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Hot from the press!

The Innovation from Innsbruck: Plant-based Allergen Recombinantly Produced in Green Alga

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Abstract: An innovation in biotechnology: for the first time, researchers from MCI Innsbruck and the University of Salzburg have manufactured and purified a plant-based allergen in a green alga and opened the door to a specific immunotherapy against allergies. Their vision: to replace the unpleasant injection with oral administration, as its production is both simple and cost-effective.

Keywords: Algal-derived edible vaccines · Birch pollen (Betula) allergen Bet v 1 · *Chlamydomonas reinhardtii* · Non-glycosylated therapeutic proteins · Peanut-induced anaphylaxis · Polymerase chain reaction (PCR) · Recombinant allergen

More and more people around the globe are suffering from allergies and asthma. Almost one third of the world's population are allergy sufferers: their number is growing faster than ever before. According to estimates, some 250 million people are suffering from food allergies. In countries with strong economies, like the USA, every fifth person is affected by allergies or asthma. Children in particular are suffering from food and respiratory allergies: every fourth school-age child in Europe shows allergic symptoms.

When Scientists Pool their Expertise

An allergy is not something we are necessarily born with, it can develop during the course of our life. The allergy is triggered when the immune system makes immunoglobulin E (IgE) antibodies to 'fight off' an allergen that wouldn't normally bother us. And this can be one of many things, for example pollen, dust, food, insect stings, animal dander, mould, medicines or latex. Allergen immunotherapy – also known as allergy shots – decreases the sensitivity to allergens, which often leads to lasting relief of allergy symptoms even after treatment is stopped. The aim is to modify the immune system so that it no longer reacts to allergens. The patient is given increasing doses of the allergen in a controlled manner until the immune system tolerates the allergen and does not 'fight' it. Immunotherapy is time-consuming, often costly and associated with certain risks. Nevertheless, it helps reduce hay fever symptoms, for instance, in about 85% of people with allergic rhinitis. As a seasonal or perennial condition, hay fever causes symptoms such as sneezing, stuffy nose, runny nose, watery eyes and itching of the nose, eyes or roof of the mouth.

“Mechanisms of Allergen Immunotherapy (AIT) include early desensitization of effector cells, the induction of anti-inflammatory T and B cells, and the modulation of the allergen-specific immunoglobulin (IgE) response including the induction of blocking antibodies”, explains **Christoph Griesbeck**. He is Professor for Biotechnology and Food Engineering and head of



Dr. Sonja Hirschl, lector and research assistant, checks the algae cultures at the MCI laboratories. Photo by Elsbeth Heinzelmann.

department and study programmes at the MCI Innsbruck and has many years of experience in researching the properties of algae as a basis for high-quality products for the pharmaceutical, food and cosmetics industries. “Recombinant allergens or derivatives thereof are an efficient alternative to allergen extracts widely employed in AIT.”

Chlamydomonas reinhardtii is a single-cell green alga about 10 micrometres in diameter, and is a known production platform for therapeutic proteins. As a haploid unicellular eukaryote, each of its cells contains a chloroplast similar to those of plants and swims with two flagella similar to those found in numerous other eukaryotic groups including mammals. Microalgae are recognized by the US Food and Drug Administration (FDA) as ‘Generally Recognized As Safe’ (GRAS), algal products being essentially free of human pathogens. Given the fact that recombinant protein production in *Chlamydomonas reinhardtii* is scalable and inexpensive, Christoph Griesbeck searched for



Claudia Ralsler is experienced in the production of recombinant allergens as therapeutic vaccines in the green alga *C. reinhardtii*. Photo by Elsbeth Heinzelmann.

a comrade-in-arms to help explore this expression system for the recombinant production of allergen products using the major birch pollen allergen Bet v 1 as a model. Birch pollen (*Betula*) is native to Central Europe, highly allergenic and active from April to June. The partner of choice was Dr. **Michael Wallner** from the Molecular Biology department at the University of Salzburg, whose research is focused on the identification, recombinant production and characterization of inhalant and associated food allergens. Also involved were the *Allergie Ambulatorium Reumannplatz* and the Medical University of Vienna.

Inspired by the Spirit of Pioneers

To ensure the efficient use of the green Alga *Chlamydomonas reinhardtii* as a novel allergen production platform, the scientists selected the major birch pollen allergen Bet v 1 as a candidate molecule. This pollen allergen has been shown to be homologous to pathogenesis-related proteins in a number of plants. The researchers synthesized a codon-optimized gene and integrated this into the micro alga. Positive transformants were identified by Polymerase Chain Reaction (PCR), cultured, after which the cells were disrupted by sonication, a process in which sound waves are used to destroy cells. Bet v 1 was purified from algal total soluble protein (TSP) by affinity chromatography and characterized physico-chemically and immunologically. “All transformants, which had integrated the genetic cassette of Bet v 1, showed expression of the recombinant allergen only in very small amounts, but we are committed to becoming even better!”, explains **Sonja Hirschl**, research assistant at MCI. Michael Wallner, who is particularly interested in the molecular mechanisms initiating allergic sensitization, sums up positively: “Algae-derived Bet v 1 displayed a secondary structure element resembling an *E. Coli*-produced reference allergen. Moreover, Bet v 1 produced in *Chlamydomonas reinhardtii* showed strong binding to human IgE as well as murine Bet v 1-specific IgG.” He continues: “Our attempt was a success: We produced a batch of recombinant Bet v 1 in micro alga.” As *Chlamydomonas reinhardtii* is generally recognized as safe (GRAS) – at least no pathogens were ever found –, the project partners are now concentrating their activities on investigations to verify whether they can use the whole algae or only some of its purified proteins. Future studies will then focus on the development of novel allergy treatment concepts such as the oral administration of allergen-containing algae for therapy.



Prof. Christoph Griesbeck, Head of Biotechnology at MCI and the director of the project, is familiar with bio- and food technology and has twelve years of experience in the field of algal biotechnology. Photo by Elsbeth Heinzelmänn.

Microalgae – Panacea for Protein-based Pharmaceuticals?

Today, glycoproteins represent the largest section of biologically-derived drugs approved by the European Medicines Agency (EMA). Here, Chinese Hamster Ovary (CHO) cells are the most widely used production platform. Non-glycosylated therapeutic proteins are typically produced in *E. coli*, followed by *S. cerevisiae*. The most common way to produce vaccines is using an egg-based manufacturing process that has been in existence for more than 70 years. In recent years significant advances have been achieved in microalgae biotechnology. The use of microalgae as bioreactors for expressing recombinant proteins is attracting growing interest. Microalgae is an attractive alternative for the production of pharmaceuticals, recombinant proteins and other high-quality products. These include, for example, vaccines, antibodies, enzymes, blood-clotting factors, immune regulators, growth factors, hormones and anticancer agents.



The goal of Christoph Griesbeck is to develop new processes for converting algae into high-grade raw materials and active agents with potential for pharmaceuticals, agriculture and the food and cosmetics industries. Photo by Elsbeth Heinzelmänn.

“The advantages of algal-based systems depend primarily on the algae strain”, comments **Claudia Ralsler**, also participating in the project. “In general, expression systems based on microalgae offer high protein folding accuracy, sufficient yields and scalable processes, thereby reducing costs. But probably the biggest advantage is the fact that many microalgae are classified as GRAS, generally regarded as safe, suitable for medicines and with no known pathogens.” Recombinant allergens seem to be an attractive alternative as they allow precise dosing and the possibility to engineer proteins with improved safety profiles. Recently, US scientists^[1] tested whether Ara h 1 and Ara h 2, two major peanut allergens, could be produced using chloroplast of the unicellular eukaryotic alga. They discovered that *C. reinhardtii* is a suitable host for producing allergens that is genetically tractable, inexpensive and easy to grow, and is able to produce more complex proteins than bacterial hosts. Their investigations show that immunotherapy using algal-produced Ara h 1 core domain confers protection from peanut-induced anaphylaxis in a murine model of peanut allergy.

There remains a lot to do

But let’s return to the hotbed for new ideas in Austria and to Christoph Griesbeck: “In this project we were able, for the first time, to generate stable transformants of *C. reinhardtii*



This project with the University of Salzburg involves a high degree of cooperation between the researchers involved, including Dr. Sonja Hirsch (left) and Claudia Ralser, here conducting research together at the sterile bench. Photo by Elsbeth Heinzlmann.

expressing an inhalant allergen, namely Bet v 1. Accordingly, a codon-optimized gene was inserted into an expression vector.” The system used has the advantage of enabling selection *via* photo autotrophy, dispensing with the need for antibiotics. After protein purification, a detailed physico-chemical comparison

between algal-derived Bet v 1 and an *E. coli*-produced batch did not reveal any differences. When looking at antibody binding in ELISA, we found that Cr Bet v 1 bound specific antibodies even more strongly than Ec Bet v 1. The results provide solid proof of the high quality of the algae-derived Bet v 1 batch. This pilot study now offers novel strategy concepts for AIT using algae-produced allergens or derivatives thereof. It is probably not necessary to purify the allergens from algal extracts. But AIT formulations based on allergen-containing algal preparations applicable for sublingual immunotherapy (SLIT) seem possible. And he concludes: “For the time being, we are at the forefront of research on algal-derived edible vaccines. Since we have only managed to produce very few proteins in algae to date, we now have to find ways and means to increase the quantities produced. Further studies are needed to evolve our concepts, but we believe in the high medical potential of algae-derived biologics.”

This contribution is based on the scientific paper ‘Expression and characterization of functional recombinant Bet v 1.0101 in the chloroplast of *Chlamydomonas reinhardtii*’, which will be submitted to the *International archives of allergy and clinical immunology* (September 2016).

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[1] <http://www.ncbi.nlm.nih.gov/pubmed/26801740>