

# Legends of allergy and immunology: Henning Løwenstein

## Major contributions

- Characterization of components in allergen extracts by Quantitative or Crossed ImmunoElectrophoresis (QIE/CIE)
- Identification of allergens by Crossed Radio ImmunoElectrophoresis (CRIE)
- Categorizing allergens into major and minor allergens
- Isolation and characterization of a number of allergens
- Allergen nomenclature
- Standardization of allergen extracts for diagnosis and treatment of allergic diseases.

When the physician Kaj Baagøe returned to Denmark from the United States in 1923 and, in collaboration with the pharmacist Paul Barfod, introduced the use of allergen extracts for allergy diagnosis to the Danish medical community, it was an incident that had a great impact on the life of Henning Løwenstein. It was pivotal for the establishment of a scientific allergology community and the company ALK, which later became the workplace of Henning Løwenstein for a major part of his scientific career.

In 1972, after finishing his training in chemistry at the University of Copenhagen, Henning Løwenstein, aged 33, initiated his studies on allergens in collaboration with the young physician Bent Weeke who used immunoelectrophoretic techniques to describe serum components. Løwenstein and Weeke implemented the techniques to the characterization of allergen extracts and invented crossed radio immunoelectrophoresis (CRIE) to identify IgE-binding components in allergen extracts.<sup>1</sup> The first results looked very promising and were presented at allergy meetings in 1973 and 1974 followed by 2 years of control experiments ensuring that no artifacts were involved. Løwenstein described the use of CIE/CRIE to understand the complexity of allergen extracts (Figure 1) and its impact on allergy diagnosis and treatment through publication of 25 scientific papers on this subject during the years 1973–1982. The technique sets new standards, and PS Norman states in his review on immunotherapy in 1978, “It appears to provide the sophistication required for a definitive analysis of numerous allergens.” Initially, Løwenstein collaborated with young Danish scientists and clinicians meeting in a growing local allergy society (Figure 2). Later the collaborations included other prominent members of the international allergy/Immunology

community, such as the characterization of ragweed allergens with David Marsh,<sup>2</sup> and many of these scientists became close friends.

Løwenstein's visit to Johns Hopkins University in the summer of 1977 fueled the international collaborations. He insisted on bringing all instruments and reagents from Denmark, and getting the reagents through customs was quite an accomplishment on its own. Access to a large quantity of myeloma-IgE (PS, generously donated by the Ishizakas), which was used to generate the anti-IgE central to CRIE, further increased the fruitful competition between Scandinavian research groups. During this period, many new allergens were identified and characterized around the world. At the 10th EAACI/WAO congress in Jerusalem in 1979, Tom Platts-Mills (UK), Kevin Turner (Australia), and Henning Løwenstein all presented data on a new house dust mite allergen, which subsequently, through collaboration and exchange of reagents, was shown to be the same allergen, Der p 1. During the following years, it was heavily debated whether pteronyssinus, microceras, or farinae was the mite species most relevant for allergy, but the many identical allergens in these mites and the pronounced immunological cross-reactivity toward the allergens have made these discussions obsolete. IgE cross-reactivity toward group 5 allergens from the related grasses was initially described by Løwenstein et al in 1978 by the use of inhibition assays. The early studies also led to the classification of major and minor allergens as well as purification of many of these using (immuno)-electrophoretic methods to guide the purification.

Danish Allergen Standardization was initiated by Løwenstein and his Danish collaborators in 1976,<sup>3</sup> and the work continued in the allergen standardization committee under IUIS with Løwenstein as president. This led to the IUIS allergen standardization guidelines reported in 1983 and the description of 8 WHO-approved international standards and even had the consequence that recommendations on immunotherapy by WHO only apply to the products based on standardized allergen extracts. Løwenstein started the committee work by inviting colleagues for a standardization course in Denmark. Scientists from around the world discussed multiple approaches, which paved the way for the work in the standardization committee.

The allergen nomenclature was an add-on to the committee work, and Løwenstein describes that the nomenclature was initially drafted at the CIA meeting in 1980 by Marsh, Platts-Mills, and himself in a rowing boat on Bodensee with a bag of beers. The draft was followed up by additional input especially from TP King, and the only major change to the original nomenclature has been to use Arabian numbers instead of Roman.<sup>4</sup>

The use of standardized allergen extracts and allergen components in clinical trials became the next step in the development of recommended procedures for immunotherapy. Changes in IgE and IgG

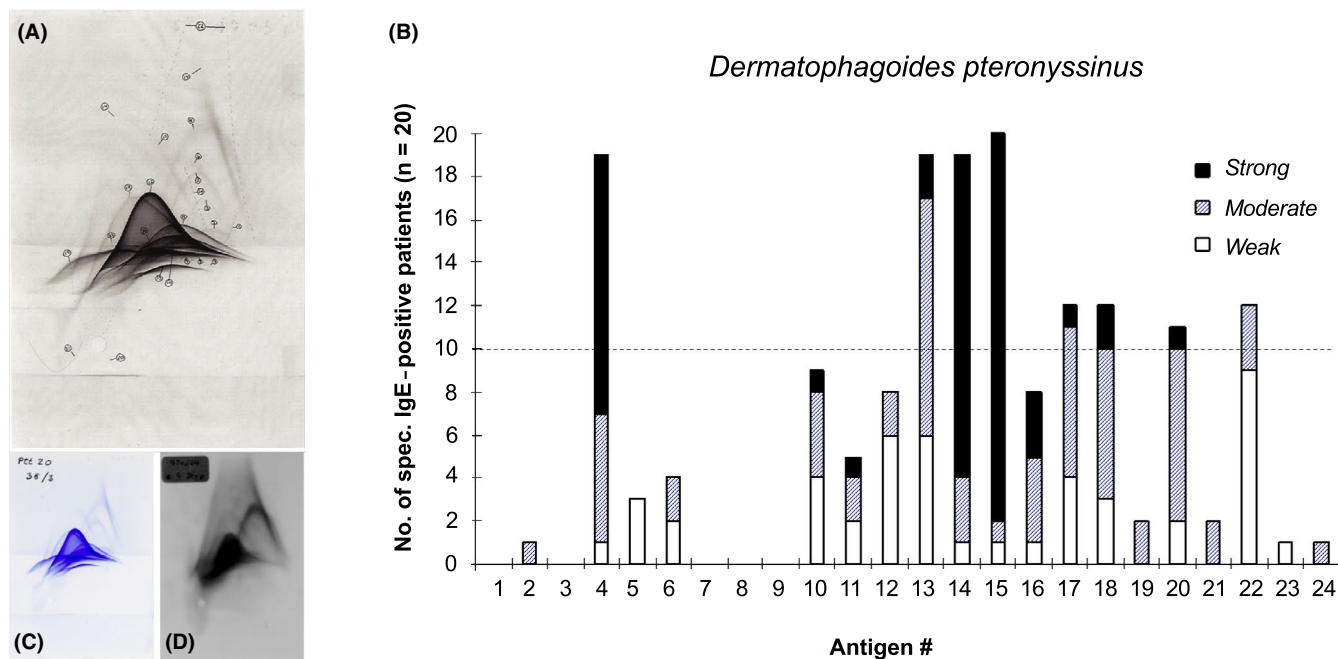


**FIGURE 1** *Dermatophagoides pteronyssinus* (Der p) reference CIE (A) and allergogram (B) based on CRIE (C/D). The IgE staining in D is directed toward multiple Der p allergens with antigen 15 (Der p 1) and antigen 4/14 (Der p 2) as the strongest for this patient serum [Colorfigure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIGURE 2** Henning Løwenstein enjoying a cup of coffee with members of his team in 1978 at The Protein Laboratory, University of Copenhagen. From left: Gitte Nordskov Hansen, Kirsten Billesbølle, Peter Lind, Kaj Danielsen, and Henning Løwenstein

responses toward extracts and individual allergens were reported by Løwenstein et al<sup>5</sup> increasing the knowledge about the immunology involved in the clinical effect of immunotherapy. The discussions about the mechanism of action also included long-term effects and whether the clinical effect could be predicted by immunological changes, which are still hot topics among scientists and clinicians. These and other similar trials further substantiated the early vaccine development, with the first standardized vaccines without alum introduced in the early 1970s and alum-based vaccines in the late 1970s in both Europe and the United States. Løwenstein reviewed allergy vaccines in 1991 with the general observation that "Allergen extracts derived from these sources will correspondingly contain 20-50 proteinic,

antigen constituents which can all be regarded as potential allergens," which is important to remember in modern vaccine development. All of these trials investigated immunotherapy toward individual allergen sources, which became the European immunotherapy tradition as opposed to the mixtures of allergens still used for immunotherapy in the United States. The clinical effect of these vaccines and allergen immunotherapy as such was heavily debated throughout the twentieth century, but the combined effort of the allergy community championed by Løwenstein and his friends moved the field forward toward today's discussions about evidence-based medicine for AIT.

The authors were introduced to the Løwenstein laboratory during the 1980s and early 1990s, and we have had the pleasure of working in a scientific environment with very high scientific standards, productive discussions, and lots of enthusiasm and energy, all hallmarks of Løwenstein's approach to science. Additional contributions from Henning Løwenstein to the allergy society are the SOSA meetings, CIA 2004 at Bornholm, and the Henning Løwenstein Research Award exemplifying his drive and the willingness to make an extra effort to bring people together for scientific discussions and to reward excellent contributions to the field.

## CONFLICTS OF INTEREST

The authors are employed by ALK, a pharma company producing allergy vaccines.

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