Cow **antibodies**

DR AZAD KUMAR KAUSHIK

Veterinarian and researcher **Dr Azad Kumar Kaushik** is studying antibodies from cattle in the hope of developing novel drugs and vaccines. Here, he reveals his fortuitous entry into the field, and the talented team members who have made his work possible

What intrigues you about the mechanisms of immunity?

As a veterinary medicine student, I was intrigued by the generation of immunity against a variety of infectious agents, especially the concept of an almost endless generation of diversity, while being tolerant to self-constituents. This led me to the Pasteur Institute to study the origin of autoantibodies against red blood cells involved in haemolytic anaemia, seen in systemic autoimmune disease.

Can you discuss some of your key findings?

We noted that the same host genetic elements encoded physiological autoantibodies, antibodies to infectious agents and pathogenic autoantibodies, using similar mechanisms. Our later research showed that impaired B-lymphocyte development, due to underlying genetic factors evident at birth, provides the trigger that gets amplified by environmental factors resulting in production of pathogenic autoantibodies seen in systemic autoimmune disease like lupus. These studies of the immune system in health and disease across species led me to bovine antibodies upon my return to veterinary profession.

Why do you focus specifically on the bovine immune system?

Given their medical significance, most studies had focused on human and mouse immune systems. As a veterinarian, I was aware that the immune system must have evolved for each species to meet varying individual host defence requirements. When taking up the position of Clinical Immunologist at the Ontario Veterinary College – a move away from the human medicine environment – I needed to embark upon a new research path. I decided to study the bovine immune system as I knew that little was understood about cattle antibody genetics.

Could you explain the need for new antibody-based vaccines?

There are many controversies surrounding vaccines in the public domain. Some of the concerns may be related to the quality of available vaccines or components; alternatively, individual host genetics might influence response to a particular vaccine. Hence, there is a need for better quality vaccines against various infectious agents that have minimal side effects and are suitable for use in large populations, including immunocompromised individuals and neonates. Furthermore, successful vaccines are yet to be developed against certain pathogens, such as HIV and *Plasmodium falciparum*.

With a world-leading team in bovine antibody genetics, what distinguishes your laboratory from others?

My laboratory is small with limited resources, but rich in human intellect and ingenuity; a nurturing environment exists for advancing scholarships, exchanging ideas and flourishing creativity. I have had the good fortune of working with some outstanding colleagues, whose passion, creativity and efforts put us in the top league of bovine immunoglobulin genetics. The commonwealth scholar, Dr Surinder S Saini, discovered the exceptionally long CDR3H in cattle antibodies, at a time when cDNA sequencing was a time-consuming challenge. Dr Farbod Shojaei discovered the single long germline IGHD gene in the cattle, capable of encoding 49 codons of CDR3H. Dr Madhuri Koti discovered the molecular basis of exceptionally long CDR3H in cattle antibodies beyond germline potential via CSNS insertion. While Dr Koti first engineered bovine scFv that neutralises BoHV-1, Ms Yfke Pasman, enhanced bovine scFv's anti-viral potency via multimerisation. Ms Pasman is currently enhancing the understanding of cattle antibody structure-function relationships for developing the next generation of vaccines, immunodiagnostics and anti-viral drugs. Such wonderful, dedicated and passionate colleagues distinguish our laboratory from others in the field.

Mega antibodies: next-generation **therapeutics**

Researchers from a world-leading laboratory at the **University of Guleph** in Canada are studying the antibodies of cows, paving the way for new vaccines and medicines to prevent and treat human disease

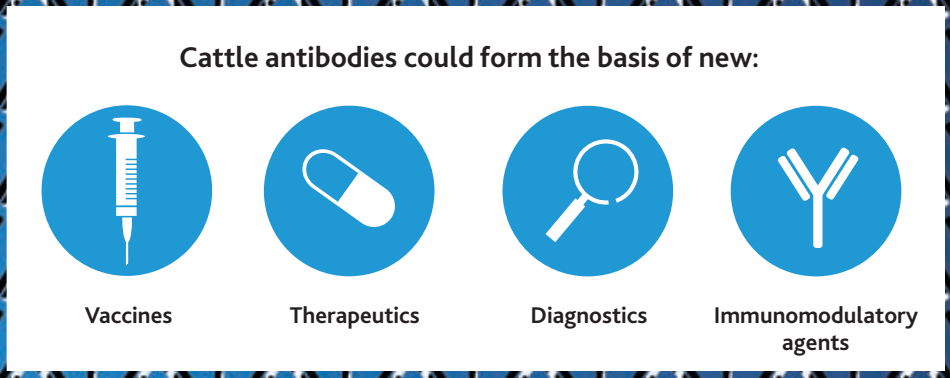
WHEN ASKED TO think about cows and the resources they provide, milk and beef might be the first to come to mind, but there is actually something else produced by cows – unique to them – that makes them a valuable therapeutic resource: antibodies. Cows produce the largest sized antibodies of the whole of the animal kingdom. These massive antibodies are distinctively poised for more effective at attacking diverse bacteria and viruses, and have the potential for use in humans as well.

Dr Azad Kumar Kaushik, Associate Professor of Immunology at the University of Guleph in Ontario, was captivated by the processes of immunity at an early age and has been working on them, in humans and animals, ever since. After years of studying the structure of immune systems across multiple species, his laboratory is now focused on the bovine immune system.

Supported by the Canadian Natural Science and Engineering Research Council (NSERC), Kaushik's investigations have identified and classified the genetic elements encoding bovine antibodies, as well as discovered novel mechanisms that enable production of such a vast array of antibodies beyond germline potential. His current research is conducted with a view to engineering antibodies for next-generation therapeutic and diagnostic tools.

OVERCOMING LIMITED DIVERSITY

Kaushik's investigations of the bovine immune system began by identifying the genetic elements that encode the antibody. The team discovered that the limited germline diversity of the cow genome has led to the creation of the largest antibodies known in any species. Exceptionally long third complementary determining regions of



the heavy chain (CD3RH) are generated, over 50 amino acids in length. As well as creating atypical antigen-binding site diversity, the size of the antibodies also makes them suitable to work with. A major barrier to successful antibody-based immunotherapies in the past has been the lack of a suitable vehicle to transfer immunity from animals to the lab, but the size and flexibility of the bovine antibody overcomes this. In the longer term, modified bovine antibodies could be used to target diseases where traditional therapies fail, helping the body to recognise and protect from pathogens that would otherwise go undetected.

The researchers went on to find that CD3RH is encoded by a single, again unusually long, gene – *IGHD* – which is capable of encoding a striking 49 codons. It does so in concert with the insertion of a conserved short nucleotide sequence (CSNS) at a specific junction of *IGHV* and *IGHD* that further increases CDR3H size. Building on these past successes, Kaushik's team is presently focused on the next stage on from genetics: structure and function.

Antibodies are multi-functional molecules, they recognise antigens, engulf pathogens, and much more. Each function is designated to a specific structure. "It is therefore possible to genetically manipulate and generate an engineered molecule for a desired function," Kaushik explains.

TACKLING A BOVINE VIRUS

Through his studies, Kaushik successfully developed powerful antibody fragments called single chain fragment variables (scFv). Importantly, these scFv are able to neutralise Bovine Herpes Virus-1 (BoHV-1), a major problem in North America that costs the cattle industry up to $100 million each year in Canada alone.

The team took genetic material from a cell producing antibodies against BoHV-1 and modified the gene producing the antibody. They inserted the new gene into yeast cells to produce the antibodies en masse, before adding them to infected cells to investigate their ability to attack the virus. Kaushik's scFv was found to neutralise the virus, preventing its attachment to host cells and subsequent replication.

> Kaushik hopes to develop drugs and vaccines for humans, particularly against common infections of the intestine

MAKING MEGABODIES

Based on these promising findings, Kaushik hopes to develop drugs and vaccines for humans, particularly against common infections of the intestine. The potential here is huge. By coupling the antibodies with drugs, they could be used to target specific locations in the body to destroy the pathogen or diseased tissue. "Our discovery of large sized bovine antibodies, which I call 'megabodies', will have a huge impact not just on veterinary science but also on human medicine," Kaushik enthuses. In the future, he hopes to capitalise on the rise in popularity of using antibodies for therapeutic purposes by further developing his research on structure-function to understand, exploit and even engineer multifunctional antibodies to prevent and treat disease, setting his sights on complex autoimmune diseases and even cancer.

INTELLIGENCE

POST GENOMIC STRUCTURAL AND FUNCTIONAL ASPECTS OF ANTIBODY IN HEATH AND DISEASE

OBJECTIVE

To advance knowledge of the development, 'structure-function' relationships and regulation of humoral immunity in health and disease aimed at designing antibody-based therapeutics, diagnostics and novel vaccines across species, including humans.

KEY COLLABORATORS

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DR AZAD KUMAR KAUSHIK has published over 85 research articles, two books and four patents on cattle antibodies. He is Consultant to several international organisations (USVIRN and CIgW in USA, and IMGT in France). He was recognised as The Esther Z Greenberg Honors Chair in Biomedical Research, Oklahoma Medical Research Foundation, USA, in 1998. He received BVSc&AH (Honors) in 1976 and MVSc (1978) from the Faculty of Veterinary Science, Hisar, India; followed by a Docteur es Science (DSc) in1987 from the Pasteur Institute (University of Paris VII), Paris, France.