collected at day 7 post infection. Wet weight (initial weight upon lung tissue removal) was quantified prior to incubation at 60 °C for 48 h to yield dry weight measurements. (**D**) Mice were instilled on day 7 with 3mg/mL of FITC-dextran and relative FITC fluorescence in plasma was assessed. Student's *t*-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. Similar results were obtained from at least three independent experiments, with $N = 5$ per group. Data are expressed as the mean ± SD.

2.3. Dysregulated Type I IFN Signaling in Aged Lung

Given the importance of antiviral signaling on host immune responses to influenza, we next examined the expression of several genes associated with the type I IFN signaling response in young and aged adult murine lung treated with PBS or influenza (H1N1 or H3N2) at days 3, 5, and 7 post infection. As shown in **Figure 3**A, there was a distinct pattern of genes elevated in both young and aged adult lung in response to each strain of influenza. On day 3, despite high Ifnα2 gene expression in aged H1N1 infected lung, we noted significantly diminished levels of IFNα present in BAL collected from H1N1 and H3N2 infected aged murine lung (**Figure 3**A–C). At day 3 post infection there was an increase in Ifnα2, Isg15, and Stat1 gene expression detected in young adult lung in response to either H1N1 or H3N2 (**Figure 3**A,C,E, and F). At day 5 of H1N1 or H3N2 infection, there was elevated expression of Ifnα2, Ifnβ1, Il-15, Mx1, and Tlr3 detected in young lung (**Figure 3**A,C,D). In response to H1N1 or H3N2, there was increased expression of Il-15, Tlr3, and Stat1 detected in young lung at day 7 of infection (**Figure 3**A,F). Examination of aged lung demonstrated differential expression patterns of genes associated with type I IFN signaling. Specifically, at day 3 post H1N1, there was increased Ifnα2 and Ifnβ1 and decreased Isg15 expression detected in aged lung (**Figure 3**A,C–E). In contrast, there was elevated expression of Isg15, Stat1, Mx1, and Tlr3 genes detected in aged lung at day 3 post H3N2 (**Figure 3**A,E,F). By days 5 and 7 of infection, there was a marked difference in gene expression with heightened expression of Mx1, Tlr3, Ifnα2, Ifnαr1, Ifnβ1, and Il-15 detected in aged lung in response to H1N1 (**Figure 3**A,C,D). In contrast, a similar elevation of Ifnα2, Ifnαr1, Ifnβ1, and Il-15 was not detected in aged lung in response to H3N2 (**Figure 3**A,C,D).
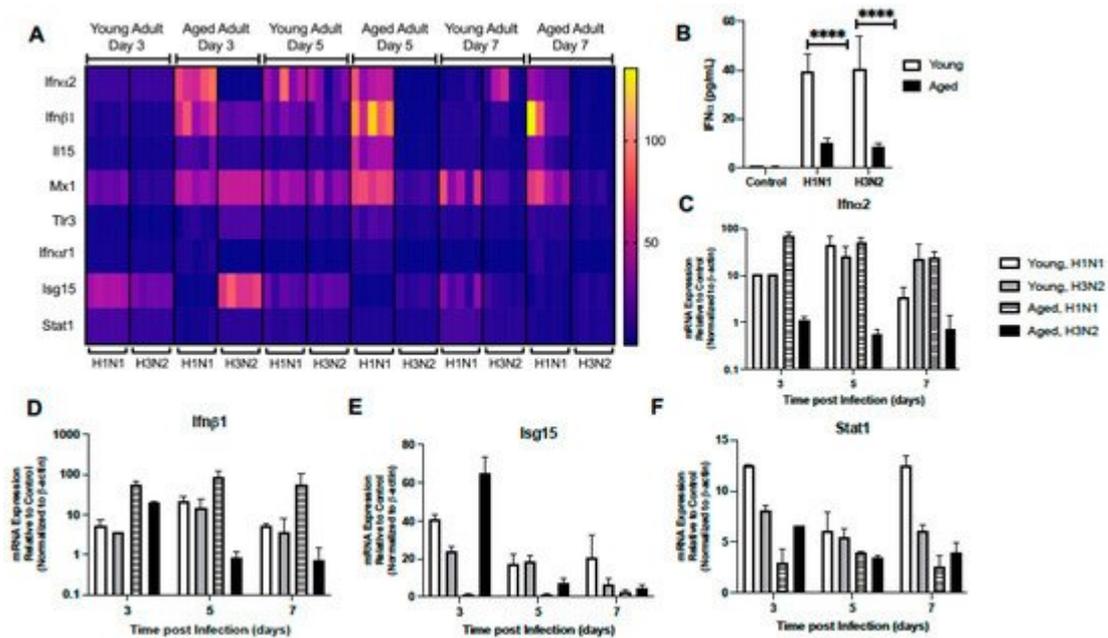
Figure 3. Dysregulated Type I IFN Signaling in Aged Lung. Young (3 months) and aged (18–20 months) adult mice were intranasally instilled with 12.5 PFU of influenza (strain: A/Puerto Rico/8/1934, PR8, H1N1) or (strain: A/Aichi/2/1968, HKx31, H3N2). (**A**) Lung tissue was collected from PBS treated or influenza infected young and aged mice at select time points post infection. Gene expression was assessed using the RT^2 Profiler PCR Array, Mouse Antiviral Response, PAMM-122Z and results were quantified using analytical software provided by Qiagen Gene Globe. (**B**) IFN-α expression in BAL was assessed by ELISA on day 3 post infection (student's *t*-test: **** $p < 0.0001$). Representative gene expression of (**C**) Ifnα2 (two-way ANOVA, $p < 0.0001$), (**D**) Ifnβ1 (two-way ANOVA, p

= 0.0361), (**E**) Isg15 (two-way ANOVA, $p < 0.0001$), and (**F**) Stat1 (two-way ANOVA, $p < 0.0001$). Similar results were obtained from at least three independent experiments, with $N = 5$ per group. Data are expressed as the mean ± SD.

Table 1. Symbol and gene names.

Symbol	Gene Name
Atg12	Autophagy-related 12
Atg5	Autophagy-related 5
Azi2	5-azacytidine induced gene 2
Casp8	Caspase 8
Ccl3	Chemokine (C-C motif) ligand 3
Ccl4	Chemokine (C-C motif) ligand 4
Ccl5	Chemokine (C-C motif) ligand 5
Cd40	CD40 antigen
Cd80	CD80 antigen
Cd86	CD86 antigen
Chuk	Conserved helix-loop-helix ubiquitous kinase
Cnpy3	Canopy 3 homolog
Ctsb	Cathepsin B
Ctsl	Cathepsin L
Ctss	Cathepsin S
Cxcl10	Chemokine (C-X-C motif) ligand 10
Cxcl9	Chemokine (C-X-C motif) ligand 9
Cyld	Cylindromatosis
Ddx3x	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3
Ddx58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
Dhx58	DEXH (Asp-Glu-X-His) box polypeptide 58

Symbol	Gene Name
Fadd	Fas (TNFRSF6)-associated via death domain
Ifih1	Interferon induced with helicase C domain 1
Ifnα2	Interferon alpha 2
Ifnαr1	Interferon (alpha and beta) receptor 1
Ifnβ1	Interferon beta 1
Ikbkb	Inhibitor of kappaB kinase beta
Il12a	Interleukin 12A
Il12b	Interleukin 12b
Il15	Interleukin 15
Il6	Interleukin 6
Irf3	Interferon regulatory factor 3
Irf7	Interferon regulatory factor 7
Isg15	ISG15 ubiquitin-like modifier
Map3k1	Mitogen-activated protein 3 kinase 1
Map3k7	Mitogen-activated protein 3 kinase 7
Mapk14	Mitogen-activated protein kinase 14
Mapk8	Mitogen-activated protein kinase 8
Mavs	Mitochondrial antiviral signaling protein
Mx1	Myxovirus (Influenza virus) resistance 1
Nfκb1	Nuclear factor of kappa light polypeptide gene enhancer in B- cells 1, p105
Nfkbia	Nuclear factor of kappa light polypeptide gene enhancer in B- cells inhibitor, alpha
Pin1	Protein (peptidyl-proyl cis/trans isomerase) NIMA-interacting 1
Rela	V-rel reticuloendotheliosis viral oncogene homolog A
Ripk1	Receptor (TNFRSF)-interacting serine-threonine kinase 1
Stat1	Signal transducer and activator of transcription 1

Symbol	Gene Name
Tbk1	TANK-binding kinase 1
Tlr3	Toll-like receptor 3
Tlr9	Toll-like receptor 9
Tnf	Tumor necrosis factor
Tradd	TNFRSF1A-associated via death domain
Traf3	Tnf receptor-associated factor 3
Traf6	Tnf receptor-associated factor 6
Trim25	Tripartite motif-containing 25

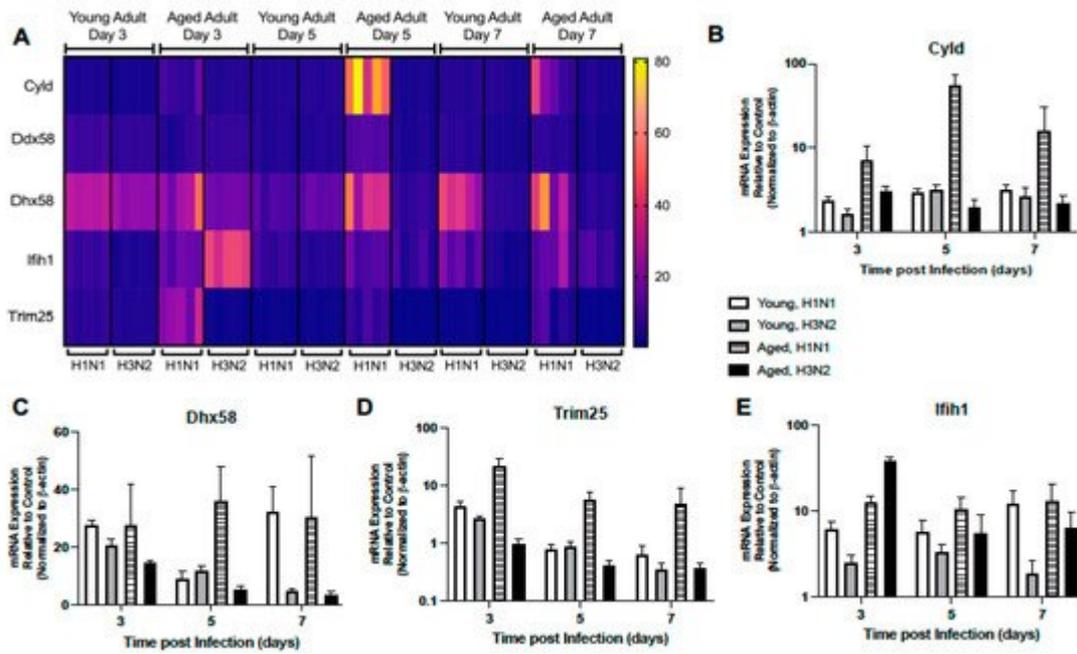## 2.4. Altered Expression of TLR Signaling Responsive Genes

We next investigated the expression pattern of TLR receptor signaling responsive genes in young and aged lung during H1N1 or H3N2 infection. In response to H1N1 or H3N2, there was a marked elevation of Ccl3, Ccl4, Ccl5, and Cxcl10 in young lung at day 3 post infection (**Figure 4**A–E). While some expression patterns remained elevated during infection, by day 5 there was increased Il-12a, Il-12b, Il-6, Cd80, and Cxcl11 expression that corresponded with elevated Il-15 levels in young adult H1N1 and H3N2 infected lung (**Figure 4**A). By day 7, expression of Ccl3, Cxcl9, Cd86, Il-6, Cd80, and Cxcl11 remained elevated in young adult lung in response to H1N1, while expression of Ccl5, Ccl4, and Cd40 remained elevated in response to either strain of influenza (**Figure 4**A–D). In aged adult lung, there was a significant upregulation of multiple TLR signaling responsive genes at day 3 post H1N1, with a marked increase in Cd80, Cxcl11, and Il-6 expression being detected (**Figure 4**A). Interestingly, by days 5 and 7, there was heightened expression of multiple genes, such as Cxcl9, Ccl3, Ccl4, Il-12a, Il-12b, Cd86, Cxcl11, and Cd80, detected in aged lung in response to H1N1 (**Figure 4**A–C).
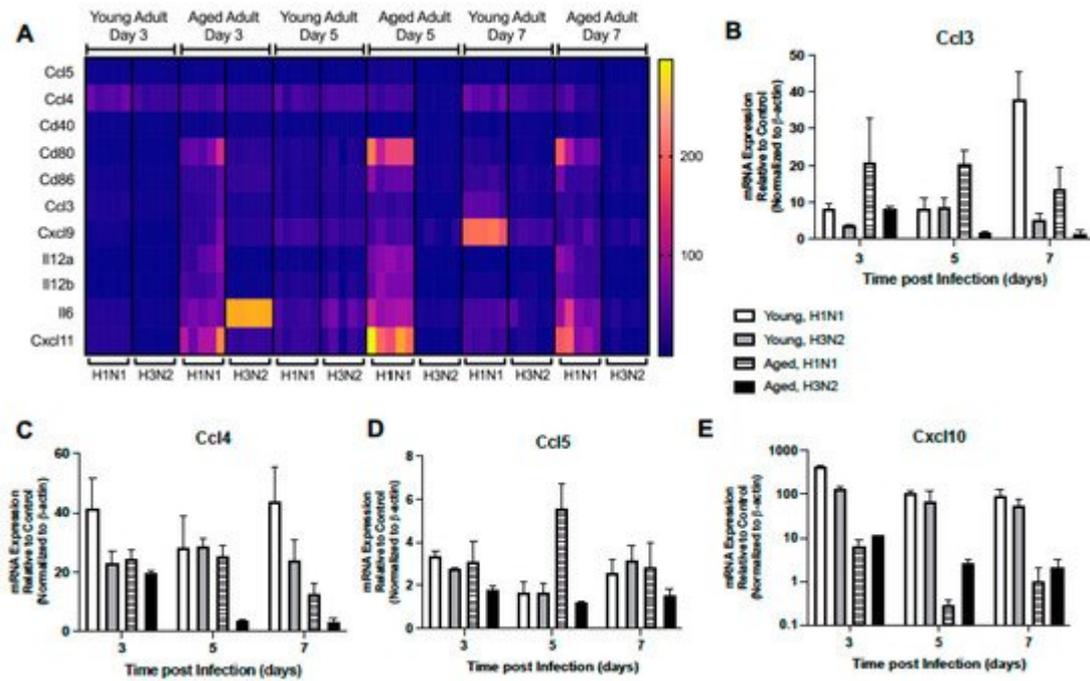
Figure 4. Altered Expression of TLR Signaling Responsive Genes. Young (3 months) and aged (18–20 months) adult mice were intranasally instilled with 12.5 PFU of influenza (strain: A/Puerto Rico/8/1934, PR8, H1N1) or (strain: A/Aichi/2/1968, HKx31, H3N2). Lung tissue was collected from PBS treated or influenza infected young and aged mice at select time points post infection. (**A**) Gene expression was assessed using the RT^2 Profiler PCR Array, Mouse Antiviral Response, PAMM-122Z and results were quantified using analytical software provided by Qiagen Gene Globe. Representative gene expression of (**B**) Ccl3 (two-way ANOVA, $p < 0.0001$), (**C**) Ccl4 (two-way ANOVA, $p < 0.0001$), (**D**) Ccl5 (two-way ANOVA, $p < 0.0001$), and (**E**) Cxcl10 ($p < 0.0001$). Similar results were obtained from at least three independent experiments, with $N = 5$ per group. Data are expressed as the mean ± SD.
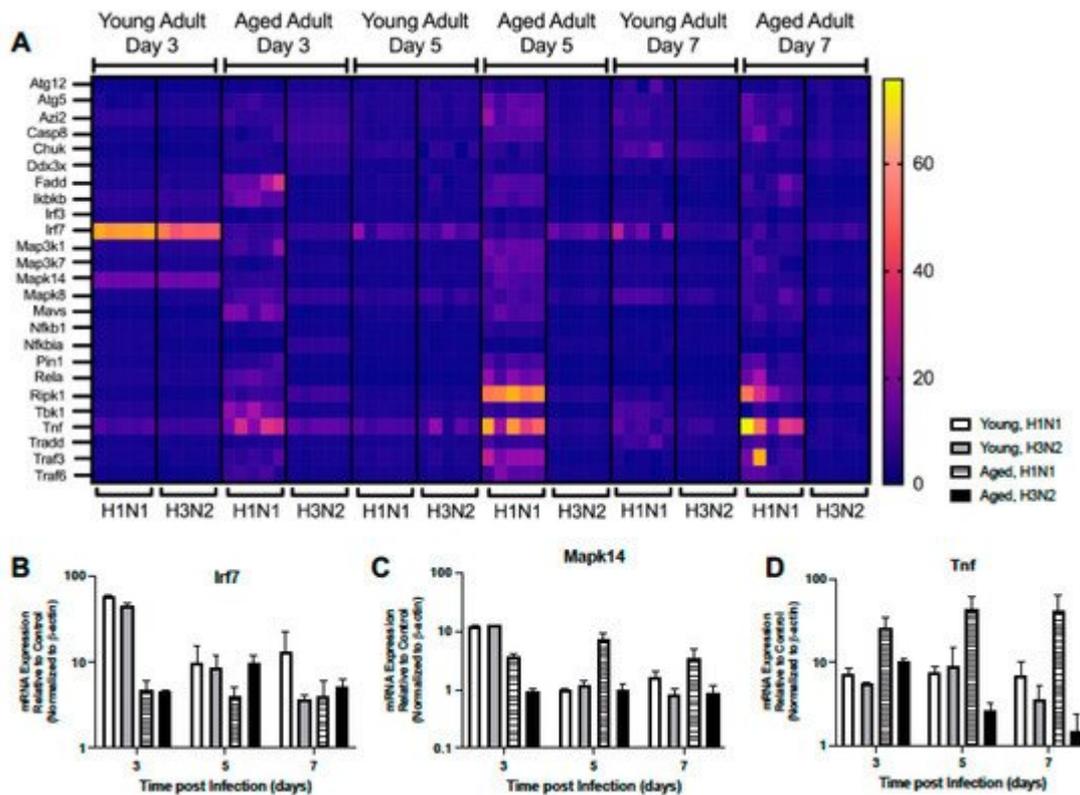
2.5. Altered Expression of RIG-I-like Receptor Signaling

Given the importance of RIG-I signaling in host mediated immune response to influenza, we next examined the impact of age on lung responses to H1N1 or H3N2 infection. In young lung, there was similar expression patterns in H1N1 and H3N2 lung, with increased expression of Ddx58, Trim25, and Dhx58 detected on day 3 and Cyld expression on day 5 post infection (**Figure 5**A–D). When compared to H3N2, by day 7, Ddx58, Dhx58, and Ifih1 expression remained elevated in young H1N1 infected lung (**Figure 5**A,C,E). In contrast, there was altered expression in aged lung on day 3 post infection, with increased Dhx58, Cyld, Trim25, and Ddx58 detectable in aged H1N1 infected lung tissue (**Figure 5**A–D). Despite increased Ifih1 gene expression on day 3 post infection, diminished RIG-I like receptor signaling was observed in aged H3N2 infected lung on day 5 and 7 post infection (**Figure 5**A–E).

Figure 5. Altered Expression of RIG-I-Like Receptor Signaling. Young (3 months) and aged (18–20 months) adult mice were intranasally instilled with 12.5 PFU of influenza (strain: A/Puerto Rico/8/1934, PR8, H1N1) or (strain: A/Aichi/2/1968, HKx31, H3N2). Lung tissue was collected from PBS treated or influenza infected young and aged mice at select time points post infection. (**A**) Gene expression was assessed using the RT^2 Profiler PCR Array, Mouse Antiviral Response, PAMM-122Z and results were quantified using analytical software provided by Qiagen Gene Globe. Representative expression of (**B**) Cyld (two-way ANOVA, $p < 0.0001$), (**C**) Dhx58 (two-way ANOVA, $p = 0.0058$), (**D**) Trim25 (two-way ANOVA, $p < 0.0001$), and (**E**) Ifih1 (two-way ANOVA, $p < 0.0001$). Similar results were obtained from at least three independent experiments, with $N = 5$ per group. Data are expressed as the mean ± SD.

We investigated the impact of aging on the expression of downstream RIG-I-like receptor signaling molecules. In young lung, there were comparable Pin1, Iκbκb, Mapk14, Map3k1, Rela, Irf7, Map3k7, and Nfκb1 expression patterns at days 3 and 5 post H1N1 or H3N2 infection (**Figure 6**A–C). In response to H1N1 or H3N2 infection, on day 5 post infection there were also similar expression patterns of Mavs, Traf3, and Traf6 detected in young lung (**Figure 6**A). Interestingly, by day 7 of infection, multiple genes were elevated in young H1N1 infected lung, such as Atg5, Mapk8, Tbk1, Casp8, and Ripk1, that were not similarly expressed in response to H3N2 (**Figure 6**A). In aged adult lung, there was a differential pattern of gene expression observed in response to H1N1 or H3N2 infection (**Figure 6**A). Specifically, at day 3, while the expression of Tbk1, Tnf, Mavs, and Traf6 were upregulated in response to H1N1, only the expression of Ddx3x, Chuk, Irf7, and Nfκbia were highly expressed in aged lung in response to H3N2 (**Figure 6**A,B,D). By days 5 and 7, heightened expression of Pin1, Traf6, Ripk1, Map3k7, Map3k1, Mapk14, Atg5, and TNF were observed in aged lung in response to H1N1 (**Figure 6**A,D).

Figure 6. Altered Expression of RIG-I-Like Receptor Signaling. Young (3 months) and aged (18–20 months) adult mice were intranasally instilled with 12.5 PFU of influenza (strain: A/Puerto Rico/8/1934, PR8, H1N1) or (strain: A/Aichi/2/1968, HKx31, H3N2). Lung tissue was collected from PBS treated or influenza infected young and aged mice at select time points post infection. (**A**) Gene expression was assessed using the RT^2 Profiler PCR Array, Mouse Antiviral Response, PAMM-122Z and results were quantified using analytical software provided by Qiagen Gene Globe. Representative expression of (**B**) Irf7 (two-way ANOVA, $p < 0.0001$), (**C**) Mapk14 (two-way ANOVA, $p < 0.0001$), and (**D**) Tnf (two-way ANOVA, $p = 0.00459$). Similar results were obtained from at least three independent experiments, with $N = 5$ per group. Data are expressed as the mean ± SD.

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