LUNG IMMUNOPATHOLOGY FOLLOWING INFLUENZA AND PNEUMOCOCCUS INFECTION: MECHANISMS OF DISEASE AND THERAPEUTIC APPROACHES

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Lung immunopathology following influenza and pneumococcus infection: mechanisms of disease and therapeutic approaches  

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ABSTRACT

Influenza is a highly contagious respiratory disease. Yearly epidemics and pandemics account for high morbidity and mortality worldwide. Lung immunopathology is a major factor causing death following influenza. In addition, secondary bacterial superinfections that occur after influenza further complicate the lung immunopathology and contribute to higher morbidity and mortality. The research presented in this thesis addressed important, understudied questions in the complicated field of tissue immunopathogenesis and host defense to influenza and pneumococcal infections. Firstly, in a model of acute respiratory influenza infection, we found that the classically proinflammatory cytokine TNF plays a dual and biphasic role at different times post-infection. While it does have pro-immune roles in the beginning stages, TNF acts as a negative type 1 immune regulator at later points of infection. TNF controls the level of immune activation and has a key role in preventing lung immunopathology and aberrant tissue remodeling. Secondly, to further investigate mechanisms of lung pathology, we elucidated the role of bacterial replication and over activated host immune responses during bacterial superinfection following influenza. In our model of pulmonary Streptococcus pneumoniae infection after influenza, we found that dual infected animals experience rapid weight loss and succumb to infection. Bacterial outgrowth, dysregulated cytokine and chemokine expression, and severe lung neutrophilia and immunopathology are linked to the poor clinical outcome. Combined treatment with both an antibiotic azithromycin and corticosteroid dexamethasone best improves clinical outcome, bacterial clearance, cellular and cytokine responses, and immunopathology. Thirdly, in our
2. Influenza

Acute pulmonary influenza infection is a very common and highly contagious respiratory disease. Annual worldwide influenza epidemics account for a significant number of deaths, particularly in high risk populations such as children, the elderly, and people with chronic illness. The influenza viruses are responsible for more than 100,000 hospitalizations and 20,000 deaths each year in the United States alone (19). Influenza viruses of varying pathogenicity not only cause annual epidemics, but also continue to cause unpredictable and perilous worldwide pandemics. These include the 1918 influenza pandemic that killed over 50 million people worldwide, the pandemic influenza strains such as the H5N1 influenza that re-emerged in 2003, and the pandemic influenza H1N1 of 2009 (20). The protective efficacy of the influenza vaccine is suboptimal and the social and economic impact of influenza infections remains high (19).

2.1 The virus

Influenza viruses are negative stranded, enveloped RNA viruses belonging to the family Orthomyxoviridae (Figure 2) (21, 22). There are three types of influenza virus (A, B and C) which are distinguished by antigenic differences in two of the internal proteins, nucleoprotein (NP) and matrix protein (M) (21). Influenza A viruses are the most pathogenic and clinically relevant, and are divided into subtype viruses based on antigenic differences in the surface molecules, haemagglutinin (HA) and neuraminidase (NA) (23). At present, there are two subtypes of influenza A (H1N1 and H3N2) as well as influenza B circulating in the community, and these viral strains compose the annual vaccine (21). The viruses preferentially replicate in the epithelial cell layers of the upper
Immunopathology and Infectious Diseases

Negative Regulation of Lung Inflammation and Immunopathology by TNF-α during Acute Influenza Infection

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Lung immunopathology is the main cause of influenza-mediated morbidity and death, and much of its molecular mechanisms remain unclear. Whereas tumor necrosis factor-α (TNF-α) is traditionally considered a proinflammatory cytokine, its role in influenza immunopathology is unresolved. We have investigated this issue by using a model of acute H1N1 influenza infection established in wild-type and TNF-α-deficient mice and evaluated lung viral clearance, inflammatory responses, and immunopathology. Whereas TNF-α was up-regulated in the lung after influenza infection, it was not required for normal influenza viral clearance. However, TNF-α deficiency led not only to a greater extent of illness but also to heightened lung immunopathology and tissue remodeling. The severe lung immunopathology was associated with increased inflammatory cell infiltration, anti-influenza adaptive immune responses, and expression of cytokines such as monocyte chemoattractant protein-1 (MCP-1) and fibrotic growth factor, TGF-β1. Thus, in vivo neutralization of MCP-1 markedly attenuated lung immunopathology and blunted TGF-β1 production following influenza infection in these hosts. On the other hand, in vivo transgenic expression of MCP-1 following influenza infection in wild-type hosts. Thus, TNF-α is dispensable for influenza clearance; however, different from the traditional belief, this cytokine is critically required for negatively regulat-
Pneumococcal respiratory infection. Strikingly, a single intranasal dose of AdIFN-α led to control of bacterial replication, lung inflammation, and improvement in clinical outcome. Our study demonstrated the protective role of type I IFN during S. pneumoniae infection, and for the first time established the successful use of adenovirus-delivered IFN-α for pulmonary pneumococcal infection.

In summary, the major findings presented in my thesis are: (i) TNF is not required for effective influenza clearance, (ii) TNF is a critical negative regulator of inflammatory responses and immunopathology during influenza, (iii) the mechanisms of severe lung immunopathology by influenza-associated bacterial superinfection are multifactorial and are in part independent of bacterial burden, (iv) overactive immune responses during bacterial superinfection contribute to deleterious lung immunopathology and death, (v) effective intervention strategies during superinfection should involve the effective control of both bacterial infection and aberrant host immune responses, (vi) IFN-α plays a protective role during S. pneumoniae infection by controlling bacterial replication, and (vii) transgenic expression of IFN-α is an effective treatment of respiratory pneumococcal infection. These major findings will be further discussed below.

2. The role of TNF in lung immunopathogenesis during influenza infection

Pulmonary influenza continues to be a common respiratory infectious disease worldwide. It is now well established that lung immunopathology is one of the main causes of influenza related morbidity and mortality (141-146). Recent studies using experimental animal models have significantly enhanced our understanding of the
complex mechanisms involved in the immunopathogenesis during influenza infection (as reviewed in Appendix I) (14). This involves acute inflammatory responses including the role of cytokines and chemokines. Current studies together suggest that carefully selected targeting of certain cytokine pathways may improve influenza immunopathology without negatively affecting viral clearance, which would be the ideal treatment. However, cytokine biology is complex, as most cytokines are required at proper levels and at specific times in the immune response, and any deviation from this well controlled expression can cause complications such as extensive lung immunopathology. Also, proper expression of immunoregulatory cytokines is essential in the return to homeostasis during the resolution phase of influenza infection.

We have shown that TNF plays an important immunoregulatory role during influenza immunopathogenesis. This finding adds to recent studies that have discovered immune suppressive roles for the classically proinflammatory cytokine (37-45, 48). We found that TNF is not required for influenza clearance and thus plays a redundant role in the early times of the infection, as immune responses are effectively initiated to clear the virus in TNF deficient hosts. However these same responses that successfully clear the virus are excessive and prolonged, culminating in lung immunopathology. Therefore we have shown that TNF plays a differential and biphasic role during the course of primary influenza infection. In the early phase of infection it is proinflammatory, and may in fact contribute to the ‘cytokine storm’ and immunopathology, seen particularly in cases of highly pathogenic influenza. However, TNF is immunoregulatory in later phases of infection after viral clearance. This role is in agreement with the pleiotropic nature of
TNF and with its role in apoptosis, which is reflected in the reduced T cell contraction following viral clearance in our model (28, 30). Recent evidence has also shown an anti-fibrotic activity of TNF in various models, including idiopathic pulmonary fibrosis (IPF) (42-44). Interestingly, we have also found that TNF is anti-fibrotic during influenza infection, through suppression of profibrotic chemokine MCP-1 expression. Thus we identified that the dysregulated MCP-1 and in turn TGF-β1 responses represent an important molecular mechanism in aberrant tissue repair and heightened immunopathology in influenza.

TNF depletion has been widely implemented as a means to counter inflammatory conditions in humans (37-41). Our results caution the clinical use of such therapeutics as they may worsen influenza-associated immunopathology during flu seasons or epidemics. Interestingly, a study found that treatment of RA patients with etanercept (a soluble TNF receptor) led to increased peripheral T cell reactivity to microbial antigens that included influenza (41). Several studies have also indicated that TNF blockade with infliximab (a monoclonal antibody) increases T cell reactivity (147, 148). As such, it has been suggested that in autoimmune diseases in which TNF blockade is therapeutic (RA, Crohn’s disease, psoriasis) the overproduction of TNF is part of the innate immune response and released by macrophages, rather than by T cells such as in diseases where neutralization of TNF worsens disease severity (MS, diabetes, mouse models of EAE and lupus) (28, 30, 37). However, to date there is little evidence showing that patients treated with TNF inhibitors exhibit more frequent or severe influenza infections. Clinical trials of the safety and immunogenicity of influenza vaccination in patients with RA have shown
that the vaccine induces an adequate humoral response and does not induce clinical exacerbation of RA (149-151). There is a lack of reports of influenza illness itself in RA patients being treated with TNF inhibitors (152). However, as mentioned above, TNF deficiency may have stimulating effects on T cell activity against microbial antigens and may assist in the generation of CTLs to clear the infection, as was shown in our model. Thus an adequate humoral response following immunization and influenza viral clearance following infection may occur in patients under TNF deficiency, which is in agreement with our model. However, the question still remains about the fate of such patients at later times post-infection after the virus is cleared which is when TNF acts as a negative regulator preventing lung immunopathology. Also, RA patients are concurrently on glucocorticoid immunosuppressive therapy used to control the disease, which may mask the effect of TNF deficiency seen in our model, where TNF deficient mice had excessive inflammatory responses including cytokine and chemokine expression that led to fibrosis and immunopathology. Nevertheless, influenza patients on TNF inhibiting medication should be more closely monitored, including at later stages after the virus has been cleared and the lung architecture is returning to homeostasis, in order to prevent complicating lung disease that may occur under TNF deficiency.

3. Control of lung immunopathology in pneumococcal superinfection

Secondary bacterial superinfections following influenza are major contributors to hospitalizations and mortality, and lung immunopathology is severely exacerbated in dual infections (14, 91, 112, 115, 153, 154). The underlying mechanisms of such excessive
REFERENCES (for Chapters 1 and 5)


