Gene Therapy

Pulmonary Infections

Gene therapy was initially conceptualized as an approach to the treatment of genetic diseases, particularly diseases that are inherited in an autosomal recessive fashion and carry a significant mortality, such as cystic fibrosis. As the field has expanded, it has taken advantage of the increasing knowledge that there are genetic components to acquired disorders such as cancer and infectious diseases.

Knowing the operative host defense mechanisms against certain pathogens may allow these pathways to be exploited for therapeutic purposes using gene transfer technology. In this article, I focus on evolving strategies to use gene-based therapies in bacterial pneumonia and tuberculosis.

Pneumonia is one of the most frequently encountered community-acquired infections that result in hospitalizations. Nosocomial pneumonia has a mortality of 20 to 40% in most series. The development of pneumonia depends on complex interactions between the host and the pathogen and involves such factors as comorbid illness, such as diabetes or alcoholism, the size of the bacterial inoculum, and virulence factors associated with the organism.

One of the first lines of host defense against bacterial invasion of the lower respiratory tract is the alveolar macrophage. Cytokine elaboration by these cells is critical to recruitment of inflammatory cells, which are necessary to clear bacteria from the lung. Cytokines have thus been proposed as therapeutic agents for a variety of pulmonary infections.

Immunotherapy with cytokines can be complicated by significant toxicity, especially when these cytokines are given systemically. This is especially true for tumor necrosis factor (TNF) and interleukins 2 and 12, which are often associated with substantial hemodynamic instability and end-organ injury when given intravenously.

In cases of pneumonia, where the disease process is focal, it seems that the local, compartmentalized delivery of specific cytokine therapy is the most rational approach to treatment. Moreover, breakthroughs in gene therapy using adenoviral, retroviral, and liposomal vectors have provided powerful tools to study the biologic effects of specific cytokine mediators as well as to develop novel and clinically applicable therapies in certain disease states.

Theodore J. Standiford of the University of Michigan and his colleagues have focused on the delivery of interleukin 12, a pleiotropic cytokine that has been increasingly recognized for its critical role in cell-mediated immunity against tumorigenesis and infection. Interleukin 12 is a heterodimeric protein consisting of p35 and p40 subunits. It promotes the development of T_H1 cells and increases the cytolytic activity of $CD8^+$ cells and natural killer cells. Importantly, IL-12 serves as the major inducer of interferon γ production by T cells and NK cells.

Recombinant IL-12 given systemically to mice has been shown to increase host resistance to a number of intracellular pathogens, including *Leishmania major*, *Toxoplasma gondii*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, and *Plasmodium chabaudi*. The increased resistance to *M. tuberculosis* in IL-12-treated animals has been shown to be interferon-dependent, whereas increased resistance to *P. chabaudi* is mediated by the cooperative effects of IFN-γ and TNF.

Moreover, IL-12 appears to play a major role in antibacterial host defense in the lung. Specifically, IL-12 mRNA and protein are detected in the lungs of mice challenged with *Klebsiella pneumoniae*. Endogenous IL-12 production is required for effective bacterial clearance in *K. pneumoniae* infection, as passive immunization with rabbit anti-murine IL-12 antibodies results in an approximately 12-fold increase in the numbers of *K. pneumoniae* colony-forming units isolated from lung homogenates 48 hours post inoculation compared to animals receiving preimmune serum. Furthermore, in vivo neutralization of IL-12 decreases survival from 45% in control animals to zero in animals treated with anti-IL-12 antibodies.

Standiford and associates have recently demonstrated that adenoviral-mediated gene transfer of IL-12 p35 and p40 cDNA's enhances both bacterial clearance and survival in mice challenged with *K. pneumoniae*. The enhanced pulmonary host defenses were attenuated if the experimental animals were pretreated with antibodies against TNF or interferon.

My colleagues and I have used adenoviral-mediated transfer of the interferon γ gene to enhance pulmonary host defenses against *Pseudomonas aeruginosa* and *K. pneumoniae*. This vector primes alveolar macrophages for enhanced TNF release, increases recruitment of activated neutrophils into the lung of bacteria-challenged animals, and enhances bacterial clearance. In this model, the enhanced anti-

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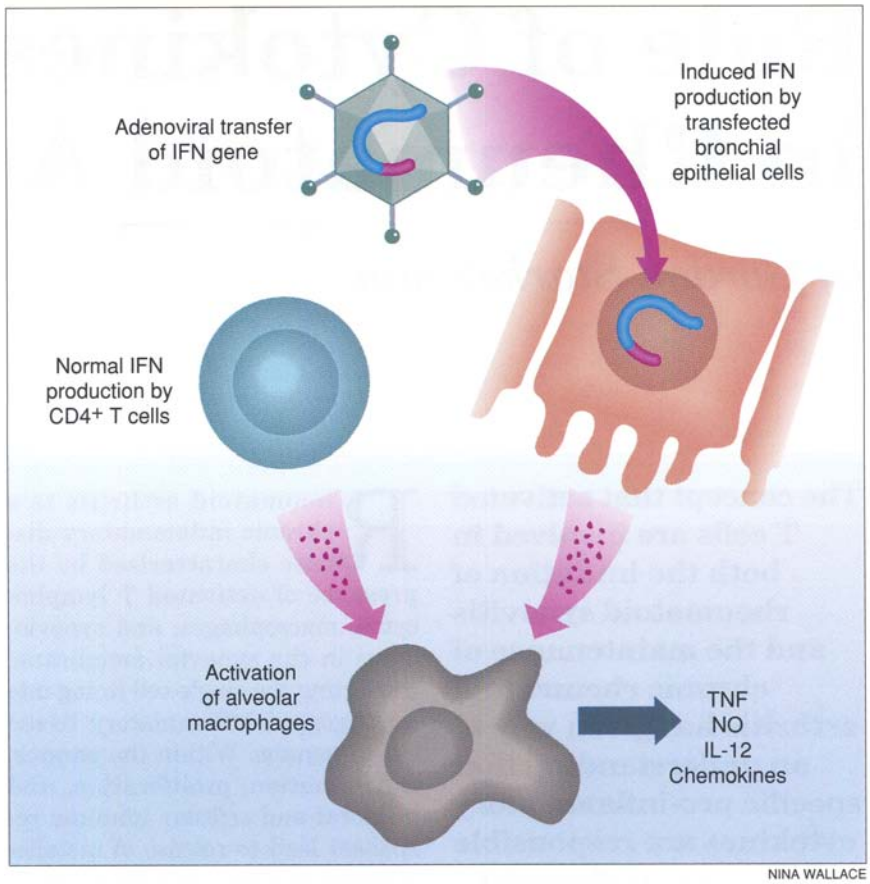
bacterial interferon is not blocked by passive immunization against TNF. However, in the lung, interferon also induces nitric oxide, and this may represent one of the TNF-independent effector pathways.

Tuberculosis remains a significant worldwide public health concern. In the United States, a steady decline of new TB cases ended in 1985, partly because of the emergence of the HIV epidemic. In fact, there have been more than 40,000 excess cases of TB during the past 10 years, and drug-resistant and multi-drug-resistant forms of TB have been emerging. Multi-drug-resistant TB is associated with significant mortality because there are no effective antimycobacterial agents.

One form of prevention is to vaccinate at-risk populations, traditionally with BCG, which is an attenuated bovine strain of TB. Although BCG is efficacious, it does not confer absolute protection, and it often causes a positive TB skin test. Thus it makes the traditional skin test less reliable in diagnosing TB later on.

Two strategies have been proposed for the use of gene-based therapies for TB. One uses mycobacterial genes in the form of DNA-based vaccines to stimulate an immune response, and the other uses cytokine gene transfer to augment host defenses.

DNA-based vaccines can be given intramuscularly and successfully generate both cellular and humoral responses. Moreover, the DNA construct can be sequenced, propagated to large amounts in bacteria, and subsequently purified of contaminating endotoxin, and thus it provides investigators with a standardized reagent with which to perform clinical trials. Efficacy of several TB antigens administered in this fashion has been demonstrated in a mouse model. Interestingly, immunizing with the proteins themselves was not effective, suggesting that production of the proteins in vivo by the DNA construct led to better T_H1 immune responses.



Interferon gene transfer as a potential therapy for tuberculosis is intended to augment normal interferon production by CD4+ T cells. Interferon stimulates alveolar macrophages to secrete TNF and other cytokines, as well as chemokines that recruit neutrophils from the bloodstream into the lung.

Cytokine gene transfer relies on the role of CD4+ lymphocytes in host defense, a function that came to be understood when HIV-infected individuals with lower than normal CD4+ cell counts were found to have a higher incidence of infections. One of the principal cytokines secreted by CD4+ lymphocytes is interferon γ. In addition to activating alveolar macrophages, interferon is necessary in tuberculosis for adequate granuloma formation, which can control mycobacterial infection.

My associates and I have found that adenoviral transfer of the murine interferon gene results in a significant attenuation of growth of tuberculosis lesions in mice given a low-dose aerosol challenge with the organism.

Investigations such as these may provide entirely new weapons in the arsenal against infectious disease. They may also improve the understanding of effective host defense mechanisms and thus may lead to novel peptide-based therapeutics. Either way, the work is critical, for it is increasingly apparent that existing antibacterial agents are less than optimally effective.

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