

Intensity of a human immunological response for allergenic properties of subunits and fractions of silver birch (*Betula pendula*) pollen proteins against the background of air pollution

The scientific problem planned to be solved within the project is to determine, whether and how the immunological and clinical reactivity of people with birch pollen allergy changes, in relation to the pollution of environment in which the studied trees have developed.

It was confirmed that chemical particles suspended in the particulate matter, especially in the urban environment, are the stress factors for plants and may straighten the expression of genes encoding proteins responsible for stress resistance of plants, interfering with the structure of the polypeptides. It results in the increase in the immunological activity of plant allergens, including the main birch pollen allergen Bet v1. The variability of the pollen protein profile may result in a different individual immune response of people with birch allergy, resulted in a variety of clinical symptoms depending on feature of the pollen source.

The main goal of the project is to estimate whether the quantitative and qualitative changes in the protein pollen components of *B. pendula* are associated with changes in their immunoreactivity and allergenicity.

The study will be carried out using plant pollen material collected from the selected sites in Kraków and in the areas of the lower particulate matter level. The time of pollen collection will be established on the basis of phenological observations of *Betula pendula* inflorescences in order to select the right time, just before the full flowers opening and the release of pollen grains. Simultaneously, the measurement of *Betula* spp. pollen concentration in the air by volumetric method in Krakow will be performed to monitor the pollen concentration within the season and to compare these data with the level of air pollution. A group of 100 persons with birch allergy confirmed on the basis of skin prick tests and a control group (20 people) not allergic to birch pollen will be included into the study.

Laboratory analyses will include:

- Estimation of the proteins concentration in pollen material using colorimetric method
- The analyses of the protein profile of *B. pendula* pollen to characterize the composition of the fraction and subunits of allergenic proteins, by the SDS-PAGE electrophoresis
- Assessment of the serum reactivity of patients allergic to birch pollen in vitro by immunoblotting to identify the most immunoreactive subunits
- Molecular diagnostics of allergen components in patients included in the study, in order to check whether the binding strength of IgE antibodies in case of the commercial birch allergens is similar to the in vitro reaction with proteins from the natural birch pollen. Four birch allergen components will be analysed: Bet v1, Bet v2, Bet v4, Bet v6.
- Human basophils activation after in vitro stimulation by the natural and commercial birch pollen extracts using Basophil Activation Test (BAT)

An novel approach of the proposed project will be a multi-subject analyses of the cause-effect relationship from the source of allergenic pollen to the patient's clinical symptoms. This process will include the study of the effect of allergenic proteins derived from natural pollen material on the in vitro immune IgE response of patient sera, taking into account the birch allergenic proteins profile and the precise diagnosis of molecular components in patients.

Undertaking interdisciplinary research will enable expanding the knowledge in the fields of aerobiology, biochemistry and plant physiology as well as immunology. The proposed study will mostly broaden the research topics of the Polish and European Aeroallergen Networks (PAN, EAN) within the concept of "molecular aerobiology" and will make a significant contribution to allergology. The project subject follows up the study on the impact of air pollution on the physiological state, protein profile and content of the main allergenic protein in birch pollen, performed in Kraków in 2017-2019.