India). Until new evidence suggests otherwise, all ZIKV strains should be considered to have the potential to cause birth defects, and many region-specific factors need to be taken into account when evaluating the population risks.

The misleading messaging about the risks of microcephaly during the ongoing ZIKV outbreaks in India stems from many failed processes: (i) the journal review process for allowing overextrapolated findings to be published, (ii) the process by which journals and researchers disseminate information to the public, (iii) the interpretation and fact-checking process by the press, leading to over-hyped and sensationalized stories (e.g.,[viii]), and (iv) the lack of robust scientific advisory committees for public health institutions. Not only should we be vigilant about the threat of future ZIKV spread, but also of the spread of misinformation that could put many vulnerable populations at risk. It is imperative that we stop trying to assign a phenotype to a virus based on its strain to diminish the perceived risks without strong epidemiological evidence. This messaging is a real disservice to control efforts. In the absence of licensed vaccines or prophylactic drugs, our most powerful weapons to combat ZIKV are mosquito control, education, and support from clinicians, community health workers, and social workers. Health policy towards ZIKV should be based on strong epidemiological evidence and open communication to empower the public.

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Resources

[i]www.pib.nic.in/PressReleseDetail.aspx?PRID=1551803

[ii]www.thehindu.com/sci-tech/health/stopping-the-virus/article25580955.ece

[iii]www.thehindu.com/opinion/op-ed/cause-to-remain-alert/article25457709.ece

[iv]https://timesofindia.indiatimes.com/city/bhopal/uganda-strain-of-zika-virus-found-in-madhya-pradesh-aiims-report/articleshow/67414003.cms

[v]https://indianexpress.com/article/india/not-so-alarming-revise-zika-alert-india-to-cdc-5514459/

[vi]http://apps.who.int/iris/handle/10665/254440

[vii]www.paho.org/hq/dmdocuments/2017/2017-phe-zika-situation-report-bra.pdf

[viii]www.latimes.com/science/sciencenow/la-sci-sn-zika-mutation-microcephaly-20170928-story.html

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Spotlight

Influenza and *Staphylococcus aureus* Coinfection: TLR9 at Play

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Bacterial lung infections are frequent causes of mortality following influenza infection, but the fundamental mechanisms remain largely unknown. A new study by Martínez-Colón *et al.* (*PLoS Pathog*. 2019;15:e1007560) now suggests that influenza-induced immune suppression of *Staphylococcus aureus* is mediated by TLR9 signaling.

Secondary bacterial pneumonia, particularly with *Streptococcus pneumoniae* and *S. aureus*, is a frequent cause of severe morbidity and mortality associated with influenza infection. Likely due to difficulties in modeling *S. aureus* infection in mice, previous *in vivo* studies were often performed with secondary *S. pneumoniae* infection. However, in recent years, the increasing incidence of fatal methicillin-resistant *S. aureus* (MRSA) pneumonia has promoted a need for direct investigations of influenza and *S. aureus* coinfection pathogenesis.

In both influenza/*S. pneumoniae* and influenza/*S. aureus* coinfection models, a

common observation is that prior influenza infection inhibits phagocyte-mediated bacterial clearance in the lung. Influenza infection promotes lung monocyte recruitment, while acute bacterial infection predominantly recruits neutrophils. Thus, both inflammatory monocytes and neutrophils are prominent in the lung during influenza and bacterial coinfection. Interestingly, mice deficient in inflammatory monocytes are resistant to secondary bacterial infection, as evidenced by improved bacterial clearance and animal survival from either influenza/*S. pneumoniae* [1] or influenza/*S. aureus* [2] coinfection. On the other hand, multiple studies suggest that influenza-induced type I IFN (IFN-I) signaling [3,4], including IFN-I/STAT1 suppression of interleukin-17 (IL-17) immunity [5], suppresses neutrophil recruitment, and thereby causes increased host susceptibility to secondary bacterial infection. These two lines of investigation reached common conclusions – influenza-induced monocyte/macrophage recruitment impairs lung bacterial control, while compensatory neutrophil accumulation promotes host resistance to secondary bacterial infection.

Compared with their relatively limited ability to bind and take up encapsulated pneumococci, macrophages and neutrophils are proficient in uptake of *S. aureus*. As a result, phagocytic clearance of *S. aureus* is mainly restricted to an intracellular killing process. For example, NADPH oxidase activity, which is likely able to overcome the antioxidant action of staphylococcal catalase, is essential for pulmonary clearance of *S. aureus* but dispensable for *S. pneumoniae* [6]. Accordingly, neutrophils are believed to be most important for phagocytic killing of *S. aureus*, in agreement with their greater oxidative burst capacity compared with macrophages. Therefore, it would be logical if the influenza-induced immune response has a differential impact on the susceptibility to these two bacterial pathogens. Recently, the findings reported by Martínez-Colón *et al*. have shed light on this prediction [7].

Toll-like receptor 9 (TLR9) recognizes unmethylated cytosine and guanine (CpG) motifs which are rich in microbial DNA. The recent study by Martínez-Colón *et al*. [7] revealed an unexpected pathologic role for TLR9 in limiting clearance of *S. aureus* after influenza infection. In contrast, TLR9 had no detectable effect on influenza-induced susceptibility to *S. pneumoniae* infection. With the use of a TLR9-deficeint ($TLR9^{-/-}$) mouse model, the authors found that there were no differences between wild-type (WT) and $TLR9^{-/-}$ mice in influenza-induced cytokine responses and immune cell recruitment into the lungs. However, despite being present in equal numbers, monocytes/macrophages from influenza-infected $TLR9^{-/-}$ mice had increased bacterial phagocytosis and intracellular killing ability. Similarly, influenza-induced STAT2 signaling has been shown to inhibit macrophage uptake and killing of *S. aureus* [8]. Furthermore, Robinson *et al.* recently reported that the ASC inflammasome inhibits bacterial clearance and thereby increases animal mortality during influenza/*S. aureus* coinfection [9]. Together, these studies demonstrate that, rather than regulating neutrophil accumulation, defective bacterial killing by macrophages/monocytes directly contributes to influenza-induced susceptibility to *S. aureus* infection.

The new results indicate that TLR9 signaling surprisingly influences susceptibility to secondary MRSA infection following influenza, but the precise mechanisms will require further study. Although the authors performed multiple experiments to show that influenza increases TLR9 expression in macrophages, they concluded that it is actually TLR9 expression in non-hematopoietic cells that regulates macrophage function. The explanation the authors provide is that TLR9 upregulation is induced by soluble factors. Further research should therefore focus on identifying these soluble factors. Arguably, it is possible that these soluble factors directly inhibit phagocytosis activity of macrophages/monocytes, with TLR9 upregulation as a byproduct. Furthermore, it remains possible that these soluble factors inhibit the phagocytosis activity of neutrophils, and that this is a primary cause for increased susceptibility to *S. aureus* infection. Another limitation of the study is that, although the authors detected the peak susceptibility to *S. aureus* on day 7 after viral infection, they focused the mechanistic studies on day 5 post-influenza. Therefore, although the present work suggests that targeting TLR9 could be a potential novel therapeutic approach for treatment of influenza and *S. aureus* coinfection, future studies will be required to determine the overall pathologic role of TLR9 under different experimental conditions.

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