Therapeutic antibodies vary depending on production system

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Differences in production methods for therapeutic antibodies can lead to variations in their structure, depending on the recombinant procedure selected. The distinctions, which are based on a number of glycosylations, even impact antibodies' stability. This was the result of a high-precision comparison of the structural properties of antibody isotopes that were manufactured in cell cultures or plants. Equipment BOKU (EQ BOKU) based at the University of Natural Resources and Life Sciences, Vienna (BOKU) employed state-of-the-art mass spectrometers to pinpoint minute differences in the glycosylation of immunoglobulins.

Antibodies are one of the most precise forms of medicine and are increasingly being used to combat cancer and other conditions. They are frequently produced recombinantly, with a range of different manufacturing processes in use. Each process creates an identical protein scaffold in the antibody, but there are differences in what is known as glycosylation, or modification by adding specific carbohydrates. Previously, little was known about the way in which these differences come about and the form they take. Identifying these subtle, but potentially medically significant distinctions requires extremely complex analysis, which is only possible using the latest mass spectrometry procedures. A BOKU team had access to such equipment at the university's EQ BOKU facility and unearthed some surprising results.

The team headed by Prof. Richard Strasser is the first to identify precise differences in the glycosylation patterns of immunoglobulin A, which

had been produced either in human cell cultures (HEK293) or plant systems (Nicotiana benthamiana). Prof. Strasser, a member of the Department of Applied Genetics and Cell Biology, commented: "Even we were surprised by how big the differences were. There were sharp contrasts between the two systems in terms of the structure of the carbohydrates used for glycosylation and their position on the proteins."

With the help of ultra-modern techniques called capillary reversed-phase chromatography and electrospray mass spectrometry provided by EQ, the team managed to analyse glycosylation in each system down to the finest detail. They found that the immunoglobulin A produced in the HEK293 cell culture had many more and also more complex N-glycans – a group of carbohydrates that bond with particular nitrogen atoms – than that produced in mammalian cell cultures. The immunoglobulin A manufactured in plants also had a significantly narrower range of structures. This was primarily due to the fact that plants have none of the metabolic pathways required for mammalian glycosylation. "But we also saw glycosylations in the antibodies manufactured in plants which can only occur in plants," Prof. Strasser added.

Although the glycosylations in the antibodies produced in N. benthamiana were purely plant-specific, the antibodies displayed the same bonding properties for antigens as those manufactured using human cells. This suggests that as far as therapeutic applications are concerned, the choice of production system makes no difference. However, further analysis by Prof. Strasser's team revealed that the stability of immunoglobulin A varies depending on the production method – a factor which could have a decisive effect on its use in treatment. The analysis involved testing the antibodies' thermal stability, which turned out to be lower in immunoglobulin A produced in plants. "We need to carry out further tests to discover how significant this is for the application of these antibodies in treatment," Prof. Strasser explained.

His team has all the necessary resources to do just that at EQ BOKU. Cutting-edge mass spectrometers, chromatography procedures and calorimeters – and the know-how of BOKU's experts – are all available to the university's research teams, as well as to users from industry and other academic institutions.

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