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Allergen component analysis as a tool in the diagnosis and management of occupational allergy

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ABSTRACT

We are now in the epoch of "molecular allergology" and numerous clinically relevant allergenic molecules are available improving the performance of *in vitro* allergen tests and allergen detection methods. This review is focusing on characterized occupational allergens and their implementation into the *in vitro* diagnosis for occupational allergy and in allergen detection methods.

More than 400 occupational agents are identified and documented as being 'respiratory sensitizers', but currently only a limited number of them are characterized on the molecular level and available for routine diagnosis as native or recombinant allergens. One exception, however, is natural rubber latex (NRL) from *Hevea brasiliensis* still remaining an important occupational allergen source. Characterization of 15 NRL allergens led to the development of assays for the determination of allergen content of NRL materials and the implementation of component-resolved diagnosis (CRD) for specific IgE antibody measurement. Microarray or singleplex using recombinant or native allergens are reliable tools for NRL allergy diagnosis. In addition, NRL allergy is an excellent model for improving extract-based specific IgE measurement by amplification of NRL extract preparation with stable recombinant major allergen rHev b 5. Despite the many efforts to characterize the occupationally relevant wheat allergens for baker's asthma, the most frequently occurring forms of occupational asthma, the results are highly diverse. Wheat sensitization profiles of bakers showed great interindividual variability and no wheat allergen could be classified as the major allergen. For diagnosis of baker's asthma, a whole wheat extract is still the best option for specific IgE determination. But single wheat allergens might help to discriminate between wheat-induced food allergy, grass pollen allergy and baker's asthma. For workplace-related allergens like coffee, wood, soybean, seafood and moulds allergens are characterized and few of them are available, but their relevance for occupational sensitization routes should be verified in the further studies.

1. Introduction

The most frequently reported occupational respiratory disease is occupational asthma (OA), with about 15–20% of the overall adult-asthma public burden, mainly associated with allergy respiratory sensitizers (de Matteis et al., 2017). Additionally rhinitis caused by work-related substances (so-called occupational rhinitis, OR) is also common, often associated or pre-existing with OA (Moscato et al., 2008). Therefore OR and OA present serious health problems in industrialized countries and the prevention, but also the diagnosis of these diseases represent a challenge. In the case of allergic OR and OA, respectively more than 400 occupational agents are identified and documented as being 'respiratory sensitizers' (Quirce and Bernstein, 2011; Cartier, 2015; Tarlo et al., 2017). These known workplace-related agents that may exacerbate or aggravate pre-existing rhinitis or asthma or induce OR or OA can be divided into high-molecular weight (HMW) and low-molecular weight (LMW) agents. Typical LMW substances are isocyanates, acid anhydrides, metals, ammonium persulfate, fumes arising from washing, bleaching and fixing agents used by hair dressers, disinfectants and medicinal drugs. In the case of an IgE-mediated mechanism it is generally assumed that the allergenicity of these LMW or metabolites of them based on a mostly covalent interaction with some carrier proteins to build a hapten-carrier complex. The most common occupational HMW agents are proteins or glycoproteins found in cereal

Abbreviations: CCD, cross-reactive carbohydrate determinant; CRD, component-resolved diagnosis; ELISA, enzyme linked immunosorbent assay; FEIA, fluorescent enzyme immunoassay; HCW, health care worker; Hev b, Hevea brasiliensis; IUIS, International Union of Immunological Societies; MW, molecular weight; NRL, natural rubber latex; nsLTP, non-specific lipid transfer protein; OA, occupational asthma; OR, occupational rhinitis; SB, spina bifida; Tri a, Triticum aestivum; WDEIA, wheat-dependent exercise-induced anaphylaxis; WHO, World Health Organization

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flour, livestock and laboratory animals, mites, fish and seafood, fodder and detergent enzymes, mould (fungi), *Hevea brasiliensis* latex and wood dust. So far only few of the HMW agents have been biochemically and molecular characterized or are produced in recombinant form, because most of the respiratory sensitizing properties of various occupational substances are being documented only as individual case reports. Based on this lack of knowledge of the allergen components and their allergenicity currently only a limited number of recombinant or native occupational relevant allergens are commercially available for the *in vitro* diagnosis. In this review the focus is on component-resolved diagnosis (CRD) with occupational allergens, their clinical significance and their implementation into the *in vitro* diagnosis for occupational allergy and in allergen detection methods.

2. Natural rubber latex

Natural rubber latex (NRL) allergy is a very good example of a 'new allergy' that suddenly arose in the late 1980s with tremendous health and economic implications, and also of an allergy which becomes history – especially in industrialized countries – in a relatively short time period based on successful primary prevention strategies by strict allergen avoidance (Raulf, 2014). The increased recognition of NRL allergy especially in the health care workers using NRL powdered gloves and spina bifida patients with operation on their first days of life initiated enhanced research on allergen characterization, quantification and improvement of allergy diagnosis. Therefore *Hevea brasiliensis*, the origin of NRL, is the best characterized occupational allergen source (Raulf and Rihs, 2017; Vandenplas and Raulf, 2017). Currently 15 allergens identified from *Hevea brasiliensis* NRL are identified and have been included in the official allergen list of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature database and assigned official numbers Hev b 1–15 (http://www.allergen.org) (Table 1). Most of the allergens are produced in recombinant form and several are available as singleplex (Raulf-Heimsoth et al., 2007a; Vandenplas et al., 2016) or multiplex tool (Ebo et al., 2010; Seyfarth et al., 2014; Chełmińska et al., 2016). These single allergens are useful for the determination of sensitization profiles. Studies demonstrated that various risk groups like patients with spina bifida (SB) and occupational latex exposed health care workers (HCWs) are sensitized by different NRL allergens based on the different route of exposure (direct blood contact versus inhalation) or as also shown in differences in the allergen levels measured between internal and external surfaces of NRL gloves (Peixinho et al., 2008). In the case of health care workers suffering from occupational latex allergy the most important NRL allergens are Hev b 5 and Hev 6.01 or Hev b 6.02, respectively. Other NRL allergens like Hev b 1 or Hev b 3 often recognized by specific IgE of spina bifida patients are only minor allergens in latex allergic health care workers (Raulf-Heimsoth et al., 2007a, b). Lamberti et al. (2015) confirmed the importance of Hev b 5 and Hev b 6.01 as clinical relevant in health care students with occupational exposure to latex for less than 5 years. They also found that the combination of Hev b 5, Hev 6.01 and Hev b 8 identified 92% of the latex-allergic subjects in their series. Positivity to rHev b 8 in their study was not an isolated IgE response and always associated with positivity to rHev b 6.01 and rHev b 5. Seyfarth et al. (2014) conducted a study to evaluate the diagnostic value of the ISAC® allergy chip in detecting latex sensitization with 40 sera from subjects with suspected NRL allergy and with positive skin prick test to NRL, positive NRL Western blots and positive results in the cellular antigen stimulation test (CAST). The sera were re-analyzed with the ISAC® chip and recombinant single NRL coupled on the ImmunoCAP. The ISAC® chip enables simultaneous determination of sIgE against five latex allergens with 20 µl sera. The authors summarized that the sensitivity of the ISAC® with respect to detection of NRL sensitization was lower compared to other methods and that the conventional ImmunoCAP k82 showed a superior sensitivity (Raulf, 2016). It is worth to mention, that NRL allergens such as Hev b 2, Hev b 6.02, Hev b 7, Hev b 8 and Hev b 12 have been discussed as being responsible for the latex-fruit cross-reactivity. In some cases, the use of recombinant single latex allergens for NRL-specific IgE mapping was helpful to discriminate between cross-reactivity and co-sensitization of latex and fruits (Raulf-Heimsoth et al., 2007b; Rihs et al., 2006). Especially in plant allergens like NRL or wood allergens (Kespohl et al., 2010) and also in insect venoms (Jappe et al., 2006) the presence of cross-reactive carbohydrate determinants (CCDs) can negatively influence the specificity of the *in vitro* diagnostic test. Therefore it is necessary to exclude glyco-epitopes (with low clinical relevance) responsible for IgE-binding. Corresponding CCD screening tools (e.g. horseradish peroxidase, bromelain, ascorbate oxidase) and/or inhibition testing can performed to clarify the origin of the IgE-binding to latex (protein epitopes versus glyco-peptides). Attention should be paid also in false-positive results with non-glycosylated recombinant allergens in patients with high levels of anti-CCD IgE antibodies (Hemmer et al., 2017). Hemmer and coworkers (Hemmer et al., 2017) demonstrated that cellulose used as solid-phase allergen carrier (e.g. Phadia ImmunoCAP platform) can contain varying amounts of CCDs responsible for false-positive results up to 2 kUA/L with non-glycosylated allergens. They recommended in addition to screen for anti-CCD and CCD inhibition easy-to-perform allergen-free dummy CAPs which are useful to identify sera with nonspecific background binding. But unfortunately, this allergen-free dummy CAP is so far not available. A

Table 1
Allergens of *Hevea brasiliensis* (para rubber tree latex) according to WHO/IUIS Allergen Nomenclature Sub-Committee.

Allergen	*Hevea brasiliensis* protein (and molecular weight (kDa))	Clinical relevance
Hev b 1[a]	Rubber elongation factor (14 kDa)	Major allergen in SB
Hev b 2	β-1,3-Glucanase (34 kDa)	Relevance under discussion[b]
Hev b 3[a]	Small rubber particle proteins (24 kDa)	Major allergen in SB
Hev b 4	Lecithinase homologue (53-55 kDa)	Minor allergen[b]
Hev b 5[a]	Acidic structural protein (16 kDa)	Major allergen in HCW and important in SB
Hev b 6.01[a]	Prohevein (20 kDa) (precursor of hevein Hev b 6.02, the major IgE binding domain)	Major allergen in HCW
Hev b 7	Patatin-like protein (esterase) from latex-B- and C-serum (44 kDa) (two isoforms: Hev b 7.01 and Hev b 7.02)	Minor allergen
Hev b 8	Profilin (actin-binding protein) (14 kDa) (several isoforms and variants)	Minor allergen
Hev b 9	Enolase (51 kDa)	Minor allergen
Hev b 10	Manganese superoxide dismutase (MnSOD) (26 kDa)	Minor allergen
Hev b 11	Class I chitinase (30 kDa)	Minor allergen
Hev b 12	Non-specific lipid transfer protein type 1 (nsLTP1) (9 kDa)	Minor allergen
Hev b 13	Esterase (42 kDa)	Relevance under discussion[b]
Hev b 14	Hevamine (30 kDa)	Minor allergen[b]
Hev b 15	Serine protease inhibitor (7.5 kDa)	Minor allergen

Legend: Hev b: *Hevea brasiliensis*; SB: spina bifida patients, HCW: health care workers.
[a] recommended for specific IgE antibody testing to verify clinical relevance of latex sensitization according to (Raulf and Rihs, 2017).
[b] not available in recombinant form; [adapted from (Raulf, 2016; Raulf et al., 2018)].