Investigations Towards The Characterisation And Standardisation Of Allergen Extracts OF Mite Cultures.

Allergen extracts for medical diagnostics and therapy are classified as drugs in German pharmaceutical law and are thus subject to according quality criteria.

Because of the non-existence of national or international standards, there are large differences in the quality of compounds of different producers. Therefore, a complex and elaborating quality assurance is required.

Quality is not a well defined, generally accepted and proofed state, hence it is subjected to the individual interpretation of the manufacturer. Internal comparisons are not required. If carried out, they would result in large quality differences. Today, the single requirement for quality assurance is manufacturing according to “the current scientific state of the art”. Only during first admission of a new drug an *in-vivo* prick-testing is required, controlling of following production charges can be done by simple *in-vitro*-testing.

Because of the non-existence of national and/or international guidelines for allergen standardisation and quality control, the global activity of allergen extracts are estimated

- on a biological basis (histamin equivalent in prick testing)
- by an *in-vitro* method like EAST-inhibition or histamin liberation

Standardisation of allergen standards on the basis of biological units are very useful since they correlate to all individual activities present. However, it is impossible to draw conclusions about individual allergen concentrations, i.e. a high allergen activity could also be achieved without major allergens.

If the allergen activity of a whole extract would be considered as the sum of the single components and every single allergen yields an individual contribution, the estimation of single allergens and the correlation to individual specific activities becomes a new meaning.

Due to this fact the following investigations were carried out in this work:

- gently pulping of mite cultures,
- physico-chemical characterisation,
- functional activity of allergen extracts and
- fragmentation and structure elucidation of the allergen components had been done.

Cultures of the house dust mite *Dermatophagoides pteronyssinus* from different suppliers and one culture of the storage mite *Lepidoglyphus destructor* were the objects of the investigation.

Results

Purified mite cultures and whole mite cultures are both suitable for characterization. Differences between the two pulping-media Cocas- and Ammonium-hydrogene-carbonat-solution were not observed.
The addition of polyethyleneglycol resulted in qualitative changes of the extract.
Dialysis for separating low-molecular components has to be avoided because of an incresed fragmentation and latent proteolytic activity.
An increased shelf life was achieved by lyophilisation which gave a shift in fragmentation pattern to low molecular weights. Otherwise an allergen pattern over the whole molecular weight range was found by specific incubation with patient sera.

A fast determination of apparent molecular weight fractions and the estimation of changes during dialysis and lyophilisation was achieved by size exclusion chromatography. Quantitative changes of the protein pattern were documented by SDS-PAGE and IEF.
Specific reactions of both examinated mite species against patient sera were in accordance to the known literature with very few exceptions. The taxonomic diversity of the two mite species were well differentiated by immunoblotting techniques. However, the high binding frequency of the major allergen Der p 1 of about 80% could not be verified.

A different range in molecular weight and source specific proteolytic activity of different sources of mite cultures (europe vs australia) were shown by casein zymographic determination.
Further investigations must clarify if these differences are based on evolutionary derivation or if they are the result of mechanical stress during production.
In conclusion, the MALDI-TOF MS (Matrix-assisted-laser-desorption-ionisation-time-of-flight-mass-spectrometry) represents a highly efficient and useful tool for the analytical characterisation of complex mite culture extracts.
Results of immunoblottings and electrophoretical separations were verified and assigned by MALDI-TOF MS.