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Navigating through the Jungle of Allergens: Features and Applications of Allergen Databases

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Keywords

Allergens · Databases · Bioinformatics · Epitopes · Protein families

Abstract

The increasing number of available data on allergenic proteins demanded the establishment of structured, freely accessible allergen databases. In this review article, features and applications of 6 of the most widely used allergen databases are discussed. The WHO/IUIS Allergen Nomenclature Database is the official resource of allergen designations. Allergome is the most comprehensive collection of data on allergens and allergen sources. AllergenOnline is aimed at providing a peer-reviewed database of allergen sequences for prediction of allergenicity of proteins, such as those planned to be inserted into genetically modified crops. The Structural Database of Allergenic Proteins (SDAP) provides a database of allergen sequences, structures, and epitopes linked to bioinformatics tools for sequence analysis and comparison. The Immune Epitope Database (IEDB) is the largest repository of T-cell, B-cell, and major histocompatibility complex protein epitopes including epitopes of allergens. AllFam classifies allergens into families of evolutionarily related proteins using definitions from the Pfam protein family database. These databases contain mostly overlapping data, but also show differences in terms of their targeted users, the criteria for including allergens, data shown for each allergen, and the availability of bioinformatics tools.

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Introduction

Since the identification, cloning, and sequencing of the first allergens in the late 1980s, hundreds of allergens from all types of sources have been identified. In the light of the rapidly growing data on allergens, their sequences, biochemical features, IgE binding, and other clinical properties as well as their evolutionary and immunologic relationships, a demand for publicly available allergen data resources arose. The ubiquitous availability of the World Wide Web since the 1990s fostered the establishment of various freely accessible allergen databases. These databases contain mostly overlapping data, but also show differences in terms of their targeted users, the criteria for including allergens, data shown for each allergen, and the availability of additional tools such as sequence comparisons.

This review article will provide a critical overview of the features, strengths, and weaknesses of the six currently most widely used, freely accessible allergen databases.

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Table 1. Basic data of the most important allergen databases

Name	Address	First public release	Maintained by	Ref.
WHO/IUIS Allergen Nomenclature Database	www.allergen.org	2000	WHO/IUIS Allergen Nomenclature Sub-Committee	3, 7, 9
Allergome	www.allergome.org	2005	Allergy Data Laboratories, Latina, Italy	10, 11
AllergenOnline	www.allergenonline.org	2005	Food Allergy Research and Resource Program, Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE, USA	19
Structural Database of Allergenic Proteins (SDAP)	fermi.utmb.edu/SDAP/	2002	Sealy Center for Structural Biology, Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX, USA	26, 27
Immune Epitope Database (IEDB)	www.iedb.org	2006	National Institute for Allergy and Infectious Diseases, Bethesda, MD, USA	36, 38
AllFam	www.meduniwien.ac.at/allfam/	2007	Division of Medical Biotechnology, Department of Pathophysiology and Allergy Research, Medical University of Vienna, Austria	55

In addition, a few examples of research questions tackled by using these databases are provided. A summary of the most important features is shown in Tables 1 and 2. This article is confined to databases of protein allergens eliciting IgE-mediated (type I) allergies and does not cover allergens responsible for delayed-type (type IV) reactions.

Allergen Databases

World Health Organization/International Union of Immunological Societies Allergen Nomenclature Database

Originally, researchers were free in naming the allergens they had identified and each researcher used a different naming scheme. The first allergens identified in the 1970s and early 1980s received nonsystematic names such as antigen E (now Amb a 1) and Ra5 (now Amb a 5) from ragweed, allergen M (now Gad m 1 and Gad c 1) from cod, or cat-1 (now Fel d 1) from cat. In some instances, different groups even used different names for the same allergens. In order to establish an official, uniform naming system, the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-Committee was founded in 1984 under the auspices of the WHO and the International Union of Immunological Societies IUIS. It currently comprises 22 leading experts in allergen characterization, structure, function, molecular biology, and bioinformatics and is responsible for maintaining and developing a unique, unambiguous, and systematic nomenclature for all proteins that induce IgE-mediated allergies in humans.

The official allergen nomenclature was first published in 1986 [1] and revised in 1994 [2–6]. This still valid revised nomenclature is based on the Linnaean binominal nomenclature identifying the genus and species of all organisms. Allergen names are composed of the first 3–4 letters of the source's genus name, a space, the first 1–2 letters of the species name, a space, a number in most cases reflecting the order of identification in the respective source, a dot, and a 4-digit number specifying isoallergens and variants. Related allergen sequences from a species are subsumed as isoallergens of the same allergen if their sequences show greater than 67% identity. Variants of an isoallergen are defined as sequences with >90% identity. For a more detailed description of the nomenclature, the reader is referred to the original nomenclature publications and several more recent articles that provide updates on certain aspects of allergen nomenclature [7–9].

The list of officially recognized allergen names has been available on the World Wide Web since 2000 (www.allergen.org). Originally, it comprised a simple table with an associated list of references. Later it was developed into a fully functional database with browse and search func-

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Table 2. Features of the most important allergen databases

Name	Number of allergens[a]	Data included	Search tools	Frequency of updates	Remarks
WHO/IUIS Allergen Nomenclature Database	869 allergens 1,250 isoallergens and variants	Official allergen name, biochemical designation, source, isoallergens, literature (first description), links to external databases (literature, sequence, structure)	Search for allergen name, biochemical name, and source	Continuous	Official reference for allergen names and corresponding sequences; new allergens are included after review by the Allergen Nomenclature Sub-Committee
Allergome	3,104 allergenic molecules and their isoforms	Allergen name, biochemical function, isoallergens, allergen source, route of exposure, diagnostic reagents, links to sequences and structures, sequence homologs, cross-reactive allergens, allergenic properties and epidemiology, extensive bibliography grouped by subject area	Text search in all database fields, sequence comparison, sophisticated literature search	Continuous	Extensive collection of data on allergens, obtained from other databases and the literature; all allergens with published data are included, without filtering by relevance; not all allergen names are officially recognized
AllergenOnline	808 allergen groups 2,035 sequences	Allergen name, type (inhalant, food, etc.) and source, sequence, selected literature	Text search, different methods of sequence comparison	Annually	List of allergens reviewed by a panel of experts, aimed at providing a reliable database of relevant allergen sequences
Structural Database of Allergenic Proteins (SDAP)	1,526 allergens and their isoforms	Allergen name and source, protein family, selected literature, links to sequences and structures	Sequence comparison, peptide comparison, links to external servers (e.g., BLAST)	Unknown[b]	Peer-reviewed allergen sequence database with collection of bioinformatics tools; emphasis on allergen structures and epitopes
Immune Epitope Database (IEDB)	8,288 epitopes from 322 allergens[c]	T and B cell epitopes of protein and nonprotein antigens, including allergens, type of assay, source antigen and organism, host organism, type of disease	Search in all database fields (e.g., organism, host, type of assay, type of epitope); tools for epitope prediction	Quarterly	Largest resource of epitope data for all types of antigens
AllFam	1,018 allergens excluding isoforms	Allergen name with links to WHO/IUIS database and AllergenOnline, route of exposure, abstracts on protein families with selected literature	List members of allergen families (filtered by source and exposure), text search for allergens, sources, and protein families	Quarterly	Classification of allergens by protein family

[a] As of March 2, 2017. [b] No updates since 2013. [c] Excluding nonpeptide epitopes.

tionality and links to other databases. New entries are added to the database by the members of the Allergen Nomenclature Sub-Committee. In order to maintain a consistent allergen nomenclature that complies with the guidelines, researchers are required to submit newly described allergens to the Allergen Nomenclature Sub-Committee before submitting their manuscript to a journal for consideration for publication. Submissions are kept confidential, and no specific information other than the name of the new allergen, its biochemical name, and basic data on its allergenicity will be disclosed on the website before publication. The submission form is available at www.allergen.org. Submissions are reviewed by at least 2 committee members. In order to be included in the database and to receive an official allergen designation, submitted allergens have to fulfil the following criteria: (i) at least partial determination of their sequence and provision of an accession number for a nucleotide or protein sequence database (although the database contains a few old entries without an associated accession number), (ii) unambiguous iden-

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tification of its expression in its source on the mRNA or protein level, and (iii) binding of IgE from at least 5 sera of patients allergic to the respective source.

In March 2017, the WHO/IUIS allergen Nomenclature Database comprised 869 allergens with 1,250 isoallergens and variants. The record of each officially recognized allergen contains its name, biochemical names, obsolete names, source data (scientific and common species names, order, and source group), and basic information on its allergenicity, including a link to the publication containing its initial description. For newer entries, the name and affiliation of the submitter is published as well. In addition, each record contains data on isoallergens and variants including isoallergen number, peptide and nucleic acid sequences, molecular mass, allergenicity of recombinant isoallergens, as well as links to allergenicity and sequence references in PubMed, nucleic acid and peptide sequences in GenBank and UniProt, and structures in the Protein Data Bank (PDB). Allergens and isoallergens eliciting allergic reactions after ingestion are labeled as food allergens. In contrast, for all other types of allergens, no indication of the route of exposure is provided. Allergens recorded in the WHO/IUIS Allergen Nomenclature Database are the main data source for most other allergen databases such as AllergenOnline, SDAP, and AllFam.

Allergome

The Allergome database (www.allergome.org) was released in 2003 and is maintained by Allergen Data Laboratories, a company located in Latina, Italy [10, 11]. It collects all data on allergen sources and allergenic molecules from the literature and from other databases. Allergome is most likely the most comprehensive collection of allergen data. In March 2017 it contained data on more than 3,500 allergen sources and 3,000 allergens (www.allergome.org/script/statistic.php). In contrast to the official nomenclature, allergens are termed "molecules" while all records (allergens and sources) are collectively termed "allergens." Data entered into the database are not filtered by relevance, but instead each allergen record includes a so-called allergenicity score, which provides a quick overview of the extent of characterization of the allergen in terms of sequence, IgE binding and activity assays, skin tests, provocation tests, and epidemiologic data. In addition to established allergens, Allergome also lists proteins that are homologs of allergens but have not been identified as allergens so far or are derived from sources without allergological relevance. Those entries are labelled "in silico."

Each allergen record contains the allergen name, a link to the allergen record in the WHO/IUIS database for officially recognized allergens, and common names. For many allergens not yet recognized by the WHO/IUIS Allergen Nomenclature Sub-Committee, IUIS-like allergen names are provided. Nevertheless, it has to be stressed that those names are not, and possibly might never be, recognized by the Allergen Nomenclature Sub-Committee and may be changed if an official name is assigned to the respective allergen. Moreover, data on the allergen source are provided including scientific name, common names in multiple languages, pictures, and links to taxonomic databases such as those provided by the National Center for Biotechnology Information (NCBI) and UniProt. Biochemical data include biological function, a list of isoforms and variants with links to sequence records in UniProt, links to structural data in the PDB, and the tissue of expression of the allergen. Allergological data listed include the routes of exposure and a table of epidemiological data extracted from the literature, which lists the inclusion criteria of the tested patient cohort, the type of test, the number of patients, and the percentage of positive results. Each allergen record contains a comprehensive list of references classified by subject area such as biochemistry/structure/function, molecular biology, or immunochemistry/allergenicity.

Allergome may be searched either using a simple text search or an advanced search that enables the specification of the database fields to be searched in. The website also provides a large array of data mining tools that allow the extraction of additional data on well-characterized allergens. Records of allergens with a known sequence have a sequences tab that contains protein sequences of all isoallergens and variants. It also lists sequence motifs generated by an algorithm originally published by Stadler and Stadler [12] to classify allergens into families and to provide a tool to predict allergenicity based on sequences. The AllergomeAligner tool allows comparing sequences and motifs with either Allergome or UniProt by using different sequence similarity search tools. Allergen records also provide the Allergome O-Ring, a graphical representation of sequence similarities and IgE cross-reactivities within groups of related allergens. The Real Time Monitoring of IgE Sensitization (ReTiME) module is a database created to acquire and store geographically resolved epidemiologic data on allergen sensitization provided by collaborating allergy clinics. The allergen-related literature can be explored by the RefArray tool. The user can search the Allergome literature database by various criteria such as allergen names, thematic categories (e.g., bio-

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chemistry, immunology/allergenicity, etc.), and standard bibliographical categories such as year of publication, journal, and authors.

Allergome has been widely used by the scientific community. By 2014, it had been cited in 308 publications, according to the Allergome website (www.allergome.org/script/about.php#database). Only a few examples of recent studies are provided hereafter. First, the most basic application of Allergome is retrieving a comprehensive list of known allergens from a specific source. Dimitrov and Doytchinova [13] used Allergome to retrieve the sequences of 13 milk and egg allergens in order to simulate their gastrointestinal digestion and peptide-HLA-binding. They predicted 3 and 4 HLA-DR and DQ alleles binding to peptides from milk and egg allergens, respectively, while several alleles did not bind to allergen-derived peptides and were hence considered to be protective against the respective allergies. Second, Allergome includes a searchable sequence database. Hence, these data were used to compare newly identified allergens with known ones or to predict, based on homology, the presence of putative allergens in sequenced proteomes, transcriptomes, or genomes. Campbell et al. [14] analyzed the complete pollen proteome and transcriptome of Johnson grass, a subtropical species. By comparing transcriptome and proteome data as well as sequences of IgE-binding proteins identified by mass spectrometry with Allergome, the authors showed that Johnson grass contained members of all pollen allergen families previously identified in other subtropical grass species, while they lacked the group 5 and 6 allergens that are restricted to pollen of temperate grasses. Third, Allergome has a unique feature in that it stores data on allergen sources including those without identified allergens. We used these data to compare the taxonomic distributions of all plant species known to elicit pollen allergy with those containing identified allergens [15]. While 178 pollen allergen sources were listed in Allergome, allergens were identified only in 52 species. Allergen data were lacking particularly for tropical and subtropical plants. These results may direct future research to plant families with a growing impact as sources of allergenic pollen given the increasing prevalence of allergy in developing countries and the effect of climate change on the geographic distribution of allergenic plants [16].

AllergenOnline

One part of the premarket safety assessment for genetically modified organisms is an evaluation of their allergenic potential as compared to their wild-type counterparts. The prototypical case of an allergenic 2S albumin introduced into genetically modified soybeans intended for animal feed (which, consequently, were never introduced into the market) fostered the development of guidelines for allergenicity assessment of novel foods [17]. These involve screening the sera of allergic patients, testing the newly introduced proteins for resistance to gastrointestinal digestion, animal studies, and bioinformatics evaluation of the similarity of newly introduced genes to known allergens [18].

The AllergenOnline database (www.allergenonline.org) was set up in 2005 by the Food Allergy Research and Resource Program in the Department of Food Science and Technology at the University of Nebraska in Lincoln, NE, USA. Its aim is providing a peer-reviewed database of allergen sequences to be used for allergenicity assessment of proteins introduced into genetically modified foods [19]. The rationale behind using sequence comparisons with known allergens is the assumption that proteins with a significant sequence similarity with allergens may also show cross-reactivity with allergen-specific IgE or T cells and thereby elicit allergic symptoms. AllergenOnline collects putative allergen sequences from the NCBI protein database, supplemented by data from the WHO/IUIS Allergen Nomenclature Database and Allergome. On the basis of peer-reviewed publications, a panel of expert reviewers then filters the list. Sequences are included in AllergenOnline only if there is clear evidence that the protein binds IgE from sera of patients allergic to the respective source and the protein is expressed in the relevant tissue of the source. The reviewer panel currently comprises 8 researchers. Details on the evaluation process can be found on the AllergenOnline website (http://www.allergenonline.org/about.shtml). AllergenOnline is updated annually. The current version 17 of January 2017 contains 2,035 sequences.

The AllergenOnline user interface includes a list of all entries that can be filtered for search terms in all database fields. For each sequence, the species (scientific and common name), the WHO/IUIS allergen designation (if available), the type of allergen, which is composed of the route of exposure and the source group (e.g., aero mite, food plant), the allergen group, the sequence length, and a link to the sequence entry in the NCBI protein database are listed. Allergen groups contain related allergens from the same species or genus with greater than 67% sequence identity. Groups defined in AllergenOnline therefore correspond mostly, but not always, with allergens as defined by the species-based WHO/IUIS nomenclature. Each group is linked to a list of references and sequence entries.

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The main purpose of AllergenOnline is to provide an allergen sequence database for sequence comparisons. Users can compare their sequences with the database using 3 methods: a standard similarity search using the FASTA program [20], a FASTA search with all possible 80 residue fragments of the query sequence, and a search for identical peptides of 8 residues in length. The latter 2 methods were recommended by regulatory authorities for testing transgenes [21], despite doubts about their scientific validity [18]. AllergenOnline was used to assess the allergenicity risk of the Cry proteins encoding *Bacillus thuringiensis* toxins frequently introduced into crops to render them resistant to insects [22] and of genes introduced into Indian mustard for pollination control [23]. In both cases, no significant similarity with known allergens was found. Moreover, AllergenOnline has been used by researchers as a reliable source of allergen sequences in various applications such as mass spectrometry-based quantification of known allergens [24] or finding homologs of newly identified allergens [25].

Structural Database of Allergenic Proteins

The Structural Database of Allergenic Proteins (SDAP; fermi.utmb.edu/SDAP/) was established in 2002 by the Sealy Center for Structural Biology, Department of Biochemistry and Molecular Biology at the University of Texas Medical Branch in Galveston, TX, USA [26, 27]. It comprises a database of allergen sequences, structures, and epitopes, and a collection of bioinformatics tools to analyze allergens and their similarities. It compiles allergen data from the WHO/IUIS Allergen Nomenclature Database supplemented by non-IUIS allergens retrieved from sequence (UniProt, NCBI, Protein Information Resource), structure (PDB), and literature (PubMed) databases. New data are entered by the SDAP team and checked by an external scientific advisory board that currently comprises 3 researchers. However, no formal criteria for including allergens have been published. SDAP was updated regularly until 2013. However, as of March 2017, the website specified February 2013 as the date of the latest update.

Allergens in SDAP are classified into 2 subsets. SDAP All contains all allergens, while SDAP Food is restricted to food allergens. All search functions can be used with both database versions. The database contents may either be browsed as an alphabetical list of allergens or searched using a text search for allergen names, sources, or allergen descriptions, optionally restricted to WHO/IUIS-approved allergens. Each allergen record lists the allergen name and type (e.g., food, mites, weed pollen), the status of approval by the WHO/IUIS Allergen Nomenclature Sub-Committee, and common names of the allergens (designated "keywords"). Moreover, the scientific and common names of the source are given. Furthermore, the record contains links to sequence databases (NCBI, UniProt, PIR), protein family databases (Superfamily, InterPro, Pfam), and key references. For allergens with known structures, a link to the PDB entry is provided. Otherwise, automatically generated homology models are provided if homologous structures exist [28]. For allergens with known epitopes, a list of epitopes with associated references is provided.

Each allergen entry includes links to various tools for sequence analysis, as there are prediction of cleavage sites for proteases or chemicals by PeptideCutter [29], search for similar sequences in SDAP using FASTA and BLAST, similarity searches against the NCBI and UniProt protein databases [30], and a search of the Prosite database for the presence of sequence motifs characteristic of protein families or functional sites [31]. For allergens classified into a protein family by comparison with the Pfam database [32], the user can view a list of SDAP allergens belonging to the same family.

Allergen sequences in SDAP can be searched by various tools. Similarity of user-provided sequences to known allergens can be tested using the methods recommended by the Food and Agriculture Organization of the United Nations (FAO) and WHO [21] by using either a full FASTA search, a FASTA search using an 80-mer sliding window, or a search for short stretches of identical sequences. Furthermore, users can compare peptide sequences (e.g., epitopes) with sequences in SDAP to identify allergens with identical sequences or sequences with predicted similar physicochemical properties and possible cross-reactivity using the Property Distance (PD) index [27]. The connection between the PD index and IgE cross-reactivity was validated experimentally by testing sera from patients allergic to the major mountain cedar pollen allergen, Jun a 1 [33]. Peptides corresponding to 3 major IgE epitopes of Jun a 1 were tested for IgE binding together with peptides with varying sequence similarities and hence PD indexes compared with the wild-type epitopes. The authors showed an association of IgE cross-reactivity with sequence similarity expressed as PD index and concluded that the PD index is suitable for predicting cross-reactivity.

SDAP has been used as a source of allergen sequences for various bioinformatics applications. In a study aiming at predicting allergenicity of proteins, Kumar and Shelokar [34] used a machine learning approach to distinguish

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allergenic from nonallergenic proteins. Their software classified proteins based on several sequence-related features. Using allergen sequences from SDAP as a test data set, they achieved an accuracy of 90% for correctly predicting allergenic and nonallergenic proteins. Similarity searches against SDAP can be used to predict the allergenicity of proteins. In a bioinformatics, biochemical, and immunologic analysis, Sharma and coworkers assessed the allergenicity of osmotin, a pathogenesis-related protein from tobacco involved in resistance to abiotic stress [35]. The gene encoding osmotin was aimed to be inserted into transgenic crops to increase their stress tolerance. However, it turned out that the protein showed considerable sequence similarities to allergenic members of the thaumatin-like protein family and also bound IgE from sera of patients allergic to apple or tomato.

Immune Epitope Database

The Immune Epitope Database (IEDB; www.iedb.org) was established in 2004 by an international team of experts and became available to the public in 2006. It is funded by a grant from the National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA [36]. The IEDB is most likely the most comprehensive resource of data on experimentally determined epitopes. The database covers peptide (both linear and conformational) and nonpeptide epitopes of adaptive immune receptors (antibodies, T-cell receptors, and major histocompatibility complex proteins) of humans and nonhuman animals. It comprises epitopes related to infectious diseases, allergy, autoimmunity, and transplantation. Epitopes of the human immunodeficiency virus (covered by the Los Alamos HIV Molecular Immunology Database; www.hiv.lanl.gov) and cancer-related epitopes are not within the scope of the IEDB.

Data entered into the IEDB are either directly submitted by the researchers or – in the majority of cases – extracted from the literature using a combination of automated searches in PubMed with manual curation of the retrieved publications by a team of expert curators following detailed guidelines specified in a comprehensive curation manual [37]. The core of IEDB comprises data on experiments describing recognition of an epitope or antigen by T cell receptors, antibodies, or MHC molecules. All experimental assays that characterize the epitope or its recognition by immune receptors are entered into the IEDB, including all negative data. These data are linked to the reference they were extracted from, the source antigen, organism from which the epitope was derived, the host organism, the disease related to that epitope, and the exposure or immunization procedure. In March 2017, the IEDB contained data on nearly 280,000 epitopes from 3,600 source organisms that were extracted from more than 18,000 publications.

The search form on the IEDB homepage presents the user with the most frequently used search criteria. Users may search all epitopes in the database for epitope type (linear, conformational, nonpeptide), type of assay (B cell, T cell, MHC), MHC restriction, antigen and source organism, host organism, and type of disease (infectious diseases, allergy, autoimmunity, transplantation). All search fields have an autocomplete function to facilitate quick identification of potentially ambiguous names, such as organism names or antigens. Search results are displayed in 4 tables showing lists of epitopes, antigens, assays, and references. The results can be refined by including additional criteria or removing existing ones. The initial search criteria may be further restricted. For instance, one can specify the exact type of assay (e.g., searching only for ELISA results or 3D structures of antibody-antigen complexes), the exact MHC restriction or a more restricted type of disease (e.g., asthma instead of all allergic diseases). The search fields are associated with a Finder tool that displays a list of hierarchically classified search terms to select from (e.g., 3D structure has subcategories such as crystallography or NMR). Search results for specific antigens can be analyzed using the Immunone Browser tool. It generates a plot showing the number of positive and negative assays as well as the frequency of positive responses of each residue in the sequence of the antigen. Location of epitopes can be visualized by the Epitope Viewer Tool, a Java application to display 3D structures. The IEDB website also hosts various tools for epitope prediction and analysis, which can also be applied to search results.

In a review article, applications of the IEDB in allergy research were outlined [38]. As of March 2017, the IEDB contained data on more than 8,000 peptide epitopes of more than 300 allergens. Epitope data are crucial for various areas of allergy research and development of clinical applications. Epitope-based diagnosis may provide more accurate results in terms of correlation with severity of symptoms [39], prediction of clinical cross-reactivity [40], and prognosis of the outgrowth of pediatric food allergies [41]. Peptide-based allergen-specific immunotherapy may be performed with immunodominant T-cell epitopes [42, 43], non-IgE-binding B-cell epitopes [44], or mimotopes, peptide mimics of conformational IgE epitopes [45], as vaccines. Knowledge of IgE epitopes is also instrumental in designing hypoallergenic allergen

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derivatives [46–48]. Moreover, following the development of IgG and IgE epitope specificities during treatment may predict the outcome of classical extract-based immunotherapy [49, 50]. Characterization of the profile of epitopes from a specific allergen source recognized by T cells may help in understanding the dynamics of the allergic immune response and identifying biomarkers or therapeutic targets for specific immunotherapy. In an immunoproteomics approach, Schulten et al. [51] found a surprisingly high proportion of T_H2 cell recognition of peptides from non-IgE-binding timothy grass proteins in grass pollen allergic patients. Moreover, recognition of some of those novel T-cell antigens correlated with outcomes of grass pollen-specific immunotherapy.

AllFam – The Database of Allergen Families

After the identification of hundreds of allergens from dozens of sources, it became clear that many allergens were evolutionarily related and showed similarities in their sequences, structures, physicochemical properties, and biochemical functions. The advances in bioinformatics enabled the classification of allergens into protein families, which was performed by several researchers using different methods, but reaching similar conclusions [12, 15, 52–54]. It turned out that most allergens belonged to a surprisingly small number of protein families. Nevertheless, it has to be kept in mind that most members of allergen-containing protein families are nonallergenic. The demand of many allergy researchers and clinicians for a simple interface to the protein family classification of allergens prompted the author of this article to establish the AllFam database of allergen families in 2007 (www.meduniwien.ac.at/allfam/; [55]).

AllFam was originally based on allergen data from Allergome. In 2016, the database underwent a major relaunch. AllFam now gets its allergen data from the WHO/IUIS Allergen Nomenclature Database supplemented by non-IUIS entries from AllergenOnline. Allergen sequences are classified into protein families by comparing them with family definitions from the Pfam database (pfam.xfam.org). Pfam is one of the largest protein family databases, with its release 30.0 of June 2016 containing 16,306 entries [32]. Pfam families are based on manually curated sequence alignments of representative family members.

The AllFam release of September 22, 2017 classifies allergens into 151 AllFam families that are based on 216 Pfam domains. AllFam families are created by merging several Pfam domains to ensure that each allergen is a member of only a single AllFam family and that all related allergens are grouped into the same family. This applies to constituent Pfam domains of multidomain proteins and multiple Pfam families representing highly divergent protein families that cannot be covered by a single Pfam family.

The user interface of AllFam comprises a database browsing function by listing allergen families filtered by allergen source group (plants, animals, fungi, bacteria), route of exposure (inhalation, ingestion, etc.) and approval by the WHO/IUIS Allergen Nomenclature Sub-Committee. Allergenic members of each family can be listed and filtered using the same options. Furthermore, the database can be searched for allergen names, sources, protein family names, protein family accession numbers (both from AllFam and Pfam), and keywords. Each allergen is linked to the respective records in the WHO/IUIS database and AllergenOnline. Each AllFam family has its family description containing short summaries of the biochemical properties and the allergological significance of its allergenic members, a list of selected key references, and links to the corresponding Pfam records and Wikipedia entries.

The protein family classification in AllFam can be used to shed light on the question of which factors are responsible for the allergenicity of certain proteins. We compared the number of Pfam domains found in allergens with an equally sized sample of randomly selected sequences and showed that the number of protein families that contained allergens was significantly lower than that in random samples [55]. This supported the view that allergens have distinct features and not every protein can become an allergen. Moreover, a biochemical classification of allergens by linking Pfam families with the Gene Ontology Database [56, 57] revealed an even more restricted distribution of allergens. The majority of allergens were hydrolases (proteases, lipases, and polysaccharide hydrolases), metal- or lipid-binding proteins, storage proteins, or components of the cytoskeleton [55]. AllFam was also used to analyze sequence similarities of allergens with parasite antigens. Tyagi et al. [58] compared Pfam protein family distributions of allergens and proteins from metazoan parasites. They revealed that nearly half of the more than 2,000 parasite proteins analyzed were members of the 10 most important families of allergens as extracted from AllFam. They concluded that the IgE response, having evolved under the selective pressure of parasite infestation, may be diverted to certain nonparasite proteins as a consequence of their similarity to parasite antigens. In a follow-up study, the same group tested this hypothesis by analyzing 13 recombinant *Schistosoma mansoni* proteins for binding of antibodies from *S. man-*

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soni-infected patients' sera [59]. All 9 proteins predicted to be antigenic based on their membership in allergen families from AllFam bound IgE from at least 10% of the tested sera. In contrast, only 2 of the 5 tested abundant *S. mansoni* proteins without allergenic homologues bound IgE. Interestingly, homologues of one of those IgE-binding antigens, aldolases, were recently described as cross-reactive allergens in fish and chicken meat [60].

Concluding Remarks

The 6 databases discussed in this review are the most widely used resources of allergen data. The WHO/IUIS database is the source of the official allergen nomenclature. A large proportion of the allergens included in other databases are derived from the WHO/IUIS database. Allergome is the most comprehensive database providing links to many other resources and powerful literature search capabilities. Allergome does not apply strict inclusion criteria for allergen, hence it is up to the user to judge the allergenic significance of proteins recorded in Allergome. The focus of AllergenOnline is providing a peer-reviewed allergen sequence database as a resource for prediction of allergenicity. SDAP focuses on allergen structures and epitopes, and includes links to many other databases and bioinformatics tools. However, SDAP appears to have ceased updates in 2013. The IEDB is the largest resource of epitope data from all fields of immunology, including allergy. Allfam provides a simple interface to explore the protein family classification of allergens.

In addition to the resources described above, there are other, less popular databases available, such as the Informall Food Allergen Database (research.bmh.manchester.ac.uk/informall/allergenic-foods/; [61]), the Allergen Database for Food Safety (allergen.nihs.go.jp/ADFS/), and AllAllergy (allallergy.net). Even more databases have been established in the past but no longer exist. The still existing databases are widely used and appreciated by the scientific community, but have to cope with various challenges. In the light of the ever-increasing rate of publication of new papers related to allergens, it is getting more demanding to keep up with timely updates and ensure adequate data quality. Maintainers of the databases will most likely rely to a greater extent on automated data mining and text mining software. Nevertheless, manual curation by experts in molecular allergology will still be indispensable. Moreover, more resources have to be diverted to technical experts to keep up with increasing Internet security demands and to create intuitive user interfaces that enable also scientists with little experience in bioinformatics to retrieve specific information from an ever-growing amount of data.

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