Published in final edited form as:

JAMA Pediatr. 2015 October ; 169(10): 956–963. doi:10.1001/jamapediatrics.2015.1387.

Influenza A Virus Infection, Innate Immunity, and Childhood

Bria M. Coates, MD, Kelly L. Staricha, Kristin M. Wiese, MD, and Karen M. Ridge, PhD

Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, Illinois (Coates, Staricha); Division of Critical Care Medicine, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois (Coates); Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois (Wiese, Ridge); Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois (Ridge).

Abstract

Infection with influenza A virus is responsible for considerable morbidity and mortality in children worldwide. While it is apparent that adequate activation of the innate immune system is essential for pathogen clearance and host survival, an excessive inflammatory response to infection is detrimental to the young host. A review of the literature indicates that innate immune responses change throughout childhood. Whether these changes are genetically programmed or triggered by environmental cues is unknown. The objectives of this review are to summarize the role of innate immunity in influenza A virus infection in the young child and to highlight possible differences between children and adults that may make children more susceptible to severe influenza A infection. A better understanding of age-related differences in innate immune signaling will be essential to improve care for this high-risk population.

Influenza A virus (IAV) is a highly contagious RNA virus that causes respiratory tract infections in humans and animals. Seasonal IAV is responsible for considerable disease worldwide, with the World Health Organization estimating 3 to 5 million severe cases of IAV each year and approximately 250 000 to 500 000 deaths.¹ Pandemics threaten to affect significantly more people. During the 1918 pandemic, it is estimated that IAV was responsible for 50 to 100 million deaths.² The burden of IAV infection is highest in children, the elderly, and persons with chronic medical conditions. Each year, IAV infection affects up to 40% of children younger than 5 years in the United States and 90 million

Corresponding Author: Bria M., Coates, MD, Division of Critical Care, Medicine, Ann & Robert H. Lurie, Children's Hospital of Chicago, 225 E. Chicago Ave, Campus Box 43., Chicago, IL 60611, (b-coates@northwestern.edu).

Author Contributions: Dr Coates had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: All authors.

Acquisition, analysis, or interpretation of data: Coates, Staricha, Wiese.

Drafting of the manuscript: Coates, Staricha, Ridge.

Critical revision of the manuscript for important intellectual content: All authors.

Obtained funding: Ridge.

Administrative, technical, or material support: Coates, Ridge.

Study supervision: Coates, Ridge.

Conflict of Interest Disclosures: None reported.

Additional Contributions: Jennifer Davis, BS (Division of Pulmonary and Critical Care, Department of Medicine, Northwestern University Feinberg School of Medicine), carefully edited the manuscript. She did not receive compensation for editing.

worldwide.³ Approximately 20 million of these children develop lower respiratory tract infections, and 1 million may experience severe, life-threatening disease.⁴ Although underlying medical conditions place certain children at increased risk of severe IAV infection, a considerable amount of morbidity and mortality occurs in healthy children. In the United States, the number of deaths each year in previously healthy children is almost equal to the number of deaths in children with chronic conditions, including asthma and prematurity. In contrast, adults who develop severe lower respiratory tract disease with IAV infection almost always have predisposing conditions that place them at increased risk of death (Figure 1).⁵ In mouse models of IAV pneumonia, increased mortality and pulmonary injury are seen in juvenile mice compared with adults despite neither having previous exposure to the virus.^{6,7} This discrepancy suggests that there may be age-related differences in the pathogenesis of IAV infection that leave children at particularly high risk of severe disease.

Mild IAV infection is characterized by fever, headache, sore throat, and coryza. Progression to lower respiratory tract disease can lead to cough, wheezing, impairment in gas exchange, and ultimately respiratory failure. Children may be particularly susceptible to severe IAV infection for various reasons. Small pediatric airways are prone to obstruction by the secretions produced during IAV infection, which can result in substantial respiratory distress.⁸ Infants also have a more cartilaginous (and therefore more compliant) chest wall, leaving the lungs more susceptible to collapse during infection and difficult to reinflate.⁸ The combination of these anatomic factors may increase the severity of symptoms once infection is established. However, why IAV is able to establish lower respiratory tract infection so effectively in otherwise healthy children is yet to be fully explained.

In addition to anatomic factors, the quality and quantity of the immune response appear to change with age. Young children with naive adaptive immune systems are dependent on their innate immunity toward off threats as adaptive immunity develops.⁹ This circumstance likely contributes to the vulnerability of young children to infection. However, immature adaptive immunity does not adequately explain the different inflammatory responses to infection seen in children and adults. Various viral infections have different outcomes depending on the age at which they are acquired,⁹ and vaccine responses vary depending on age.¹⁰ The mechanisms underlying these age-related differences in immune signaling are incompletely understood. Therefore, a better understanding of innate immune responses in children is crucial for developing targeted therapeutic strategies and improved vaccine design to support this population.

Pathogenesis of IAV Infection

Influenza A virus is a single-stranded, enveloped RNA virus with 8 RNA segments that encode 12 proteins. Type A influenza is classified into subtypes depending on which versions of 2 different proteins are present on the surface of the virus: hemagglutinin (HA) and neuraminidase (NA). There are 17 versions of HA and 10 versions of NA. Therefore, a virus with version 1 of the HA protein and version 2 of the NA protein is classified as influenza A subtype H1N2 (H1N2). The influenza A subtypes are further classified into strains, and the names of the virus strains include the place where the strain was first found

and the year of discovery. Therefore, an H1N1 strain isolated in California in 2009 is referred to as A/California/7/2009 (H1N1). There are approximately 170 different combinations of the HA and NA proteins possible, but, in practice, only a few circulate through the human population at any given time. A recent analysis of patients infected with H1N1 virus from 2009 to 2014 revealed that the H1N1 virus has drifted from the prototype A/California/7/2009 virus by approximately 1.3%.¹¹ Subtypes H1N1 and H3N2 are in general circulation worldwide. Other combinations circulate in animals (eg, the H5N1 virus found in birds). The subtypes that exist within a population change over time. For example, the H2N2 subtype, which infected people between 1957 and 1968, is no longer found in humans.

Accumulation of mutations over time allows IAV to evade recognition by the adaptive immune system, necessitating yearly modification of anti-influenza vaccines and annual immunization. This phenomenon is known as antigenic drift and is responsible for seasonal influenza epidemics. Alternatively, antigenic shift occurs when there is exchange of RNA between human and nonhuman IAV strains, which results in a virus previously unseen by the human population and therefore capable of causing pandemics.¹² Influenza B viruses (IBVs) are capable of causing the same spectrum of disease as IAV, but their limited host range is thought to prevent frequent reassortment and thus emergence of widely varying strains capable of causing pandemics. Although IBV can be an important pathogen in young children, research on its pathogenesis in children is limited, so IAV will be the focus of this review.

The initial target of IAV infection is the respiratory epithelium. Influenza A virus binds to sialic acid residues through its HA protein, which triggers endocytosis of the virus.¹³ Once internalized, acidification of the endosome leads to dissociation of the viral ribonucleoprotein complex, which is transported to the nucleus, where viral replication occurs.¹⁴ Following replication, new virions are assembled at the cell surface and released through cleavage of HA–sialic acid linkages by the viral NA.¹⁵ Influenza A virus may be detected at multiple points along the pathway of viral internalization, replication, and release. Detection of IAV leads to a coordinated response by the innate immune system to produce proinflammatory cytokines and induce antiviral signaling.¹² Proinflammatory cytokines recruit effector cells for viral clearance and cells of the adaptive immune system to develop antigen-specific responses and immune memory. Antiviral signaling combats intracellular infection through type I interferons (IFNs) and transcription of hundreds of IFN-regulated genes.¹⁶

Innate Immune Mechanisms Against IAV

Detection of IAV first occurs within the infected respiratory epithelial cell through recognition of the virus by germline-encoded receptors of the innate immune system known as pattern recognition receptors (PRRs).¹⁷ There are 3 main classes of PRRs involved in IAV detection, including the Toll-like receptors (TLRs), the retinoic acid-inducible gene (RIG)–like receptors, and the nucleotide-binding oligomerization domain (NOD)–like receptors.^{12,16} The first class probes the extracellular and endosomal compartments, whereas the latter 2 classes scan the cytosol. Activation of these PRRs leads to cytokine production

that initiates antimicrobial programming and recruits effector immune cells. The fact that the germline encodes PRRs has led many researchers to conclude that the innate immune system is present at birth and does not change throughout life. However, there is increasing evidence that the downstream signaling events after identification of microbes by PRRs are not equal across age groups.^{18–21}

The TLRs are the first family of PRRs to recognize infection of the respiratory epithelium by IAV.¹² Eleven TLRs have been described in humans, and at least 2 are involved in IAV infection. As IAV replicates within the epithelial cell, it creates double-stranded RNA recognizable by TLR3.²² As the infection spreads from epithelial cells to alveolar macrophages and dendritic cells (DCs), TLR7 can identify single-stranded IAV RNA.²³ Signaling through TLR3 and TLR7 culminates in production of proinflammatory cytokines and chemokines, in particular interleukin 6 (IL-6), IL-12, and tumor necrosis factor (TNF),²⁴ as well as antiviral IFNs (Figure 2).

As IAV replicates in alveolar macrophages and epithelial cells, it can also be identified by RIG-I, a member of the RIG-like helicase receptor family. Retinoic acid-inducible gene I has been shown to detect single-stranded IAV RNA bearing the 5'-triphosphate moiety that is generated during viral replication.²⁵ Once activated, RIG-I binds to its adaptor mitochondrial antiviral signaling protein to initiate nuclear factor κ B (NF- κ B) and IFN regulatory factor 3 signaling to result in proinflammatory cytokine production and type I IFN production, respectively. RIG-I activation is key to efficient IFN production and therefore control of IAV replication.²⁵ Mice deficient in RIG-I are highly susceptible to IAV-induced lung injury.^{26,27}

Respiratory epithelial cells, macrophages, and DCs also contain NOD-like receptors (NLRs) for detection of microbial invasion and cellular injury. Activation of certain NLRs leads to inflammasome assembly. Inflammasomes are multimeric protein complexes that assemble to activate caspase 1, which is required for the proteolytic maturation and release of the proinflammatory cytokines IL-1 β and IL-18. The NLR family, pyrin domain-containing 3 (NLRP3) inflammasome comprises a PRR (NLRP3), the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and an effector protein (caspase 1). The NLRP3 inflammasome assembly has recently been shown to require the intermediate filament vimentin, which may act as a scaffold on which inflammasome components can converge.²⁸ Two signals are required for inflammasome activation, including a priming signal that induces production of the precursor cytokines pro-IL-1 β and pro-IL-18 via the transcription factor NF- κ B and a second signal that leads to inflammasome assembly and cleavage of these precursors to their mature forms. In the case of IAV infection, the first signal is thought to occur through TLR3 or TLR7 recognition of the virus, leading to NF- κ B activation and production of pro-IL-1 β and pro-IL-18. The second signal appears to result from changes in the intracellular ionic concentrations detected by NLRP3, although other indicators of cellular stress have been implicated in inflammasome assembly, including reactive oxygen species, potassium flux, and lysosomal rupture.²⁹ The influenza virus proton channel, M2, has been shown to be sufficient and necessary to induce inflammasome activation and release of mature IL-1 β and IL-18.³⁰

Recently, the IAV protein polymerase B1-F2 (which was a key virulence factor during the 1918 pandemic) was also shown to activate the NLRP3 inflammasome.³¹

The NLRP3 inflammasome is necessary for survival in lethal mouse models of IAV infection.^{32–34} Loss of NLRP3, ASC, or caspase 1 in mice leads to decreased IL-1 β and IL-18 secretion and increases mortality from IAV. In sublethal models, NLRP3 may be dispensable for survival.³² Absence of the IL-1 receptor significantly decreases survival after IAV infection.³⁵ Alternatively, excessive inflammasome activation may also contribute to immunopathologic changes seen in lethal IAV infection.³¹ Therefore, inflammasome signaling must be tightly controlled to promote eradication of the virus while limiting collateral damage to the host.

Developmental Dependent Changes in PRR Signaling

Of the PRR families, the TLRs are the best studied, and their maturation throughout the first years of life are the best characterized. Many investigators have shown equal amounts of TLR expression in children and adults as well as an ability in children to upregulate TLRs in response to infection.^{36–39} However, the downstream effects of TLR activation appear to differ by age (Table).⁴⁰ Possibly important for IAV infection are the observations that young children may produce large amounts of IL-6²¹ and IL-10^{18,40} in response to TLR stimulation and decreased amounts of IL-12,²¹ type I IFNs,⁴¹ and IFN- γ .²¹ How these differences in cytokine production may influence IAV pathogenesis in children is discussed below in the Age-Dependent Differences in Susceptibility to IAV subsection.

Little is known about the ontogeny of the NLR response and even less about changes in RIG-like receptor (RLR) signaling over time. A study⁴² of NLRP3 inflammasome stimulation in whole blood from children in Papua New Guinea showed that IL-1 β production was highest in infants younger than 3 months and steadily decreased until 10 months of age. Interleukin 1 β has been shown to be elevated in pediatric patients with severe influenza, but so has its antagonist.^{43,44} In addition, IL-1 receptor antagonist polymorphisms have been associated with severe community-acquired pneumonia in children.⁴⁵ How inflammasome activation and RLR signaling differ between children and adults is unknown and represents an important area of future investigation.

Age-Dependent Differences in Susceptibility to IAV

A few studies have attempted to investigate the apparent vulnerability of young children to IAV infection. Increased mortality has been replicated in juvenile mice,^{6,7} but the mechanism of this age-dependent susceptibility is unclear. A major outstanding question is whether such pathologic conditions seen in young animals and children are secondary to enhanced viral replication, impaired viral clearance, excessive inflammation, or increased susceptibility to secondary bacterial infections.

Viral Replication and Clearance

Alterations in the kinetics of viral replication and clearance have been shown in animal models of juvenile IAV infection. Compared with primary airway epithelial cells from adult

naturally occurring IAV infection in newborns, it could lead to increased virus burden and contribute to the susceptibility of young infants to IAV.

An alternative explanation for increased viral replication in these animal and ex vivo models of pediatric IAV infection could be successful viral evasion of the immune response. To replicate successfully and establish widespread infection, IAV has developed multiple strategies to combat recognition by the innate and adaptive immune systems.¹² To our knowledge, the effectiveness of viral evasion strategies is not known to differ between children and adults, but it has not been carefully explored and may deserve further investigation.

Excessive Inflammation

There is now contrasting evidence challenging the conclusions that children have a deficient inflammatory response, an impaired type I IFN response, and an inability to limit IAV replication. Instead, evidence is emerging regarding a pathologic role for an excessive inflammatory response to IAV in children. Multiple studies^{44,57,58} have correlated elevations in the proinflammatory cytokine IL-6 with the severity of symptoms in pediatric patients, and the IL-6 antagonist tocilizumab was incidentally seen to decrease the duration of symptoms during IAV infection.⁵⁹ Increased IL-6 was associated with more severe lung injury and death in a ferret model of IAV infection.⁶⁰ However, complete absence of IL-6 may be harmful, with IL-6 knockout mice having increased viral titers and less likelihood of survival following IAV infection.^{61,62}

A recent prospective study⁵⁸ of natural IAV infection also challenges the hypothesis that an impaired innate immune response to IAV is responsible for increased severity of illness in children. In that study, viral load was equal between children and adults and did not correlate with clinical outcomes. In addition, the presence of neutralizing antibodies was not predicted by age nor did it correlate with cytokine secretion. Infants and young children had significantly higher levels of inflammatory nasal cytokines and type I IFNs than adults. These findings are consistent with a recent study⁶³ that correlated excessive type I IFN production with immunopathologic changes during IAV infection in mice. Disease severity in children was predicted by the presence of a mix of inflammatory and anti-inflammatory proteins (elevated IFN- α 2, monocyte chemotactic protein 3, and IL-10).⁵⁸ Based on their findings, the authors concluded that children have an immunologic predisposition to enhanced inflammation during IAV infection and not an inability to control viral replication. They proposed that this inflammation is responsible for the morbidity seen in this population.

Relative Immunosuppression and Secondary Bacterial Infection

The elevated level of nasal IL-10 seen in natural IAV infection in children is consistent with a body of literature exploring PRR responses in young children that demonstrates robust IL-10 production by peripheral blood mononuclear cells in response to PRR stimulation (Table). Classically, IL-10 is regarded as a regulatory cytokine, with a role in immune tolerance and limiting infection-associated immunopathologic conditions. Evidence suggests that the IL-10-mediated downregulation of inflammation may have deleterious effects in

early IAV infection by lessening the efficacy of the helper T-cell subtype 1 (T_H1) immune response needed to combat infection.⁶⁴ In a mouse model, IL-10 deficiency was associated with increased influenza-specific antibody titers in BALF, improved viral clearance, and longer survival compared with wild-type mice.⁶⁵ The authors proposed that IL-10 diminishes the CD4⁺ T-cell response that is crucial for production of virus-specific antibodies, thus inhibiting viral clearance. There was no appreciable difference in the extent of pulmonary inflammation on histologic examination after IAV infection between the 2 groups, suggesting that a lack of IL-10 does not significantly increase lung inflammation. Compatible with these findings, the addition of supplemental IL-10 to wild-type mice in early influenza resulted in increased mortality.⁶⁶ This evidence suggests that infants with a dominant primary IL-10 response may be at risk for worse outcomes after IAV infection.

In addition to preventing an effective T_H1 response to IAV, elevated IL-10 may contribute to immunoparalysis after IAV infection and increase the susceptibility to secondary bacterial infections. Children who did not survive influenza A (H1N1) virus infection had increases in the inflammatory proteins IL-6, IL-8, IFN-inducible protein 10, granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein 1, and macrophage inflammatory protein 1 α .⁴⁴ However, there were also increases in the anti-inflammatory proteins IL-1 receptor antagonist and IL-10 in these non-survivors, but this trend did not reach significance. In addition, ex vivo TNF secretion from macrophages was significantly depressed. The authors speculated that this relative immunosuppression left children susceptible to secondary bacterial infection and contributed to mortality in their cohort. Data are limited on the incidence of bacterial pneumonia in pediatric IAV infection, but bacterial coinfection has been reported in 43% to 75% of fatal cases^{44,67} and is likely an important contributor to IAV-induced morbidity and mortality.

Conclusions

Healthy children develop severe IAV infection at a rate that is out of proportion to that of their adult counterparts. This epidemiologic observation may be related to age-dependent changes in the innate immune response to pathogen detection. The consequence of developmental differences in PRR signaling during IAV infection may manifest as impaired viral clearance, excessive inflammation, increased susceptibility to secondary infection, or a combination of these factors. The most consistent finding in the literature to date is that young children produce decreased amounts of the cytokines IL-12 and IFN- γ , increased amounts of IL-6 and IL-1 β , and greater amounts of the anti-inflammatory cytokine IL-10 (Figure 2).⁴¹ Data are contradictory regarding the role of type I IFNs in pediatric susceptibility to severe IAV infection. Although animal models have suggested impaired viral clearance in the young host, emerging data in humans suggest that a pathologic role of the host inflammatory response, and not unchecked viral replication, may be responsible for the severity of disease in children. In addition, a subsequent anti-inflammatory response may predispose the young host to secondary bacterial infection and contribute to mortality. Defining the relative contributions of (1) a child's ability to control IAV replication, (2) the tendency to produce excessive inflammation, and (3) a predisposition to a state of relative immunosuppression will be important in developing innovative therapies to support children with severe IAV infection. Because infections are the primary cause of death in children

worldwide, elucidation of the details of innate immune system development will aid in establishing strategies to protect this vulnerable population and tailor therapeutics.

REFERENCES

1. World Health Organization. [Accessed June 19, 2015] Influenza (seasonal). <http://www.who.int/mediacentre/factsheets/fs211/en/>. Published March 2014
2. Johnson NP, Mueller J. Updating the accounts: global mortality of the 1918–1920 “Spanish” influenza pandemic. *Bull Hist Med.* 2002; 76(1):105–115. [PubMed: 11875246]
3. Pickering, LK.; Baker, CJ.; Long, SS.; Kimberlin, DW., editors. Influenza. Red Book: 2012 Report of the Committee on Infectious Diseases. 29th. Elk Grove Village, IL: American Academy of Pediatrics; 2012. p. 439–453.
4. Nair H, Brooks WA, Katz M, et al. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. *Lancet.* 2011; 378(9807):1917–1930. [PubMed: 22078723]
5. Centers for Disease Control and Prevention. [Accessed August 10, 2013] FluView: Influenza Hospitalization Surveillance Network. <http://gis.cdc.gov/grasp/fluview/FluHospChars.html>. Updated June 13, 2015
6. Sun S, Zhao G, Xiao W, et al. Age-related sensitivity and pathological differences in infections by 2009 pandemic influenza A (H1N1) virus. *Virology.* 2011; 8:52. [PubMed: 21299904]
7. Yasui H, Kiyoshima J, Hori T. Reduction of influenza virus titer and protection against influenza virus infection in infant mice fed *Lactobacillus casei* Shirota. *Clin Diagn Lab Immunol.* 2004; 11(4):675–679. [PubMed: 15242940]
8. D’Agelis, CA.; Coalson, JJ.; Ryan, RM. Structure of the respiratory system: lower respiratory tract. In: Fuhrman, BP.; Zimmerman, JJ., editors. *Pediatric Critical Care.* 4th. Vol. chap 36. Philadelphia, PA: Elsevier; 2011.
9. Prendergast AJ, Klenerman P, Goulder PJ. The impact of differential antiviral immunity in children and adults. *Nat Rev Immunol.* 2012; 12(9):636–648. [PubMed: 22918466]
10. Philbin VJ, Levy O. Developmental biology of the innate immune response: implications for neonatal and infant vaccine development. *Pediatr Res.* 2009; 65(5, pt 2):98R–105R.
11. Jalovaara P, Mishel P, Kallio-Kokko H, et al. Complete genome sequences of influenza A/H1N1 strains isolated from patients during the 2013–2014 epidemic season in Finland. *Genome Announc.* 2015; 3(2) pii: e01523-14.
12. van de Sandt CE, Kreijtz JH, Rimmelzwaan GF. Evasion of influenza A viruses from innate and adaptive immune responses. *Viruses.* 2012; 4(9):1438–1476. [PubMed: 23170167]
13. Rust MJ, Lakadamyali M, Zhang F, Zhuang X. Assembly of endocytic machinery around individual influenza viruses during viral entry. *Nat Struct Mol Biol.* 2004; 11(6):567–573. [PubMed: 15122347]
14. Portela A, Digard P. The influenza virus nucleoprotein: a multifunctional RNA-binding protein pivotal to virus replication. *J Gen Virol.* 2002; 83(pt 4):723–734. [PubMed: 11907320]
15. Rossman JS, Lamb RA. Influenza virus assembly and budding. *Virology.* 2011; 411(2):229–236. [PubMed: 21237476]
16. Iwasaki A, Pillai PS. Innate immunity to influenza virus infection. *Nat Rev Immunol.* 2014; 14(5): 315–328. [PubMed: 24762827]
17. Janeway CA Jr, Medzhitov R. Innate immune recognition. *Annu Rev Immunol.* 2002; 20:197–216. [PubMed: 11861602]
18. Belderbos ME, van Bleek GM, Levy O, et al. Skewed pattern of Toll-like receptor 4–mediated cytokine production in human neonatal blood: low LPS-induced IL-12p70 and high IL-10 persist throughout the first month of life. *Clin Immunol.* 2009; 133(2):228–237. [PubMed: 19648060]
19. Burl S, Townend J, Njie-Jobe J, et al. Age-dependent maturation of Toll-like receptor–mediated cytokine responses in Gambian infants. *PLoS One.* 2011; 6(4):e18185. [PubMed: 21533209]
20. Kollmann TR, Crabtree J, Rein-Weston A, et al. Neonatal innate TLR-mediated responses are distinct from those of adults. *J Immunol.* 2009; 183(11):7150–7160. [PubMed: 19917677]

21. Corbett NP, Blimkie D, Ho KC, et al. Ontogeny of Toll-like receptor mediated cytokine responses of human blood mononuclear cells. *PLoS One*. 2010; 5(11):e15041. [PubMed: 21152080]
22. Guillot L, Le Goffic R, Bloch S, et al. Involvement of Toll-like receptor 3 in the immune response of lung epithelial cells to double-stranded RNA and influenza A virus. *J Biol Chem*. 2005; 280(7): 5571–5580. [PubMed: 15579900]
23. Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science*. 2004; 303(5663):1529–1531. [PubMed: 14976261]
24. Medzhitov R, Janeway C Jr. Innate immunity. *N Engl J Med*. 2000; 343(5):338–344. [PubMed: 10922424]
25. Rehwinkel J, Tan CP, Goubau D, et al. RIG-I detects viral genomic RNA during negative-strand RNA virus infection. *Cell*. 2010; 140(3):397–408. [PubMed: 20144762]
26. Kato H, Takeuchi O, Sato S, et al. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature*. 2006; 441(7089):101–105. [PubMed: 16625202]
27. Loo YM, Fornek J, Crochet N, et al. Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity. *J Virol*. 2008; 82(1):335–345. [PubMed: 17942531]
28. dos Santos G, Rogel MR, Baker MA, et al. Vimentin regulates activation of the NLRP3 inflammasome. *Nat Commun*. 2015; 6:6574. [PubMed: 25762200]
29. Schroder K, Tschopp J. The inflammasomes. *Cell*. 2010; 140(6):821–832. [PubMed: 20303873]
30. Ichinohe T, Pang IK, Iwasaki A. Influenza virus activates inflammasomes via its intracellular M2 ion channel. *Nat Immunol*. 2010; 11(5):404–410. [PubMed: 20383149]
31. McAuley JL, Tate MD, MacKenzie-Kludas CJ, et al. Activation of the NLRP3 inflammasome by IAV virulence protein PB1-F2 contributes to severe pathophysiology and disease. *PLoS Pathog*. 2013; 9(5):e1003392. [PubMed: 23737748]
32. Ichinohe T, Lee HK, Ogura Y, Flavell R, Iwasaki A. Inflammasome recognition of influenza virus is essential for adaptive immune responses. *J Exp Med*. 2009; 206(1):79–87. [PubMed: 19139171]
33. Allen IC, Scull MA, Moore CB, et al. The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. *Immunity*. 2009; 30(4):556–565. [PubMed: 19362020]
34. Thomas PG, Dash P, Aldridge JR Jr, et al. The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. *Immunity*. 2009; 30(4): 566–575. [PubMed: 19362023]
35. Schmitz N, Kurrer M, Bachmann MF, Kopf M. Interleukin-1 is responsible for acute lung immunopathology but increases survival of respiratory influenza virus infection. *J Virol*. 2005; 79(10):6441–6448. [PubMed: 15858027]
36. Zhang JP, Yang Y, Levy O, Chen C. Human neonatal peripheral blood leukocytes demonstrate pathogen-specific coordinate expression of TLR2, TLR4/MD2, and MyD88 during bacterial infection in vivo. *Pediatr Res*. 2010; 68(6):479–483. [PubMed: 20805788]
37. Tulic MK, Hodder M, Forsberg A, et al. Differences in innate immune function between allergic and nonallergic children: new insights into immune ontogeny. *J Allergy Clin Immunol*. 2011; 127(2):470–478. e1. [PubMed: 21093030]
38. Strunk T, Prosser A, Levy O, et al. Responsiveness of human monocytes to the commensal bacterium *Staphylococcus epidermidis* develops late in gestation. *Pediatr Res*. 2012; 72(1):10–18. [PubMed: 22460219]
39. Dasari P, Zola H, Nicholson IC. Expression of Toll-like receptors by neonatal leukocytes. *Pediatr Allergy Immunol*. 2011; 22(2):221–228. [PubMed: 21054549]
40. Yerkovich ST, Wikström ME, Suriyaarachchi D, Prescott SL, Upham JW, Holt PG. Postnatal development of monocyte cytokine responses to bacterial lipopolysaccharide. *Pediatr Res*. 2007; 62(5):547–552. [PubMed: 17805207]
41. Kollmann TR, Levy O, Montgomery RR, Goriely S. Innate immune function by Toll-like receptors: distinct responses in newborns and the elderly. *Immunity*. 2012; 37(5):771–783. [PubMed: 23159225]

42. Lisciandro JG, Prescott SL, Nadal-Sims MG, et al. Ontogeny of Toll-like and NOD-like receptor-mediated innate immune responses in Papua New Guinean infants. *PLoS One*. 2012; 7(5):e36793. [PubMed: 22649499]
43. Chiaretti A, Pulitanò S, Barone G, et al. IL-1 β and IL-6 upregulation in children with H1N1 influenza virus infection. *Mediators Inflamm*. 2013; 2013:495848. [PubMed: 23737648]
44. Hall MW, Geyer SM, Guo CY, et al. Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network PICFlu Study Investigators. Innate immune function and mortality in critically ill children with influenza: a multicenter study. *Crit Care Med*. 2013; 41(1):224–236. [PubMed: 23222256]
45. Patwari PP, O'Cain P, Goodman DM, et al. Interleukin-1 receptor antagonist intron 2 variable number of tandem repeats polymorphism and respiratory failure in children with community-acquired pneumonia. *Pediatr Crit Care Med*. 2008; 9(6):553–559. [PubMed: 18838927]
46. Clay CC, Reader JR, Gerriets JE, Wang TT, Harrod KS, Miller LA. Enhanced viral replication and modulated innate immune responses in infant airway epithelium following H1N1 infection. *J Virol*. 2014; 88(13):7412–7425. [PubMed: 24741104]
47. Tsurita M, Kurokawa M, Imakita M, Fukuda Y, Watanabe Y, Shiraki K. Early augmentation of interleukin (IL)-12 level in the airway of mice administered orally with clarithromycin or intranasally with IL-12 results in alleviation of influenza. *J Pharmacol Exp Ther*. 2001; 298(1):362–368. [PubMed: 11408563]
48. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon- γ : an overview of signals, mechanisms and functions. *J Leukoc Biol*. 2004; 75(2):163–189. [PubMed: 14525967]
49. Fenton MJ, Vermeulen MW, Kim S, Burdick M, Strieter RM, Kornfeld H. Induction of gamma interferon production in human alveolar macrophages by *Mycobacterium tuberculosis*. *Infect Immun*. 1997; 65(12):5149–5156. [PubMed: 9393809]
50. Whitmire JK, Tan JT, Whitton JL. Interferon- γ acts directly on CD8⁺ T cells to increase their abundance during virus infection. *J Exp Med*. 2005; 201(7):1053–1059. [PubMed: 15809350]
51. You D, Ripple M, Balakrishna S, et al. Inchoate CD8⁺ T cell responses in neonatal mice permit influenza-induced persistent pulmonary dysfunction. *J Immunol*. 2008; 181(5):3486–3494. [PubMed: 18714021]
52. Welliver TP, Garofalo RP, Hosakote Y, et al. Severe human lower respiratory tract illness caused by respiratory syncytial virus and influenza virus is characterized by the absence of pulmonary cytotoxic lymphocyte responses. *J Infect Dis*. 2007; 195(8):1126–1136. [PubMed: 17357048]
53. Copenhaver CC, Gern JE, Li Z, et al. Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. *Am J Respir Crit Care Med*. 2004; 170(2):175–180. [PubMed: 15087299]
54. Lin SJ, Cheng PJ, Lin TY, Lee PT, Hsiao HS, Kuo ML. Effect of influenza A infection on umbilical cord blood natural killer function regulation with interleukin-15. *J Infect Dis*. 2012; 205(5):745–756. [PubMed: 22262794]
55. Li J, Li H, Mao H, et al. Impaired NK cell antiviral cytokine response against influenza virus in small-for-gestational-age neonates. *Cell Mol Immunol*. 2013; 10(5):437–443. [PubMed: 23872919]
56. Lin SJ, Lee YC. Effect of influenza A infection on maturation and function of neonatal monocyte-derived dendritic cells. *Viral Immunol*. 2014; 27(6):277–284. [PubMed: 24834787]
57. Kim YH, Kim JE, Hyun MC. Cytokine response in pediatric patients with pandemic influenza H1N1 2009 virus infection and pneumonia: comparison with pediatric pneumonia without H1N1 2009 infection. *Pediatr Pulmonol*. 2011; 46(12):1233–1239. [PubMed: 21626718]
58. Oshansky CM, Gartland AJ, Wong SS, et al. Mucosal immune responses predict clinical outcomes during influenza infection independently of age and viral load. *Am J Respir Crit Care Med*. 2014; 189(4):449–462. [PubMed: 24308446]
59. Carvalho LM, de Paula FE, Silvestre RVD, et al. Prospective surveillance study of acute respiratory infections, influenza-like illness and seasonal influenza vaccine in a cohort of juvenile idiopathic arthritis patients. *Pediatr Rheumatol Online J*. 2013; 11:10. [PubMed: 23510667]

60. Svitek N, Rudd PA, Obojes K, Pillet S, von Messling V. Severe seasonal influenza in ferrets correlates with reduced interferon and increased IL-6 induction. *Virology*. 2008; 376(1):53–59. [PubMed: 18420248]
61. Dienz O, Rud JG, Eaton SM, et al. Essential role of IL-6 in protection against H1N1 influenza virus by promoting neutrophil survival in the lung. *Mucosal Immunol*. 2012; 5(3):258–266. [PubMed: 22294047]
62. Lauder SN, Jones E, Smart K, et al. Interleukin-6 limits influenza-induced inflammation and protects against fatal lung pathology. *Eur J Immunol*. 2013; 43(10):2613–2625. [PubMed: 23857287]
63. Davidson S, Crotta S, McCabe TM, Wack A. Pathogenic potential of interferon $\alpha\beta$ in acute influenza infection. *Nat Commun*. 2014; 5:3864. [PubMed: 24844667]
64. McKinstry KK, Strutt TM, Buck A, et al. IL-10 deficiency unleashes an influenza-specific Th17 response and enhances survival against high-dose challenge. *J Immunol*. 2009; 182(12):7353–7363. [PubMed: 19494257]
65. Sun K, Torres L, Metzger DW. A detrimental effect of interleukin-10 on protective pulmonary humoral immunity during primary influenza A virus infection. *J Virol*. 2010; 84(10):5007–5014. [PubMed: 20200252]
66. Dutta A, Huang CT, Chen TC, et al. IL-10 inhibits neuraminidase-activated TGF- β and facilitates Th1 phenotype during early phase of infection. *Nat Commun*. 2015; 6:6374. [PubMed: 25728041]
67. Centers for Disease Control and Prevention (CDC). Surveillance for pediatric deaths associated with 2009 pandemic influenza A (H1N1) virus infection: United States, April–August 2009. *MMWR Morb Mortal Wkly Rep*. 2009; 58(34):941–947. [PubMed: 19730406]

At a Glance

- Influenza A virus can cause life-threatening disease in otherwise healthy children.
- The innate immune response to infection changes throughout childhood and may predispose children to severe influenza infection.
- Excessive inflammation, and not unchecked viral replication, likely contributes to severe influenza infection in children.
- A compensatory anti-inflammatory response may then predispose to secondary bacterial infections.
- Because children and adults appear to respond to influenza infection differently, the development of therapies specific for children is necessary.
- Better understanding of the susceptibility of young children to influenza will be essential to developing targeted therapies to protect this vulnerable population.

Figure 1.
Prevalence of Comorbid Conditions Among Patients Requiring Hospitalization for Influenza A Virus Infection During the 2012–2013 Influenza Season
Children who were hospitalized with influenza were significantly less likely to have had a preexisting medical condition before their hospitalization compared with the adult population. Data are adapted from the Centers for Disease Control and Prevention FluView website.⁵

Figure 2.

How The Innate Immune Response to Influenza A Virus (IAV) Infection May Differ Between Children and Adults

Viral RNA is recognized by Toll-like receptors located in the endosome, primarily TLR3 and TLR7, which activate the adaptor proteins Toll/interleukin (IL)–1 receptor domain–containing adapter-inducing interferon β (TRIF) and myeloid differentiation primary response gene 88 (MyD88), respectively. Activated TRIF and MyD88 act downstream on interferon regulatory factor 3 (IRF3), IRF7, and nuclear factor κ B (NF- κ B). Activated IRF3

and IRF7 translocate to the nucleus, where they stimulate transcription of the type I interferons (IFN- α/β). Nuclear factor κ B serves as a transcription factor promoting production of proinflammatory cytokines and chemokines, including pro-interleukin-1 β (pro-IL-1 β), IL-6, IL-10, and IL-12. Alternatively, cytosolic viral RNA is recognized by retinoic acid-inducible gene I (RIG-I), which binds to its adaptor mitochondrial antiviral signaling protein (MAVS), stimulating IRF3 and NF- κ B and their downstream transcription products IFN- α/β and proinflammatory cytokines. Nuclear factor κ B activation, in addition to a second signal indicating cellular stress (changes in intracellular ionic concentrations, reactive oxygen species [ROS], potassium flux, etc), induces assembly of the multimeric nucleotide-binding oligomerization domain (NOD)-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome protein complex. The NLRP3 inflammasome activates caspase 1, which cleaves the proinflammatory cytokines to release their mature forms. Upward and downward arrows indicate the relative cytokine response in the young pediatric population compared with adults. The overall consequence may move away from a helper T-cell subtype 1 (T_H1) response and toward a T_H2- and T_H17-predominant response that is more effective against extracellular pathogen clearance, leaving the young host at risk for the intracellular influenza pathogen. dsRNA indicates double-stranded RNA; ssRNA, single-stranded RNA. Illustration by Jacqueline Schaffer, MS, medical illustrator.

Relative Cytokine Response to Pattern Recognition Receptor (PRR) Activation in Children Compared With Adults^a

Table

PRR Agonist	Age, mo	IL-6	IL-10	IL-12 (p70)	IL-23	IL-1 β	IFN- γ	IFN- α	Source
TLR3									
Poly I:C	0		↑	↓					Belderbos et al, ¹⁸ 2009
	1		↑	=					
	0						↓↓		Burl et al, ¹⁹ 2011
	0	=	=	↓↓↓	=	↓↓↓	↓		Corbett et al, ²¹ 2010
	12	=	=	↓↓↓	=	↓↓↓	↓↓		
	24	=	=	↓↓	=	↓↓↓	↓↓↓		
	0	↑	↑	↓			↓		Kollman et al, ²⁰ 2009
TLR7									
Loxoribine	0		=				↓↓		Belderbos et al, ¹⁸ 2009
	1		↑				=		
Guardiquimod	0	=	=		↓↓	↓↓			Burl et al, ¹⁹ 2011
	1	↑	↑↑			↓			
	12	↑↑	↑			↓			
TLR 7/8									
3M-003	0	=	↑↑↑	↓↓↓	↑↑↑	=	↓↓	↓	Corbett et al, ²¹ 2010
	12	↓↓	↑↑	↓↓↓	↑	↓↓↓	↓↓↓	↑	
	24	↓↓↓	=	↓↓↓	=	↓↓↓	↓↓↓	=	
	0	↑	↑	↓	↑	↑	↓	↓	Kollman et al, ²⁰ 2009

Abbreviations: IFN, interferon; IL, interleukin; Poly I:C, polyinosinic-polycytidylic acid; TLR, Toll-like receptor.

^a Arrows indicate cytokine levels after PRR activation. ↓ Indicates decreased; ↓↓, moderately decreased; ↓↓↓, greatly decreased; ↑, increased; ↑↑, moderately increased; ↑↑↑, greatly increased; and =, similar levels between children and adults. Empty cells indicate no testing.