Immune cells, alleles and biochips

Dr Richard Bucala elaborates on his research into how an individual’s genetic makeup may predispose them to various types of infectious disease and autoimmune disorders.

Could you offer an insight into the background of your work and the primary objectives of the research?

Our primary interest arose from our desire to better understand how the immune response, while essential for survival and long-term protection from infection, can sometimes contribute to severe disease and death. For example, while the vast majority of people who contract such common infections as influenza or tuberculosis control their infection and survive, a small percentage develop disseminated disease or lethal complications. Our investigations therefore became directed at the cells and genes that regulate the means by which an infected individual defends against pathogens.

Who have been the main collaborators on the project?

Our investigations are by necessity multidisciplinary, and have required the collaboration of geneticists, molecular biologists, biochemists, and public health workers. Most importantly, we have had to form strong partnerships with local hospitals and field sites in Africa. Our studies have been supported by the US NIH, the Fulbright Foundation, and the Yale Down’s Fellowship.

How did you discover the unusual prevalence of different forms of the MIF gene in the African community?

We sampled the DNA of individuals in populations around the world. We believe that there is a strong skewing of variant MIF genes in Africa - specifically, a high prevalence of low-expression forms or alleles of the gene - because Africa is the continent where the human population first arose and has resided longest under the threat of malaria infection.

While our research indicates that commonly-occurring MIF alleles in Africa confer protection against lethal malaria, the MIF allelic system also influences human susceptibility to other diseases with an immune component. We already know that the same MIF alleles that protect from malaria also confer protection against some autoimmune inflammatory diseases, such as rheumatoid arthritis, common in the developed world, and that MIF alleles influence the incidence of certain cancers. In a recent study of almost 1800 subjects in the United States, we found that among 14 different polymorphic immune genes, MIF alone influences the outcome of bacterial pneumonia leading to sepsis and septic shock. Since many of these lethal pneumonias develop after a viral illness, such as influenza, the determination of MIF alleles may have important bearing on a person’s susceptibility to influenza, SARS, and other important respiratory pathogens.

Could you explain the link between the low expression of MIF alleles and autoimmune inflammatory diseases such as asthma and arthritis?

Autoimmunity and inflammatory diseases such as asthma are significant health problems in the developed world. As an example, rheumatoid arthritis is an autoimmune disease that affects upwards of one per cent of the U.S. population. MIF alleles that have evolved to protect against the lethal, inflammatory complications of severe malaria infection also influence the expression of the inflammation that destroys tissues and kills patients with autoimmune disorders. For instance, if patients first diagnosed with rheumatoid arthritis are found to have a low-expression MIF allele, they have an eight-fold greater chance of having mild, less-erosive joint disease. A similar protective effect of low expression MIF alleles has been found in asthma and even in the neurodevelopmental disorder autism.

How has your research contributed to antibiotic-resistant gene detection and how can this help in the development of future treatments?

We’ve developed a facile and inexpensive biochip methodology for the determination of MIF alleles in rural field settings that is applicable to any polymorphic gene system, irrespective of whether the DNA is human, from an insect vector, or a pathogen. Being able to track the emergence and the spread of antibiotic resistance in malaria parasites, whether isolated from infected patients or from mosquitoes in the field, is very important, and we can contribute to a rapid and low cost means by which to do this.

What are your plans for the future of research into the genetic propensity of severe malaria? How soon do you hope to get the microchip into clinical use?

We are developing therapeutics that can influence the MIF pathway in the host; these may be applied for the treatment of infectious or autoimmune diseases. MIF-directed therapies can be selectively administered to those individuals who, on a genetic basis, stand to benefit most.

Biochip methods are undergoing optimisation in field studies. We hope that they may also be applied in the studies of candidate malaria vaccines as they may assist in determining the genetic basis by which some individuals respond well or poorly to vaccines.
Genetic susceptibility to malaria

A project led by Dr Richard Bucala has found that an exciting new technology has significant implications for combating death from malaria and other infectious diseases in the developing world.

POPULATION GENETICISTS CONSIDER that most of the world’s population derives from an initial migration of about 100 individuals who left northern Africa approximately 60,000 years ago. The descendants of these founding individuals radiated throughout the world, with their genomes evolving and adapting in order to survive in different environments. Consequently, genetic susceptibility to various types of infection is observed to vary from one population to the next.

Malaria is a leading cause of death from infectious disease and globally it accounts for 1-2 million deaths a year, mostly in children under the age of five. The disease is caused by a mosquito-borne parasite that invades the red blood cell, and death ensues from the infectious complications of cerebral disease leading to coma and a severe, refractory anaemia. Malaria affects disproportionately those countries with few public health resources, not only contributing to the overall burden of disease and poverty in the developing world, but hindering societal development and economic advancement. The parasite’s emerging resistance to new antibiotics, the growth and migration of human populations, and global warming, threaten current efforts to restrict the spread of malaria. Fresh approaches to the prevention and treatment of the disease are critically needed and have become a renewed priority for the international community.

VARIANCE BREEDS RESILIENCE

It is now well understood that lethal malaria results from an excessive or uncontrolled immune response by the host. While an immune response is necessary for an infected individual to eliminate an invasive pathogen, an excessive or over-exuberant response can itself produce tissue injury and lethal end-organ damage. The genes that encode the immune response comprise the most highly-variable components of the human genome. All individuals carry different variants of many immune response genes and, depending on one’s encounter with different pathogens or environmental stimuli, some of these variants may have a predisposition to autoimmunity, while others may influence the ability to fight off different infections. The presence of such genetic variation ensures that when a given population encounters an infectious agent, some individuals will survive and eventually re-constitute the population.

Dr Richard Bucala is Professor of Medicine, Pathology, and Epidemiology and Public Health at Yale University. He focuses his research on the role of host immunity in infectious diseases and the host-pathogen interaction. Through their current project - Defining Susceptibility to Lethal Malaria - Bucala and researchers from the Macha Malaria Research Institute, Zambia, the University Teaching Hospital in Lusaka, and the Kenya Medical Research Institute, hope to benefit health delivery in the developing world.

“The objective of our research,” explains Bucala, “is to understand at the molecular level why malaria infection kills some individuals but not others. It has been estimated that a child dies of malaria every 45-60 seconds. A better understanding of individual susceptibility to lethal malaria can allow children who are at high risk of death to be identified in a timely fashion and treated more aggressively by closer monitoring, hospitalisation, antibiotics, and blood transfusion.”

UNDERSTANDING MIF

In 1993, Bucala’s team successfully cloned the immune protein known as MIF, or macrophage migration inhibitory factor, which had long escaped identification and established its role in the host’s immune response. “MIF is an immune protein, or cytokine, and one of the first mediators to be produced by the host to combat infection,” outlines Bucala.

Building on these findings, in 2001, Bucala and his colleagues sequenced the human MIF gene in 10 laboratory workers and found that different individuals had DNA sequence variations in the regulatory region of the gene. These variations were identified as short DNA repeat units that influence the expression of the gene, such that...
DEFINING GENETIC SUSCEPTIBILITY TO SEVERE MALARIA INFECTION

OBJECTIVES

This work focuses on the role of host immunity in malaria and the host-pathogen interaction.

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MD PhD is Professor of Medicine, Pathology, and Epidemiology and Public Health at Yale University. He focuses his research on the role of host immunity in infectious diseases and the host-pathogen interaction. He directs genetic epidemiology studies to examine the role of innate immunity in the susceptibility and manifestations of different infections. Ongoing studies are underway in severe malaria and in leishmaniasis. Among his research accomplishments are the discovery of the circulating fibrocyte and the cloning of the innate cytokine, MIF. Dr Bucala has developed low-cost and robust biosensor chips that are suitable for genetic analyses of pathogen or host DNA in rural field settings.

individuals who have more repeats produce more MIF. “Depending on the individual,” continues Bucala, “this repeat can be present in five to eight copies; the more copies that are present, the more the gene that is expressed and the greater the inflammatory response during an infection.” By extending this DNA-sequencing analysis to a few hundred individuals, the team found that people of African-American ancestry are more likely to have a low number of repeats in their MIF gene and as a result, to produce less MIF protein during an immune response. This work led directly to the hypothesis that these low-expression variants of the MIF gene, or alleles, offer a selective advantage to people living in Africa and there exposed to chronic infections, the most prevalent and lethal being malaria.

Bucala notes that the human population has co-existed with malaria for the longest period of time in Africa, suggesting that low-expression MIF alleles were favoured and selected for in order to allow individuals to escape the lethal inflammatory consequences of malaria infection. “We modelled this idea in a controlled laboratory infection and found that genetically-engineered mice lacking the MIF gene show enhanced survival during lethal malaria,” Bucala explains. His team has since verified this idea by completing a genetic epidemiology study in a malaria-susceptible population in Africa, observing a significant protective effect of low-expression MIF alleles in the development of severe malarial anaemia.

RAPID GENOTYPING

Genotype determination normally requires costly and sophisticated analytical instrumentation that is available in only a few specialised centres in Africa. Ordinarily, samples would have to be collected, shipped to a laboratory for processing, analysed by a genetic analyser, and the results then reported back to the healthcare providers. This is a process that normally takes many days to weeks, even in developed countries. But Bucala’s team has developed a facile and inexpensive ‘biochip’ that, within two hours, enables direct visualisation of a patient’s MIF genotype without the need for sophisticated instrumentation. “All too often,” says Bucala, “exciting discoveries or technologies are developed that are not economically practical for widespread application. Witness the problem in providing adequate care for patients infected with HIV or tuberculosis.”

The benefits of being able to genotype patients at the bedside are clear: as well as the cost being very low, the methodology is robust, the needs for training and laboratory facilities are minimised, and patients can be quickly identified and ‘stratified’ according to their susceptibility to lethal disease. This enables quicker referrals to hospitals for monitoring and aggressive treatments, such as blood transfusions and antibiotics, which saves lives and conserves precious medical resources.

LOOKING TO THE FUTURE

Given the central role of MIF in the immune response to malaria, the rapid genotyping of MIF, or other genetic polymorphisms of interest may prove useful in field studies of malaria vaccines to different antigens. Such genetic information may help to predict vaccine responsiveness in different populations, as candidate vaccines progress through clinical trials. “Although we developed this biochip methodology specifically to allow for the genotyping of MIF variant alleles,” continues Bucala, “it is broadly applicable to any genetic polymorphism of interest, and there is ongoing work to adapt this technique for the study of other genes, both for malaria and other prevalent infectious diseases such as leishmaniasis.”

Low-expression MIF alleles are associated in Western populations with protection against autoimmune inflammatory diseases such as arthritis, asthma, and inflammatory bowel disease. Investigation of a polymorphic genetic locus that appears to have evolved in response to lethal malaria infection therefore also provides one explanation for susceptibility to the development of autoimmune disorders prevalent in many Western countries.