

A patent on the synthesis of arabino-oligosaccharides for regulation of immune responses in the lung

The patent summarized in this commentary describes the synthesis of arabino-oligosaccharides that were designed to prevent the development of allergic asthma.¹

Since the prevalence of allergic disorders has increased over the last decades, there is an urgent need for prophylactic strategies. A westernized lifestyle with a high hygienic standard, less infections, and small family size was identified as one important factor for the increased allergic sensitization. Sensitization against allergens starts in the first years of life and in the case of respiratory allergens often evolves to allergic rhinitis or even asthma. Until now, there are no approved treatment options available to prevent this “allergic march.”²

The only treatment option showing some benefit in prevention of asthma is immunotherapy. For example, the GAP investigator group has recently shown that sublingual immunotherapy reduced the risk of experiencing asthma symptoms and using asthma medication in young children suffering from grass pollen-induced rhinoconjunctivitis.³ However, there is still an unmet need for an early prophylactic strategy to prevent development of sensitization in high-risk atopic children.

The idea behind the invention described in this patent is derived from epidemiological data showing that children who grew up in the rural environment of a traditional farm are protected against the

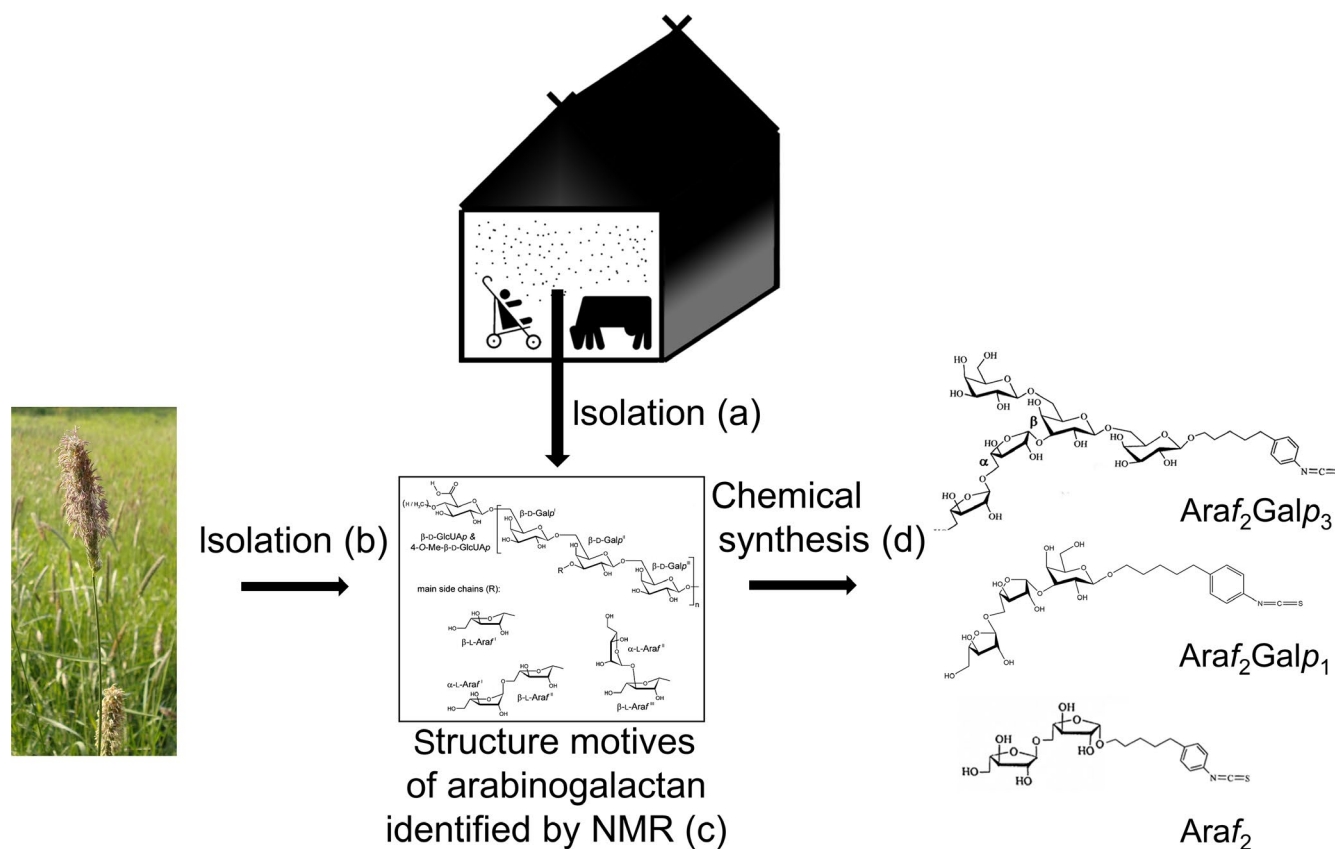


FIGURE 1 Living on a farm protects against allergic sensitization and asthma development. Arabinogalactan (AG) was detected in and isolated from the inhalable dust from the cowsheds of these farms (a). Subsequently, AG was also identified and isolated from forage plants such as the meadow foxtail (b). In murine model systems, AG was proven to be protective against allergic asthma. Moreover, chemical analysis revealed the structure of AG (c). To mimic the structure of AG, we synthesized three different partial structures consisting of a di-L-arabinose coupled to one or three D-galactose molecules and a spacer with an amino-reactive isothiocyanate group, namely Araf₂, Araf₂Galp₁, and Araf₂Galp₃ (d)

development of atopic diseases such as allergic asthma.⁴ It is assumed that allergy protection is partially caused by the inhalation of dust in the stables of the farms. In the search for protective substances from the stable environment, we were able to prove that the effect can be mediated by immunomodulatory "cowshed dust extract" (CDE). This was demonstrated in experiments using a mouse asthma model where inhalation of CDE has a protective effect on the development of bronchial hyper-reactivity and eosinophilia in the respiratory tract.⁵ With the aim of identifying the responsible immunomodulatory molecules, the next step was to characterize the chemical composition of the extract. Arabinogalactan (AG) was isolated as one of the active substances from the dusts consisting mainly of plant material derived from the abundant animal fed in the cowsheds. Subsequently, the polysaccharide was isolated from forage plants such as the meadow foxtail, the timothy grass, and the meadow grass. AG from all these different sources was shown to be protective in murine models of asthma. Intriguingly, further experiments showed that AG also led to modification of the behavior of human dendritic cells. We showed that AG binds to c-type lectin

receptors on human DCs leads to the down-regulation of cellular activation by extracellular stimuli like for instance lipopolysaccharide. Subsequently, DCs have reduced ability to stimulate T lymphocytes.⁶ Therefore, it was discussed that inhalation of AG could result in a dampened Th2 cell activation in the lung, thus leading to a reduced allergic sensitization via the airways.

Nuclear magnetic resonance analysis showed that AG consists of a backbone of $-(1 \rightarrow 6)$ -linked D-galactose, which has many substitutions with short arabinose side chains in different configurations. The structure shown in Figure 1C was disclosed in a patent describing the isolation and use of AG to prevent allergic sensitization.⁷ The AG molecule is large with a molecular mass of 127 kDa.⁶ Due to its high mass and structural complexity, the complete chemical synthesis of this molecule is impossible. However, experiments, in which the arabinose side chains were cleaved, suggested that these residues are critically involved in the immune modulatory activity of the molecule. These experiments showed that a significant part of the allergy-protective effect of AG is lost due to the reduced arabinose content.⁸ Due to these results, we suspected the arabinose

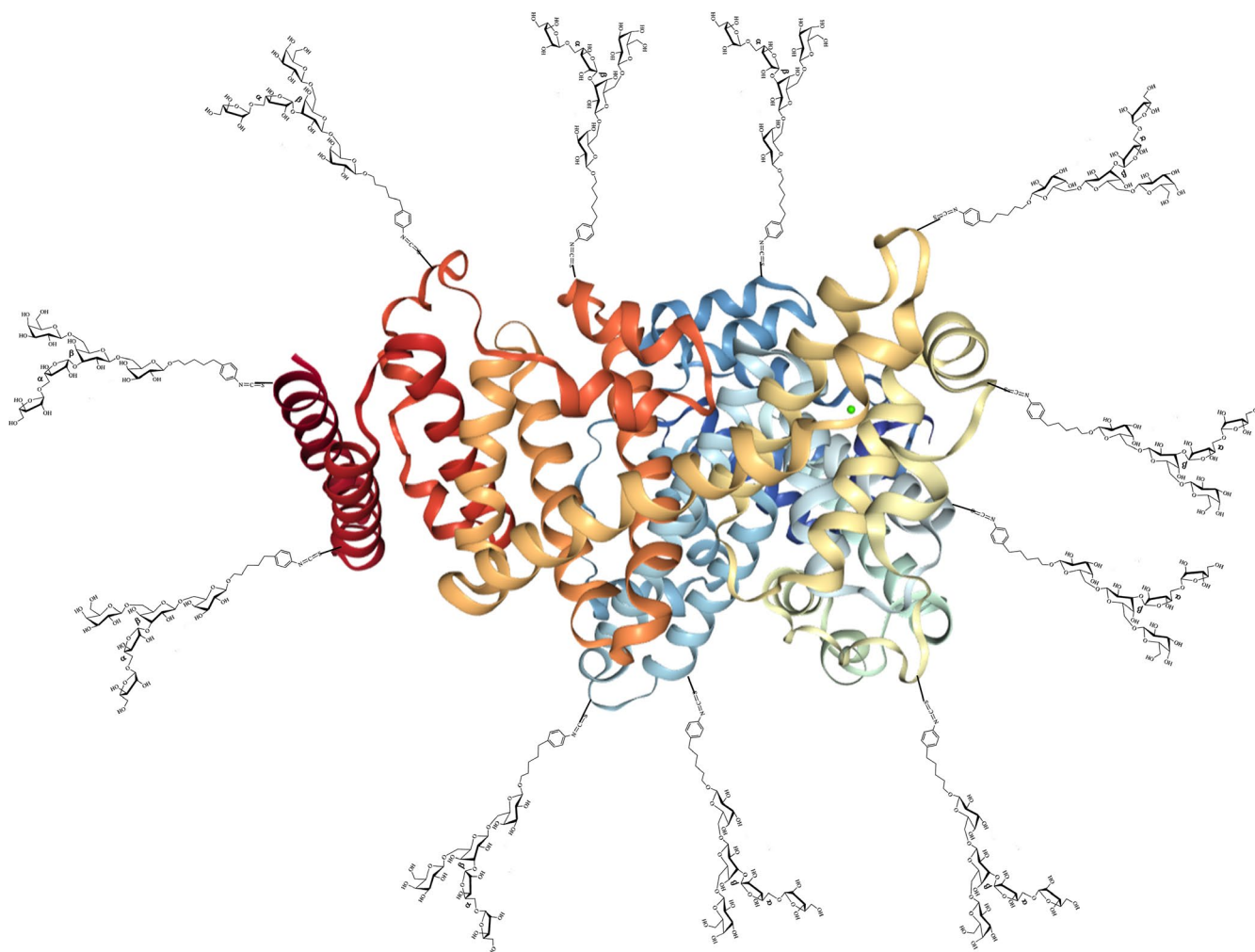


FIGURE 2 Schematic depiction of the neoglycoprotein that was composed by coupling the synthetic oligosaccharide Araf₂Galp₃ to the carrier protein bovine serum albumin (BSA). Inhalative administration of this molecule during sensitization and challenge protects against allergic disease in a mouse model of asthma. The ribbon structure of BSA was reproduced from data deposited in the Protein Data Bank (Research Collaboratory for Structural Bioinformatics)

side chains to be involved in the protection against allergic disease. Therefore, we started chemical synthesis of these arabino-oligosaccharide structures containing galactose and arabinose keeping in mind that arabinogalactan is a large molecule that is likely to contain several of these side chains. Thus, we connected the side chains to an amino-reactive spacer to have the opportunity to couple the substance to a larger carrier protein, namely bovine serum albumin (BSA). In the examples shown in the patent, the use of this substance for the prevention of allergic sensitization and the development of allergic asthma is described.¹ Treatment with the neoglycoprotein, schematically shown in Figure 2, protects against allergic sensitization and airway inflammation in a mouse model of asthma. Interestingly, coupling of the oligosaccharides to the carrier protein was essential for the efficacy of the treatment. The usage of the different L-arabinose-containing oligosaccharides shown in Figure 1 revealed that an $-(1 \rightarrow 5)$ -linked di-arabinose (Araf_2) is the smallest unit still conferring protection against allergic disease, though, with reduced activity when compared to the larger molecules containing one or three galactose molecules in addition such as $\text{Araf}_2\text{Galp}_1$ and $\text{Araf}_2\text{Galp}_3$. The most obvious pharmaceutical application of this invention will be the use for the prophylactic treatment of allergic sensitization by inhalative treatment during early infancy, when sensitization toward allergens occurs. However, other possible applications would be conceivable such as the prevention of the development of other hypersensitivity reactions or even autoimmune disease. In particular, for the treatment of multiple sclerosis this could be promising since it was shown that the autoaggressive neuroreactive T cells that are driving the disease become activated in the lung, before they are licensed to pass the blood-brain barrier.⁹ Therefore, it is tempting to speculate that the reactivity of these cells could be dampened due to treatment with the neoglycoproteins.

Today, we have a lot of evidence that a reduced stimulation of the immune system during early infancy caused by a high hygienic standard results in a hypersensitive immune response. The consequence is increased allergic sensitization and subsequently manifestation of diseases such as allergic rhinitis or asthma. The aim of our invention was to prevent this hypersensitive immune response. By preventive treatment with a L-arabino-oligosaccharide coupled to a carrier protein, allergic sensitization and development of asthma are suppressed at least in mice. Since it is feasible to produce these compounds under GMP conditions, it will be of high interest to test in clinical trials whether inhalation of these molecules will prevent the development of allergic sensitization also in human.

CONFLICTS OF INTEREST

Dr. Peters reports grants from Deutsche Forschungsgemeinschaft, grants from Gesellschaft für Pädiatrische Allergologie und

Umweltmedizin, during the conduct of the study; In addition, Dr. Peters has a patent WO2017093276A1 issued.

KEYWORDS

allergy treatment, environment and hygiene hypothesis, immunotherapy and tolerance induction, pharmacology and pharmacogenomics, prevention

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