

Efforts to eradicate asthma

Dr Barack Abonyo and his team at Florida A and M University's College of Pharmacy and Pharmaceutical Sciences are passionate about the importance of developing better treatments for asthma sufferers

DR BARACK ABONYO



How crucial is research in the fight to treat asthma?

The fight to develop effective therapeutic agents is crucial because current medicines only alleviate temporary symptoms. Put simply: these medicines do not eradicate asthma, they simply tame it. Asthma has been here for too long and it is crucial to develop cures using new knowledge acquired through state-of-the-art molecular biology technology.

Which factors do you believe aggravate cellular inflammatory events and subsequent inflammatory attacks?

Conditions and actions that aggravate asthma include exposure to aeroallergens, pollution, viral infection and hereditary factors. These triggers cause airway inflammation by activating mast cells, followed by infiltration of the eosinophils and other leucocytes into the airway.

Asthma derives from complex interactions, so why have many laboratories concentrated on studying the mechanisms of chemokines?

We have concentrated on chemokines because after the trigger of inflammation, they attract eosinophils into the airway. The eosinophils then produce other inflammatory products, including chemokines that attract further eosinophils and cause them to proliferate, clogging the airway. Eosinophils attraction and activation is, therefore, the major factor causing more damage. Understanding the mechanisms by which chemokines operate is key in limiting eosinophil access to the airway, and could be critical for solving progression of asthmatic inflammation.

How do eotaxins differ from other chemokines in their behaviour during an inflammatory attack?

Eotaxins are notably specific in their mechanism of action – they are unusual chemokines, because they carry out their high affinity signalling via a single receptor, CCR3. This interaction appears to be very specific in attracting eosinophils from the blood stream to the site of inflammation. To further complicate this process, the binding of eotaxins to these receptors triggers a chain of signal transduction events, including: calcium ion mobilisation, activation of adenylyl cyclase, mitogen-activated protein (MAP) kinase, protein tyrosine kinases (PTK), protein kinase C (PKC) and Jun-N-terminal kinase (JNK). These second messengers are known to be involved in eotaxin-induced eosinophilic degranulation, eosinophil migration and eotaxin-mediated respiratory burst. But how one eotaxin can induce all these activities is unclear: we believe they may be involved in inducing expression or release of proteins specific for each process. These proteins or their trigger mechanisms remain a subject for research.

Can you provide an update as to the stage of your invention disclosure?

The disclosure submission claims that the sequences encoding CCR3, CCL11, 24 and

CCL26-siRNA are capable of inhibiting target genes, resulting in reduction of asthma related symptoms including eosinophil chemotraction. It is approved by the Florida A and M University Technical Transfer Committee and is currently being analysed by patent lawyers before being forwarded to the United States Patent and Trademark office.

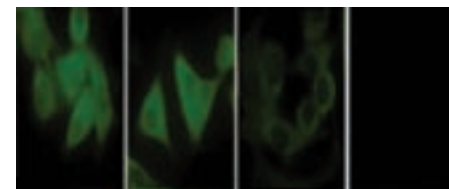
How significant do you consider your hypotheses on CCL26?

Our hypothesis is that CCL26 released by alveolar epithelium may bind to its receptor on the same cell and regulate the expression and the release of cytokines/chemokines. The significance of conducting experiments on the global effect of CCL26 on a variety of inflammatory proteins and genes using microarray technology is crucial in understanding its autoregulatory role. The results obtained could lead to new gene/protein targets that could be used for formulating asthma or other lung inflammation disease therapies.

Can you describe your short- and long-term treatment goals around CCL26 research?

In the short-term, we aim to identify cell differentiation and proliferation genes and proteins regulated by CCL26, as these proteins may have a role in repairing airway epithelium damage caused by lung inflammatory diseases. Long-term, we would like to elucidate the signal transduction mechanisms by which CCL26 regulate these and eventually create CCL26 knockout animal models.

FIGURE 1. CCL211, 24, 26 AND CCR3 ARE CONSTITUTIVELY EXPRESSED BY ALVEOLAR TYPE II EPITHELIAL CELLS



With bated breath

While asthma claims lives every day, **Dr Barack Abonyo's** team are a key part of the international push to treat the condition

WITH WORLD ASTHMA Day having passed on May 4th 2010, the chronic inflammatory condition is an ever-present and increasing issue to health authorities across the globe. Its debilitating symptoms include wheezing, breathlessness, chest tightness, and coughing; it claims 5,500 lives a year in the US alone (approximately 11 people each day); and it accounts for one quarter of all emergency room visits. Since 1980 the asthma death rate overall has increased by 50 per cent, and the ever-increasing cost of managing it reached \$14 billion in 2004. Dr Barack Abonyo – a senior researcher in molecular biology technology – attributes this increasing cost to the continually growing number of instances, especially in built up areas in which more and more of the population is choosing to reside: “The cost of asthma management increase in the United States has risen because of new cases – it is believed these are generally in cities where pollution is rampant”. As asthma perpetuates as a major health issue, a correspondingly

aggressive scientific search for therapeutic agents has emerged – of which Abonyo and his team at Florida A and M University's College of Pharmacy and Pharmaceutical Sciences are an energetic and focused part.

NOT A SINGLE DISEASE

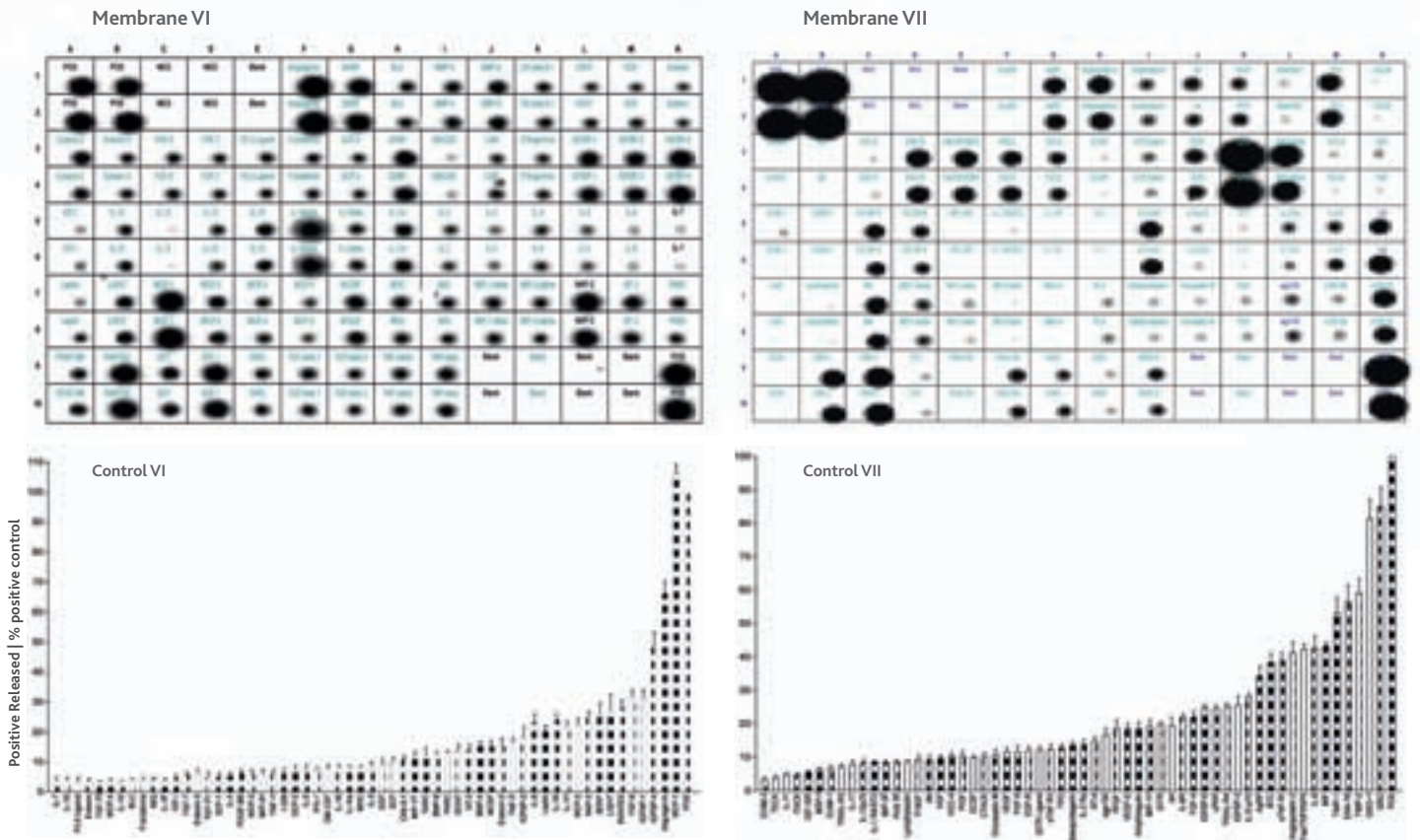
But asthma is not a single disease: it results from complex cellular inflammatory events and interactions between neural mechanisms, inflammatory cells, inflammation mediators and intrinsic abnormalities of the arachidonic acid pathway with smooth muscle cells. Molecular and cellular events leading to the infiltration and eventual activation of eosinophils (white blood cells in the immune system responsible for combating parasites and certain infections) in the airway appear to contribute to the development of the disease. This process is such that a trigger – immunologic or non-immunologic – activates T helper (TH2) lymphocytes to release cytokines, activating other cells and eosinophils, which



further release eosinophil chemoattractants: chemokines. In turn, this induces further eosinophil attraction and their proliferation, resulting in airway clogging and constriction.

Among chemokines, eotaxins are very specific to eosinophil attraction and activation: they belong to the C-C (cysteine-cysteine) group of chemokines in which the first two conserved cysteine residues are adjacent to each other. Those known for chemoattraction of eosinophils include CCL11 (eotaxin), CCL24 (eotaxin-2), and CCL26 (eotaxin-3). Eotaxins are unusual chemokines because they carry out high affinity signalling through a single receptor – CCR3 – although they have recently been shown to bind and induce some effects through CCR2 and

FIGURE 2. A549 ALVEOLAR EPITHELIAL LIKE CELLS CONSTITUTIVELY RELEASE A VARIETY OF CYTOKINES AND CHEMOKINES



CCR5. Many laboratories have concentrated on the mechanisms of these proteins with a view to circumventing their role in eosinophil migration into the airway during inflammation.

UNDERSTANDING MOLECULAR MECHANISMS

The principle objective of Abonyo's research, however, is understanding these molecular mechanisms and developing non-toxic molecular biology-based therapeutic agents to target genes contributing to progression of asthma pathology. They have focused on the respiratory zone (alveolar epithelium) because the airway epithelium is the terminal destination for uncleared allergens produced locally, and those originating from the conducting zone (bronchial epithelium). Abonyo points out the other unique facets that make the respiratory zone the focus of the programme: "This region is endowed with extensive blood supply, so it can be a site for extensive eosinophil migration into the airway." He goes on: "The epithelium is also composed of specialised cells – alveolar type I and alveolar type II – known to play a critical role in recovery from other inflammatory diseases, including ARDS". This recovery process has been associated with regulation of release and synthesis of cytokines and chemokines, so could prove important steps toward treatments.

The main focus has been to discover whether alveolar epithelial cells release eotaxins which could attract and activate eosinophils. In 2005, Abonyo's team demonstrated that human A549 alveolar type II epithelium-like cells, when stimulated with tumour necrosis-factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta), and IL-4, cytokines (known to be elevated in the airways of asthmatics), released CCL11, CCL24 and CCL26. While CCL11 and 24 were constitutively released, CCL26 was only released during airway cytokine stimulation. The team also demonstrated that these cells constitutively expressed CCR3 receptors, which are the principle receptors for these chemokines.

UNDER EVERY ROCK A SPRING

The discovery that CCL26 inhibits its own expression, by Abonyo's team, implies that it is essential for progression or inhibition of inflammatory process and may have a unique autoregulatory capacity. Furthermore, the protein was required for migration of eosinophils as well as induction of superoxide release by eosinophils. Abonyo's team also demonstrated that processes were significantly inhibited by siRNA (small interfering RNA) of both CCL26 and its receptor CCR3. Because these sequences have the potential of acting as therapeutic agents, Abonyo's team have submitted an invention disclosure claiming that the sequences encoding CCR3, CCL11, 24 and CCL26-siRNA are capable of inhibiting target genes, resulting in reduction of asthma-related symptoms, including eosinophil chemotraction.

The mechanisms by which eotaxins, especially CCL26 binding to CCR3 receptors, cause such heterogeneous responses in cells was initially perplexing to the team. Although existing reports suggested that alternating pathways of G-protein activation or heterodimerization are responsible for these activities, previous research could not explain all the effects of eotaxins. Abonyo believed it could involve the ability of CCL26 to regulate expression of specific genes and proteins. His team hypothesised that CCL26, released by alveolar epithelium, may bind to its receptor on the same cell and regulate the expression and release of cytokines/chemokines. This, he proposed, could lead to discovery of new targets that could be used to formulate therapies for asthma or other lung inflammation diseases.

Underpinning the team's work, Abonyo's 'Life Philosophy' states: "We are not into those who give up" – a phrase borrowed from the Apostle Paul. He explains how this applies in a research context as a philosophy of positivity and perseverance: "We never give up on an idea, irrespective of undesired outcomes, and are never discouraged by naysayers. Experimental diversion to us is simply the discovery of a new road. We keep our hummer hitting the rocks until we find water, because under every rock is a spring". The dedication and collective nature of the team – consisting of Rukia Marijani (PhD), A S Heimann (PhD), D Lebyy (PhD), M S Alexander (MS), D M Bauer (MS), Emmanuela Rony, Mimminih Tuma, and Naomi Nash – has been key to the developments made in their research.

LATEST DISCOVERIES AND FUTURE PLANS

Using protein arrays, the team has demonstrated that A549 cells constitutively secrete over 84 cytokines/chemokines at a 30 per cent level (compared to the positive control). Treating A549 cells with cytokine IL4, followed by treatment with CCL26 inhibited the release of some cytokines and enhanced others. It appears the list of proteins regulated by CCL26 under normal or inflammatory conditions is endless. Interestingly, CCL26 enhanced the expression of particular proteins, while at the same time inhibiting their release. There are a number of possible reasons as to why this occurs, so the next task for Abonyo's team is to concentrate on proteins highly regulated by CCL26.

In the future, the team plans to carry out investigations exclusively on proteins that have roles on progression of lung inflammation, alveolar epithelial cell differentiation and proliferation. This will enable Abonyo to narrow down the proteins that are of high target value in developing therapeutic agents for lung inflammatory diseases. The group's latest discovery is that CCL26 strongly regulates cancer related genes/proteins, including p53, Retinoblastoma protein, BCL2-related ovarian killer (BOK) and others, so applications to a broader range of conditions may well be on the horizon.

INTELLIGENCE

IDENTIFICATION OF EOTAXIN3-REGULATED PROTEINS IN ALVEOLAR TYPE II CELLS

Research in preventing inflammatory asthma attacks

OBJECTIVES

- To develop non toxic molecular biology - based therapeutic agents to target a variety of proteins/genes contributing to progression of asthma pathology
- To gain a better understanding of the mechanisms by which chemokines operate in asthmatic inflammation

KEY FIGURES

College of Pharmacy and Pharmaceutical Sciences, Florida A and M University:
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DR BARACK O ABONYO currently serves as an Associate Professor of Pharmacology at Florida A and M University College of Pharmacy and Pharmaceutical Sciences. His most significant research contribution is related to applications of molecular techniques in circumventing lung inflammatory diseases. His discoveries are paving the way to the development of non-toxic asthma and lung cancer therapeutic agents.

