

SUMMARY

It is generally assumed that the incidence of allergies in the Western world has been rising over the past few decades. It is against this background that the European Commission decided to put food allergy on their research agenda. This thesis is part of a multi center collaborative study, the SAFE project, funded by the EU Fifth Framework Program. The acronym SAFE stands for “Plant food allergies: field to table strategies for reducing their incidence in Europe”. Apple allergy was chosen as a model system because apples are widely consumed in Europe and they frequently (1-2% of the population in Europe) cause allergic reactions with a variable degree of severity. In Northern and Central Europe, apple allergy has always been described as a mild disease (itching and swelling of lips, mouth and throat). More recent observations from Southern European countries indicate that apple can induce severe systemic reactions as well. The SAFE project aimed at addressing the various aspects of food allergy from sensitization to clinical presentation, as well as at evaluating strategies for prevention and treatment.

Chapter 2 describes the patient population studied. Patients from four countries in Europe were included, specifically from Austria, Italy, Spain and The Netherlands. Different sensitization patterns were studied. Detailed IgE serology was performed using pollen extracts, food extracts and purified apple allergens. The results of these analyses were compared to clinical histories and skin tests. Our studies in four European countries have confirmed that the presence of birch trees is a dominant factor in sensitization to the major apple allergen Mal d 1. Primary sensitization to the major birch pollen allergen Bet v 1 is at the basis of IgE recognition of Mal d 1 in Northern and Central Europe. In Madrid where birch trees are virtually absent the mean IgE response to Mal d 1 was low. The explanation for

the absence of Bet v 1-related fruit allergies in Spain is thus straight forward (i.e. the absence of birch trees). However, it is less clear why sensitization to lipid transfer protein (LTP) occurs almost exclusively in Mediterranean countries. Apple allergy in Spain is most likely a result of primary sensitization to peach. Whether the virtual absence of LTP-related food allergy in The Netherlands and Austria is caused by lower consumption levels of fruits like peach is still unclear. It can also not be ruled out completely that specific pollen in the Mediterranean are at the basis of LTP sensitization.

In *Chapters 3 and 4* two novel forms of cross-reactive food allergies among birch pollen allergic patients are presented, i.e. to jackfruit and sharonfruit. In both cases patients reacted on the first exposure to the fruit involved and more severely than they used to do on apple and other fruits. These studies suggest that some food homologs of Bet v 1 might be more stable than the extremely labile Mal d 1 from apple. Jackfruit and sharonfruit allergy can be added to the list of birch pollen related food allergies. Increased consumption of these fruits will result in a rise in allergic symptoms.

Treatment of food allergic patients is primarily based on avoidance diets. Such diets can have a considerable social impact and lead to nutritional deficiencies.

Chapter 5 and 6 describes possibilities to develop hypoallergenic fruit.

In *Chapter 5* the allergenicity of different apple cultivars was assessed by SPT and double-blind, placebo controlled food challenge. Golden Delicious has shown to be a representative of apple cultivars with high allergenicity and Santana consistently demonstrated the lowest allergenicity. It has also been found that allergenicity increased during storage and that this increase could partly be prevented by reducing oxygen to 2.5% during storage.

Chapter 6 describes the development of a hypoallergenic apple plant in which the Mal d 1 gene was knocked out by RNA interference. Using leaf material of young plantlets for SPT and immunoblot, it was demonstrated that Mal d 1 activity was indeed reduced significantly in the mutant plantlets. Normally it takes around five years to grow trees that produce fruits, so over 5 years we would be able to test using the golden standard the double-blind, placebo controlled food challenge to verify, if the mutant apple is indeed hypoallergenic.

Public opinion on genetically modified foods has proven to be perhaps a bigger hurdle. The appearance of genetically modified foods in the marketplace has resulted in an emotional and fierce public debate and scientific discussion with broad media coverage. The attitude of the allergic patients towards hypoallergenic genetically modified foods was investigated and described in *Chapter 7*. Allergic participants in the study identified a number of benefits to themselves, such as being able to eat the food again to which they are allergic, and being able to eat all food without worries and not to have to carefully check labels.

Finally, two chapters focus on strategies for allergen-specific immunotherapy. For the treatment of inhalant allergies specific immunotherapy was already described in 1900 and is now widely used as an effective treatment of pollinosis, animal, mite and insect venom allergy. In contrast, specific immunotherapy is not used to treat food allergies. A major problem of immunotherapy using food allergens is the risk of severe side effects, including anaphylactic shock.

In *Chapter 8*, a study using birch pollen immunotherapy trial is described in which the effect on cross-reactive apple allergy is evaluated by SPT and DBPCFC. Our clinical trial supports earlier claims that birch pollen immunotherapy has a

beneficial effect on cross-reactive food allergies. For the first time the clinical improvement was assessed using double-blind, placebo controlled food challenge.

In *Chapter 9*, a recently developed hypoallergenic mutant of the major apple allergen Mal d 1 is evaluated *in vivo* using SPT and DBPCFC. It was demonstrated that the mutant indeed behaved as a hypoallergen. This was also confirmed by *in vitro* experiments. Hypoallergenic mutants are potential candidates for future use in immunotherapy.