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RESEARCH PAPER

Antibody repertoire profiling with mimotope arrays

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ABSTRACT
Large-scale profiling and monitoring of antibody repertoires is possible through next generation sequencing (NGS), phage display libraries and microarrays. These methods can be combined in a pipeline, which ultimately maps the antibody reactivities onto defined arrays of structures - peptides or carbohydrates. The arrays can help analyze the individual specificities or can be used as complex patterns. In any case, the targets recognized should formally be considered mimotopes unless they are proven to be epitopes driving the antibody synthesis. Here, the advantages and disadvantages of the major profiling techniques as well as their current and future application in disease prediction and vaccination are discussed.

KEYWORDS
antibody repertoire; glycan; microarray; NGS; peptide; phage display

Introduction

The highly diversified repertoire of antibody specificities, up to 10^{11} different clones,[1,2] is a rich source of information about past and current immune activities attracting increasingly interest as a potential predictor of future disease.[3-9] The immunoglobulin diversity can be interrogated either through sequencing their variable regions or through summarizing their patterns of binding to sets of diverse structures. The latter could be known antigens, individually or as mixtures prepared ex vivo and resolved in Western blot; the proteome in general synthesized on a chip; artificially synthesized molecular diversities like peptide libraries or large sets of glycans, etc. Each one of these target arrays has its limitations but they all sample the space of shapes bound by the antibodies and help build hypotheses about the structure and the ontogeny of the repertoire. The reactivity profiles observed, sometimes called immunosignatures, have been shown to predict the onset of an infection, correlate with vaccine efficacy or to distinguish cancer stages.[10,11]

Probing of the serum antibody reactivities with peptide libraries has the advantage of an easy and less expensive synthesis of large sets of targets. Being structurally related to the proteins, peptides represent natural, easily interpreted binding sites with the restriction of being linear in nature unlike the typical B cell epitopes, which are conformational. Better understanding of the antibody repertoire to carbohydrate structures is also important as glycans have significant role in microbial infections and tumor development and their unique signatures point to interesting vaccination targets.[12-15] Although anti-carbohydrate antibodies can be probed with peptides as mimotopes, the cross-reactivities are hard to interpret so it is preferable to study this compartment of the repertoire with sets of glycans as its natural epitopes.

This review summarizes current approaches in the research of the antibody repertoires as a novel methodology with a focus on profiling using peptides and carbohydrates. These techniques promote the development of novel subunit protein and carbohydrate-based vaccines opening new venues for system level immunodiagnostics.

Structure of the antibody repertoire

The antibody paratope doesn't "know" about the chemical nature of the antigen, binding a special configuration of interacting groups. Yet, the antibody repertoire is composed of specificities selected mostly by self and non-self protein or carbohydrate structures and the immune system distinguishes biologically protein from carbohydrate epitopes compartmentalizing the respective B cell clones. The sources of these epitopes can be microbial antigens,[7,16] tumor-associated antigens (TACAs),[7,17,18] host attachment sites,[7] blood group antigens,[7,18,19] xeno-antigens[7,19] and many more. Extracting information from the whole repertoire is very similar to monitoring humoral immune responses yet it has several key differences. At least 25% of the LPS responsive B cells in the mouse spleen are polyspecific and moderately autoreactive,[20,21] and these are predominantly IgM producing B cells - mostly T cell independent. About 80% of the serum IgM has the same properties and is referred to as natural antibodies (nAbs) but its source may overlap only partially with the polyspecific splenic B cells. In mice, most of the natural antibodies seem to be produced by IgM plasmacytes residing in a unique IL-5 dependent bone marrow niche and derived from B1 related lymphocytes of fetal origin.[22] This part of the repertoire apparently reflects changes in the internal environment and, thus, it may not be too far-fetched to probe it as a natural biosensor of the internal environment. Even after considering the well-known differences between the human and the mouse immune system it is

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increasingly clear that the human antibody repertoire has a similar structure.

Natural antibodies of different isotypes have been observed to target various xeno- and autologous antigens[23-27] and carbohydrates,[18,28] and to be an essential, non-redundant component of the host defense against viral infections.[29]

Apart from natural antibodies, which do not need any antigenic stimulation, another major group of antibodies is that of the induced (or adaptive) antibodies, which are induced upon an immunogenic stimulus.[30] Typically, these are high-affinity / high-specificity antibodies to peptide structures, as proteins are T cell-dependent antigens. Contradicting the long-standing dogma, stating that carbohydrates are T cell-independent antigens, adaptive antibodies can be generated also against carbohydrate structures.[31] The conversion of T cell-independent into T cell-dependent antigens might occur in case of the presence of certain protein- or lipid-carbohydrate combinations, e.g. if the carbohydrate is presented as a glycopeptide or a glycolipid.,[12,14,32] In accordance, several studies describe the presence of non-IgG2 antibodies, which are specific to carbohydrates.[7,16] Interestingly, a recent study revealed dependencies of the immunogenicity on the structure of a carbohydrate.[7] A glycan array analysis of pooled IgG from thousands of healthy donors revealed a universal architecture of the anti-carbohydrate repertoire with high reactivities against carbohydrates with certain structural features, such as terminal galactose or fucose structures.[7] In contrast, carbohydrates with terminal sialic acids were poorly recognized, reflecting poor immunogenicity.[7]

Depending on the type of assay, it might be hard, however, to discriminate between nAbs and induced antibodies, as e.g., sera-screening assess the whole, heterogeneous population of antibodies and do not differentiate nAbs from induced antibodies. While nAbs are predominantly polyspecific IgM antibodies, the opposite is true for the induced antibodies, the majority of which is of IgG and IgA isotypes and only minor part is IgM. While the potential role of natural polyspecific IgM repertoire as a biosensor of the internal environment is just a bit more than an exciting speculation, the induced antibody repertoire is clearly a record of the immune history of the individual.

Sequencing the immunoglobulin variable regions

The information from the whole antibody repertoire – adaptive T cell-dependent and T cell-independent responses, natural antibody fluctuations, autoantibodies, etc. can be described by the set of sequences coding for the antibody specificity determining regions and the frequency of each species. Direct identification of millions of antibodies through DNA sequencing of the peripheral B cells' variable regions is a demanding task. Next generation sequencing (NGS) is being increasingly used for that purpose and the method is sometimes referred to as Rep-Seq or Ig-Seq[33] although, strictly, this is BCR-Seq when peripheral memory B cells or circulating plasma blasts are used while Ig-Seq[34] should probably be used rather to denote the proteomic analysis of the serum antibodies by mass spectrometry (for review on these methods see refs.[33,34]). Both approaches give insight into the diversity of different antibody compartments and show that the secreted repertoire is 10–1000 times more restricted than that of the circulating B cells BCR.[34] These conclusions are related to the clones involved in the adaptive IgG immune responses. Since the IgM B cell repertoire is considerably more diverse[1] and the secretion of IgM antibodies, including natural antibodies, is governed by somewhat different factors as compared with high affinity/high specificity IgG responses, it is conceivable that the diversity of serum IgM antibodies should be higher than that of the serum IgG and, having in mind the difference in half-life, more dynamic as it responds to antigenic and inflammatory signals. A major challenge of high throughput sequencing techniques is the control of errors introduced during library preparation and sequencing. Recently, Khan et al. argued that, unless an appropriate amplification fingerprinting is used, the antibody diversity can be overestimated by 5000-fold.[35] Similar improvements make possible also detailed analysis of intraclonal diversity and in-depth screening for clones with particular properties.

Indirect antibody repertoire studies

Apart from sequencing the V genes and their secreted products, another approach for studying the diversity of antibodies is by analyzing their binding specificities. It is an indirect but technically easier way for retrieval of useful information from the repertoire provided the set of probes samples the structure space adequately. The patterns of binding observed should be interpreted with caution because even the most specific antibodies can bind to a range of different structures and one structure may be bound by different antibodies.[36] When serum is used the interaction with diverse serum components, competition and complex formation should not be excluded and these artifacts can be controlled to some extent by adjusting the binding assay conditions. One of the possible strategies to solve the structure space sampling problem is to reduce the complexity of the probes (e.g., - by using shorter peptides and/or higher density of the probes to detect low avidity[37]) but it leads to even stronger convolution of the repertoire specificities. Therefore, the binding pattern can be a rich source of information about the repertoire but, in general, it does not represent a one to one image of paratope complementarities but rather the overall avidity of the repertoire for the test structures.

The techniques for the indirect antibody repertoire profiling have evolved from immunoblot through phage libraries to microarrays.

Semiquantitative immunoblots

Two decades ago, investigators of the global antibody repertoire used the quantitative immunoblotting technique, based on staining of both the antibody activity and the separated pool of proteins with subsequent densitometric quantification of the immunoreactivity and the amount of the relevant recognized protein/proteins.[38] This technique had the capacity to reveal IgM and IgG reactivity patterns to self (homologous) and non-self (bacterial) antigens.[3,4,6,39,40] It was shown that different inbred mouse strains have distinct IgM reactivity profiles to homologous antigens,[6] that after immunization of rats with auto-antigen, the IgM repertoire follows characteristic pathology associated dynamic[3] and that the regeneration of the IgM repertoire, after gamma-irradiation and subsequentinoculation

of bone marrow or fetal liver cells, is a robust redevelopment of the normal repertoire.[40]

Using the same immunoblot assay, another set of experiments demonstrated that sera from healthy young males have quite homogeneous IgM and IgG profiles to self-antigens[4,5] but the total IgG reactivity with bacterial antigens in healthy people is considerably diversified both between individuals and as a function of age.[4]

These early demonstrations of immunoreactivity patterns, although limited in terms of stringent description of the binding ligands, represent a successful attempt to extract knowledge from a crude assay based on multi-ligand / multi-receptor interactions.

Protein microarray with the "immunological homunculus"

An obvious, although more expensive, improvement to the immunoblot technique was the use of protein microarrays. Early in the 90-ties Irun Cohen proposed the hypothesis of the "immunological homunculus" – a set of autoantigens preferred by the antibodies both in autoimmunity and in physiology as a result of positive/negative selection and network interactions.[41] Building upon previous studies (e.g., – by the groups of Stratis Avrameas and Antonio Coutinho at the Pasteur Institute[21,42,43]), as well as his own hypothesis, Irun Cohen and his team published a series of intriguing results with libraries of 2–3 $\times 10^2$ "self" proteins in the form of a microarray. Francisco Quintana studied the more mainstream application of the repertoire of natural and autoantibodies and showed that, what they refer to as immunomics studies, could predict reliably autoimmune disease susceptibility in mice.[44] Furthermore, he proposed statistical tools and approaches for studying repertoire profiles.[45,46] In further studies, Asaf Madi and Yifat Merbl extended this research to demonstrate the applicability of immunomics not only in basic studies of the repertoire structure and development in the ontogeny,[47-49] but also its applicability as a diagnostic tool in oncology[50] distinguishing between different tumor models and stages of the disease. An interesting conclusion, among others, was that IgM profiles may be more informative when self-reactivities are probed. This finding was supported also by more recent results indicating that IgM has the richest repertoire of specificities as compare with other isotypes.[1]

The works of the Irun Cohen lab considerably reinforced the expectation that antibody repertoire profiles can be used as biomarkers and that their application would go beyond infection and autoimmunity. Although a rationally designed autoantigen protein microarray is much more analytical and better defined than the semiquantitative immunblots, it still suffers from lack of structural definition of the reactivities. This becomes necessary when theoretical conclusions have to be drawn about the structure of the repertoire - a shortcoming that can be addressed to some extent by using peptides.

Phage display peptide libraries

Higher resolution serum antibody reactivity profiling is possible with phage libraries displaying random short peptides as a fusion protein with one of the phage capsid proteins (P3 or P8). The phage-expression cDNA library is a non-random variant of such library.[51,52] In the case of tumor tissue, probing the repertoire with cDNA library products constitutes the widely used approach for establishing tumor antigens known as SEREX (serological analysis of autologous tumor cDNA expression cloning).[53]

The simple relation of an antibody binding site (or paratope) to the bound epitope is a theoretical abstraction based on the concept of specificity. The latter, though, has been increasingly viewed as relative and is more generally described as polyspecificity.[36,54,55] Thus, the nominal epitope can be easily defined (even operationally in the case of immunization) only for some antibodies but not for others like the clones with germline variable regions. Going back to the panning experiments with peptide libraries, the antibodies select peptides binding to them with biologically relevant affinities. For antibodies that have undergone antigen driven evolution to high specificity, the peptides selected often share with the nominal epitopes parts of the binding footprint and are, thus, referred to as mimotopes. When used as antigens, mimotopes can often elicit immune response cross-reactive with the nominal epitope of the template.[56,57] For variable regions with few or no mutations, the polyspecificity is an essential property of their binding and the "mimotopes" selected from a peptide library should be viewed rather as members of the set of epitopes the template paratope maps to. Also, the very existence of mimotopes even for highly mutated antibodies is a proof that antibody polyspecificity is the general concept. The traditional view of antibody monospecificity is just its convenient approximation on a limited set of testing antigens. Polyspecificity seems an important assumption should repertoires be probed with peptide libraries. The resultant "igome" image then represents a convolution of the epitope sets complementary to each B cell's clone product in the mixture.

The practical application of phage display peptide libraries goes beyond identifying antigens. Panning of the serum antibodies from patient against the library will affinity select a characteristic mix of peptides that contains mimotopes corresponding to the tested disease (e.g., – cancer). After identification of this subset, it may be further applied for the diagnosis of sera from individuals with similar disease.[58,59] Such immunoselected phage-mimotopes were spotted on glass slides in the form of microarray (the fusion proteins remain accessible to serum antibodies after immobilization) and were used successfully for discrimination between cancer and healthy donor sera.[58] The phage microarray is a high-throughput format for profiling polyclonal serum antibodies which may be influenced by some drawbacks of the phage display diversity maintenance. The sequential immunoselection steps for enrichment of binding peptides specifically recognized by patients sera could reduce the diversity of the phage library due to competition.[51] Another source of diversity reduction is the need for amplification of the library in bacteria.[60] Depending on the bacterial strain used the amplification steps may lead to a collapse of the diversity.[60,61]

Overcoming of this problem is necessary and possible[61] as the phage display technology offers peptide diversity of up to 10^{11} random peptides that no other technique can provide. Carefully designed panning experiments make possible the affinity selection of thousands of random peptides/mimotopes. Amplification in emulsion avoids the competition during the bacterial infection and reduces the overgrowth by parasitic

sequences.[62] With subsequent Next Generation Sequencing (NGS) the sequences of hundreds of thousands of affinity selected peptides can be obtained in a single sequencing run,[61] (A.Pashov – unpublished results). The combination of phage display and NGS technologies, called Deep Panning, has successfully been used for epitope mapping of monoclonal antibodies or in the hunt for appropriate epitopes. For instance Deep Panning was used for the affinity selection of 18 random peptides by purified IgG from a polyclonal serum of HIV-infected individual.[61] These 18 peptides align to HIV envelope protein gp160 and probably are parts of structural motifs recognized by the polyclonal antibodies toward HIV, thus demonstrating the diagnostic potential of repertoire profiling.

Random peptide microarrays

The most popular binding assay to match the data derived from phage library panning is the peptide microarray. Peptide microarrays display from several thousand to hundreds of thousands short random peptides attached to a surface – usually glass. This is a simple and sensitive technique for global antibody repertoire analysis. The peptides can be deposited by spotting or synthesized directly on the support. Even when spotted, the peptides can be attached to the support through functional groups resulting in a better defined, directional arrays. Very recently a new volume was published with an up to date collection of methods and protocols of peptide arrays applications covering also technological aspects of their production.[63] When peptides are spotted in high local concentration the signal is high so even low-affinity interactions are easily detected[37] generating identifiable immunosignatures. Apart from studying sets of mimotopes derived from phage display panning, microarrays are also directly loaded with libraries of peptides either designed by some other criteria or generated as random sequences of defined length. Monoclonal antibodies bind to about 2–3% of the random peptides producing distinguishable binding patterns on a typical high ligand density random peptide microarray with diversity of $\sim 10^4$.,[37,64] Only 10% of the bound peptides share sequence similarity with the nominal epitope. Among numerous low-affinity mimotopes usually there are also some binding with affinity higher than the cognate epitope.[37] The redundancy of this polyspecificity is facilitated by the density of the probes, which encourages avidity binding increasing by several orders of magnitude the apparent affinity. This helps detect easily characteristic changes in the repertoire not only after immunization but also in different disease states – notably in cancer. When used as a repertoire probe, immunosignaturing successfully distinguishes patients with different types or grades of brain tumors[10] confirming previous results of Merbl et al.[50]

It is important to note, though, that the high density of the surface immobilized ligand amplifies spurious interactions and, thus, reduces the capacity of the method to yield mechanistic insight in the antibody/mimotope binding. Another drawback of random peptide microarrays is the relatively low number of peptides that can be attached to a single 25×75 mm glass. Although 10^4 random peptides are reported to characterize different immune responses,[10,65,66] the closer the number of peptides to the number of antibody specificities in serum ($\sim 10^7$–10^8) the better would be the resolution of disease signatures. In the context of random peptide libraries, this problem is addressed using a brute force approach by increasing the number of sequences.

Legutki et al. report also results with in situ synthesized peptide arrays that contain 330 000 distinct peptides.[65] Using this array, the authors successfully discriminate different cancer cohorts but the respective immunosignatures determnined consist of only 50 peptide reactivities. Nevertheless, the same 330K random peptide array is also reported to detect diverse antibody responses to vaccines and other intentional and unintentional perturbations of the immunity with different immunosignatures.[9]

Rationally designed peptide arrays for repertoire studies

Random peptides used in the phage display and microarray formats are almost entirely mimotopes of natural epitopes. Used for immunosignature assays, the microarray format has the advantage of being conceptually simple and straightforward but seems less cost efficient than phage display since from each random library only a small subset of mimotopes are useful and even this is achieved after amplifying the signal by allowing for high avidity interactions. Screening an oriented random peptide library of 4×10^3 15-mers (PepPERPRINT, Heidelberg) at a molecular density excluding nonspecific binding with 0.7 μM intravenous immunoglobulins, representative of the human IgG repertoire, yielded no signal at all (unpublished observation). Indeed, Sykes et al. confirm this difference between their microarrays and those of PepPERPRINT arguing that immunosignatures should be studied at a lower affinity range.[66] On the other hand, the PepPERPRINT chips apparently have a good avidity for IgM, immunosignatures of which may be even more interesting with respect to the role of natural antibodies in homeostasis.

An alternative view on the principles of constructing an immunosignature assay library is the rational design of sequences that optimally probe the mimotope sequence space. There are at least 2 obvious approaches for constructing a database of similar sequences, which can also be combined for better results.

The first relies on the above described igome analysis of the repertoire using Deep Panning. With *a* 7-mer library of diversity 10^9 (New England Biolabs) the sequence space of relatively simple linear mimo(epi)topes is sampled completely. The second approach involves *in silico* design of mimotopes based on the solid body of data both from mimotopes and prior igome experiments. At first glance any algorithm for predicting linear B cell epitopes (like lbtope[67] or Bepipred of IEDB) should be applicable. In fact, our recent studies show that the high IgM binders from a tumor antigen peptide library (PepPERPRINT) score very low in the lbpred, and a predictor constructed on their basis does not discriminate the IEDB linear epitope teaching set differences. The discrepancy is of a magnitude excluding the inefficiency of B cell epitope predictors as an explanation. This is not unexpected since even linear B cell epitopes have structural restrictions like solvent exposure. Mimotopes, on the other hand, sample larger sequence space. It is worth noting that the mimotopes used are at the same time derived from natural autologous protein sequences so the differences cannot be attributed to the artificial design of the peptides. In this aspect

the library used is actually restricted to natural structures but free with respect to secondary and tertiary structure propensities. The goal of the rationally designed peptide libraries would be to increase by a factor of 2 or 3 the frequency of immunosignature mimotopes in a testing library and to provide interpretable structural information about the nature of mimotope binding.

Glycan array technology

During the last decades, the study of complex carbohydrates (glycans) has become a major interest in different fields of life science, due to the involvement of glycans in various different cellular processes, ranging from cell signaling, to cell adhesion and other biological processes.[68-72] By deciphering protein-carbohydrate interactions, certain draw backs and limitations in glycovaccination might be understood: Whereas many glycovaccinations contributed significantly to the prevention of a variety of human diseases, still, hyporeactivity, non-response and immunogenicity issues represent major obstacles on the path to successful glycovaccines.[14,73,74] The study of glycoimmunology and functional glycomics is further complicated by the diversity and complexity of glycans, as well as challenges in terms of glycan-synthesis and characterization,[75,76] and thus demands efforts by various interdisciplinary scientific fields.[77] Traditional methodological approaches include *in silico* methods, solid-state structural analysis, solution analysis, circular dichroism, mass spectrometry, chromatography, nuclear magnetic resonance (NMR) spectroscopy, affinity chromatography, lectin blots and many more.[77] The recent development of glycan arrays as high-throughput technology has gained remarkable attention as this methodology provided significant insights into protein-carbohydrate interactions at a molecular and system level, the latter including immunosignatures that might be relevant for glycovaccination.[7,16,18,78-81]

A glycan array consists of various molecules bound onto a solid phase, usually glass, in order to enable high-throughput screening in ELISA-like assays.[77] As such, glycan arrays provide an useful tool to investigate glycan-binding proteins (GBP), such as selectins, siglecs or antibodies.[82,7,80,83,84] Traditional microarrays use well-characterized carbohydrates, which are often synthesized using chemical or combinatorial chemoenzymatic synthesis approaches.[81,83,85] Various designs of glycan microarrays exist in terms of glycan synthesis, coupling and glycan density.[86] While different coupling chemistry and glycan density could affect the assay outcome, further attention has to be drawn to the type of the solid phase or the linkage of the glycans to the solid phase. Some arrays link glycans using a primary amine to an NHS-derivatized glass slide,[81] while others couple the glycans to bovine serum albumin (BSA) or horse serum albumin (HSA) via non-naturally occurring linkages.[86,87] A major limitation of traditional glycan arrays therefore might be discrepancy between the different array-designs, which may influence the biological activity of the probes and finally the assay-outcome.[77,86] In contrast to traditional glycan arrays, shotgun glycan microarrays try to overcome the limitation of traditional arrays by assessing the entire, native glycome of a known source (e.g., a cell), thereby avoiding critical synthesis steps.[83,88] However, the purified glycans might be uncharacterized or only partially characterized, calling for a multidimensional chromatography-driven glycan-characterization in order to interpret specific results.[88,83] Often, due to the overwhelming amount of glycans, a big portion of the glycans in shotgun approaches remains uncharacterized.

Current and future glycovaccination strategies

A number of carbohydrate-based vaccines are licensed for clinical use, including vaccines for *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis, Salmonella typhi* and several strains of *Streptococcus pneumonia*.[14,89-91] Besides vaccines for infectious diseases, carbohydrate-based vaccines for several types of cancer including melanoma, breast cancer and prostate cancer are in development.[14,92-96]

A recent study using glycan array technology found an association between structural features of glycans and their immunogenicity.[7] This study revealed low immunogenicity of terminal neuraminic acid moieties, whereas galactose- or glucose-terminated structures were observed to trigger more pronounced IgG responses in humans.[7] While these insights might have important implications for the design of carbohydrate-based vaccines, they might also explain limitations in vaccination efficacy.[89,91] The composition of microbial carbohydrate antigens contained in the glycovaccine might explain observed differences in serotype-specific responses. For instance, the multivalent capsular polysaccharide-CRM197 conjugate vaccine for Streptococcus pneumoniae induced lower antibody responses to serotype 4 than to other serotypes, including serotype 18C.[91] Notably, the serotype 4 repeating unit tetrasacharide consists of terminal N-acetyl-D-mannosamine (ManNac),[97] a precursor of neuraminic acid, whereas the serotype 18C consist of a tetrasacharide backbone with galactose, highly branched by glucose and glycerol phosphate.[98] Given that currently licensed carbohydrate-based vaccines, such as meningococcal vaccines, contain neuraminic acid-terminated structures (Council of Europe. European Pharmacopoeia. 8th edition (2014), Strasbourg), the question must be raised if the vaccine efficacy could be improved by substitution with more immunogenic glycans. Results from glycan array studies [16,7,18,78-81] revealed candidate glycan antigens with high immunogenicity for more efficient vaccination strategies for infectious disease and cancer.

Summary

With the advent of technologies like NGS and Rep-Seq, combined with phage display and microarrays, the old interest in antibody repertoire profiling is coming of age as a new immunodiagnostic paradigm. It is also a path immunology can follow as it joins the unfolding effort called systems biology. Peptides and glycans seem the structures best suited to construct the necessary arrays of probes that are at the same time highly diverse, accessible and also natural targets of the antibodies. The first results of these studies show the existence in human serum of carbohydrate-specific adaptive antibodies with modular organization. Analyzing the way immunogenicity of glycans depends on the carbohydrate structure has implications for the prediction of vaccine-induced immunity and the development of novel carbohydrate based vaccines. As an even more exciting tool, this new paradigm offers the

discovery that repertoire immunosignatures have diagnostic potential even outside of the domain of traditional immunobiology and immunopathology.

Abbreviations

B1	self-renewing B lymphocytes with fetal origin
BCR-Seq	B Cell Receptor Sequencing
BSA	Bovine Serume Albumine
ELISA	Enzyme Linked Immuno Sorbent Assay
GBP	Glycan Binding Proteins
HSA	Horse (Human) Serum Albumin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Ig-Seq	Immunoglobulin Sequencing
IL-5	Interleukin 5
ManNac	N-acetyl-D-mannosamine
nAbs	natural Antibodies
NGS	Next Generation Sequencing
NHS	*N*-Hydroxysuccinimide
n-mers-	peptide that consist of n aminoacid residues
NMR	Nuclear Magnetic Resonance
Rep-Seq	Repertoire Sequencing
TACA	Tumor Associated Carbohydrate Antigens

Disclosure of potential conflicts of interest

Pashova, Schneider, Prof. von Gunten and Dr. Pashov report no biomedical financial interests or potential conflicts of interest.

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