Pathogen Replication, Host Inflammation, and Disease in the Upper Respiratory Tract

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Influenza virus and respiratory bacterial synergy, a term often used when describing the relationship between these two highly divergent phyla of respiratory tract pathogens, has been known and documented for nearly a century (1–3). The first conclusive reports date back to 1931, when a young scientist, Richard Shope, and his mentor, Paul Lewis, demonstrated that, by infecting pigs initially with an influenza virus and then with *Haemophilus influenzae*, they could induce disease more severe than that seen with either of the two pathogens in isolation (3, 4). These experiments clarified at least in part the seemingly contradictory findings of Richard Pfeiffer with his discovery in 1892 of *H. influenzae*, which he believed was the causative agent of influenza (5), and of Olitsky and Gates, who postulated in 1921 that the causative agent of influenza was indeed a virus (6). In hindsight, Pfeiffer was probably more correct than he was given credit for, with his finding that a bacterial pathogen was associated with the severe disease often seen in influenza virus infections.

Since then, many clinical and laboratory investigations have been reported, demonstrating incontrovertibly that the influenza A virus induces increased susceptibility to secondary bacterial coinfections, most notably by *Streptococcus pneumoniae* (the pneumococcus) (7–14). With unanimous agreement that this relationship exists, more recent reports have moved to delineate the mechanism responsible for the severe and often lethal lower respiratory infections (LRIs) and invasive pneumococcal disease (IPD) that follow influenza virus-bacterial coinfections (9, 10, 15, 16). With a strong focus on LRIs and IPD, these studies have largely neglected the more subtle effects of the relationship between viral replication and bacteria within the upper respiratory tract (URT) and middle ear. Indeed, when introducing the topic of influenza and pneumococcal interactions, a quick review of the literature makes it seem almost compulsory that any paper on the topic must open with an overview of the lethal role that the pneumococcus plays in the excess mortality seen during the 1918 influenza pandemic. While it is especially important that we understand the mechanisms contributing to severe disease following influenza virus-bacterial coinfections, it is easy to neglect the outcomes and mechanisms of diseases resulting from such a synergy that are not potentially lethal but continue to contribute immensely to the overall level of morbidity due to infectious disease, particularly in young children (e.g., acute otitis media, rhinitis, and sinusitis). The overwhelming interest in mortality and severe disease as an endpoint has led to a focus on highly virulent influenza virus and pneumococcal strains that are not necessarily relevant to the more common, less invasive strains currently circulating and colonizing individuals on a daily basis. Further, most of these investigations have focused on the initial doses and overall effects on morbidity, immunopathology, and mortality but often do not consider the effects of localized within-host replication and viral or bacterial densities as significant in the mechanisms underlying the synergy. This is not to say, however, that studies on nonlethal effects of influenza-pneumococcal synergy do not exist. Indeed, there is a small but growing trend to look at these issues.

Nakamura et al. have recently reported a role for type I interferons (IFN) following influenza virus infection in promotion of pneumococcal colonization in mice, suggesting that a synergistic increase in alpha IFN (IFN-α) disrupts appropriate CCL2-mediated macrophage recruitment, and thus proper clearance of pneumococcal colonization is inhibited (17). Diamatopoulos and colleagues have previously demonstrated a role for influenza A virus (IAV) in pneumococcal transmission between infant mice, demonstrating that IAV infection of both pneumococcus-colonized (donor) and pneumococcus-naïve (recipient) mice is essential for transmission of pneumococci from donor to recipient (12). Similarly, McCullers et al. have reported on a significant role for IAV infections in the transmissibility and acquisition of pneumococci between ferrets and for the development of pneumococcal acute otitis media (AOM) (18). In that series of experiments, the authors also demonstrated an important role for the pneumococcal capsular serotype in those interactions with IAV where, for example, influenza virus infection led to greater rates of colonization and AOM development following pneumococcal infection with the colonizing serotype 19F versus the more invasive serotype 7F. Previously, the same group had also demonstrated that the ability of IAV to enhance pneumococcal sinusitis and AOM is a consequence of an IAV strain-dependent process such that H3N2 viruses confer significantly greater induction of bacterial colonization and disease than either H1N1 or influenza B viruses (14).

In this issue of *Infection and Immunity*, Short and colleagues begin to unravel the mechanisms underlying increased susceptibility to pneumococcal AOM following H3N2 versus H1N1 viruses and add significant insight into the often-overlooked effect of within-host, organ-specific pathogen replication and density on localized inflammation, and the role of inflammation in bacterial migration, density, and URT disease (19). Utilizing a panel of clinical and carefully crafted recombinant influenza viruses,
Short et al. demonstrate that it is the influenza virus hemagglutinin (HA) that is indirectly responsible for the differential effects on pneumococcal AOM following influenza virus infection. At first glance, this finding seems in contrast to a study by McCullers et al. demonstrating that influenza neuraminidase (NA) is responsible for influenza virus-pneumococcus synergy through cleavage of sialic acid residues and subsequent exposure of receptors for pneumococcal adherence (15). However, the findings by McCullers were aimed toward an understanding of lower respiratory tract infections and invasive disease, and while it may seem appropriate to assume similar mechanisms of action between the viral-bacterial interactions in the lower and upper respiratory tracts and middle ears, the current report by Short demonstrates that the interactions are more complex (19). The authors elegantly demonstrate that it is the viral HA, responsible for initial virus binding of sialic acid residues and subsequent internalization, that dictates the location and magnitude of viral replication within the middle ear in the mouse model. Using a series of H3N2, H1N1, and recombinant H3N1 or H1N2 virus pairs, isogenic except for the respective HA or NA, Short et al. show that viruses containing an H3 HA replicate within the middle ear to significantly greater titers than their H1 HA counterparts while finding no statistically significant differences in viral replication between strains containing N1 NAs and those containing N2 NAs. Interestingly, the differential effects of the HA were demonstrated only within the middle ear, with no differences in replication due to HA type within the nasopharynx (NP). The localized effect further clarifies the otherwise contradictory results of McCullers indicating that it is the viral NA that is responsible for increased pneumococcal synergy in the respiratory epithelial tract. In this case, the mechanisms underlying increased viral growth are site specific, with HA dictating viral replication, but only within the middle ear epithelium. Setting aside the synergistic relationship with the pneumococcus, this finding is important in its own right, as it can begin to inform development of specific therapies aimed at reducing or preventing influenza-mediated episodes of acute otitis media.

Correspondingly, the authors demonstrate a significantly greater inflammatory response within the middle ear following infection by H3 versus H1 viruses, and further show that it is the enhanced inflammatory response that instigates pneumococcal growth and subsequent episodes of bacterial AOM. Inflammation has long been known to be a double-edged sword in the human response to infectious organisms. On the one hand, a robust inflammatory response is required to mobilize cellular and humoral immunity to clear foreign invaders (20). While such a response may be carried out in the absence of adverse sequelae, an innate immune response disproportionate to that required to fend off the pathogen can often result in more harm than good (21, 22). Further, it has been shown that the magnitude of the response itself scales with the pathogenic insult in a dose- or replication-dependent manner (23). Thus, given a large enough invasion, significant immunopathology becomes a necessary and unavoidable byproduct of an appropriate and proportionate response. In the case of influenza virus-pneumococcus synergy, it is this immunopathologic response, be it inflammation-mediated epithelial denudation (15, 24) or a viral preoccupation of the innate immune system (8–10), that may provide a more hospitable environment for pneumococcal outgrowth and disease. Although in the present study by Short et al. the findings implicate the H3 HA of the influenza virus as predisposing to bacterial proliferation and AOM, the authors go on to show that the true culprit rests in the inflammatory response to viral replication, with the HA3 type important insofar as it seems important for middle ear viral replication. This underlying relationship between viral replication, density, and inflammation adds to a growing body of work implicating within-host pathogen replication as a major driver of pathology and disease, as a result of pathogen density-dependent induction of inflammation, and the immunopathologic consequences that follow. Carrol et al. have previously reported findings that increased pneumococcal DNA loads in blood and cerebrospinal fluid (CSF) are associated with greater systemic cytokine responses and worse patient outcomes (25). Similarly, Rello et al. have shown that pneumococcal bacterial load in whole blood is associated with increased risk of septic shock and death (26). de Jong et al. analyzed samples from 18 influenza H5N1 virus-infected patients, 13 of whom died from the infection. They found positive correlations between high viral replication and hypercytokinemia which, unsurprisingly, were inversely associated with probability of survival (27). In contrast, cold-adapted live attenuated influenza vaccines (LAIV), which fail to replicate with any efficiency in the warmer environment of the lower respiratory tract, are deemed safe due to their ability to stimulate only a mild inflammatory response while failing to generate detectable immunopathology within the lower lungs (28).

Besides demonstrating that increased inflammation follows increased replicative capacity, the findings of Short and colleagues bring to light an equally important topic, namely, the heterogeneous and organ-specific nature of the immune response. Inflammation is often viewed as a systemic response, highlighted by the common practice of measuring serum cytokines as a gauge of inflammation and immunity following infection or vaccination. The current findings by Short et al. remind us that inflammation is a highly heterogeneous and localized process, with significant implications for immunopathologic consequences and secondary coinfections. Inoculating mice with two influenza virus strains that are isogenic except for the HA, the authors demonstrated the specificity and heterogeneity of the inflammatory response following viral replication, or a lack thereof, in the middle ear. While the two viruses replicate with equal efficiency in the nasopharynx, likely inducing similar inflammatory responses, only the H3 viruses replicate with any efficiency in the nearby middle ear, and, despite its proximity to the NP, the local inflammatory responses within the middle ear are dramatically different following H3 versus H1 HA virus infections. Similarly, utilizing a panel of 11 unique viruses (5 H1 and 6 H3 viruses), they demonstrate that all 6 H3-containing viruses generate significantly greater inflammation within the middle ear than any of the H1 viruses, despite their similar growth patterns in the nearby NP. These findings highlight the site-specific heterogeneity in the inflammatory response following introduction of an infectious organism, and add credence to the notion that it is not simply the initial infectious dose that is important; equally important to the degree of inflammation and immunopathologic consequences is the magnitude of within-host replication. Interestingly, these findings are corroborated by a recent investigation by Ramakrishnan et al., who measured serum cytokine responses in humans following vaccination with injected trivalent inactivated influenza vaccine (TIV) versus intranasal LAIV (29). Although the study by Ramakrishnan utilized small
sample sizes and failed to detect a majority of cytokines within the serum of the vaccinated subjects, significant changes in levels of serum interleukin-8 (IL-8), IL-10, and tumor necrosis factor alpha (TNF-α) were measured following vaccination with TIV alone, with no change in serum cytokine levels detected following the intranasal vaccination. These results suggest that, since cold-adapted LAIV are designed for viral replication in the cooler temperature of the nasopharynx, any protective (or pathological) immunologic response following LAIV administration may well be isolated to the nasopharynx. Even so, they demonstrate efficacy against homologous and heterologous systemic influenza virus infections (30–32).

As Short et al. demonstrate, the implications of specific and localized inflammatory responses extend beyond the primary infection and immunopathology to secondary infections that follow. Inflammation following H3 influenza virus strain replication enhances susceptibility to bacterial migration into and replication within the middle ear, inducing bacterial AOM. By showing the results of instillation of exogenous bacterial lipopolysaccharide (LPS) directly into the middle ear, they demonstrate that the increase in bacterial replication and disease can be explained by inflammation alone. Thus, while it is the more efficient replication of the H3 HA-containing virus strains within the middle ear that appears to induce the increase in bacterial disease, the underlying mechanism rests on the shoulders of the double-edged sword of the innate immune response.

The study by Short et al. has provided further insight into the mechanisms responsible for influenza virus and bacterial AOM, which will prove useful in advancing our understanding and, thus, ability to prevent this more mild but far more common adverse sequela of influenza virus-pneumococcus coinfection. Further, the report highlights the importance of with-in-host pathogen density and replication for inflammation and the degree of immunopathology that may follow. While they have shown that inflammation, irrespective of the cause, increases bacterial replication in a site-specific manner, further investigations are required to understand the specific inflammatory components responsible for increased bacterial replication and disease. Undoubtedly, the story will be complex. With the many redundant pathways and pathogen density-dependent shifts in immune activation versus regulation, among other dynamic processes at hand, there will exist no single silver bullet, except perhaps the prevention of the initial introduction of the pathogens themselves into the host.

REFERENCES