tive potential, particularly in case of polyclonal antibodies, is more reliably assessed by inhibition tests than by direct binding tests.

Cross-reactivity and allergenicity

For various reasons, often related to regulatory safety issues, a discussion is ongoing on prediction of allergenicity. This involves both prediction of de novo allergenicity as well as prediction of cross-reactivity. The latter, prediction of allergen cross-reactivity, is the topic of this communication, with emphasis on quantitative and methodological aspects.

Clinically, allergic cross-reactivity is often encountered as symptoms without prior exposure. Another common clinical situation is the occurrence of symptoms upon exposure to allergenic sources that are unlikely to sensitise, such as apples. In Northern Europe it is rare to find apple allergy in the absence of birch allergy. The major birch pollen allergen acts as the sensitizer or primary allergen, which by definition is able to trigger the immune system to produce IgE antibodies. The homologous protein in apple Mal d 1 is an incomplete allergen, because it is unable (or: extremely inefficient) to induce IgE antibodies, but is able to elicit symptoms due to its ability to trigger mast cells loaded with IgE anti-Bet v 1.

Cross-reactivity is sometimes seen as a property of a subgroup of antibodies: antibodies to some epitopes (recurring epitopes such as cross-reactive carbohydrate determinants (CCDs [2]) are more likely to be cross-reactive than antibodies to other epitopes. However, it is often more appropriate to use cross-reactivity to describe a relation between two allergens (which I will refer to as Ag1 and Ag2; alternatively, I will use the birch allergen Bet v 1 and the cross-reactive apple allergen Mal d 1 as examples): the closer the similarity between two allergens, the more likely it is to find a cross-reactive antibody. In either case, the concept of cross-reactivity concerns (at least) three rather than two reagents: two allergens and an antibody. Since it is impossible to test all antibodies, we have to live with the frustrating thought that it is impossible to prove that two allergens completely lack cross-reactivity. Conversely, it is also impossible to prove that they are fully cross-reactive. It is all a matter of probability, which in many cases is either very close to 0 or very close to 1. In many other cases it is, however, easy to demonstrate some cross-reactivity.

Prediction of cross-reactivity from the amino acid sequence

Antibodies largely bind to surface patches of folded proteins (epitopes), so knowledge of the full 3D structures of the target protein and the related allergens would clearly be an advantage for such a prediction and now more commonly available (or the 3D structures can be reliably predicted). Undoubtedly, cross-reactivity prediction algorithms will be developed in which such information is incorporated. However, sufficiently reliable information on the relevant 3D structures is often not available and the prediction has to be based on the linear amino acid sequence. One of the points of debate is whether short stretches of 6–8 fully identical amino acids are reliable predictors and should be used in combination with partial amino acid identity of longer stretches (typically more than 35% identity between stretches of 80 amino acids [3]).

Other issues related to prediction of cross-reactivity, such as the repertoire-modifying effect of a human homologue, the contribution of post-translational modification (particularly non-mammalian glycosylation patterns), the possibilities and limitations of peptides as epitope mimics and the intriguing question whether IgE antibodies tend to be more cross-reactive than IgG antibodies, with its possible link with positive and negative regulation of B cells by IgE versus IgG antibodies, have been discussed elsewhere [4-6].

Symmetric versus asymmetric cross-reactivity

Some situations of allergen cross-reactivity are almost trivial, such as the cross-reactivity between major allergens of botanically-related grasses [7] and between major dust mite allergens. Without information on allergen exposure it is then virtually impossible to decide which allergen is the sensitizer. Symmetric cross-reactivity a likely possibility: both allergens in the couple can sensitize and both can largely (but not completely) inhibit the binding of IgE to the other allergen (figure 1A). In the birch/apple situation the situation is different (at least in Northern Europe) [8]. Cross-reactivity is asymmetric (figure 1B), as can be demonstrated in vitro by reciprocal IgE antibody neutralization. The usual finding is that birch allergen inhibits IgE binding to the apple allergen similar to or even better than the inhibition found by using equimolar amounts of the apple allergen as inhibitor, whereas the apple allergen only partially inhibits IgE binding to the birch allergen.

Issues on the quantification of the degree of cross-reactivity of two allergens

First, a semantic issue: polyclonal versus monoclonal cross-reactivity. The word "cross-reactivity" is used differently in the polyclonal situation and the monoclonal situation. Traditionally, it is used to describe the *polyclonal* situation as encountered in the body. Many different antibodies recognize Ag1 (for example: Bet v 1). Some of these antibodies react with Ag2 (Mal d 1). The degree of cross-reactivity can be expressed as the fraction of anti-Bet v 1 antibodies that react with Mal d 1. This fraction will vary between individuals and usually varies within one indi-