

Stimulating natural defences

Dr Burton F Dickey's research into the inducible innate resistance of the lung epithelium has exciting implications for preventing respiratory infection and disease

Could you begin by giving an overview of your expertise and work?

I am a pulmonary physician who has run a basic biology laboratory for 30 years. My principal area of scientific expertise is intracellular vesicular transport, and for the past 12 years I have focused on airway mucin secretion.

When my laboratory moved from Baylor College of Medicine to the MD Anderson Cancer Center, I began to think about pneumonia because of its high incidence and morbidity in cancer patients. In studying airway mucus, I had been struck by the remarkable change in phenotype of the airway epithelium during allergic mucous metaplasia, suggesting that the epithelium senses and rapidly responds to its environment. I wondered whether it would be possible to provide an appropriate stimulus that would cause the epithelium to become resistant to microbial infection.

Our initial experiment was to aerosolise a bacterial lysate to mice, then challenge the mice with live bacteria of a different species. It appeared to confirm our hypothesis: all of the control animals died from the pneumococcal challenge, while all of the animals that had been treated four hours earlier with the bacterial lysate survived.

Which of the lung's defence mechanisms are you focusing on?

We focus on innate defense mechanisms. My principal area of study is mucociliary

clearance, whereby inhaled pathogens are cleared from the lungs when they land on the airway mucus layer that is continuously moved out of the lungs by ciliary action. Alveolar macrophages are capable of engulfing pathogens and killing or removing them, though we don't study these. In addition, lung epithelial cells release antimicrobial peptides and reactive oxygen species into the lung lining fluid that kill pathogens, and this is a second area of study of our group, and the subject of this article.

How has inducible innate resistance been used in your research?

Our work with the aerosolised bacterial lysate uncovered the very high degree of inducibility of innate defences, which we believe is mostly due to antimicrobial peptides and reactive oxygen, and is reviewed in Evans S E, Xu Y, Dickey B F, 'Inducible innate resistance of lung epithelium to infection', *Annual Review of Physiology* 2010, **72**:413-35 (PMID 20148683). Our subsequent work has been directed to moving from proof-of-principle to a practical drug.

How can potentially protective responses in the lung epithelium be boosted by microbes?

The phenomenon of inducible innate resistance (IIR) presumably evolved as part of the host's response to an evolving infection. However, in the setting of a strong microbial challenge such as serious pneumonia in a cancer patient, which we mimic in our live bacterial aerosol



challenge studies in mice, the body ramps up its innate defences in response to the infection too slowly to save the host. That is why providing an exogenous strong stimulus in the form of a therapeutic aerosol early in the course of infection provides such benefit.

What does your role as co-founder of Pulmotect, Inc. entail? Why was the company founded and how has it developed over the years?

In order to translate the implications of our initial experiment into a clinical therapeutic, we needed a commercial entity to develop the technology. No pharmaceutical or biotechnology company expressed interest when contacted by the MD Anderson Office of Technology Commercialization, which is unsurprising considering the early stage of development of the technology. My colleagues and I concluded we would need to found a company ourselves to develop the technology. MD Anderson has strict limits on involvement by its faculty in commercial entities, so after two years I was required to resign from the Board of Directors and simply serve in a scientific advisory capacity. However, by then we had recruited a very capable scientist and businessman, Brenton Scott, PhD, MBA, to run the company, and partnered with an angel incubator, AlphaDev of Houston, to provide seed funding and management expertise. We have been very successful in raising funds through competitive grant awards that have financed most of our product development.

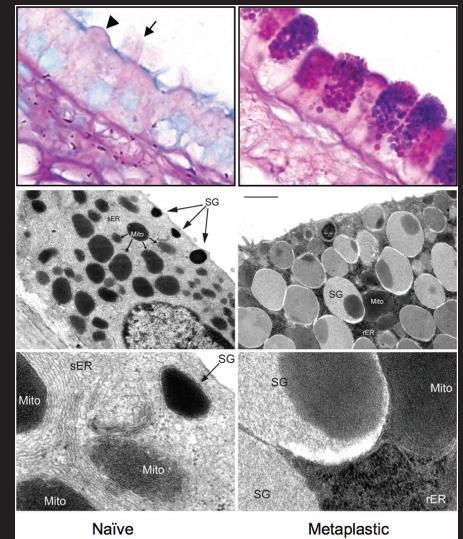
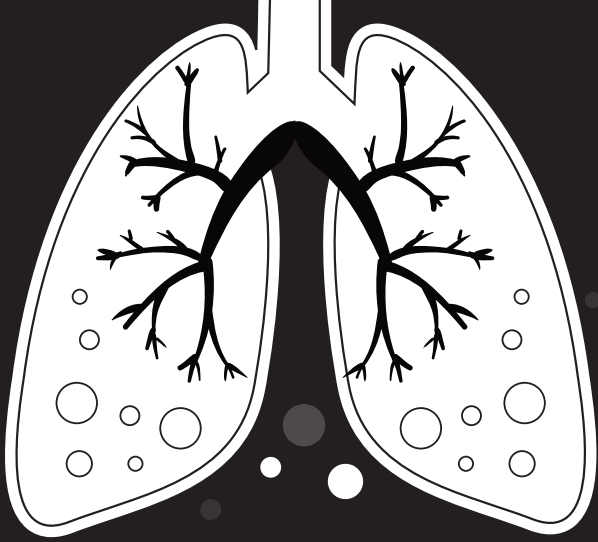


FIGURE 1. Airway epithelial structural plasticity. Light microscopy (top) and electron microscopy (EM) (middle, low magnification; bottom, high magnification) images of airway epithelial cells. On the left are images from the airway of a healthy mouse without airway inflammation (naive), and on the right are images from the airway of a mouse with mucous metaplasia three days after the onset of allergic inflammation, as described (Evans CM, et al., *Am J Respir Cell Mol Biol*, 31:382, 2004). Cells in the top row are stained with Alcian Blue and periodic acid Schiff reagent (AB-PAS), and on the left show alternating ciliated (arrow) and domed secretory (arrowhead) cells, and on the right show prominent mucin granules in secretory cells. Cells in the EM images on the left show small numbers of electron-dense secretory granules (SG) near the apical membrane, numerous mitochondria (Mito), and abundant smooth endoplasmic reticulum (sER). Cells in the EM images on the right show numerous electron-lucent SG containing mucins and an electron-dense core, and abundant rough ER (rER). Scale bar in the right middle panel is 10 μ m for top row, 1 μ m for middle row, 150 nm for bottom row.

adaptive immune response, and considering that the stimulus was different from the challenge bacterium, the team was able to infer that the protection resulted from the stimulation of an innate resistance mechanism.

Other studies in influenza models reveal that inducible innate resistance (IIR) had a similar protective effect against the usually lethal influenza pneumonia. Fascinatingly, the research has shown that the protection is still effective even if the treatment is administered up to 24 hours after exposure to the virus. This stands in stark contrast to induced protection against the bacteria, which is most effective if treatment is given before, or up to two hours after, exposure. Dickey believes the reason for this difference lies in the way that viral and bacterial infections cause disease. Bacteria rapidly penetrate the lining of the lung before moving into the bloodstream, meaning that IIR must be activated in advance in order to kill the bacteria before they can invade and proliferate. By contrast, viral respiratory infections tend to spread along the epithelial lining of the lung; IIR prevents this spread, thereby protecting the host. Further studies show that the lung

Resistance is vital

Based at the University of Texas MD Anderson Cancer Center, the **Inducible Innate Resistance of the Lungs to Microbial Pathogens** project promises a fascinating new therapeutic strategy against lung infection

LUNG INFECTIONS ARE the second leading cause of death globally. With a large surface area of around 100 m² and their direct exposure to the environment, the lungs are particularly vulnerable to infection. The physical architecture necessary for processing some 10,000 litres of air per day precludes the protective strategies employed by some other organs such as the skin, which is encased in an impermeable sheath.

Despite its vulnerabilities, the lung has multiple levels of defence, including mucociliary clearance, whereby inhaled pathogens which land on the airway's mucous layer are cleared from the lungs by ciliary action; alveolar macrophages capable of engulfing pathogens and killing or removing them; and lung epithelial cells which release antimicrobial peptides and reactive oxygen species into the lung lining fluid, killing pathogens. Crucially, these latter defences are highly inducible.

REMARKABLE PLASTICITY

At the University of Texas' MD Anderson Cancer Center, Dr Burton F Dickey coordinates 'The Inducible Innate Resistance of the Lungs to Bioterror Pathogens' – an ongoing research programme aiming to improve our understanding of the cellular mechanisms involved in inducible innate resistance.

"Cancer patients are particularly vulnerable to infection as chemotherapy often results in temporary suppression of the bone marrow, with a loss of protective white blood cells," Dickey outlines. "In addition, the vulnerability of the lungs to infection means that they are by far the most likely target of a bioterror attack, such as with anthrax."

Dickey is hopeful that the programme will lead to the development of a broad-spectrum therapeutic that could serve as an effective countermeasure to conventional, emerging and bioterror respiratory infections. During allergic inflammation, the epithelium of the airway exhibits a remarkable structural, molecular and functional plasticity; it was this characteristic which led Dickey to study inducible resistance in the lung epithelium. He hypothesised that it might be possible to rapidly boost innate protective responses with the use of microbial components – an idea supported by the work of Jules Hoffman and colleagues, which shows that fruit fly epithelial cells sense microbial molecular patterns and respond with antimicrobial proteins. The observation led Dickey to question whether similar epithelial defences were retained during mammalian evolution, or if their immune defences had been entirely outsourced to leukocytes, which, in evolutionary terms, appeared much later.

LETHAL INFECTION

Dickey's team prepared a crude lysate of a common respiratory bacterium, reasoning that it would stimulate the lung's epithelial lining defences with a mixture of pathogen-associated molecular patterns in proportions reflecting natural exposure. They then used a nebuliser to treat mice with the aerosolised lysate by inhalation, subsequently challenging them with exposure to *Streptococcus pneumoniae*. The experiment was a success: the treated mice exhibited high levels of protection against what would otherwise have been a lethal infection, with peak protection some four hours after stimulation. Remaining high for 24 hours before slowly declining, the protection was mirrored by bacterial killing within the lungs indicative of a resistance mechanism. Because the onset of protection was too rapid to be an

INTELLIGENCE

INDUCIBLE INNATE RESISTANCE OF THE LUNGS TO MICROBIAL PATHOGENS

OBJECTIVES

Firstly, to determine whether the innate immune mechanisms for resistance to microbial pathogens are as highly inducible in mammalian lung epithelial cells as they are in insects. Then, to determine the mechanisms of microbial killing, and to develop a practical aerosolised therapeutic to protect immunocompromised patients against opportunistic lung infections (eg. cancer patients), and normal hosts against virulent pathogens (eg. bioterror attack or pandemic influenza).

PARTNERS

Pulmotect, Inc.

KEY COLLABORATORS

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BURTON DICKEY received a bachelor's degree from Columbia University summa cum laude, and a medical degree from the University of Connecticut. He did residency training in Internal Medicine at Temple University Hospital in Philadelphia, and fellowship training in Pulmonary and Critical Care Medicine and in Biochemistry at Boston University Medical Center. He is Chair of the Department of Medicine at the University of Texas MD Anderson Cancer Center, where he has a clinical practice focused on inflammatory diseases of the airways and pneumonia. His research is focused on airway epithelial cells, which show great plasticity in structure, function, and gene expression in response to inflammation.

epithelium is predominantly responsible for this effect and that it is local. IIR does not require the involvement of immune cells; mice deficient in these cells demonstrate effective resistance, and when mouse and human lung epithelial cells are grown as cell cultures *in vitro*, they kill microbes more effectively than macrophages. The principal mechanism appears to be localised, non-specific killing by the production of reactive oxygen species and antimicrobial peptides.

TOLL-LIKE RECEPTORS

At the same time as Dickey and his team were formulating their hypothesis around the airway epithelium's ability to sense and respond to its environment, fellow scientist Jules Hoffman and his colleagues in France were studying the innate immune system of fruit flies, and identified the ability of their epithelial cells to sense microbial products through a receptor called Toll, and to respond with the production of antimicrobial peptides. Hoffman won the Nobel Prize for his work and shared it with Bruce Beutler, who showed that mammalian homologues of Toll, called Toll-like receptors (TLR), perform similar functions in mammalian cells.

Inspired by this work, Dickey and his colleagues sought to determine the role of TLR in the protective response they had induced. They examined mice in which the genes responsible for encoding TLR adaptors had been deleted, and one of these—MyD88—was found to be essential in inducing the protective response, which meant that a subset of TLR played a dominant role. The team then screened commercially-available TLR agonists, alone and in combination, for their ability to recapitulate the protective response.

"One combination stood out—Pam2CSK4—an agonist for the TLR2/6 heterodimer," Dickey reveals, "together with a Class C ODN, an agonist for the TLR9 homodimer. We then optimised the ratio in numerous challenge experiments and named this combination drug PUL-042."

COMPLEMENTARY RESEARCH

Key partnerships have been crucial to Dickey's work. The Institute of Biosciences and Technology at the Texas Medical Center is also home to the lab of colleague Magnus Höök, PhD, whose complementary research into

microbial pathogenesis has led to a close and continuing collaboration.

"Magnus's insights were essential in choosing the direction of our research and in founding the company, and nothing I've done in the lab could have been accomplished without my longstanding scientific partner Michael Tuvim, PhD," Dickey admits. "My colleague Scott Evans, MD did the key experiments that identified the role of MyD88 and the combination of TLR ligands with high protective efficacy, while Brian Gilbert, PhD in influenza virus infection, and Johnny Petersen, PhD in bioterror agent infection, have been essential in determining the breadth of activity of inducible resistance."

The team has recently completed IND-enabling animal studies and hopes to begin phase I safety and tolerability studies in 2014, before proceeding to a phase II trial in leukaemia patients.

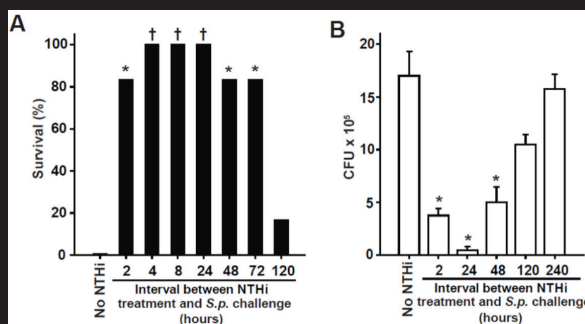


FIGURE 2. Inducible innate resistance – the phenomenon. Mice were exposed to an aerosolised lysate of non-typeable (unencapsulated) *Haemophilus influenzae* (NTHi), then challenged with aerosolised live *Streptococcus pneumoniae*. (A) Host survival. Mice not pretreated with the NTHi lysate all died, whereas mice pretreated 2–72 hours before challenge had high rates of survival. (B) Lung bacterial counts expressed as colony forming units (CFU) immediately after challenge. The time course of host survival is mirrored by the efficiency of bacterial killing within the host's lungs. * $p < 0.05$ compared to no NTHi treatment, † $p < 0.01$ compared to no NTHi treatment. (Adapted from Clement CG, et al., *Am J Respir Crit Care Med*, 177:1322, 2008)

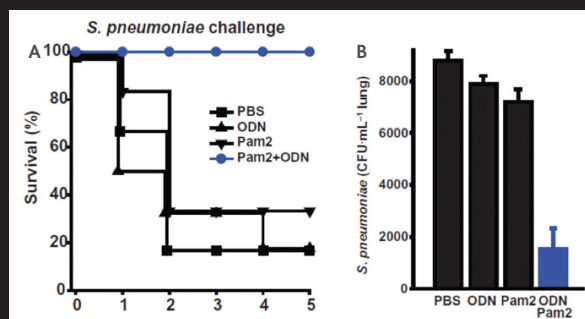


FIGURE 3. Inducible innate resistance – a drug. Mice were exposed by aerosol to the TLR ligands Pam2CSK4 (Pam2), ODN M362 (ODN), alone and in combination, then challenged 24 hours later with aerosolised live *S. pneumoniae*. (A) Host survival. Mice treated with single TLR ligands showed no difference in survival from those given vehicle alone (PBS), whereas mice treated with the combination of TLR ligands all survived. (B) Lung bacterial counts expressed as colony forming units (CFU) immediately after challenge. The rate of host survival is mirrored by the efficiency of bacterial killing within the host's lungs. (Adapted from Duggan JM, et al., *J Immunol* 186:5916, 2011)