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Review article

Autoantigenomics: Holistic characterization of autoantigen repertoires for a better understanding of autoimmune diseases



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ABSTRACT

Autoimmune diseases are mostly characterized by autoantibodies in the patients' serum or cerebrospinal fluid, representing diagnostic or prognostic biomarkers. For decades, research has focused on single autoantigens or panels of single autoantigens. In this article, we advocate to broaden the focus by addressing the entire autoantigen repertoire in a systemic “omics-like” way. This approach aims to capture the enormous biodiversity in the sets of targeted antigens and pave the way toward a more holistic understanding of the concerted character of antibody-related humoral immune responses. Ongoing technological progress permits high-throughput screenings of thousands of autoantigens in parallel, e.g., via protein microarrays, phage display, or immunoprecipitation with mass spectrometry. We argue that the time is right for combining omics and autoantibody screening approaches into “autoantigenomics” as a novel omics subcategory. In this article, we introduce the concept of autoantigenomics, describe its roots and application options, and demarcate the method from related holistic approaches such as systems serology or immune-related transcriptomics and proteomics. We suggest the following extendable method set to be applied to autoantigen repertoires: (1) principal component analysis, (2) hierarchical cluster analysis, (3) partial least-square discriminant analysis or orthogonal projections to latent structures discriminant analysis, (4) analysis of the repertoire sizes in disease groups and clinical subgroups, (5) overrepresentation analyses using databases like those of Gene Ontology, Reactome Pathway, or DisGeNET, (6) analysis of pathways that are significantly targeted by specific repertoires, and (7) machine learning approaches. In an unsupervised way, these methods can identify clusters of autoantigens sharing certain functional or spatial properties, or clusters of patients comprising clinical subgroups potentially useful for patient stratification. In a supervised way, these methods can lead to prediction models that may eventually assist diagnosis and prognosis. The untargeted autoantigenomics approach allows for the systematic survey of antibody-related humoral immune responses. This may enhance our understanding of autoimmune diseases in a more comprehensive way compared to current single or panel autoantibodies approaches.

1. Introduction

Autoimmune diseases comprise ≥ 80 diseases that share the common definition of targeting the patient's own body in an immune-

mediated manner [1]. Although individually rare in most cases, autoimmune diseases may be prevalent in 7.6–9.4% of the population [2], many of which are assumed to be increasing in frequency in the industrialized countries [1]. Autoimmune diseases are frequently

Abbreviations: CSF, cerebrospinal fluid; ELISA, Enzyme-linked immunosorbent assay; IP + MS, Immunoprecipitation and mass spectrometry; SLE, Systemic lupus erythematosus; SERPA, serological proteome analysis; MS, Mass spectrometry; ANA, Antinuclear antigen; TLR, toll-like receptor; PLS-DA, Partial least-square discriminant analysis; OPLS-DA, orthogonal projections to latent structures discriminant analysis; PCA, Principal component analysis; HCA, Hierarchical cluster analysis

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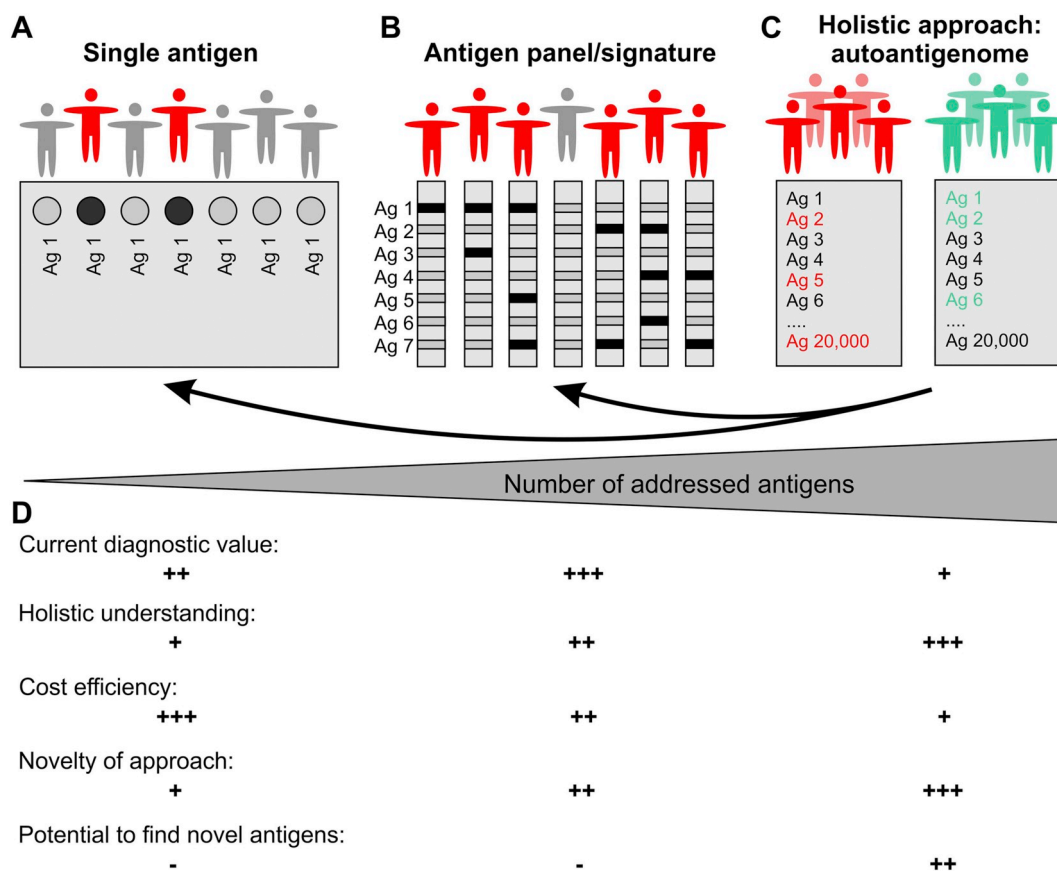


Fig. 1. Three simplified types of autoantibody-based approaches from single candidate to systemic approaches. A, Reactivity with single autoantigens defines a subset of patients. B, Individual reactivities with a panel of autoantigens define one or more subsets of patients. C, Individual or group-wise reactivities with thousands of bait proteins. The set of targeted autoantigens defines the autoantigenome. A-C, Arrows indicate that single or panels of autoantigen candidates can result from the autoantigenome approach. D, Evaluation of five characteristics of the three approaches. Ag: antigen.

characterized by autoantibodies in the patients' serum or cerebrospinal fluid (CSF), representing biomarkers with diagnostic and potentially even prognostic value [3–9].

While research has successfully focused on single autoantigens for decades, a newer perspective is addressing the *repertoire* of targeted antigens. Two intriguing articles have led us to consider whether larger parts of entire repertoires of autoantibodies may play (patho)physiological roles: Firstly, a diversity of 180 different autoantibodies have been found in systemic lupus erythematosus (SLE) patients making it the autoimmune disease with the largest number of reported autoantibodies to date, described by the authors as a “volcanic explosion of autoantibodies” [10]. At least partial correlation with disease severity was assumed for 122 of them. Secondly, Meyer and colleagues found that their cohort of 81 autoimmune regulator-deficient patients collectively harbored antibodies toward a surprisingly high number of > 3700 human proteins, comprising a subset of presumably protective autoantibodies [11]. Hence, the time might be right for the community to think more holistically about autoantigen repertoires, in addition to the single antigen centric way.

2. From single-antibody to holistic approaches

In 2013, Cohen stressed the importance of implementing a “systems immunology”, wondering “how to turn information into understanding” [12]. In 2009, Plebani and colleagues addressed the question of extending the “omics” field to the autoimmune domain, stating that “the ultimate aim, for research and clinical purposes is, to study the entire autoimmune process rather than its individual components” [13]. This holistic perspective is met by the recent technological

progress enabling high-throughput screenings for thousands of autoantigens in parallel, e.g., via peptide [14] or protein microarrays [15–17], immunoprecipitation and mass spectrometry (IP + MS) [18,19], or phage-display assays [20]. This unleashes options for combining omics and autoantibody screening approaches to a novel omics subcategory: autoantigenomics.

In our eyes, there are vague margins between the single-antigen or multiple-antigen concept on one side and the holistic concept capturing the autoantigenomic approach on the other side. So far, the terms in the literature are not clearly defined by the community. We suggest three rough categories (Fig. 1):

2.1. Single autoantigen approach

Autoantibodies against single antigens have been known and analyzed as biomarkers or pathogenetic agents for decades (Fig. 1A). The first ones were discovered in the 1940s as lupus erythematosus cell-inducing factor or “Rheumatoid factor”, albeit the antigens were not known at that time [21]. Promising examples of autoantibodies are reported to be detectable before disease onset, making them powerful tools for diagnostics [22]. Some of the most prominent autoantigens may be NMDAR [23] and SS-A/Ro [24]. Autoantibodies against those and other antigens have a solid diagnostic value and are increasingly used in clinical routines. Highly standardized and cost-effective methods, such as enzyme-linked immunosorbent assay (ELISA)-based methods, immunofluorescence assays, or cell-based assays, are commonly used to detect those single antibodies in sera and CSF (Fig. 1D).

2.2. Autoantigen panel/signature approach

The terms “panels” [25–27], “patterns” [28], “profiles” [12,25–27,29,30], “signatures” [25,28,31,32], or “parallel sets” [33] are often synonymously used for a set of single antibodies that can be detected in parallel (Fig. 1B). Parallelization is realized for example by highly standardized line immunoassays [34]. Due to parallel testing, the diagnostic value – but also the costs – are higher than for addressing single antibodies (Fig. 1D). Also sets of not yet diagnostically used but suggested antibody candidates resulting from quasi-untargeted approaches, such as protein microarrays, are frequently called signatures in the literature. One cause of vague discrimination between the single and multiple autoantigen approach is the common designation of entities like the nucleus or mitochondria as “antigens”. However, autoantibodies against such entities, like the “antinuclear antibodies” (ANAs), are mostly targeting a panel of different nuclear antigens [35].

2.3. The autoantigenomic approach

This approach does not address single nor panels of autoantibodies. Instead, it addresses the repertoire of autoantibodies in an “omics”-like view (Fig. 1C). This means that the repertoire is analyzed holistically, i.e., with the goal to find systemic differences instead of single/multiple candidates. The direct diagnostic value of such an approach may be currently still lower than that of single or panel approaches. However, this novel approach allows for finding clusters of autoantigens and patients sharing similar patterns. Clusters of autoantigens sharing certain functional or spatial properties offer an understanding of the antibody repertoires in a more comprehensive way. For example, over-represented cellular components or pathways being targeted by a set of autoantibodies may be identified. Clusters of patients may lead to the identification of subgroups in an apparently homogeneous disease, which might support stratification or treatment prediction. At the same time, autoantigenomic approaches can be the starting point for identifying novel single or panel candidates. A downside of autoantigenomics is the higher experimental cost compared with the single and panel approaches.

3. The concept of autoantigenomics

The terms “autoantigenome” or “autoantigenomics” have been used before. For example, the group of Joshua LaBaer has “expanded the current [type 1 diabetes] autoantigenome” using protein microarrays [36]. Meinke et al. defined the “antigenome” for bacterial antigens [37]. Yang and Bloch [38] have used the term “autoantigenomics” for screening sera from patients with anti-P-body autoantibodies using protein microarrays resulting in the identification of a novel P-body protein. However, all of these examples used the term for the identification rather than the holistic characterization of the set of autoantigens.

The concept of autoantigenomics hypothesizes that there may be systematic differences in the sets of targeted antigens between patients and controls or between disease subgroups. The type of targeted protein categories (such as specific cellular components) or the pattern of targeted protein (used for clustering patients with similar patterns) are examples for systemic features that can differ among study groups. One goal of autoantigenomics is to identify those differing features via bioinformatical methods. The concept of autoantigenomics may have its roots in the 1990 – without calling it like that – when bioinformatic tools were used to understand complex patterns of immunobands or small antigen sets. Systematic changes of the autoantigen repertoires may first have been hinted at in the 1990s. Without even knowing the identity of the targeted antigens, it was recognized via multiparametric analyses that the immunoband patterns can differ between different disease states and healthy controls [39–41].

Probably the earliest example of studying a small repertoire of potential antigens in a more systematic way was published by the group of

Irwin Cohen where they tested the reactivity set of 87 distinct proteins with sera from 20 healthy persons and 20 persons with type 1 diabetes mellitus [42]. The authors analyzed patterns of autoantibodies rather than single species of autoantibodies, performing cluster and principal component analysis to find patterns that were significantly different between healthy controls and patients.

A gel-based approach sometimes called “serological proteome analysis” (SERPA), can be used for autoantigenomics in principle [43,44]. In this method, two-dimensional gel electrophoresis is coupled with immunoblotting using human sera to detect reactivities and followed by mass spectrometry or Edman sequencing to identify the corresponding antigens. Today, despite classical issues of 2D gel-based proteomics [45], SERPA is occasionally used for antigen biomarker detection [44,46] (also in its 1D version [130]), but barely for holistic analyses of the autoantigenome [47].

Regarding all of these examples, we conclude that there is little value in leading semantic discussions on where a systemic “omics” analysis of the autoantigenome begins, in terms of the number of antigens and the set of methods taken into consideration. It is more valuable to appreciate these examples and to extend them by motivating the community to take a holistic look into the system, in addition to searching for single or panels of antibodies.

Examples of recent high-throughput autoantigenomics are still rare, but if realized, lead to highly interesting results as represented by the following two publications. Lewis et al. [48] used protein microarrays to identify the autoantigen repertoire of SLE patients. Hierarchical cluster analysis (HCA) revealed four patient subgroups with different disease characteristics regarding frequency of arthritis, or pulmonary, neurological, and renal involvement. Protein interaction network analysis revealed that the largest network of one of the patient subgroups was centered around SMAD and TGF- β signalling proteins. Arthritis was overrepresented in this subgroup. Another patient subgroup was characterized by autoantibodies targeting a protein network involving toll-like receptor (TLR) signalling and NF- κ B activation. In this subgroup, individuals showed differences in medication usage (lower prednisolone, higher warfarin usage).

Bussone et al. [47] detected patient-specific autoantigen repertoires of systemic sclerosis via SERPA. Pathway annotation revealed that most of these proteins were involved in the TGF- β pathway. Interestingly, the analyzed patients had unidentified ANAs.

Both examples indicate that holistic approaches enable the detection of systemic peculiarities like potential immunomodulatory effects. Such systemic peculiarities may improve patient diagnosis or stratification into subgroups.

In recent years, the holistic perspective may have become even more interesting and timely. We have recently witnessed how the development of advanced high-technology methods paved the way for the multiple omics fields: for example next-generation sequencing for transcriptomics or mass spectrometry for proteomics. Similarly, developments in the application of protein microarrays [15,16], phage display technologies [49], and IP + MS [50,51] have the potential to catalyze similar advances in autoantigenomics. In each of those methods, a broad set of bait proteins are presented to the antibodies in the serum or CSF of a patient. The targeted proteins are identified based on the position on the array, the genetic information packaged in the phage, or via mass spectrometry (Fig. 2AB).

4. Benefit of autoantigenomics

We see four main benefits of autoantigenomics over the use of single or panel autoantibodies:

4.1. Identification of subgroups of patients sharing particular features

Autoantigenomics aims at discovering systemic differences in the antigen repertoire of patients versus controls, but also among potential

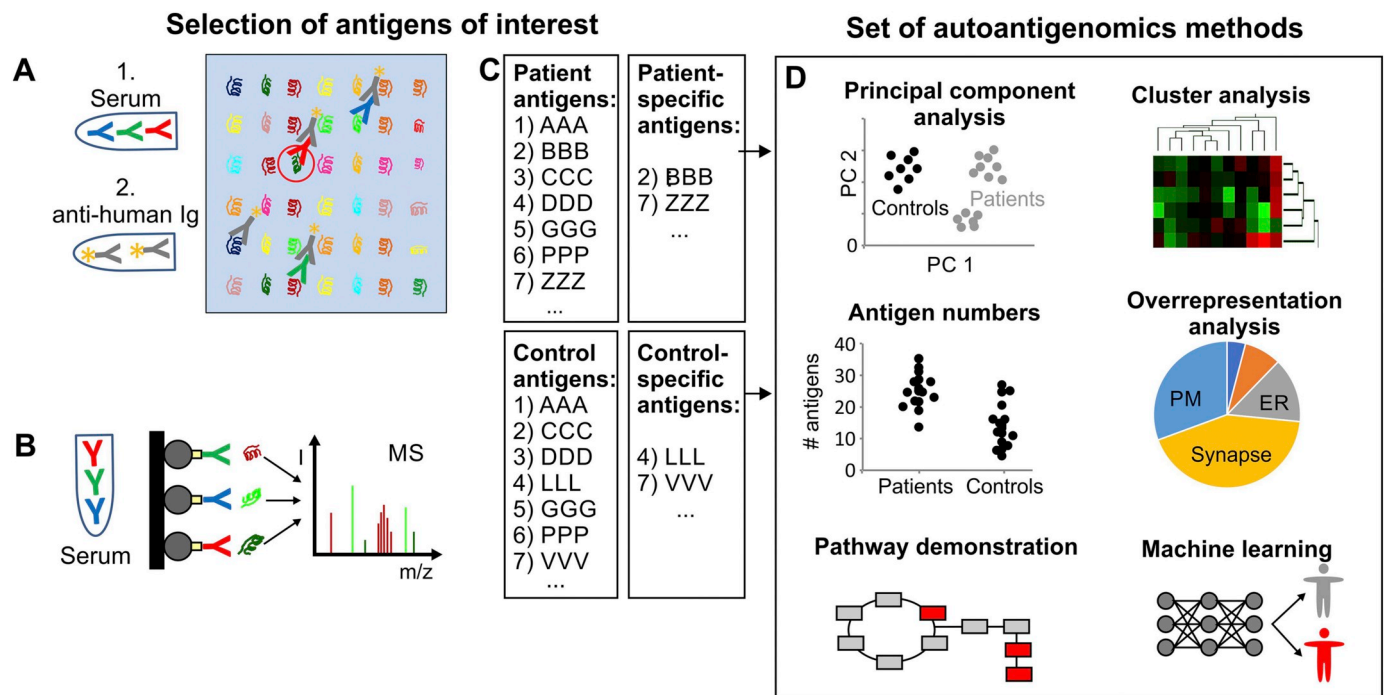


Fig. 2. Schemes of antigen list selection and the method repertoire. A-C, Detection of the targeted antigens of serum antibodies of patients and controls, using protein arrays (A), immunoprecipitation and mass spectrometry (MS) (B), or other methods. Only those antigens specific for a subject group (e.g., disease-specific antigens emerge only in patients and not in controls) are defined as the group-specific repertoire, yielding autoantigen lists (C) for systematic analyses. D, Extendable set of methods suggested for the systematic analyses (autoantigenomics) of autoantigen repertoires.

patient subgroups. e.g., based on treatment response, disease severity, or disease prognosis. In an example mentioned above, HCA identified four SLE patient groups with different clinical peculiarities and possible implications for treatment options [48]. Hence, autoantigenomics methods may be considered as a tool for patient stratification for clinical studies.

4.2. Identification of clusters of targeted autoantigens sharing particular features

Holistic analyses of the autoantigenome are able to identify antigen clusters with particular features. For example, overrepresented sets of antigens belonging to particular cellular components or signalling pathways [48] can improve the understanding of the disease and can identify potential therapeutic targets.

4.3. Identification of novel single or panels of autoantigens as biomarkers for clinical diagnostics or prognosis

High-throughput approaches are mostly untargeted, permitting an unbiased way of finding novel biomarkers. For example, anti-fibroblast growth factor receptor 3 (FGFR3) antibodies have been identified in a subgroup of sensory neuropathy patients via protein microarrays [52]. Peptide microarrays revealed the N-terminal portion of the P antigen (PAGE) protein family [53] and chloride-channel protein anoctamin 2 (ANO2) [54] as autoantigens in subsets of patients with first-episode psychosis and multiple sclerosis, respectively. Furthermore, data modelling can identify panels of autoantigens that serve as better clinical markers than conventional methods. Via logistic regression analysis for example, Lewis et al. found a set of 26 autoantigens serving as a superior diagnostic biomarker for SLE, as compared to standard ANA and dsDNA antibody assays and a combined ANA + dsDNA regression model [48].

4.4. Potential computer assistance for diagnostics and clinical decisions

By capturing the set of targeted antigens comprehensively, autoantigenomics in combination with artificial intelligence may turn out to be a better predictor of diseases and their prognosis or treatment chances than single or panel biomarkers currently are. Due to current high costs and limited automatization of data interpretation, the direct diagnostic use of autoantigenomics is not yet possible. For this purpose, quantifying single or multiple autoantibody levels still is the method of choice. Nevertheless, provided that the costs of protein arrays, IP + MS, or phage display approaches will decrease, and that interpretation will be automated based on machine learning algorithms, we assume that the autoantigenome may be incorporated in future diagnostics. Although we are aware that similar expectations for proteomics have not yet been completely fulfilled over the last 25 years [55] (but see [56]), we hypothesize that autoantigenomics is advantageous as it focuses on a more targeted set of proteins, compared to the entire serum proteome. Furthermore, the presence of autoantibodies targeting an antigen may be closer to autoimmune-related pathogenetic mechanisms than the changes in the profile of other serum proteins.

5. Bioinformatical methods for autoantigenomics

We suggest extracting the repertoires of significantly targeted autoantigens from patient and control cohorts. It is recommendable to use a healthy cohort as one of the control groups, as it is becoming increasingly clear that also healthy individuals have a nonnegligible autoantibody repertoire [57–59]. Then, the repertoires of the patient and control groups should be compared systematically using bioinformatic method sets, as known from proteomics, metabolomics, or transcriptomics [60, 131, 132] (Fig. 2). Most of the methods can be classified as either supervised or unsupervised (Table 1). Supervised methods need an allocation of data sets to specific classes, such as disease groups, and are creating models that predict the allocation of novel data sets. Unsupervised methods need no allocation to a class and

Table 1
Extendable set of bioinformatical methods for autoantigenomics.

Method	Central goal	Supervised method	Unsupervised method	Free tools
Principal component analysis	Finding patient or antigen clusters via dimensionality reduction		x	MeV (multiple experimental viewer), several packages for R or Python
Partial least-square discriminant analysis or Orthogonal projections to latent structures discriminant analysis	Identifying the variables with the largest discriminatory power between two groups and identify potential biomarkers.	x		Several packages for R or Python
Hierarchical cluster analysis	Finding patient or antigen clusters via dendrograms based on pattern similarity		x	MeV (multiple experimental viewer), Hierarchical Clustering Explorer, several packages for R or Python
Repertoire size analysis	Determining the number of targeted autoantigens per patient or per study group			
Composition of the repertoires	Finding differentially targeted protein categories via overrepresentation analyses	x		GeneOntology (GORilla tool), PantherDB
Protein network and pathway analysis	Finding and visualizing proteins interacting physically or belonging to the same pathway	x		Reactome, KEGG, STRING, IntAct, BioGRID
Machine learning	Generating prediction models that can be used for diagnostics or prognosis	x		Several packages for R or Python

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are hence exploratory or hypothesis-creating, aiming at finding auto-antigen patterns that might detect and distinguish novel subgroups of patients.

5.1. Principal component analysis (PCA) and 5.2 hierarchical cluster analysis

Both methods allow for clustering the patient and/or control cohort into subgroups of patients based on clinical features and technical biases via dimensionality reduction or similarity dendrograms [61,62]. Both methods are unsupervised and are thus able to suggest novel clusters of antigens or patients. This may be a potent holistic way for patient stratification (separation of patient groups for clinical trials), or for finding clusters of targeted antigens with similar reaction patterns over the tested patients. It may also help with uncovering batch effects, by virtue of clustering patients according to subgroups built for study feasibility, or finding clusters of targeted antigens with similar reaction patterns over the tested patients.

5.3. Partial least-square discriminant analysis (PLS-DA) or orthogonal projections to latent structures discriminant analysis (OPLS-DA)

PLS-DA and OPLS-DA are supervised methods used for patient classification [63]. Unlike PCA, these methods are suitable for detecting differences between two predefined groups. They can discover which variables have the largest discriminatory power and identify potential biomarkers. The most frequently used method is OPLS-DA which can be considered as a modification of the traditional PLS-DA, with integral orthogonal signal correction filter. The OPLS-DA plots enable the extraction of such statistically significant variables and identification of shared/unique structures in the groups. A potential limitation of these methods is the possible over-fitting of the models.

5.4. Analysis of the repertoire sizes in disease groups and clinical subgroups

This simple quantification permits, for example, to test the hypothesis that the number of targeted antigens may be disease-defining, as suggested for autoimmune regulator-deficient patients [11]. Up to now, there are no clear standards of how to define the entire set of targeted antigens. Strict statistics are necessary, but simple statistical hypothesis testing for differences of means (such as student *t*-tests) might be inappropriate since many autoantibodies are not necessarily characterized by a significant difference of the mean concentration in a patient versus control group, but rather by high levels in a small subgroup of the patients (anti-MOG in multiple sclerosis and some other demyelinating diseases [64], anti-contactin 1 and anti-neurofascin 155 in CIDP [65], anti-FGFR3 in sensory neuropathies [52, 129]). Hence, standardization¹ and z-score cutoffs may be more appropriate in order to find antigens with significantly high levels in a subgroup, as used in Meyer et al. [11]. Nevertheless, the exact way of using z-statistics is under current discussion [66,67].

5.5. Characterization of the composition of the repertoire

This approach aims to identify protein categories being over- or underrepresented (targeted more/less likely) in patients compared with controls. To do so, each single protein must be automatically annotated via an algorithm retrieving information from a data base, associating each protein with its corresponding protein categories. These are cellular components, cellular processes, or biological functions in the case of Gene Ontology [68], cellular pathways in the case of Reactome Pathway [69], and disease-relations in the case of DisGeNET [70]. All of these tools are openly accessible.

¹ Actually “studentization”, to be mathematically correct.

5.6. Analysis of targeted protein networks or pathways

This method likewise relies on pathway and interaction databases and enables the visualization of the targeted proteins of selected pathways and the discovery of striking accumulations. This visualization can be of value for those pathways that have turned out to be significantly overrepresented in a particular study group. Open-access tools are for example available within Reactome Pathway [71] and STRING [72].

5.7. Machine learning

This approach comprises a large set of methods, all benefitting from the availability of large data sets from high-throughput analyses [73]. Machine learning is increasingly used in medical research as it is expected to enable computers to assist humans in the analysis of large, complex data sets by recognizing features and patterns, to describe networks of interactions [74–77]. In supervised machine learning approaches, a training data set, for example the autoantigenomes of a cohort of patients of interest and controls, is usually used to train the model. The autoantigenomes of a second set of patients and controls, the testing data set, is then used to verify the quality of the model and its predictions [76].

Machine learning approaches have already been successfully applied for other omics data sets in immunology [78], but to our knowledge not for autoantigenomic data sets. By automating the detection of autoantigenome patterns that are reproducibly defining a disease or a patient subgroup, e.g., treatment responders, machine learning approaches might assist physicians in decisions regarding diagnosis or prognosis in the next 10 years that could guide the care of patients.

These methods may result in enhanced understanding of the autoantigen repertoire at the systemic level. Each of the methods relies on uncovering significant patterns in the complex system of targeted autoantigens, which may be used to identify patients or subgroups of patients. For example, one might imagine that patients responding to immunomodulatory therapies, such as intravenous immunoglobulin treatment, show a different autoantigen pattern compared with non-responders. Similarly, the severity of a disease might depend on the specific autoantigen pattern. These patterns might be detected and used to predict models via artificial intelligence, which could assist the clinician's decisions.

6. Other omics approaches in immunology

Other holistic approaches, such as transcriptomics and sub-categories of proteomics have been successfully applied in different fields of immunology.

6.1. Transcriptomics

Regarding transcriptomics, genome-wide microarray analyses [79] and more recently next-generation sequencing [80] have enabled comprehensive analysis of gene expression signatures, the latter method even covering non-coding parts of RNA. Thanks to advances in sensitivity, these methods even can be applied at the single cell level, and have led to valuable insights into mechanisms of autoimmunity [81]. A single-cell transcriptomic atlas has been published for different cell types and developmental stages of the thymus [82]. Furthermore, as interferons are able to induce expression of hundreds of genes via a complex pathways [83], transcriptomics is a promising method to characterize interferonopathies, a disease group defined by enhanced interferon levels or activities. The set of interferon-induced transcripts is referred to as IFN signature. This signature, identified by transcriptomics, can serve as a biomarker for interferonopathies, its subgroups, or other diseases [81,84]. Transcriptomic signatures were also

able to distinguish different diseases with similar symptoms, making them interesting for clinical diagnostics, prognosis, or patient stratification [85]. Nevertheless, to our knowledge none of the holistic transcriptome analyses have made it to clinics so far [81].

Interestingly, transcriptomics methods have been used to support the identification of autoantigens. High-throughput sequencing of the variable regions of the B and T cell receptors enable the identification of clonal receptor families [81,86–88]. In a next step, recombinant antibodies derived from these clonal B cell receptor families can be used to identify the corresponding antigen(s), which, however, does not yet work in a high-throughput manner. Being used on single-cell level, this method may be more sensitive than methods based on bait proteins. The latter, however, are closer to reality as they rely on real binding and also encompass interactions dependent on conformational epitopes.

6.2. Proteomics

Apart from transcriptomics, multiple proteomic approaches have been applied to analysing the immune system.

Advances in MS, but also in other technologies [89, 128], enabled a comprehensive look into the human plasma proteome, resulting in 3509 confidently detected proteins [90]. Secretome profiling identified patterns of anti-inflammatory cytokines along with trophic factors that may support remission of arthritic manifestations [91]. Mass-spectrometry analyses have been performed to reveal the proteomic signature regulating Th17 cell differentiation and function in humans [92]. Single-cell proteomics approaches enabling systems-wide analyses of immune cell frequencies and cell type-specific expression patterns of 12 immune checkpoints in ulcerative colitis have led to the identification of immune checkpoint signatures and therapeutic targets [93]. In an attempt to comprehensively assess exhausted CD8 T cells, single-cell proteomic profiling has identified 9 distinct cell clusters, partly linked to disease severity [94].

6.2.1. Cytokinomics

Cytokines with their 7 families comprising > 120 proteins in total represent a very targeted but powerful subset of the proteome. This subset, sometimes referred to as “cytokinome” [95], is commonly defined with multiplex assays quantifying dozens of cytokines in parallel. The cytokinome can be a characteristic and useful correlate for specific disease states in sepsis [96], chronic fatigue syndrome patients [97], Cytomegalovirus infection [98], common variable immunodeficiency [99], or inflammatory bowel diseases [100].

6.2.2. Antibody-omics

As antibodies are proteins, the holistic approaches addressing the set of antibodies can be seen as a sub-category of proteomics. The approach of analysing the antibody set is called “systems serology” or “antibody-omics”. It represents – like autoantigenomics – an analytical framework combining experimental and computational analysis aiming at holistically understanding the pattern of multiple antibody variants, Fc domain subclass, antibody glycosylation, functional qualities, and the effect on their target (e.g., neutralization, complement fixation, phagocytosis) [101]. Antibody-omics significantly contributed to understanding the profile of the humoral response to vaccines, e.g., in the HIV field [101,102]. Large data sets and machine learning approaches benefitted this holistic approach [103]. Apart from vaccine development, it has recently been suggested that antibody-omics can be applied more broadly in defining mechanisms of immunity [104]. Its predictive character has been demonstrated [103], showing that patterns of antibody features detected via holistic methods can be used to classify the tested patient groups and to use these patterns even to classify future patients. The development of high-throughput biophysical antibody profiling approaches for such antibody characterization [102,105] paved the way for antibody-omics.

6.3. Demarcation of autoantigenomics from other omics approaches

While antibody-omics characterizes the serum antibodies in a holistic way, autoantigenomics addresses the pattern of targeted *autoantigens* and hence addresses another comprehensive level on the complex multi-layered system of autoimmunity. This level does not replace the other omics approaches, but it may complement them by addressing the autoantigenome as a very specific set of proteins that cannot be addressed by transcriptomic or classical proteomic approaches. Autoantigenomics may rather represent a subcategory of affinity proteomics. While the latter comprises protein sets showing binding properties to any other molecule, e.g., protein-protein interactions [106] or aptamer-protein interaction [107], the autoantigenome as described in this article comprises the autoantibody-targeted antigens only.

7. Applications of autoantigenomics outside of autoimmunity

Apart from autoimmune diseases, autoantigenomics may be a promising approach for other diseases or other applications.

7.1. Cancer

In cancer for example, corresponding autoantibodies have been known for five decades [108] and today are valuable biomarkers or biomarker candidates for multiple cancer types [109–112], some of which may even function as cancer risk marker [113]. Although it is not clear if the repertoire of autoantibodies changed as a result of cancer is large enough to perform autoantigenomics, it is encouraging that currently naturally appearing antibodies are discussed as potential therapeutic target for cancer therapy [114]. For example, a study screening > 300 patients against 58 antigens found 4 antigens whose reactivities were significantly associated with shorter survival of human papillomavirus-negative head and neck squamous cell carcinoma patients [115,116]. Even more interestingly, different antigen reactivity clusters could distinguish patient groups regarding their status of human papillomavirus [116]. Findings like those suggest that holistic autoantigenomics approaches might find systemic differences in cancer patients.

7.2. Infections

Viral or bacterial infections can be promising use cases of autoantigenomics. Upon infection, the set of targeted autoantigens may be systemically affected due to cross-reactivities of anti-viral or -bacterial antibodies with human antigens. Apart from *autoantigenomics*, an adapted form of holistically capturing the targeted *bacterial* or *viral* antigenome is conceivable [117].

7.3. Vaccinations

Various features concerning properties of serum antibodies are used to assess the humoral responses to vaccinations via systems serology (discussed above, [101,104,118]). Adding information on the targeted autoantigenome may serve as a helpful novel feature to holistically understand vaccination reactions.

8. Current limitations of autoantigenomics

We see the following current limitations in the application of autoantigenomic approaches.

1) Due to currently high costs (list prices often exceeding \$1000 per unit for commercial protein microarrays) and limited automatization of data interpretation, the direct diagnostic use autoantigenomics is not yet feasible.

- 2) How to define antigen repertoires methodologically and statistically has not yet been defined by the community and is under current discussion [66,67].
- 3) The current high-throughput methods to detect the set of targeted autoantigens do not permit parallel characterization of interesting features of the corresponding set of autoantibodies in a likewise high-throughput manner. For example, it would be beneficial to learn about the pathogenic potential, affinity [119], glycosylation pattern [120], or other post-translational modifications of all autoantibodies targeting the autoantigenome. However, these analyses are currently possible only in approaches addressing single antibodies (e.g., regarding pathogenicity [121–123]). Currently, there is no standardized way to combine autoantigenomic screenings with high-throughput affinity tests or post-translational modification screenings.
- 4) A further limitation is that known and established antigens as disease biomarkers are often not found as positive results in high-throughput methods. This may be due to the fact that they can be conformation-specific [124,125] while methods like protein arrays partly cover proteins in non-native conformations. Producers of protein arrays or phage display approaches, however, have understood this issue and are developing improvements which will further strengthen autoantigenomics.
- 5) Direct comparisons of protein arrays, phage display assays, and IP + MS using the same patient sera including positive controls with known antibodies would be valuable in order to assess sensitivity, variability, and ability to detect conformation-specific antigens. However, such comparison has not been done to our knowledge, probably due to the costs involved.

9. Conclusion

We conclude that the community should broaden their view from single autoantigen candidates to a more holistic view of autoantigen repertoires. Our suggested set of methods can be seen as an extendable framework for future autoantigenomic experiments. We find it appealing that hundreds of data sets may be stored on many servers of scientists worldwide, once used to identify single autoantigen candidates but still ready to be mined in an “autoantigenomic” way. Hence, we suggest that all researchers who have performed autoantigen screenings in a single-autoantigen-centric perspective, may see their large data sets as a mine to dig for systemic characteristics. This novel level of holistic analysis can complement other omics analyses, such as transcriptomics, proteomics, or autoantibody-omics. Future bioinformatical approaches will need to find intelligent algorithms capable of assisting diagnosis or subgroup identification and of integrating the different omics levels [126,127].

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Author contributions

CPM drafted the manuscript; SP, OS, YT, JPC, and JCA revised the manuscript critically for important intellectual content.

Declaration of Competing Interest

CPM received travel grants from CSL Behring (France), outside of the submitted work. OS is an employee of Cambridge Protein Arrays Ltd. YT reports personal fees from The Binding Site and Alexion, outside of the submitted work. JPC reports personal fees for lectures, consulting, writing of articles, or training courses from Akcea, Alnylam,

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