YALE JOURNAL OF BIOLOGY AND MEDICINE 85 (2012), pp.127-132.

REVIEW

The Complication of Coinfection

Lesley Pasman

Department of Immunobiology, Yale Graduate School of Arts & Sciences, New Haven, Connecticut

Infectious disease remains one of the largest burdens on humankind. Even with modern medical and public health standards, infectious disease remained the No. 1 killer worldwide at the turn of the century. Often, the most costly disease burdens come from multiple infections at once, i.e., coinfection. Influenza, an annual infection often considered relatively harmless, can increase susceptibility to both deadly bacterial pneumonia and childhood ear infections. Major health threat HIV rarely kills a patient on its own, but instead allows for opportunistic infections and re-emergence of infections such as tuberculosis. What generates these unique relationships is not well understood; herein, we examine in detail three types of coinfection and the unique interactions between infectious agents as well as with the host in each setting. We also begin to address how we may aid further understanding of coinfection and what questions need to be addressed to help direct future treatments.

INTRODUCTION

Of the various assaults our bodies face throughout our lifetimes, infectious disease remains one of the main killers across the globe. At the turn of the century, infectious disease classified as the second leading killer in the world, behind only cardiovascular diseases [1]. However, in most scientific and medical investigations of prominent infections, these causal agents are studied in isolation. The immune state of the infected host is largely assumed to be a blank slate, when this is often not the case. Our bodies are in constant contact with the outside environment and, therefore, at constant risk of infection. It is consequentially predictable that many individuals may experience combined infections. These com-

To whom all correspondence should be addressed: Lesley Pasman, Department of Immunobiology, Yale Graduate School of Arts & Sciences, New Haven, CT; Tele: 203-785-6090; Email: lesley.pasman@yale.edu.

†Abbreviations: HIV, human immunodeficiency virus; Mtb, *Mycobacterium tuberculosis*; RSV, respiratory syncytial virus; TLR, toll-like receptor; PRRs, pattern-recognition receptors.

Keywords: coinfection, HIV, tuberculosis, bacterial pneumonia, influenza, flu, polymicrobial infection, secondary infection, Mtb

binations, called polymicrobial or coinfection, represent some of our most difficult infectious diseases to battle, even as they remain largely understudied in their combined state.

Below, we examine three common settings that can fall under the coinfection umbrella, how this situation affects our immune system's handling of each infection, and how we can better address these infections to increase our understanding of their natural course and perhaps also improve medical treatment.

COINFECTION, A DEFINITION

There are several different scenarios in the umbrella of coinfection. Infections can be concurrent or closely sequential, as well as involving both acute and chronic infections. Each of these combinations has representations in modern health care. A major example of concurrent acute infections is the increased susceptibility to respiratory bacterial infection during an ongoing influenza infection; in fact, this susceptibility is the main cause of death by influenza infection, especially in the elderly [2-4]. Through complications with bacterial pneumonia, influenza infection kills on average 20,000 individuals a year [2]. The immune response can also remain suppressed following resolution of infection, thus allowing secondary infection in an altered host state. This can be exemplified by childhood ear infection and is also seen as a variation of secondary bacterial pneumonia after influenza [5]. Lastly, an ongoing chronic infection can be complicated by and increase susceptibility for concurrent acute infections. A prime example plaguing our current health care is human immunodeficiency virus (HIV†) infection, as patients ultimately succumb to opportunistic acute infections that can take hold only due to the overwhelming immune suppression caused by the virus [6].

Concurrent acute: bacterial pneumonia complicating flu infection

Although we have annual vaccinations, influenza virus is one of the main infectious disease killers worldwide [1]. Of the 25 percent of deaths caused by infectious diseases, acute respiratory infections (for the majority, influenza and pneumonia) constitute 30 percent. Even in the years of mild seasonal flu, this infection remains a threat to the elderly [3]. Yet this virus rarely kills on its own. In most cases, death by influenza virus actually occurs due to the onset of bacterial pneumonia. Often, these are bacterial species that have colonized the nasal and upper respiratory systems, known as "commensal flora," and considered un-harmful and asymptomatic in these locations. These bacteria, upon weakening of host defenses by influenza, can migrate down the respiratory tract and expand in new bacterially naïve niches, where they can become harmful to the host. The most common complicating bacterial agents include *Staphylococcus aureus, Streptococcus pneumonia*, and *haemophilus influenzae*, among others [7-10]. In fact, due to high rates of bacterial complications, *haemophilus influenzae* was given its name upon discovery because researchers originally thought it was the cause of flu-like symptoms (viruses had yet to be discovered) [11].

Upon the onset of viral infection, several processes occur that researchers believe may make the respiratory system susceptible to bacterial infiltration. One leading thought holds that influenza neuraminidase, the viral enzyme responsible for removing sialic acids from host cell surfaces and viral anchoring proteins, thus allowing new virion release from cells, also may remove sialic acids that usually block bacterial anchor sites, allowing new niches for bacteria to grow [12,13]. In concurrent coinfection, bacterial expansion is typically more elevated than in singular infection alone, potentially because of increased binding sites [13]. Host parameters also may aid in priming sensitivity to coinfection. Recent reports indicate that specific host signaling molecules from the antiviral response may upregulate host bacterial-sensing signaling components [14]. Virtually all cells are equipped with specific receptors known as pattern-recognition receptors (PRRs) to detect the presence of pathogens such as

viruses and bacteria. Virally infected cells produce cytokines, host proteins secreted for intercellular communication. Upon receptor binding of these cytokines, receiving cells are shown to increase expression of bacterial-sensing PRRs such as the NOD proteins, allowing for amplified immune responses seen in coinfection [14]. It is typical in concurrent coinfection that combined infection is amplified often in pathogen amount and immune response as compared to singular infections [14-16].

Concurrent coinfection causes some difficulty in treating either infection. While antiviral treatment helps reduce viral burden, the efficacy of many of these treatments against concurrent bacterial infection is still unknown [17], and treatment to counter host pro-inflammatory molecules that may ameliorate flu symptoms may have unknown consequences on bacterial infection [16]. These interactions also can work against the underlying viral infection, exemplified with the use of glucocorticoids, hormones produced by the host to dampen inflammation. Recent work has demonstrated that blocking flu-induced glucocorticoid production helps block bacterial spread by maintaining active inflammation [18]. However, this treatment has negative consequences on viral infection, allowing for more host pathology [18]. Preventive treatment such as flu vaccination seems the immediate option currently available, and antibiotics and antivirals are often prescribed after coinfection [19].

Secondary/sequential infection: respiratory viruses + commensal bacteria

Two infections also do not need to overlap directly in time in order to affect each other. Bacterial pneumonia in conjunction with influenza can occur soon after viral clearance, a situation slightly different from concurrent infection. This is also seen in other respiratory coinfections, such as bacterial infection leading to ear infections, known as otitis media [5]. Otitis media is especially common in small children and usually caused by bacterial *S. pneumoniae* or *H. influenzae* following coronavirus, respiratory syncytial virus (RSV), or adenoviral infection [5,20]. Like bacterial pneumonia and influenza, otitis media can often be a concurrent polymicrobial infection [21].

Focusing on bacterial pneumonia associated with influenza infection, there are various differences noted in secondary bacterial infection versus concurrent [7]. These include altered cytokine expression patterns *in vivo*, especially an increase in immune-suppressive IL-10 versus pro-inflammatory interferons as seen in concurrent infections [22]. Moreover, a common feature seen soon after clearance of initial infection is the suppression of toll-like receptor (TLR) signaling, also seen post-influenza infection [23]. The TLR family of receptors is a member of the previously described PRRs and includes receptors to virus and bacterial products. This is distinct from concurrent infection, where active TLR signaling continues. In this case, TLR responsiveness is decreased, thereby having the immune system respond less actively than usual upon bacterial infection, potentially allowing the bacteria to take hold without immune deterrent [23].

In the situation of delayed secondary infection, there are both similar difficulties with treatment and differences from concurrent infection. In this case, there is no direct interaction with the virus. Instead, one must consider the condition of the host tissue post viral infection, which may be experiencing immune dampening and increased thresholds for activating signals. Therefore, giving immune-stimulating agents to assist in bacterial clearance may not be as effective as in isolated infection. Giving direct antibiotics can likely help, but giving specific cytokines to help boost immunity may not [7]. While most current treatments work through direct assault on the pathogen, another useful angle may be to target ways to help return tissue homeostasis, such as helping induce angiogenesis or increased barrier protections such as mucus production [24]. Further study into the timing of returned immune sensitivity and tissue homeostasis will be especially important for these delayed situations of secondary coinfection.

Concurrent with chronic infection: HIV

A third scenario that allows for coinfection is an already underlying chronic infection. HIV is a systemic viral infection that infects and destroys both innate and adaptive immune cells [25]. Through its manipulation of the immune system, HIV can disarm several defensive strategies of the host [26]. Upon exposure, HIV virus infects both T cells and macrophages at different stages of the infection, leading to a net loss in both of these immune defense cell types. Moreover, through the loss of these cells, T cells can no longer help activate B cells, the cells responsible for producing protective antibodies, proteins that can neutralize virus and help remove virally infected cells. Loss of macrophages also breaks a crucial step in the activation of other immune responses as well, as macrophages serve as important initial sensors of infection [25,26]. Continual immune activation also allows for characteristic changes that are different from acute infection, including non-antigen-specific polyclonal B cell activation [27], enhanced T cell turnover [28], and maintained increased levels of inflammatory cyto- and chemokines [26,29].

This active immuno-suppression and destruction of immune cells is continually ongoing during an HIV infection, allowing for the onset of another infection in an already weakened host. Complicating agents that take hold during HIV infection are often opportunistic, in that under "normal" immune-competent conditions, these agents would not be infective. These agents are often already a part of our normal flora, either commensal or suppressed when immune-competent, and include agents such as salmonella, toxoplasma, herpes infection, tuberculosis, candida albicans, and cytomegalovirus. For sake of concision, this review will focus on tuberculosis as the example of HIV coinfection. Tuberculosis, caused by bacterium *Mycobacterium tuberculosis* (Mtb), has seen a recent rise in global infection rates predominantly due to concurrent infection with HIV [30]. Patients who previously have been infected by asymptomatic Mtb (about 90 percent of 2 billion Mtb infections worldwide) can now see a reawakening of infection due to weakened immune state from HIV [31].

Opportunistic infections during HIV infection are often described as secondary infections, but HIV is still ongoing. This is an important distinction because it means a continual immune response and immune activation remain, rather than a delayed immune dampening following the resolution of an initial infection. There are several dilemmas for treatment of concurrent coinfection with an underlying chronic infection. A recent study has highlighted the potential for HIV to selectively destroy a class of T cells activated against Mtb among its targeted activated CD4+ T cells, thereby removing the adaptive response against Mtb [32]. In that light, any immune-enhancing treatment against Mtb that acts through the adaptive response would likely be less effective in HIV+ patients. Conversely, if left untreated, preliminary studies indicate that tuberculosis may expedite the course of HIV infection, showing associations with increased HIV replication rates and enhanced viral entry into host cells *in vitro* [6]. Underlying HIV infection can modulate Mtb infection to allow further dissemination of the bacteria, where HIV+ patients have higher rates of extra-pulmonary Mtb than those who are HIV- [6,33]. Luckily, conventional antiretroviral treatment of HIV also helps lower levels of Mtb infection [6]. The improved understanding of how these infections modulate each other allows more specific determination of appropriate therapeutic and preventive treatments.

FUTURE RESEARCH

From these examples, it is clear that the study of individual infectious agents against a "clean slate" host may be insufficient to fully capture the underlying issues of physiological infection. Additional focus on the tissue state of the host before, during, and after common "priming" infections such as respiratory viral infections or systemic chronic infections will help clarify the state of host tissue environment upon contraction

of the coinfecting agent. Moreover, for individual infections, it is important that scientists follow through after infectious clearance to determine the time delay and processes involved in returning host tissue to homeostasis.

Respiratory viral infections are notorious for secondary complications, but we still do not understand the unique relationships between initial viral infections and secondary bacterial complicators. Why does influenza allow for complicating bacterial pneumonia, but RSV more commonly complicates bacterial otitis media? Both secondary infections are often caused by the host's own commensal nasal flora, but the main agents differ in co-viruses as well as where they colonize in their pathological state. Clearer delineation of tissue alterations during and following viral infection can help clarify the logic behind these pairings.

How an infectious agent behaves may differ depending on the host environment it finds itself within. It is reasonable to imagine that a secondary infection entering into an environment already inflamed with increased cytokine levels and an ongoing adaptive response may activate a different collection of virulence genes, infect different cell types and tissues, or use different strategies than it would upon infecting a "clean slate." This type of difference is seen in complicating cases of tuberculosis secondary to HIV infection, in which Mtb can more easily spread to extra-pulmonary sites. In this case, it becomes equally important to study Mtb infection within an already suppressed immune response as separate from Mtb infection alone, because isolated infection may not accurately predict how to treat complicated Mtb for HIV+ patients.

CONCLUSION

Studies of infectious agents can be performed in various levels of "complication" and all are important. Basic *in vitro* studies are important to understand the exact functioning of individual components of the infectious agent and allow scientists to delineate requirements and interactions of different components that would be too obscured in more complicated in vivo settings. Oftentimes, *in vivo* studies may not even be possible when we do not know how infection is established, relevant hosts may not be available for study, agents may not be infectious for common laboratory animals, or understanding of how chronic infections are maintained may not be well enough understood to recapitulate as of yet. However, especially for key killers such as acute and chronic viral infections, additional research is starting and should be continued in the settings of more complicated polymicrobial infections. This can be examined both *in vitro* and *in vivo* and may help clarify what activities, both from the pathogen and the host, allow for these increased susceptibilities and how we can better address these in preventive and therapeutic care.

On a scientific level, these studies also will increase our understanding of the interactions between different infectious agents and how they may aid each other in their life cycles within the complex setting of a larger host. Interaction with another infectious agent during one's own natural infectious cycle can have large alterations on that agent that we do not yet understand. As our technological capacities expand, so does our ability to delineate the complex interactions between living things, and we can step up from studies of one-to-one relationships between organisms to combinatorially and web-like interactions, slowly progressing toward a more full, physiologically accurate understanding.

REFERENCES

1. WHO. World Health Organization Report on Infectious Disease: Removing Obstacles to Healthy Development [Internet]. 1999. Available from: http://www.who.int/infectious-disease-report/index-rpt99.html.
2. Bakaletz LO. Developing animal models for polymicrobial diseases. Nature Reviews Microbiology. 2004;2(7):552-68.
3. WHO Public Health Research Agenda for Influenza. Geneva: World Health Organization; 2010. p. 1-19.
4. Cabre M. Pneumonia in the elderly. Curr Opin Pulm Med. 2009;15(3):223-9.
5. Vergison A. Microbiology of otitis media: a moving target. Vaccine. 2008;26(Suppl 7):G5-10.

6. Kwan CK, Ernst JD. HIV and Tuberculosis: a Deadly Human Syndemic. Clin Microbiol Rev. 2011;24(2):351-76.
7. van der Sluijs KF, van der Poll T, Lutter R, Juffermans NP, Schultz MJ. Bench-to-bedside review: bacterial pneumonia with influenza — pathogenesis and clinical implications. Crit Care. 2010;14(2):219.
8. Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. J Infect Dis. 2008;198(7):962-70.
9. CDC. Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) — United States, May-August 2009. MMWR Morb Mortal Wkly Rep. 2009;58(38):1071-4.
10. Dhanoa A, Fang NC, Hassan SS, Kaniappan P, Rajasekaram G. Epidemiology and clinical characteristics of hospitalized patients with pandemic influenza A (H1N1) 2009 infections: the effects of bacterial coinfection. Virol J. 2011;8:501.
11. Kolata G. FLU: The story of the great influenza pandemic of 1918 and the search for the virus that caused it. New York: Touchstone; 1999.
12. McCullers JA, Bartmess KC. Role of neuraminidase in lethal synergism between influenza virus and Streptococcus pneumoniae. J Infect Dis. 2003;187(6):1000-9.
13. Peltola VT, Murti KG, McCullers JA. Influenza virus neuraminidase contributes to secondary bacterial pneumonia. J Infect Dis. 2005;192(2):249-57.
14. Kim Y-G, Park J-H, Reimer T, Baker Darren P, Kawai T, Kumar H, et al. Viral Infection Augments Nod1/2 Signaling to Potentiate Lethality Associated with Secondary Bacterial Infections. Cell Host Microbe. 2011;9(6):496-507.
15. Palacios G, Hornig M, Cisterna D, Savji N, Bussetti AV, Kapoor V, et al. Streptococcus pneumoniae coinfection is correlated with the severity of H1N1 pandemic influenza. PLoS ONE. 2009;4(12):e8540.
16. Kudva A, Scheller EV, Robinson KM, Crowe CR, Choi SM, Slight SR, et al. Influenza A Inhibits Th17-Mediated Host Defense against Bacterial Pneumonia in Mice. J Immunol. 2011;186(3):1666-74.
17. Mccullers JA. Effect of antiviral treatment on the outcome of secondary bacterial pneumonia after influenza. J Infect Dis. 2004;190(3):519-26.
18. Jamieson AM, Yu S, Annicelli CH, Medzhitov R. Influenza Virus-Induced Glucocorticoids Compromise Innate Host Defense against a Secondary Bacterial Infection. Cell Host Microbe. 2010;7(2):103-14.
19. Gupta RK, George R, Nguyen-Van-Tam JS. Bacterial pneumonia and pandemic influenza planning. Emerg Infect Dis. 2008;14(8):1187-92.
20. Chonmaitree T, Revai K, Grady JJ, Clos A, Patel JA, Nair S, et al. Viral upper respiratory tract infection and otitis media complication in young children. Clin Infect Dis. 2008;46(6):815-23.
21. McGillivary G, Mason KM, Jurcisek JA, Peeples ME, Bakaletz LO. Respiratory syncytial virus-induced dysregulation of expression of a mucosal beta-defensin augments colonization of the upper airway by non-typeable Haemophilus influenzae. Cell Microbiol. 2009;11(9):1399-408.
22. van der Sluijs KF, van Elden LJ, Nijhuis M, Schuurman R, Pater JM, Florquin S, et al. IL-10 is an important mediator of the enhanced susceptibility to pneumococcal pneumonia after influenza infection. J Immunol. 2004;172(12):7603-9.
23. Didierlaurent A, Goulding J, Patel S, Snelgrove R, Low L, Bebien M, et al. Sustained desensitization to bacterial Toll-like receptor ligands after resolution of respiratory influenza infection. J Exp Med. 2008;205(2):323-9.
24. Didierlaurent A, Goulding J, Hussell T. The impact of successive infections on the lung microenvironment. Immunology. 2007;122(4):457-65.
25. Piguet V, Trono D. Living in oblivion: HIV immune evasion. Seminars in Immunology. 2001;13(1):51-7.
26. Douek DC, Roederer M, Koup RA. Emerging Concepts in the Immunopathogenesis of AIDS. Annu Rev Med. 2009;60(1):471-84.
27. Shirai A, Cosentino M, Leitman-Klinman SF, Klinman DM. Human immunodeficiency virus infection induces both polyclonal and virus-specific B cell activation. J Clin Invest. 1992;89(2):561-6.
28. Hellerstein M, Hanley MB, Cesar D, Siler S, Papageorgopoulos C, Wieder E, et al. Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. Nat Med. 1999;5(1):83-9.
29. Valdez H, Lederman MM. Cytokines and cytokine therapies in HIV infection. AIDS Clin Rev. 1997:187-228.
30. UNAIDS. AIDS epidemic update: November 2009. Geneva, Switzerland: UNAIDS; 2009.
31. Perry S, Hussain R, Parsonnet J. The impact of mucosal infections on acquisition and progression of tuberculosis. Mucosal Immunology. 2011;4(3):246-51.
32. Geldmacher C, Ngwenyama N, Schuetz A, Petrovas C, Reither K, Heeregrave EJ, et al. Preferential infection and depletion of Mycobacterium tuberculosis-specific CD4 T cells after HIV-1 infection. J Exp Med. 2010;207(13):2869-81.
33. Barnes PF, Bloch AB, Davidson PT, Snider DE Jr. Tuberculosis in patients with human immunodeficiency virus infection. N Engl J Med. 1991;324(23):1644-50.