

## Review Article

# Toll-Like Receptors Dysregulation after Influenza Virus Infection: Insights into Pathogenesis of Subsequent Bacterial Pneumonia

Yoav Keynan,<sup>1,2,3</sup> Keith R. Fowke,<sup>1,2,3</sup> T. Blake Ball,<sup>1,3,4</sup> and Adrienne F. A. Meyers<sup>1,3,4</sup>

<sup>1</sup>Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada R3T 2N2

<sup>2</sup>Department of Community Health Sciences, University of Manitoba, Winnipeg, MB, Canada R3T 2N2

<sup>3</sup>Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya

<sup>4</sup>National Laboratory for HIV Viral Immunology and National HIV and Retrovirology Laboratories, Public Health Agency of Canada, 1015 Arlington Street Winnipeg, MB, Canada R3E 3R2

Correspondence should be addressed to Yoav Keynan, keynany@yahoo.com

Received 23 March 2011; Accepted 20 April 2011

Academic Editors: T. Goldmann and E. Lecuona

Copyright © 2011 Yoav Keynan et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The innate immune system utilizes an intricate network to aid in fighting foreign invaders. Recent insight and understanding of toll-like receptors (TLRs) has been critical in providing key information about early responses to infection, and more recently, understanding dysregulation of TLRs has shed light on pathogenic states. This paper addresses the importance of innate immunity and TLR regulation of immune responses to the presence of influenza infection and its role in the subsequent bacterial infections.

## 1. Introduction

**1.1. Toll-Like Receptors.** Toll-like receptors (TLRs) are members of the interleukin-1-receptor superfamily and comprise a family of transmembrane pattern recognition receptors (PRRs) that recognize highly conserved microbial molecules known as pathogen-associated molecular patterns (PAMPs) [1–3]. First identified in *Drosophila*, 13 members of the TLR family have been described with 10 being functional in humans and 12 in mice [4–7]. These receptors are key components of both innate and adaptive immunity and enable the distinction between self and nonself via specific recognition mechanisms that ultimately result in signaling cascades [8–10].

TLRs are type 1 transmembrane proteins with extracellular leucine-rich repeat domains (LRRs) for recognition of PAMPs, transmembrane regions, and intracellular Toll/IL-1 receptor (TIR) domains that are critical for downstream signaling [11]. Downstream signalling events take place by virtue of 1 of 2 pathways: a MyD88-mediated pathway resulting in activation of NF $\kappa$ B and inflammatory responses (all TLRs except TLR3) or a MyD88-independent pathway leading to the production of IFN- $\alpha$  and IFN- $\beta$  and an antiviral state (TLRs 3, 4) (reviewed in [12, 13]).

TLRs expressed on the cell surface (TLRs 1, 2, 4, 5, 6, 10) primarily recognize bacterial products as their PAMPs, while those expressed within the confines of the plasma membrane in the ER, endosomes, and lysosomes (TLRs 3, 7, 8, 9) are critical for the recognition of viral products and nucleic acids [8, 14–23].

The sensing of the environment by TLRs and subsequent cellular response to infection via signaling pathways are some of the earliest events in immune response. The most diverse repertoire and strongest levels of TLRs have been detected in antigen expressing cells such as macrophages and dendritic cells. Macrophage response to infection is one of the first steps [24–26]. Expression of TLRs is modulated by several factors including cytokines and pathogens, but stimulation and activation of TLRs in turn respond by the activation of genes and subsequent production and release of cytokines and chemokines important for inflammatory responses [27, 28]. This response, and the specificity of the response, plays an important role in determining the types and numbers of cells that are further recruited in the immune response to infection.

In addition to the immune cells, somatic cells can also respond to TLR stimulation. Most somatic cells express some TLRs, including epithelial [29, 30], endothelial [31, 32], cells

of the genital tract [33], intestinal tract [34], and respiratory tract [35, 36].

The recent work has revealed interesting details about immune responses and pathogenesis profiles involved in TLR signalling and influenza infection. Initially, it was believed that TLRs in plasmacytoid dendritic cells alone were important for antiviral responses, but the recent work suggests that TLR expression in other cell types is also key in mediating innate immune responses against viruses.

## 2. Postinfluenza Bacterial Pneumonia

Influenza viruses are members of the family *Orthomyxoviridae* and are responsible each year for almost half a million deaths. The virions infect epithelial cells of the upper respiratory tract and bronchi. A self-limited respiratory illness is the most common manifestation of infection with influenza, and there is a wide range of effects that can be seen, depending on many factors such as virus strain and immunocompetence of the infected host. Both seasonal and pandemic strains of influenza lead to significant morbidity and mortality with various estimates suggesting that approximately 36000 influenza-associated deaths occur annually in the United States [37–40]. Several days after resolution of the viral illness, some individuals will go on to develop a recurrence of fever accompanied by a productive cough and shortness of breath signalling the development of a superimposed bacterial infection. Although estimating the exact contribution of bacterial pneumonia is difficult, it is clear that bacterial pneumonia is an important cause of morbidity and mortality [41–43], and the most likely causative agents are *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae*. The mechanisms by which prior influenza infection predisposes to subsequent development of bacterial pneumonia are numerous with varying levels of evidence supporting each of these. The predisposition involves changes in epithelial defences and changes in epithelial cell wall, brought about by the cytolytic effects of the virus, leading to increased bacterial adherence and ability to invade. Immune changes involve several alterations of innate mechanisms affecting several aspects of function, including the ability of the innate cells to recognize the pathogen, impaired migration, or inability of macrophages and other immune cells to eradicate the invading organism [44–46]. Assessing the magnitude of the risk is complicated by variable effects attributed to particular strains of influenza virus and the degree of local epithelial damage and immune dysregulation the viral infection induces as well as the virulence of the secondary infecting bacterial organism.

## 3. Innate Immune Responses: TLRs in Influenza

Mucosal surfaces are faced with a continuous barrage of pathogens and need to differentiate innocuous from serious threats and respond appropriately. As part of the innate immune response to respiratory tract influenza infections, neutrophils, NK cells, and macrophages are recruited, and proinflammatory cytokine/chemokine production is initiated [47]. A favourable outcome following infection is

largely dependant upon the recruitment of proinflammatory leukocytes and induction of chemokine release at the site of infection [48, 49]. The ensuing adaptive responses are of paramount importance in clearance of the virus and repair of the tissue damage.

As seen for many other viruses, TLRs are an important part of the innate immune response to influenza infection [20, 50, 51]. Acidification of endosomes to allow TLR7/8-mediated signalling and inflammatory cytokine production is required for adequate responses in dendritic cells and neutrophils. Dendritic cells deficient for TLR7 have reduced ssRNA responses and IFN- $\alpha$  or TNF- $\alpha$  release.

TLR7/MyD88 recognition of influenza virus is essential for the induction of protective immune response to the dominant antigens (hemagglutinin HA) [52, 53]. It is of interest that a viral protein, rather than traditional nucleic acid (ssRNA) or glycoprotein, is capable of stimulating an immune response via TLR signalling. The HA plays an important role in the pathogenicity associated with the 1918 pandemic strain of influenza.

Influenza viral RNA was detected by distinct host receptors, TLRs and retinoic-acid-inducible-gene-like receptors (RLRs), and mediated by their interaction with adaptor molecules MyD88 (TLRs) and IPS-1 (RLRs). Likely a combined effort of several receptors and signalling pathways is involved in the regulation of the innate immune response to influenza infection. Strong evidence suggesting that non-immune type cells (epithelial, myeloid cells) generate type 1 interferons via IPS-1 induction, whereas TLR expression on immune cells (pcDCs, B cells) mediate type 1 IFN response via MyD88 interaction [50, 54–57]. MyD88-mediated signalling also appears to play a role in the generation of an adaptive immune response during the course of influenza infection. Therefore, TLR signalling is important for vaccine production (TLR7/MyD88 signalling via immunodominant Ag) and preventative as well as therapeutic modality development (adaptive immune responses via B cell-mediated antibody production and influenza nucleoprotein-specific CD4 production of IFN- $\gamma$ ). Interestingly, following infection with influenza, the TLR3 and TLR7 pathways were not important for CD4 or CD8 T cell proliferation, activation, or effector functions [58]. Furthermore, a lack of TLR3 had no impact on the humoral response to influenza infection. The lack of TLR7, however, did have an effect on Ab isotype-switching specific for influenza with enhanced levels of IgG1 titers.

One report describes the constitutive expression of TLR3 in human alveolar and bronchial epithelial cells, key cells encountering influenza virus during infection [59]. This paper went on to demonstrate the importance of TLR3 signalling in mounting an effective respiratory epithelial cell immune response when encountering dsRNA and influenza A virus.

The recent work has also taken into consideration the role of the adaptor molecules such as MyD88 in stimulating an immune response [60, 61]. One study describes the enhanced immunogenicity and protection of a DNA vaccine against influenza virus when adaptor molecules are included in the vaccinogen.

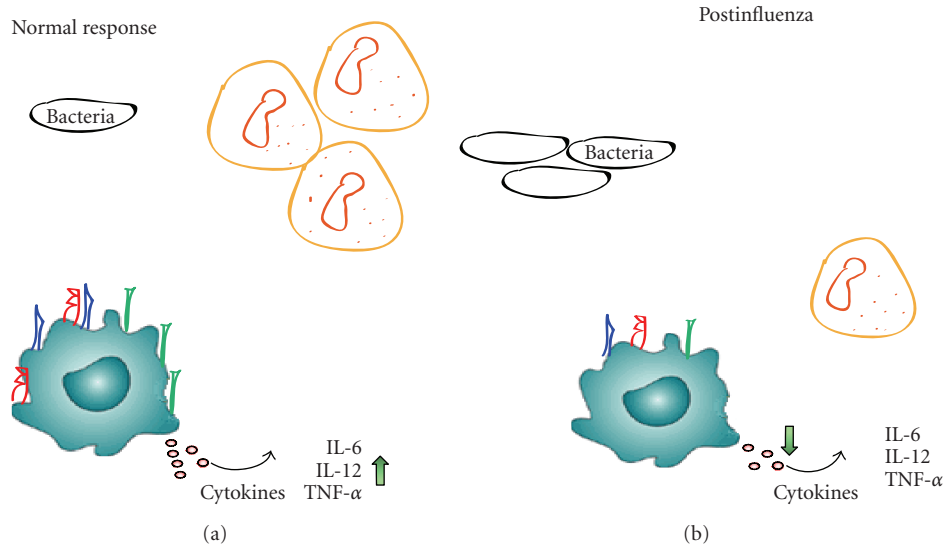


FIGURE 1: TLR downregulation leads to decreased bacterial clearance. (a) During normal response, TLR 2, 4, 5 on dendritic cells in the lung recognize bacterial ligands, leading to a signaling cascade that results in cytokine production. These cytokines increase neutrophil migration into the respiratory tract with subsequent decline in bacterial burden. (b) Postinfluenza infection-decreased TLR expression leads to decreased cytokine production, which in turn reduces chemotaxis of neutrophils into the lung with a resulting increase in bacterial burden.

#### 4. TLRs and Pathogenesis of Postinfluenza Bacterial Infection

The role of TLR receptors in determining the outcome of successive infection is only starting to be appreciated. The recently reported desensitization of airway macrophages to bacterial TLR agonists reported by Didierlaurent et al. [44] may help to shed light on the TLR dysfunction and its role as a mediator of increased susceptibility to bacterial pneumonia. The authors demonstrated that 2–6 weeks after influenza infection in mice, neutrophil recruitment in response to TLR2, TLR4, and TLR5 agonists was impaired, associated with decreased TLR-induced cytokine production, and was correlated with higher Gram-positive and Gram-negative bacterial loads. They also demonstrated that the mechanism behind the decreased neutrophil content was decreased transmigration, which is thought to be attributed to the reduced chemotactic signals. Heltzer et al. studied children with severe influenza and compared their responses to TLR stimulation *ex vivo* to that of children with moderate influenza, respiratory syncytial virus infection, and noninfected controls. They stimulated peripheral blood mononuclear cells using TLR ligands and assayed for TNF and IFN production. The responses to TLR ligands were comparable in RSV-infected children and controls. TLR responses in influenza patients were significantly lower than controls, and the responses were normalized when retested, suggesting a transient alteration. The diminished TLR responses did not translate to a decrease in plasma cytokines as these were elevated [62]. Higher rates of postinfluenza complications including bacterial infections occur among the elderly and may be attributed to higher prevalence of comorbidities with advancing age along with decreased antibody responses to

influenza vaccine [63–65]. However, recent data documented a decrease in TNF- $\alpha$ , IL-6, and IL-12p40 production following stimulation with a range of TLR ligands. The decreased cytokine production was observed for TLR 1, 2, 3, 5, 6, 7, 8 and involved both myeloid DCs and plasmacytoid DCs from older compared with young individuals. These differences occurred despite the absence of comorbidities in 80% of the older adults. The functional significance of these findings was further strengthened by the finding that the decreases in TLR-induced cytokine production were strongly associated with the absence of protective Ab response to the trivalent inactivated influenza vaccine [66]. Whether this dysfunction contributes to increased postinfluenza pneumonia was not addressed by the study. A decreased TLR expression caused by influenza may attenuate the inflammatory injury induced by the virus; however, it may persist beyond the resolution of viral infection and may play a role in the establishment of superimposed bacterial pneumonia.

Figure 1 illustrates the proposed mechanism by which TLR dysregulation after influenza infection leads to bacterial pneumonia.

#### 5. Therapeutic Targeting of TLRs in Influenza Infection

Understanding of the immune dysregulation caused by influenza is compounded by the variation of effects between different viral strains, pre-existing immunity, comorbidities, and age of the individuals. However, ameliorating the observed decrease response to TLR stimulation and/or shortening its duration may improve the outcome of postinfluenza bacterial pneumonia.

## 6. Summary

TLR-mediated signalling events are important for the generation of host response and resistance to infection. These receptors respond to influenza virus and contribute to the recruitment of innate cells as well as to the generation of the subsequent adaptive response. The limited information available suggests that innate dysregulation may occur during influenza infection and that such dysfunction may persist well beyond the viral presence in the respiratory tract. TLR dysregulation may be involved in setting the stage for subsequent bacterial infection, by altering one of the most important and early responders to bacterial invasion into the respiratory tract. Understanding the magnitude of TLR dysregulation and its contribution to the development of bacterial complications in the aftermath of influenza infection may open the door for novel therapeutic interventions.

## References

- [1] R. Medzhitov and C. A. Janeway Jr., "Innate immunity: the virtues of a nonclonal system of recognition," *Cell*, vol. 91, no. 3, pp. 295–298, 1997.
- [2] S. Akira, K. Takeda, and T. Kaisho, "Toll-like receptors: critical proteins linking innate and acquired immunity," *Nature Immunology*, vol. 2, no. 8, pp. 675–680, 2001.
- [3] C. A. Janeway Jr. and R. Medzhitov, "Innate immune recognition," *Annual Review of Immunology*, vol. 20, pp. 197–216, 2002.
- [4] B. Lemaitre, E. Nicolas, L. Michaut, J. M. Reichhart, and J. A. Hoffmann, "The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults," *Cell*, vol. 86, no. 6, pp. 973–983, 1996.
- [5] J. A. Hoffmann and J. M. Reichhart, "Drosophila innate immunity: an evolutionary perspective," *Nature Immunology*, vol. 3, no. 2, pp. 121–126, 2002.
- [6] S. Akira, "Mammalian Toll-like receptors," *Current Opinion in Immunology*, vol. 15, no. 1, pp. 5–11, 2003.
- [7] D. Zhang, G. Zhang, M. S. Hayden et al., "A Toll-like receptor that prevents infection by uropathogenic bacteria," *Science*, vol. 303, no. 5663, pp. 1522–1526, 2004.
- [8] S. Akira, S. Uematsu, and O. Takeuchi, "Pathogen recognition and innate immunity," *Cell*, vol. 124, no. 4, pp. 783–801, 2006.
- [9] K. Takeda and S. Akira, "TLR signaling pathways," *Seminars in Immunology*, vol. 16, no. 1, pp. 3–9, 2004.
- [10] T. Kawai and S. Akira, "Innate immune recognition of viral infection," *Nature Immunology*, vol. 7, no. 2, pp. 131–137, 2006.
- [11] B. Beutler and M. Rehli, "Evolution of the TIR, tolls and TLRs: functional inferences from computational biology," *Current Topics in Microbiology and Immunology*, vol. 270, pp. 1–21, 2002.
- [12] O. Takeuchi and S. Akira, "Innate immunity to virus infection," *Immunological Reviews*, vol. 227, no. 1, pp. 75–86, 2009.
- [13] S. Akashi-Takamura and K. Miyake, "TLR accessory molecules," *Current Opinion in Immunology*, vol. 20, no. 4, pp. 420–425, 2008.
- [14] J. Choe, M. S. Kelker, and I. A. Wilson, "Structural biology: crystal structure of human Toll-like receptor 3 (TLR3) ectodomain," *Science*, vol. 309, no. 5734, pp. 581–585, 2005.
- [15] J. K. Bell, J. Askins, P. R. Hall, D. R. Davies, and D. M. Segal, "The dsRNA binding site of human Toll-like receptor 3," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 23, pp. 8792–8797, 2006.
- [16] T. Kawai and S. Akira, "Toll-like receptor and RIG-1-like receptor signaling," *Annals of the New York Academy of Sciences*, vol. 1143, pp. 1–20, 2008.
- [17] S. Y. Zhang, E. Jouanguy, S. Ugolini et al., "TLR3 deficiency in patients with herpes simplex encephalitis," *Science*, vol. 317, no. 5844, pp. 1522–1527, 2007.
- [18] V. Hornung, M. Guenther-Biller, C. Bourquin et al., "Sequence-specific potent induction of IFN- $\alpha$  by short interfering RNA in plasmacytoid dendritic cells through TLR7," *Nature Medicine*, vol. 11, no. 3, pp. 263–270, 2005.
- [19] L. Alexopoulou, A. C. Holt, R. Medzhitov, and R. A. Flavell, "Recognition of double-stranded RNA and activation of NF- $\kappa$ B by Toll-like receptor 3," *Nature*, vol. 413, no. 6857, pp. 732–738, 2001.
- [20] F. Heil, P. Ahmad-Nejad, H. Hemmi et al., "The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily," *European Journal of Immunology*, vol. 33, no. 11, pp. 2987–2997, 2003.
- [21] S. S. Diebold, T. Kaisho, H. Hemmi, S. Akira, and C. Reis e Sousa, "Innate antiviral responses by means of TLR-7 mediated recognition of single-stranded RNA," *Science*, vol. 303, no. 5663, pp. 1529–1531, 2004.
- [22] A. Krug, G. D. Luker, W. Barchet, D. A. Leib, S. Akira, and M. Colonna, "Herpes simplex virus type 1 activates murine natural interferon-producing cells through toll-like receptor 9," *Blood*, vol. 103, no. 4, pp. 1433–1437, 2004.
- [23] J. Lund, A. Sato, S. Akira, R. Medzhitov, and A. Iwasaki, "Toll-like receptor 9—mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells," *Journal of Experimental Medicine*, vol. 198, no. 3, pp. 513–520, 2003.
- [24] K. A. Zarembek and P. J. Godowski, "Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines," *Journal of Immunology*, vol. 168, no. 2, pp. 554–561, 2002.
- [25] V. Hornung, S. Rothenfusser, S. Britsch et al., "Quantitative expression of Toll-like receptor 1–10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides," *Journal of Immunology*, vol. 168, no. 9, pp. 4531–4537, 2002.
- [26] M. Muzio, D. Bosisio, N. Polentarutti et al., "Differential expression and regulation of Toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells," *Journal of Immunology*, vol. 164, no. 11, pp. 5998–6004, 2000.
- [27] Z. M. Wang, C. Liu, and R. Dziarski, "Chemokines are the main proinflammatory mediators in human monocytes activated by *Staphylococcus aureus*, peptidoglycan, and endotoxin," *Journal of Biological Chemistry*, vol. 275, no. 27, pp. 20260–20267, 2000.
- [28] M. Ritter, D. Mennerich, A. Weith, and P. Seither, "Characterization of Toll-like receptors in primary lung epithelial cells: strong impact of the TLR3 ligand poly(I:C) on the regulation of Toll-like receptors, adaptor proteins and inflammatory response," *Journal of Inflammation*, vol. 2, article 16, 2005.
- [29] A. Pivarcsi, L. Bodai, B. Rethi et al., "Expression and function of Toll-like receptors 2 and 4 in human keratinocytes," *International Immunology*, vol. 15, no. 6, pp. 721–730, 2003.
- [30] G. Kollisch, B. N. Kalali, V. Voelcker et al., "Various members of the Toll-like receptor family contribute to the innate



- immune response of human epidermal keratinocytes," *Immunology*, vol. 114, no. 4, pp. 531–541, 2005.
- [31] E. Faure, O. Equils, P. A. Sieling et al., "Bacterial lipopolysaccharide activates NF-kappaB through Toll-like receptor 4 (TLR-4) in cultured human dermal endothelial cells. Differential expression of TLR-4 and TLR-2 in endothelial cells," *The Journal of Biological Chemistry*, vol. 275, no. 15, pp. 11058–11063, 2000.
- [32] J. Tissari, J. Siren, S. Meri, I. Julkunen, and S. Matikainen, "IFN-alpha enhances TLR3-mediated antiviral cytokine expression in human endothelial and epithelial cells by up-regulating TLR3 expression," *Journal of Immunology*, vol. 174, no. 7, pp. 4289–4294, 2005.
- [33] A. Fazeli, C. Bruce, and D. O. Anumba, "Characterization of Toll-like receptors in the female reproductive tract in humans," *Human Reproduction*, vol. 20, no. 5, pp. 1372–1378, 2005.
- [34] M. T. Abreu, M. Fukata, and M. Arditi, "TLR signaling in the gut in health and disease," *Journal of Immunology*, vol. 174, no. 8, pp. 4453–4460, 2005.
- [35] D. Droemann, T. Goldmann, D. Branscheid et al., "Toll-like receptor 2 is expressed by alveolar epithelial cells type II and macrophages in the human lung," *Histochemistry and Cell Biology*, vol. 119, no. 2, pp. 103–108, 2003.
- [36] L. Guillott, S. Medjane, K. Le-Barillec et al., "Response of human pulmonary epithelial cells to lipopolysaccharide involves Toll-like receptor 4 (TLR4)-dependent signaling pathways: evidence for an intracellular compartmentalization of TLR4," *The Journal of Biological Chemistry*, vol. 279, no. 4, pp. 2712–2718, 2004.
- [37] World Health Organization, "Influenza (seasonal)," 2009, Fact sheet no. 211. <http://www.who.int/mediacentre/factsheets/fs211/en/index.html>.
- [38] N. P. Johnson and J. Mueller, "Updating the accounts: global mortality of the 1918–1920 "Spanish" influenza pandemic," *Bulletin of the History of Medicine*, vol. 76, no. 1, pp. 105–115, 2002.
- [39] W. W. Thompson, M. R. Moore, E. Weintraub et al., "Estimating influenza-associated deaths in the United States," *American Journal of Public Health*, vol. 99, supplement 2, pp. S225–S230, 2009.
- [40] W. W. Thompson, D. K. Shay, E. Weintraub et al., "Mortality associated with influenza and respiratory syncytial virus in the United States," *Journal of the American Medical Association*, vol. 289, no. 2, pp. 179–186, 2003.
- [41] D. M. Morens, J. K. Taubenberger, and A. S. Fauci, "Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness," *Journal of Infectious Diseases*, vol. 198, no. 7, pp. 962–970, 2008.
- [42] M. N. Ballinger and T. J. Standiford, "Postinfluenza bacterial pneumonia: host defenses gone awry," *Journal of Interferon & Cytokine Research*, vol. 30, no. 9, pp. 643–652, 2010.
- [43] R. K. Gupta, R. George, and J. S. Nguyen-Van-Tam, "Bacterial pneumonia and pandemic influenza planning," *Emerging Infectious Diseases*, vol. 14, no. 8, pp. 1187–1192, 2008.
- [44] A. Didierlaurent, J. Goulding, and T. Hussell, "The impact of successive infections on the lung microenvironment," *Immunology*, vol. 122, no. 4, pp. 457–465, 2007.
- [45] H. Mao, W. Tu, Y. Liu et al., "Inhibition of human natural killer cell activity by influenza virions and hemagglutinin," *Journal of Virology*, vol. 84, no. 9, pp. 4148–4157, 2010.
- [46] I. Meunier, S. Pillet, J. N. Simonsen, and V. von Messling, "Influenza pathogenesis: lessons learned from animal studies with H5N1, H1N1 Spanish and pandemic H1N1 2009 influenza," *Critical Care Medicine*, vol. 38, supplement 4, pp. e21–e29, 2010.
- [47] J. P. Wang, G. N. Bowen, C. Padden et al., "Toll-like receptor-mediated activation of neutrophils by influenza A virus," *Blood*, vol. 112, no. 5, pp. 2028–2034, 2008.
- [48] M. D. Wareing, A. B. Lyon, B. Lu, C. Gerard, and S. R. Sarawar, "Chemokine expression during the development and resolution of a pulmonary leukocyte response to influenza A virus infection in mice," *Journal of Leukocyte Biology*, vol. 76, no. 4, pp. 886–895, 2004.
- [49] M. D. de Jong, C. P. Simmons, T. T. Thanh et al., "Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia," *Nature Medicine*, vol. 12, no. 10, pp. 1203–1207, 2006.
- [50] S. S. Diebold, T. Kaisho, H. Hemmi, S. Akira, and C. Reis e Sousa, "Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA," *Science*, vol. 303, no. 5663, pp. 1529–1531, 2004.
- [51] J. Lee, T. H. Chuang, V. Redecke et al., "Molecular basis for the immunostimulatory activity of guanine nucleoside analogs: activation of Toll-like receptor 7," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 11, pp. 6646–6651, 2003.
- [52] E. A. Kurt-Jones, L. Mandell, C. Whitney et al., "Role of Toll-like receptor 2 (TLR2) in neutrophil activation: GM-CSF enhances TLR2 expression and TLR2-mediated interleukin 8 responses in neutrophils," *Blood*, vol. 100, no. 5, pp. 1860–1868, 2002.
- [53] J. P. Wang, P. Liu, E. Latz, D. T. Golenbock, R. W. Finberg, and D. H. Libraty, "Flavivirus activation of plasmacytoid dendritic cells delineates key elements of TLR7 signaling beyond endosomal recognition," *Journal of Immunology*, vol. 177, no. 10, pp. 7114–7121, 2006.
- [54] J. M. Lund, L. Alexopoulou, A. Sato et al., "Recognition of single-stranded RNA viruses by Toll-like receptor 7," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 15, pp. 5598–5603, 2004.
- [55] W. Barchet, A. Krug, M. Cella et al., "Dendritic cells respond to influenza virus through TLR7- and PKR-independent pathways," *European Journal of Immunology*, vol. 35, no. 1, pp. 236–242, 2005.
- [56] S. Koyama, K. J. Ishii, H. Kumar et al., "Differential role of TLR- and RLR-signaling in the immune responses to influenza A virus infection and vaccination," *Journal of Immunology*, vol. 179, no. 7, pp. 4711–4720, 2007.
- [57] H. Kato, O. Takeuchi, S. Sato et al., "Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses," *Nature*, vol. 441, no. 7089, pp. 101–105, 2006.
- [58] A. K. Heer, A. Shamshiev, A. Donda et al., "TLR signaling fine-tunes anti-influenza B cell responses without regulating effector T cell responses," *Journal of Immunology*, vol. 178, no. 4, pp. 2182–2191, 2007.
- [59] L. Guillot, R. Le Goffic, S. Bloch et al., "Involvement of Toll-like receptor 3 in the immune response of lung epithelial cells to double-stranded RNA and influenza A virus," *The Journal of Biological Chemistry*, vol. 280, no. 7, pp. 5571–5580, 2005.
- [60] F. Takeshita, T. Tanaka, T. Matsuda et al., "Toll-like receptor adaptor molecules enhance DNA-raised adaptive immune responses against influenza and tumors through activation of innate immunity," *Journal of Virology*, vol. 80, no. 13, pp. 6218–6224, 2006.
- [61] K. Kobiyama, F. Takeshita, K. J. Ishii et al., "A signaling polypeptide derived from an innate immune adaptor molecule

- can be harnessed as a new class of vaccine adjuvant,” *Journal of Immunology*, vol. 182, no. 3, pp. 1593–1601, 2009.
- [62] M. L. Heltzer, S. E. Coffin, K. Maurer et al., “Immune dysregulation in severe influenza,” *Journal of Leukocyte Biology*, vol. 85, no. 6, pp. 1036–1043, 2009.
- [63] B. Grubeck-Loebenstien and G. Wick, “The aging of the immune system,” *Advances in Immunology*, vol. 80, pp. 243–284, 2002.
- [64] T. T. Yoshikawa, “Epidemiology and unique aspects of aging and infectious diseases,” *Clinical Infectious Diseases*, vol. 30, no. 6, pp. 931–933, 2000.
- [65] J. E. McElhaney, “Influenza vaccine responses in older adults,” *Ageing Research Reviews*, vol. 10, no. 3, pp. 379–388, 2011.
- [66] A. Panda, F. Qian, S. Mohanty et al., “Age-associated decrease in TLR function in primary human dendritic cells predicts influenza vaccine response,” *Journal of Immunology*, vol. 184, no. 5, pp. 2518–2527, 2010.