Aus der Klinik für Pädiatrie mit Schwerpunkt Pneumologie und Immunologie der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Evolution of the IgE response to Dermatophagoides pteronyssinus allergenic molecules in childhood

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

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1. ABSTRACT

1.1. English Abstract

Background: The molecular evolution of the IgE response to *Dermatophagoides pteronyssinus* (*D pteronyssinus*) and its relationship with clinical symptoms are still matters of debate.

Objectives: The study aimed at investigating the development of the IgE response to 12 molecules of *D pteronyssinus* and describing the associated risk factors and the clinical role.

Methods: 722 participants in the German Multicenter Allergy Study, a birth cohort started in 1990, were included in the study. Current allergic rhinitis related to mite allergy and asthma were defined according to clinical yearly interviews from the ages of 1 to 13 years and at 20 years. Sera collected at ages 1, 2, 3, 5, 6, 7, 10, 13, and 20 years were tested for sIgE to an extract of *D pteronyssinus.* The sera positive for sIgE ($\geq$0.35 kU/L) were further tested for the presence of IgE to 12 molecules of *D pteronyssinus* by means of microarray technology. Exposure to mites was determined by collecting house dust samples at age 6 and 18 months and evaluating the Der p 1 weight/weight concentration.

Results: Out of 722 participants, 191 (26.5%) had IgE positivity to the extract of *D pteronyssinus* at least once during the observation period. At age 20 years, the most frequently molecules of *D pteronyssinus,* recognized by IgE antibodies, in declining order of prevalence, were: Der p 2, Der p 1, and Der p 23 (defined as group A molecules; prevalence, >40%); Der p 5, Der p 7, Der p 4, and Der p 21 (group B, prevalence 15- 30%); and Der p 11, Der p 18, clone 16, Der p 14, and Der p 15 (group C, prevalence <10%). In the 97.6% of the cases, IgE response to *D pteronyssinus* molecules started as sensitization to group “A”, and can proceed to group “B” and eventually to group “C”. Patterns of broader polymolecular IgE sensitization were associated with earlier first detection of a sIgE positive response, parental hay fever, and higher exposure. A significantly higher risk of mite-related allergic rhinitis and asthma was observed in children with the broadest IgE sensitization stage (i.e., ABC) when compared with not sensitized ones. Sensitization to Der p 1 or Der p 23 during the pre-school age period was predictive of asthma after the 6 years of age.

Conclusions: The patterns of molecular IgE response against *D pteronyssinus* observed in the MAS cohort are extremely heterogeneous. During childhood in most, but not all sensitized participants, the IgE response expand progressively as sequential sensitization to groups A-B-C molecules in a process defined as “molecular spreading”. Atopic heredity and exposure during infancy are associated with IgE polysensitization to *D pteronyssinus* molecules, which is, in turn, correlated with current mite-related allergic rhinitis and current and future asthma. Results might be used to develop algorithms and strategies to prevent the evolution of IgE sensitization to *D pteronyssinus* into childhood allergy.

1.2. German Abstract

Hintergrund: Die molekulare Evolution der IgE-Antwort auf Dermatophagoides pteronyssinus (D. pt.) und ihre Beziehung zu klinischen Symptomen wird kontrovers diskutiert.

Zielsetzung: Ziel der Studie war es, die Entwicklung der IgE-Antwort gegen 12 Allergenmoleküle der Hausstaubmilbe D. pt., sowie deren klinische Bedeutung und Risikofaktoren zu untersuchen.

Methoden: 772 Teilnehmer der deutschen Multizentrischen Allergie Studie (MAS), eine Geburtenkohorte aus dem Jahr 1990, wurden in die Studie aufgenommen. Allergische Rhinitis auf Hausstaubmilben und Asthma wurden gemäß jährlicher klinischer Fragebögen im Alter von 1 bis 13 Jahren und 20 Jahren definiert. Seren, die im Alter von 1, 2, 3, 5, 6, 7, 10, 13 und 20 Jahren gesammelt wurden, wurden auf sIgE gegen Hausstaubmilbenextrakt getestet. Seren, die für sIgE (>0.35 kU / L) positiv getestet wurden, durchliefen einen Mikroarray-Test auf spezifisches IgE gegen 12 Allergenmoleküle der D. pt. Die Exposition gegenüber Milben wurde durch die Sammlung von Hausstaubproben im Alter von 6- 18 Monaten und die Beurteilung der darin enthaltenen Der p 1 –Konzentrationen bestimmt.

Ergebnisse: Von 722 Teilnehmern zeigten 191 (26,5%) mindestens einmal im Beobachtungszeitraum eine IgE-Antwort auf den Extrakt der D. pteronyssinus. Im Alter von 20 Jahren waren die am häufigsten erkannten Moleküle in abnehmender Prävalenz: Der p 2, Der p 1 und Der p 23 (definiert als Moleküle der Gruppe A, Prävalenz > 40%); Der p 5, Der p 7, Der p 4 und Der p 21 (Gruppe B, Prävalenz 15-30%); und Der p 11, Der p 18, Clone 16, Der p 14 und Der p 15 (Gruppe C, Prävalenz <10%). In 97.6% begann die IgE-Antwort gegen D. Pt.-Moleküle als Sensibilisierung gegen Moleküle der Gruppe "A", erweiterte sich in Teilen zur Gruppe "B", sowie in manchen Fällen zur Gruppe "C". Das Gesamtbild einer breiteren polymolekularen IgE-Sensibilisierung war mit einem früheren ersten Nachweis von sIgE, mit Heuschnupfen der Eltern sowie mit einer ausgeprägteren Exposition assoziiert. Ein signifikant höheres Risiko für allergische Rhinitis auf Hausstaubmilben und Asthma wurde bei Kindern mit der breitesten IgE-Sensibilisierungsstufe (d. H. ABC) im Vergleich zu nicht sensibilisierten Probanden beobachtet. Die Sensibilisierung gegen Der p 1 oder Der p 23 während der Vorschulzeit war prädiktiv für Asthma nach dem 6. Lebensjahr.

Schlussfolgerungen: Die verschiedenen molekularen IgE-Antworten auf D pteronyssinus, wie sie in der MAS-Kohorte beobachtet wurden, sind extrem heterogen. Während der Kindheit expandiert die IgE-Reaktion bei den meisten, jedoch nicht allen, sensibilisierten Teilnehmern, progressiv als sequentielle Sensibilisierung auf Moleküle der Gruppen A-B-C; ein Prozess, der als "molecular spreading" definiert wird. Die erbliche atopische Belastung und Allergenexposition im Kindesalter sind mit einer IgE-Polysensibilisierung gegenüber D.Pt.-Molekülen verbunden. Diese korreliert wiederum mit allergischer Rhinitis auf Hausstaubmilben und aktuellem, sowie zukünftigem Asthma. Auf der Basis dieses Wissens können möglicherweise Strategien zur Vermeidung des Fortschreitens einer IgE-Sensibilisierung hin zum klinischen Bild einer Hausstaubmilbenallergie im Kindesalter entwickelt werden.

2. AFFIDAVIT/ DECLARATION OF CONTRIBUTION

2.1. Affidavit

I, Daniela Posa, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic „Evolution of the IgE response to Dermatophagoides pteronyssinus allergenic molecules in childhood“ I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The section on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) corresponds to the URM (s.o) and are answered by me. My contribution in the selected publication for this dissertation corresponds to those that are specified in the following joint declaration with the responsible person and supervisor.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

_____ _____

Date Signature

2.2. Detailed Declaration of Contribution

2.2.1 Publication

Daniela Posa had the following share in the following publication:

Posa D, Perna S, Resch Y, Lupinek C, Panetta V, Hofmaier S, Rohrbach A, Hatzler L, Grabenhenrich L, Tsilochristou O, Chen KW, Bauer CP, Hoffman U, Forster J, Zepp F, Schuster A, Wahn U, Keil T, Lau S, Vrtala S, Valenta R, Matricardi PM.

Evolution and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens during the first 2 decades of life.

J Allergy Clin Immunol. 2017;139:541-549.e8. doi: 10.1016/j.jaci.2016.08.014. Epub 2016 Oct 25.

2.2.2 Contribution in Detail

Overview

The scientific publication selected for the present doctoral thesis was developed under the supervision of PD Dr. med. Paolo Maria Matricardi, leader of the Working Group "Molecular Allergology and Immunomodulation" in the “Department of Paediatric Pneumology and Immunology” of the Charité – Universitätsmedizin Berlin. Dr Matricardi closely supervised the whole project during daily meetings. The research was conducted in the context of the German Multicenter Allergy Study (MAS), a birth cohort study started in 1990, and it aimed at investigating the influence of genetic and environmental factors on the allergy development. Overall, 1314 newborns of five German cities and their families were recruited and followed-up until the age of 20 years at regular intervals for clinical data-collection, blood and skin tests, and lung function measurements.

In the aforementioned article, I identified and described the evolution and the clinical role of the IgE response to the *Dermatophagoides pteronyssinus.*

The work organized in the following phases:

- literature research;
- laboratory activity (with the technical assistance of Alexander Rohrbach);
- database and data analyses (carried out in cooperation with the statistician Serena Perena);
- preparation and publication of the manuscript;
- presentation of the results in meetings and publication of further articles.

Literature Research

I carried out a thorough literature research about the state of the art of the mite allergy.

I first focused on the technical aspects of mite molecular allergology: allergenic source and molecules, Singleplex IgE assay, composition of extracts, multiplex IgE assay, cross-reactivity. Then, I integrated the previous examination with the biological study of the *Dermatophagoides pteronyssinus*. This preliminary research phase paved the way for the following laboratory activity.

Subsequently, I studied the clinical aspects of mite allergy including allergic rhinoconjunctivitis, asthma and their risk factors

Afterwards, I examined the methodological and epidemiological aspects of the MAS birth cohort and of the Manchester asthma and allergy study (MAAS) and Children, Allergy, Milieu, Stockholm, Epidemiology (BAMSE) cohorts.

The results of this research were recently published in a scientific review article (i.e., Posa *et al*. 2017 [1]).

Laboratory Activity

During the laboratory activity, according to the inclusion criteria we defined, first, we selected the sera to be tested with microarray from the MAS database. Then, I sorted the corresponding probes in the laboratory from the MAS sera-databank.

Subsequently, I performed the IgE multiplex tests (developed by Thermo Fisher Scientific) accordingly the following steps:

- Binding of the IgE antibodies from the patients' samples to the biochip through a procedure of several consecutive washing and incubation stages.
- Scanning of the biochips with the laser-scanner (the laser-scanner Lux-Scan-10K/A produced by CapitalBio);
- Image analysis through the use of the software Phadia Microarray Image Analysis developed by Thermo Fisher Scientific®;
- Extraction of the raw and processed data;
- Database assembling and data management.

Moreover, since we handled an innovative biochip customized for research use only, during the laboratory activity, I worked with the scientific support of the manufacturer.

Database and Data Analyses

According to the study design, I selected the necessary clinical, serological and expositional data of the participants from birth up to 20 years of age. Consequently, after the approval of the MAS-principal investigators Prof Dr. Thomas Keil and Prof Dr. Susanne Lau, I obtained the corresponding variables from the global database of the cohort.

Then I merged the abovementioned data with the molecular IgE ones produced during the laboratory phase. The so-created aggregate database represented the basis for the subsequent analyses.

I preliminary conducted statistical data-analyses to find significant parameters to express the molecular IgE response. The results of such analyses were used to define the relevant parameters for the subsequent investigations and to improve the study methodology.

I, then, cooperated with Serena Perna, statistician of the working group, for the more complex statistical analyses. During periodical meetings we developed advanced statistical procedures and progressively outlined the tables and the figures of the manuscript.

[1] Posa D *et al.* Natural Evolution of IgE Responses to Mite Allergens and Relationship to Progression of Allergic Disease: a Review. Curr Allergy Asthma Rep. 2017;17:28.

Preparation and Publication of the Manuscript

During the writing process, I initially wrote the first draft of the manuscript, whose single sections were discussed with the other co-authors. Afterwards, I produced several revised versions to share the modification up to the definition of the exact contents with all the colleagues.

At the beginning of April 2016, I submitted the manuscript to the “Journal of Allergy and Clinical Immunology", managing all the formal submission process. As the article was initially accepted with revisions, I prepared the new version and an extensive and accurate reply to answer to the comments and queries of the reviewers. The final revised form was accepted at the end of August. After the acceptance, we received from the editorial office of the “Journal of Allergy and Clinical Immunology" the invitation to make known to the media the publication of the article.We cooperated with the Charité press office for producing a press release. The article was published on-line on the 25th October 2016. The article obtained in a few days more than 40 mentions on websites and newspapers in different countries. In February 2017, the article was finally published in the “Journal of Allergy and Clinical Immunology". The American Academy Allergy Asthma Immunology (AAAAI) cited the article and reported its summary in the “latest research” section on the AAAAI website. Currently, the article has been in cited in 7 scientific publications.

Meetings and Other Publications

During these years, I attended several seminars and meetings in the Charité – Universitätsmedizin. I presented the results of the study during the weekly Journal Club of the Department of Pediatric Pneumology and Immunology and during one of the monthly Comprehensive Allergy Center Charitè meetings. I also showed the progressive results of our project in several national and international meetings in German, English and Italian.

For this project, I obtained a scholarship at the European Academy of Allergy and Clinical Immunology (EAACI) Congress 2015 in Barcelona. Furthermore, two posters, showing the results of our project, were prized in the Junior Member and Affiliates (JMA) Poster Session at the EAACI Congress 2016 in Vienna, and at the EAACI Allergy School e-PAD in Moscow.

At the same time I was working on the project of my thesis, I participated as co-author to other scientific publications on molecular allergology: (see: LIST OF PUBLICATIONS).

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

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3. EXCERPT OF THE JOURNAL SUMMARY LIST

(ISI Web of Knowledge SM)

InCites Journal Citation Reports — Clarivate Analytics

Journal Data Filtered By: Selected JCR Year: 2016 Selected Editions: SCIE,SSCI Selected Categories: 'ALLERGY' Selected Category Scheme: WoS

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY	46,218	13.081	0.083210
2	ALLERGY	16,206	7.361	0.025050
3	Journal of Allergy and Clinical Immunology-In Practice	1,653	5.317	0.006190
4	CLINICAL AND EXPERIMENTAL ALLERGY	10,959	5.264	0.015390
5	CLINICAL REVIEWS IN ALLERGY & IMMUNOLOGY	2,403	5.263	0.005400
6	CONTACT DERMATITIS	5,712	4.335	0.004280
7	PEDIATRIC ALLERGY AND IMMUNOLOGY	3,787	3.775	0.006840
8	CURRENT ALLERGY AND ASTHMA REPORTS	2,071	3.735	0.005170
9	ANNALS OF ALLERGY ASTHMA & IMMUNOLOGY	6,970	3.728	0.008690
10	IMMUNOLOGY AND ALLERGY CLINICS OF NORTH AMERICA	1,463	3.610	0.002560
11	Current Opinion in Allergy and Clinical Immunology	2,861	3.463	0.006330
12	Clinical and Translational Allergy	636	3.239	0.002340
13	ALLERGOLOGY INTERNATIONAL	1,487	3.194	0.003270
14	JOURNAL OF INVESTIGATIONAL ALLERGOLOGY AND CLINICAL IMMUNOLOGY	2,073	3.094	0.002550
15	Allergy Asthma & Immunology Research	1,094	2.957	0.003140
16	Allergy Asthma and Clinical Immunology	850	2.869	0.002140
17	INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY	5,175	2.720	0.006600
18	ALLERGY AND ASTHMA PROCEEDINGS	1,937	2.614	0.003140
19	JOURNAL OF ASTHMA	3,201	1.746	0.005060
20	Postepy Dermatologii i Alergologii	418	1.683	0.000910
21	ALLERGOLOGIA ET IMMUNOPATHOLOGIA	954	1.439	0.001550
22	ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY	698	1.011	0.001040
23	Pediatric Allergy Immunology and Pulmonology	171	0.958	0.000640
24	Iranian Journal of Allergy Asthma and Immunology	457	0.812	0.000850
25	Revue Francaise d Allergologie	276	0.363	0.000130
26	ALLERGOLOGIE	187	0.311	0.000080

https://clarivate.com/products/journal-citation-reports/ 06. October 2017

4. THE SELECTED PUBLICATION

Posa D, Perna S, Resch Y, Lupinek C, Panetta V, Hofmaier S, Rohrbach A, Hatzler L, Grabenhenrich L, Tsilochristou O, Chen KW, Bauer CP, Hoffman U, Forster J, Zepp F, Schuster A, Wahn U, Keil T, Lau S, Vrtala S, Valenta R, Matricardi PM.

Evolution and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens during the first 2 decades of life.

J Allergy Clin Immunol. 2017;139:541-549.e8. Epub 2016 Oct 25.
https:/doi.org/10.1016/j.jaci.2016.08.014

5. CURRICULUM VITAE

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

6. LIST OF PUBLICATIONS

As first author:

1. **Posa D**, Pizzulli A, Wagner P, Perna S, Hofmaier S, Matricardi P M, *et al*. Efficacy and usability of a novel nebulizer targeting both upper and lower airways. Ital J Pediatr. 2017; 43:89 **(Impact Factor: 1.668)**
2. **Posa D**, Hofmaier S, Arasi S, Matricardi PM. Natural Evolution of IgE Responses to Mite Allergens and Relationship to Progression of Allergic Disease: a Review. Curr Allergy Asthma Rep. 2017; 17:28. Review. **(Impact Factor: 3.735)**
3. **Posa D**, Perna S, Resch Y, Lupinek C, Panetta V, Hofmaier S, *et al*. Evolution and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens during the first 2 decades of life. J Allergy Clin Immunol. 2017; 139:541-49. **(Impact Factor: 13.081)**

As co-author:

4. Mastrorilli C, **Posa D**, Cipriani F, Caffarelli C. Asthma and Allergic Rhinitis in childhood: what's new. Pediatr Allergy Immunol. 2016; 27:795-803. Review. **(Impact Factor: 3.775)**
5. Wert A F, **Posa D**, Tsilochristou O, Schwerk N. Treatment of allergic children - Where is the progress (for the practicing allergist)? Pediatr Allergy Immunol. 2016; 27:671-81. **(Impact Factor: 3.775)**
6. Matricardi P M, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, Aalberse RC, Agache I, Asero R, Ballmer-Weber B, Barber D, Beyer K, Biedermann T, Bilò MB, Blank S, Bohle B, Bosshard PP, Breiteneder H, Brough HA, Caraballo L, Caubet JC, Crameri R, Davies JM, Douladiris N, Ebisawa M, EIgenmann PA, Fernandez-Rivas M, Ferreira F, Gadermaier G, Glatz M, Hamilton RG, Hawranek T, Hellings P, Hoffmann-Sommergruber K, Jakob T, Jappe U, Jutel M, Kamath SD, Knol EF, Korosec P, Kuehn A, Lack G, Lopata AL, Mäkelä M, Morisset M, Niederberger V, Nowak-Węgrzyn AH, Papadopoulos NG, Pastorello EA, Pauli G, Platts-Mills T, **Posa D**, Poulsen LK, Raulf M, Sastre J, Scala E, Schmid JM, Schmid-Grendelmeier P, van Hage M, van Ree R, Vieths S, Weber R, Wickman M, Muraro A, Ollert M. EAACI Molecular Allergology User's Guide. Pediatr Allergy Immunol. 2016; 27 Suppl 23:1-250. **(Impact Factor: 3.775)**
7. Ahrens B, **Posa D**. Prävention von Allergien im Kindesalter – wo stehen wir heute? Allergologie, 2016; 39:145- 59 **(Impact Factor: 0.311)**
8. Comberiati P, Cipriani F, Schwarz A, **Posa D**, Host C, Peroni DG. Diagnosis and treatment of pediatric food allergy: an update. Ital J Pediatr. 2015; 19;41:13. **(Impact Factor: 1.668)**

7. ACKNOWLEDGMENTS

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I am grateful to Prof. Rudolf Valenta of the Medical University of Vienna and his working group for the precious and fruitful cooperation.

A big thank goes to Serena Perna for her methodical approach and her deep desire for knowledge. She performed the work with cleverness and accuracy.

I thank Laura Hatzler for the data she provided me, and all the other co-authors for the valuable contribution.

I want also to thank all my colleagues of the Molecular Allergology Working Group: Carla for her smartness and availability; Stefania for her encouragement; Alex for his fundamental support; Eissa for the wise suggestions; Steffi, Francesca, Alina, Bahar, Sveva, Petra, Marco, Xinyuan, for these incredible years.

'Old' friends because they followed my wonderful research adventure from everywhere and 'new' friends in Berlin for the wonderful time spent together.

A very special thank to Dr. Tiziana Susca for her expertise, honesty, and patience.

A special thank Alessandro and his family because they really trusted me and incited me to achieve my goals.

A final thank to my mother, my father, my brother and his family because they motivated me to never give up

"Cominciate col fare ciò che è necessario, poi ciò che è possibile. E all'improvviso vi sorprenderete a fare l'impossibile." Francesco d'Assisi

"Start by doing what's necessary; then do what's possible; and suddenly you are doing the impossible." Francis of Assisi

„Tu zuerst das Notwendige, dann das Mögliche, und plötzlich schaffst du das Unmögliche." Franz von Assisi