FEBS *Letters*

Review

Profiling the IgOme: Meeting the challenge ☆

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ABSTRACT

The entire repertoire of antibodies in our serum, the IgOme, is a historical record of our past experiences and a reflection of our immune status at any given moment. Understanding the dynamics of the IgOme and how the diversity and specificities of serum antibodies change in response to disease and maintenance of homeostasis can directly impact the ability to design and develop novel vaccines, diagnostics and therapeutics. Here we review both direct and indirect methodologies that are being developed to map the complexity and specificities of the antibodies in polyclonal serum – the IgOme.

1. Introduction

Over a Century has passed since the first Nobel Prize in Medicine (1901) was awarded to Emil von Behring for "his work on serum therapy ... by which he opened a new road in the domain of medical science and thereby placed in the hands of the physician a victorious weapon against illness and death" [1]. Serum is indeed a "victorious weapon" able to neutralize pathogens through the exquisite specificity of its antibodies that seem endless in their capacity to discriminate and bind the vast structural complexities found in nature. Susumu Tonegawa's Nobel Prize (1987) recognized the elucidation of the genetic principles for the generation of this antibody diversity [2]. Application of these principles allows the derivation of the total "potential repertoire" of antibodies in humans; the combinatorial multiplication of all the V, D, and J segments of the Heavy (H) chains times the combined product of the V and J segments of the kappa and lambda Light (L) chains as well as the contributions of the N and P nucleotides associated with the junctional complexity – leading to an extraordinary vast theoretical number, 10^{11}–10^{12}, that far exceeds the total number of B-cells in a person's body [34,65]. More realistic is the "available reper-

toire" of variant B-cells that has been calculated to be in the order of 10^7 per person [9]. How many of these B-cells subtypes are actually utilized for the production of distinct antibodies probably does not exceed more than tens of thousands in a person's life time. Surprisingly, whereas pathogens come and go as they are cleared from our bodies, the antibodies generated in response to immunological insults are archived in our memory B-cells, the cells that orchestrate the continuous production of antibodies found in serum over the course of our lives. This entire utilized repertoire of antibodies in our serum, the IgOme, is a historical record of our past experiences and a reflection of our immune status at any given moment.

Understanding the IgOme, how homeostasis is maintained, how "serum memory" is affected by immunization, boosts, encounters with pathogens, physiology and old age are all fundamental questions of great interest. The answers to these questions bear directly on the development of novel vaccines, diagnostics and therapeutics and in order to meet these challenges one must be able to profile the IgOme. We need to be able to describe the IgOme in its entirety at single antibody resolution in a manner that is cost effective and expedient. One could then imagine running IgOme screens routinely in the course of personalized medical diagnosis and treatment. Obviously, the challenge is formidable – just considering the diversity of antibodies and the dynamics of their expression at any given moment and as a result to any given stimulus. Here we review a variety of technical approaches designed to profile serum antibody diversity in the quest of describing the complexity and composition of the human IgOme.

2. Direct and indirect analysis of the IgOme

Two basically different approaches have been employed in the analysis of the antibody repertoire of serum. The "Direct" approach goes to profile the antibodies or antibody-producing cells themselves. For this, one can catalogue antibodies based on proteomic sequencing or achieve the same by genomic sequencing of antibody transcripts derived from peripheral B-cells. The ultimate goal would be to rank the antibodies by relative concentration and categorize them by antigen specificity. The "Indirect" approach uses surrogate measures to extrapolate antibody species based on proteins or peptides they bind. Hence, the IgOme profile would be a list of inferred antibody specificities. First we address the "Direct" analysis of the IgOme.

3. Direct IgOme profiling

3.1. Phage display of antibodies

Conceptually, profiling the IgOme in its entirety would be complete were one able to systematically clone the antibodies in polyclonal serum, one by one and catalogue them by frequency of usage along with their corresponding antigen specificities. Clearly, classical methods for the production of monoclonal Antibodies (mAbs) are not suitable for such massive comprehensive screening, not in mice and definitely not for humans (see for example reviews [40,58,97]).

The introduction of phage display libraries of antibodies, however, provided a breakthrough in that one could, in theory, preserve the entire repertoire of the Heavy (H) and Light (L) chains of a person to be later screened for various antigen specificities [16,43a,60]. Two types of antibody phage display libraries can be constructed: "naïve" and "immunized" libraries. Both types of libraries are generated to discover details of the immune response to a given pathogen, autoimmune disease or cancer [5,20,21,26,46,56,86,102]. Naïve libraries generated from random donors are presumed to represent the "healthy" human repertoire of antibodies in general. In principle, naïve libraries offer the possibility of selection of high affinity antibodies of any desired specificity without the need for selectively stimulating the immune response with a specific antigen. Using naïve libraries, there has been considerable success for isolating mAbs against various pathogens, such as SARS-CoV [69,86], WNV [37], and HBV [49]. The second class of library, the "immunized phage-displayed antibody library" is constructed from the mRNA from a donor who was exposed to infection/vaccination with a defined antigen, therefore his immune response has been specifically stimulated. For immunized libraries, it is generally preferable to use a donor with a high serum antibody titer for the antigen/pathogen of interest. A high serum titer is presumed to reflect relatively high levels of Ab production and therefore higher levels of specific mRNA should be obtainable for the generation of the library [17]. This approach has been successful for isolating mAbs specific for diverse pathogens, such as, H5N1 [46,90], foot and mouth disease [31] and HIV [6,23,41,64,101,102].

In the matter of HIV-1, isolation of potent neutralizing mAbs has only been successful using immunized libraries (as compared to naïve libraries). These have been constructed from selected donors that have proven neutralizing activity. A case in point is the study by Burton and Barbas where an antibody library was produced from a 31-year old, HIV-1 positive, homosexual male, who had been asymptomatic for six years [5,16]. A Fab library was constructed on the surface of filamentous phage which comprised 10^7 members. This library was then screened against monomeric glycosylated HIV-1 gp120$_{IIIB}$ from which a collection of 20 phage displayed mAbs was isolated. The most potent was mAb b12, which competes for the binding site of HIV-1 gp120 receptor (CD4) and thus prevents virus binding to its target cell. Co-crystallization of this mAb with core gp120 confirmed the epitope overlap with the CD4 binding site [105]. Moreover, these b12 studies were revealing of two major drawbacks of the phage display technology: (i) This methodology is still relatively time consuming, tedious, and somewhat inefficient. Typically in standard experiments only tens of mAbs are isolated which are specific towards the antigen against which they were screened. (ii) The natural pairing of immunoglobulin H and L chains is lost in the construction of the library, thus the vast majority of the antibodies produced are the result of random, arbitrary pairing which does not reflect the natural H:L pair of the B-cell clone that produced them. Indeed, the co-crystallization of mAb b12 shows that the L chain does not contribute to gp120 recognition at all. All the contacts are made exclusively *via* the H chain [105]. Thus, indicating that the L chain used is most probably not the H chain's natural partner. Generally, it has been estimated that when constructing an Ab phage display library from total H and L chain cDNAs derived from Peripheral Blood Mononuclear Cells (PBMCs) and generating a complexity of 10^8 phage displayed antibodies, only 10 000 mAbs are expected to maintain their native H:L pair [40].

Another drawback of the phage display antibody system is that some VH:VL pairs may be toxic and impair bacterial growth. This in turn may lead to a very biased representation of antibodies when phage display is used for antibody expression [75].

Nonetheless, the power of phage-displayed antibody libraries is the ability to immortalize the entirety of the available naïve potential of H and L chains and even expand upon it by generating novel pairs that do not naturally exist in the donor's repertoire.

3.2. Next Generation Sequencing of antibody H and L chain mRNAs

The premise for these analyses is that one can apply Next Generation Sequencing (NGS) to catalogue the entire complexity of antibody related transcripts of a given individual [13,34,96]. For this, high throughput sequencing is typically performed using the 454 pyrosequencing analyses that currently generate $>10^6$ sequences of 400–500 bp long. Thus, one can read beyond the three CDRs of a chain in a single run [3,8,13,34,96,100,106].

When total PBMCs are used, such analyses typically address the available repertoire and not necessarily the utilized repertoire. Thus for example, Glanville et al. conducted their analyses on pooled phage displayed IgM antibodies derived from 654 healthy human donors [34]. This study serves as a comprehensive and detailed analysis of the "available IgOme" and produces a baseline of sorts for the "average" human repertoire. The total diversity of the combinatorial Ab library was calculated to be 3.5×10^{10}. This library, when screened using 16 different antigens, produced a collection of antigen specific antibodies that when analyzed using NGS, were found to comprise all the human germline VDJ and VJ segments as expected. Moreover, this analysis provides insights to the relative frequency of the usage of V segments.

Application of NGS to the study of antibodies has increased our understanding of the general physical traits of antibodies produced in humans, such as: prevalence of usage of various germline V, D and J segments, for a given class of antigen [100]. Another subject of interest has been the specific focus on the characterization of HCDR3 loops, regarding their amino acid lengths and the number of "in-dels" detected in the generation of extended CDR3 loops [3,14,20,34,100]. Moreover, it emphasizes the power of NGS technology that provides a quality assurance to identify and correct biases that may be introduced during the procedure of library construction such as over representation or the absence of a specific segment [34].

Furthermore, in conducting such analyses, one can identify clonotypes of B-cells which have been defined as those transcripts that have identical germline V and J segment usage, identical CDR3 amino acid length and at least 80% amino acid homology [62,68,98]. The clonotypes can then be ranked based on their frequency in any given serum sample. Moreover, it has been suggested that by ranking the frequency of H clonotypes vs. L clonotypes, one can infer natural H:L pairs [70].

For analysis of the "utilized repertoire" one must resort to other methods, such as sorting memory B-cells as the source of the mRNA transcripts, or comparing transcripts corresponding to IgM vs. IgG [11,51–53,67]. An example is the study of Wu et al. [100] in which NGS analyses of two HIV infected individuals (ca. >220 000 sequences for each) were utilized to identify VH sequences which resemble the sequence of the highly potent neutralizing mAb VRC01 [99]. By comparing the sequences obtained from both patients to the VRC01 sequence, they could follow the affinity maturation pathway leading to this unique class of antibodies. Similar studies using NGS and addressing the maturation of HIV specific mAbs have been reported by others [14,20,100,106].

Although such NGS analyses of total RNA do not allow natural pairing of the VH and VL chains or determine antibody specificity, they do teach general structural characteristics of the antibody repertoire in a given person and the response in a given clinical status. Thus for example, sera of people infected by specific pathogens or suffering from cancer or autoimmunity disease, can be compared to control-sera which might lead to the identification of specific markers which can be used, for example, in diagnostic applications [13,25,57,100].

3.3. Single cell cloning

Since 2009, several new and improved techniques for isolation of human mAbs have emerged. Within a very limited period, there has been a burst of innovation that has driven the field from the isolation of a limited few to the production of hundreds of mAbs [38,77,79,94,99]. These all start from sorting of antigen-specific memory B-cells, the ability to perform single cell PCR transcript amplification, and eventual effective cloning and expression of Ig H and Ig L genes derived from individual B-cells. The success of these novel technologies is based primarily on two major developments and improvements:

(i) *The source of mRNA* – using single cell memory B-cells as the source of RNA. Of particular significance is the work of Lanzavecchia and co-workers who discovered that memory B-cells proliferate and secrete antibody in response to CpG oligonucleotides, without the need of antigenic stimulation [52,91]. Furthermore, memory B-cells can be efficiently sorted based on the surface markers they display such as: IgG+ or IgA+, CD19+, CD27+ and lack of ABCB1 [11,51–53,67]. This has paved the way to what has proven to be the most effective method for selecting antigen specific mAbs from a given individual [12,38,61,77,94,99]. A case in point is the study of Walker et al. [94] who first screened 1800 HIV infected individuals for potent serum cross neutralizing activity so to identify a particularly effective donor of memory B cells (a clade A-infected African patient, as opposed to Glanville et al. [34] who used 654 donors pooled all together). They then used a high-throughput neutralization screen of antibody-containing culture supernatants from approximately 30 000 activated memory B cells from the donor. This led to the isolation of two novel broadly cross neutralizing mAbs, PG9 and PG16.

(ii) *Improved design of primer*s – Broadly cross neutralizing mAbs against HIV have been discovered to contain exceptionally high levels of point mutations acquired during the course of somatic hypermutation and affinity maturation [106]. As a result, this hypervariability can interfere with the isolation of mAb transcripts. In order to overcome this problem, Scheid et al. redesigned the set of primers so that the 5′ primer is set further upstream to avoid the hyper-mutated regions [79].

Applying this approach led to the successful cloning, isolation and characterization of 576 new antibodies of HIV envelope binding memory B cells, from four HIV infected individuals. Hence, a remarkable improvement in the efficiency of mAb isolation was realized [79]. Obviously, by dramatically increasing the number of antibodies isolated with a distinct affinity along with their sequencing, one can identify generalities and how the immune system responds and is able to neutralize a given virus.

Since the study of Walker et al., a number of groups have reported variations conforming to a general paradigm where one screens the affinity selected memory B-cells followed by single cell cloning of their cognate H and L chains that then guide the construction of their functional antibodies via recombinant DNA technologies [38,61,77,99].

Whereas many of the analyses discussed thus far have dealt with the genetic bases for Ab production and expression they do not necessarily address the actual physical nature of antibodies expressed in the serum. Hence, combining proteomics with NGS has been the next step.

3.4. Combining proteomics with NGS

Despite the elegance of single cell cloning of natural mAbs from memory B-cells, these efforts go to produce select antigen specific mAbs rather than provide insights to the general diversity of antibodies physically present and "used" in the serum.

To address the challenge of deconvoluting the diversity of Abs present in polyclonal serum, two groups have successfully proven feasibility in combining proteomics with NGS techniques for the analysis of the serum antibodies response, although still to specific antigens [22,98]. Cheung et al. conducted a study to investigate the mAb composition of polyclonal serum of rabbits immunized with progesterone A/B (PR A/B) peptides [22]. While Wine et al. aimed to deconvolute the polyclonal serum of a rabbit immunized with *Concholepas concholepas* hemocyanin (CCH) as opposed to an unimmunized rabbit [98]. In both cases the following general scheme was used:

- Serum immunoglobulins are purified over protein A columns and then subjected to antigen affinity chromatography to isolate antigen specific IgGs.
- The purified Ab fraction is analyzed by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS).
- Parallel to this effort, high-throughput DNA sequencing of the immunized animal's memory B-cells of both the H and the L chains is performed to create a comprehensive data base of all immunoglobulins that have been expressed and archived.

By comparing these two data sets one can draw a number of conclusions: (i) One can ascribe the CDR3s defined by proteomic analysis to full length VH chains derived from the NGS data, (ii) Once the full length VH sequences have been identified, these can be ranked based on their frequencies of the CDR3s mapped by proteomic analysis, and (iii) Using different strategies, one can reconstitute antibodies based on the most frequent VH chains identified.

These groups have pioneered the physical characterization of the antibodies represented by the IgOme in serum. They have accomplished this by combining both proteomic and NGS data sets thus illustrating that this general approach is feasible. Whereas these studies are extremely important, the technology has yet to be further developed in order to allow the transition from antigen based IgOme analysis to the general comprehensive analyses of the total IgOme in a given person.

4. Indirect IgOme profiling

Most certainly the production and analyses of antibodies has come a long way since Köhler and Milstein introduced their murine hybridoma technology [50], the recent isolation of target-specific human mAbs derived from single memory B-cell clones [78] and the ultimate comprehensive proteomic/NGS screens described by Cheung and Wine [22,98]. However, all these efforts are still very much single antigen oriented and do not provide a broader appreciation of the diversity of antibody specificities actually found in serum. For this, combinatorial peptide and protein arrays have been proposed and perfected. Here we will focus on the use of phage display peptide libraries. For reviews dealing with synthetic peptide arrays and antigen arrays the reader is referred to specific reviews [33,72].

4.1. Random phage display libraries

George Smith pioneered the concept of using random peptides displayed on filamentous bacteriophages to interrogate antibodies [84]. The initial application of such studies was to map a mAb's specific epitope *via* the peptides it was able to affinity select from a vast collection of random short peptides (6–15 amino acids long) [15,32,81,84]. This exercise first taught us the striking realization that despite our notion of exquisite specificity of mAbs, in reality it turns out that any given mAb most often can bind a rather larger diversity of peptides that often show no obvious homology with the linear amino acid sequence of that mAb's cognate antigen [15,89]. This is due to the fact that antibodies bind protein surfaces that are comprised of discontinuous residues brought together through protein folding [73]. Hence the typical epitope is both conformational and discontinuous. Thus, more than often the peptides fished out of a random peptide library through specific binding are peptido-mimetics that in some fashion represent complex surfaces of the antigen recognized by the mAb.

One must consider, however, that antibodies do not always bind protein epitopes. Thus, for example probes for phosphotyrosine [35] as opposed to phosphoserine or phospothreonine [36,71] can be developed illustrating that some antibodies can target the modified amino acid. Moreover, antibodies are often specific for glycomoieties as is the case for the immune response to the ABO blood types [93]. Phage display peptide libraries, expressed in bacteria obviously cannot account for these post translational modifications of protein. Nonetheless, phage display peptide analyses of sugar specific antibodies have proven feasible as often the affinity purified peptide is a mimetic of a non-amino acid structure [7,82,88].

However, random peptide analyses generate collections of affinity purified peptides that represent a given mAb's epitope [15,89]. Thus, a number of computational methods have been developed in which the peptides that have been affinity selected by a given mAb can be used as a database to predict its corresponding epitope, provided an atomic structure of the antigen is available [15,18,27,32,39,59,63,80,89]. A case in point is the application of the predictive algorithm Mapitope which has been applied for the prediction of components of the epitope for the HIV neutralizing mAb b12 (mentioned above) and for the

SARS-CoV neutralizing epitope, 80R [15, 89]. These predictions have then subsequently been confirmed by X-ray analyses of antibody-antigen co-crystals [105, 43b].

Folorgi et al. were the first to demonstrate the use of phage display libraries for the analysis of disease associated polyclonal serum [30]. These investigators screened a library of random nonapeptides using human sera taken from individuals vaccinated with hepatitis B virus surface antigen (HBsAg) and compared these to sera taken from healthy donors. They illustrated that panels of peptides could be produced and corresponded with the viral antigen.

Since these studies, the dissection and interrogation of the humoral response seen in the polyclonal serum by random phage display has become a tool for epitope mapping, diagnostics and prognostics of various clinical states, as detailed in Table 1.

As has been previously discussed for the use of phage display antibodies, here too the screening process itself can be a long and tedious process which results in the selection of only tens to hundreds of affinity selected peptides after weeks of screening. Moreover, as any given mAb can select a diversity of tens of peptides on its own, the generation of panels of even hundreds of peptides by serum cannot faithfully represent the complexity of its polyclonal composition.

4.2. Deep-Panning

In order to address these issues Deep-Panning has been proposed; a method in which the power of NGS is combined with the flexibility of random peptide phage display for the analysis of polyclonal serum [74]. Conceptually polyclonal serum is used to screen a phage displayed random peptide library followed by NGS of all the affinity captured phages. The output of such an analysis is a vast collection of thousands to millions of peptide sequences that have been affinity purified by the antibodies in a given serum sample. The frequency a specific peptide is isolated, indicates the titer and affinity of its corresponding antibody. In Deep-Panning there is no isolation or cloning of single phages, rather the entire complexity of all phages harvested in a panning experiment are directly sequenced, all at once. Moreover, deep-sequencing allows parallel sequencing of tens of samples; each sample is discriminated by a short DNA barcode (5 bases) introduced during the PCR sample preparation.

An example of the use of Deep-Panning has been in the analysis of HIV-1 infected polyclonal serum. A phage display library of 7 mer peptides was used to biopan a sample of purified human IgG obtained from HIV-1 infected individuals (HIVIG). After three rounds of biopanning against the HIVIG, the eluted phages were used as templates for PCR amplification. The PCR products were deep-sequenced using the Illumina system [10]. A total of 163,400 peptides were obtained of which 7799 were unique sequences. Of these, peptides could be demonstrated that correspond to HIV envelope showing that Deep-Panning of disease related serum can generate a clear diagnostic signal indicating specific epitopes associated with the pathogen.

Thus, Deep-Panning illustrates the compatibility of combinatorial phage displayed libraries with NGS [74]. Comprehensive panels of hundreds of thousands of affinity selected phage display peptides are produced and their frequencies and motif relevance enable one to categorize the specificities of a given polyclonal serum. Further development of computational algorithms able to handle the massive data generated from random peptide libraries and catalogue the peptides into motifs could lead to the ability to associate these with defined epitopes and antigens. Thus, Deep-Panning should become a practical tool for the profiling of the IgOme.

Table 1
The usage of random peptide library for the analysis of polyclonal serum.

Disease	Aim	References
HIV	Selection of specific mimotopes by plasma samples which defined Long Term Non Progressors (LTNPs) HIV infected individuals as opposed to progressor HIV infected patients	[42]
HIV	Analysis of polyclonal serum from HIV-1 infected individuals corresponding to the pentameric loop of gp41	[28]
HIV	Identification of antigenic and immunogenic mimotopes of HIV-1 epitopes	[76]
SIV	Differentiation of a passive vaccine and the humoral immune response towards infection	[83]
SIV	Identification of epitopes associated with vaccine protection in rhesus macaques	[4]
SARS	Discovery of disease-specific B cell epitopes for SARS-CoV	[55]
HCV	Discovery of disease-specific epitopes for HCV infected patients	[87]
AS	Selection of specific serum biomarker for ankylosing spondylitis (AS)	[95]
MS	Characterization of the antigenic epitopes specifically targeted by the sera of patients with multiple sclerosis (MS)	[44]
Cancer	Discovery of potential serum biomarkers for early primary Hepatocellular carcinoma (HCC)	[103]

4.3. Gene-fragment phage display library

In contrast to random peptide display, gene-fragment phage display libraries offer an alternative that can simplify analyses especially when one focuses on a specific antigen(s). The gene-fragment phage display library can represent a gene [29], a cDNA [19,24,29,45,85,104] or even a total genome [47,54] that encodes the ligand of interest. The DNA is reduced to smaller gene fragments (50–400 bp), usually by DNaseI-mediated random digestion or by gene fragment synthesis [47,66,92]. These are cloned into the 5′ terminus of the coat protein gene of the phage. The gene-fragment phage display library is then used for biopanning against polyclonal serum or specific mAbs, and has resulted in the isolation of peptides which enable one to determine the corresponding epitopes of the Abs tested. These selected peptides can also be utilized as biomarkers for various applications. Novel biomarkers, such as circulating (auto) antibody signatures, may improve diagnosis, prognosis and treatment of cancer [19,104], autoimmune [54,85] and infectious-diseases [48]. For example, Chatterjee et al. utilized a cDNA phage display library to identify diagnostic markers for ovarian cancer [19]. Their 65 selected clones interacted with sera from 32 ovarian cancer patients but not with sera from 25 healthy women or 14 patients suffering from other benign or malignant gynecologic diseases. Thus, the panel of biomarkers was found efficient for detection of ovarian cancer. However, this platform has limitations as well. The abundance of fragments that are cloned are often out of reading frame, consequently the identification of the epitopes is not straightforward. For example, only 12 out of the 65 clones, selected by Chatterjee et al., could clearly be shown to correspond to linear segments of known antigens. The remaining clones were either unidentified or had weak homology with known proteins and thus were assumed to represent mimetics of more complicated conformational epitopes.

Although these libraries are of limited diversity (as opposed to random peptide libraries), this approach has been proven as inexpensive and high-throughput. Moreover, this method allows for direct mapping of the epitope on the antigen sequence, as opposed to phage display random peptide libraries in which epitope mapping is not straight forward.

A tour de force demonstration regarding gene fragments is the study of Larman et al. [54] who emphasized the strength of combining deep-sequencing with phage display. These investigators constructed a complete human proteome in the form of tiled overlapping peptides. For the synthetic representation of the proteome, they generated phage display libraries which were made from 413611 DNA sequences encoding 36 amino acid peptides that span 24239 unique open reading frames. The DNA oligonucleotides were robotically synthesized and cloned using the T7 phage display system. The final library obtained, was screened against spinal fluids of patients suffering from neurologic autoimmune disease. These screens resulted in the identification of 16 autoantigen

candidates of which four are considered potential diagnostic markers. The authors propose that further improvement of their methodology may be realized by construction of peptides of greater lengths which could extend the analyses to more conformational epitopes.

5. Summary

Direct and Indirect methods are being used to profile the complexity and composition of antibodies in polyclonal serum. For the present, most of these technologies have proven efficient when applied to the humoral response towards specific antigens or pathogens. Nonetheless, the examples discussed serve as practical illustrations of how we should be able to cope with the vast diversity of antibodies that comprise the IgOme. Much progress has been made both in antibody isolation and molecular characterization of the antigens/peptides they bind. Further refinement of the methods described should lead, in the not too distant future, to a comprehensive portrayal of the human IgOme.

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