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Prospects from systems serology research

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Summary

Antibodies are highly functional glycoproteins capable of providing immune protection through multiple mechanisms, including direct pathogen neutralization and the engagement of their Fc portions with surrounding effector immune cells that induce anti-pathogenic responses. Small modifications to multiple antibody biophysical features induced by vaccines can significantly alter functional immune outcomes, though it is difficult to predict which combinations confer protective immunity. In order to give insight into the highly complex and dynamic processes that drive an effective humoral immune response, here we discuss recent applications of 'Systems Serology', a new approach that uses data-driven (also called 'machine learning') computational analysis and high-throughput experimental data to infer networks of important antibody features associated with protective humoral immunity and/or Fc functional activity. This approach offers the ability to understand humoral immunity beyond single correlates of protection, assessing the relative importance of multiple biophysical modifications to antibody features with multivariate computational approaches. Systems Serology has the exciting potential to help identify novel correlates of protection from infection and may generate a more comprehensive understanding of the mechanisms behind protection, including key relationships between specific Fc functions and antibody biophysical features (e.g. antigen recognition, isotype, subclass and/or glycosylation events). Reviewed here are some of the experimental and computational technologies available for Systems Serology research and evidence that the application has broad relevance to multiple different infectious diseases including viruses, bacteria, fungi and parasites.

Keywords: antibody; Fab; Fc; Fc receptors; vaccine.

Introduction

In 1796, Edward Jenner inoculated a child with matter from a cowpox sore on a milkmaid's hand, and noted that the child was then protected against smallpox infection.[1] This event was the beginning of modern-day vaccines, which have transformed society and saved millions of lives. As the success of vaccines has been wonderfully beneficial, it has influenced our approach to the study and treatment of infectious diseases. Vaccination methods today remain largely based on broad single-target approaches, similar to those first employed by Jenner more than 200 years ago.[2] More specifically, many of the currently licensed vaccines focus on inducing a single immune correlate, with the detection of total binding antigen-specific antibodies or neutralizing antibodies being the most common assessment for protection against pathogens including polio virus, influenza virus, yellow fever virus, hepatitis viruses, human papillomavirus, *Bordetella pertussis* and pneumococci.[3,4] However, for many of the world's deadliest pathogens, including Ebola virus, *Plasmodium falciparum* (malaria) and human immunodeficiency virus (HIV), the development of an effective vaccine has been hindered largely by our inability to elucidate the immune correlates of protection by traditional approaches.

The importance of Fc-mediated functional antibodies for protection and control of diseases

Antibodies are highly functional glycoproteins that are a vital immune component for protection and control of infectious diseases. For a number of vaccines (e.g. polio, influenza, tetanus) neutralizing antibodies against the pathogen or toxins have been identified as the correlates

of protection. Interestingly, for many other vaccines (eg. hepatitis A), total pathogen-specific binding antibodies have been identified as correlates of protection, yet the specific mechanisms behind these pathogen-specific binding antibodies remain unclear.[4] Beyond neutralization, antibodies are capable of providing immune protection through multiple additional mechanisms, via engagement of their Fc (Fragment crystallizable) portions. To date, only one licensed human vaccine (that for pneumococcus) has identified Fc-mediated functional antibodies as a correlate of protection.[5] However, there is growing evidence that supports the role for Fc functional antibodies in the control of a wide range of pathogens including bacterial, viral, fungal and parasitic infections. These antibodies have the unique capacity to bridge the gap between innate and adaptive immunity, by harnessing both the specificity of the humoral adaptive immune response provided by the antibody's Fab (Fragment antigen-binding) region, which recognizes the pathogen, as well as by rapidly activating Fc Receptor (FcR) innate immune effector cell responses (e.g. complement) via the antibody's Fc region. Activation can induce a range of anti-pathogenic immune responses including but not limited to antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), antibody-dependent complement activity and antibody-dependent cytokine, chemokine and/or enzyme release (Fig. 1). Importantly, FcR innate immune effector cells are abundantly located throughout the body and can be recruited by these non-neutralizing antibodies without any need for prior antigen sensitization.[6,7]

Emerging evidence from multiple infectious disease models strongly suggest that functional antibodies are important for mediating control and/or protection against viral, bacterial, fungal and parasitic pathogens. Moreover, the fact that several bacterial (e.g. Streptococcus[8]) and viral (e.g. herpes simplex virus[9]) pathogens have evolved to encode proteins that specifically protect them from Fc-mediated antibody functions,[10] further supports the notion that these non-neutralizing anti-microbial properties of antibodies play a vital role in protection from infectious diseases. Examples of the importance of Fc functional antibodies in the control and/or protection of different pathogens are summarized in Table 1.

Lessons learned from HIV vaccines trials

Despite three decades of intense research, the development of an effective vaccine against HIV continues to produce lacklustre results. To date, only one human Phase III HIV vaccine trial has shown a modest, but significant, level of efficacy (31·2%).[11] Surprisingly, this RV144 vaccine trial did not induce $CD8^+$ T-cell cellular immunity, broadly neutralizing antibody responses or high antigen-specific antibody-binding levels.[11,12] Instead immune correlates analysis identified the importance of antibodies targeting the V1V2 region of the HIV envelope and ADCC activity, in the absence of high levels of IgA.[12,13] Follow-up analyses discovered additional features of the humoral immune response associated with protection, including the preferential induction of IgG3 responses,[14,15] which were able to mediated multiple antibody effector functions including ADCC, antibody-mediated cytokine and chemokine production from natural killer cells and ADCP in a coordinated manner, otherwise known as polyfunctional antibody immunity.[15]

Furthermore, multiple non-human primate (NHP) simian immunodeficiency virus (SIV)/ simian–human immunodeficiency virus (SHIV) vaccine studies have recently been conducted highlighting the complexity of potential correlates of protection. Administration of an adenovirus vector 26 (AD26) prime followed by an envelope protein boost in NHP was able to provide 50% protection against repetitive SIV challenges.[16] Interestingly, protective efficacy was not associated with a neutralization, but instead polyfunctional antibody immune responses (incorporating six different antibody Fc functions) were associated with protection.[16] Similarly, other NHP studies have correlated both ADCP and antibody-dependent complement deposition with protective efficacy.[17] More recently, partial protection from SHIV infection was observed in NHP when administered with a canary pox prime (ALVAC)/ recombinant pentavalent envelope protein vaccine.[18] Multiple humoral immune correlates were associated with decreased risk of infection, including plasma antibody binding to HIV-infected cells, ADCC antibody titres, natural killer cell-mediated ADCC and antibody-mediated activation of macrophage inflammatory protein-1β.[18]

Figure 1. Dynamic complexity of the humoral immune response. (a) The functional capacity of the humoral immune response is determined by complex biophysical antibody features including (i) the pathogen being targeted and the ability of the antibody's Fab to recognize different antigens, (ii) an antibody's Fc region's diversity, which in turn can modulate the antibodies capacity to engage with (iii) Fc receptor/immune molecules and (iv) availability of the Fc receptors on different effector cells/immune molecules in the surrounding environment. (b). The combination of the pathogen targeted (e.g. infected cell versus small infectious particles) and binding by an antibody's Fab determines opsonization, neutralization and immune complex formation. The composition of the Fc-regions of these antibodies can in turn modulate the functional immune response by surrounding effector cells/immune molecules potentially inducing a range of functions including but not limited to ADCC, antibody-mediated secretion of cytokines, antibody-mediated enzyme release/NET (neutrophil extracellular trap) formation, antibody-dependent phagocytosis, antibody-mediated complement activity, mucus trapping etc., dependent on the cellular Fc receptor expression or immune components available.

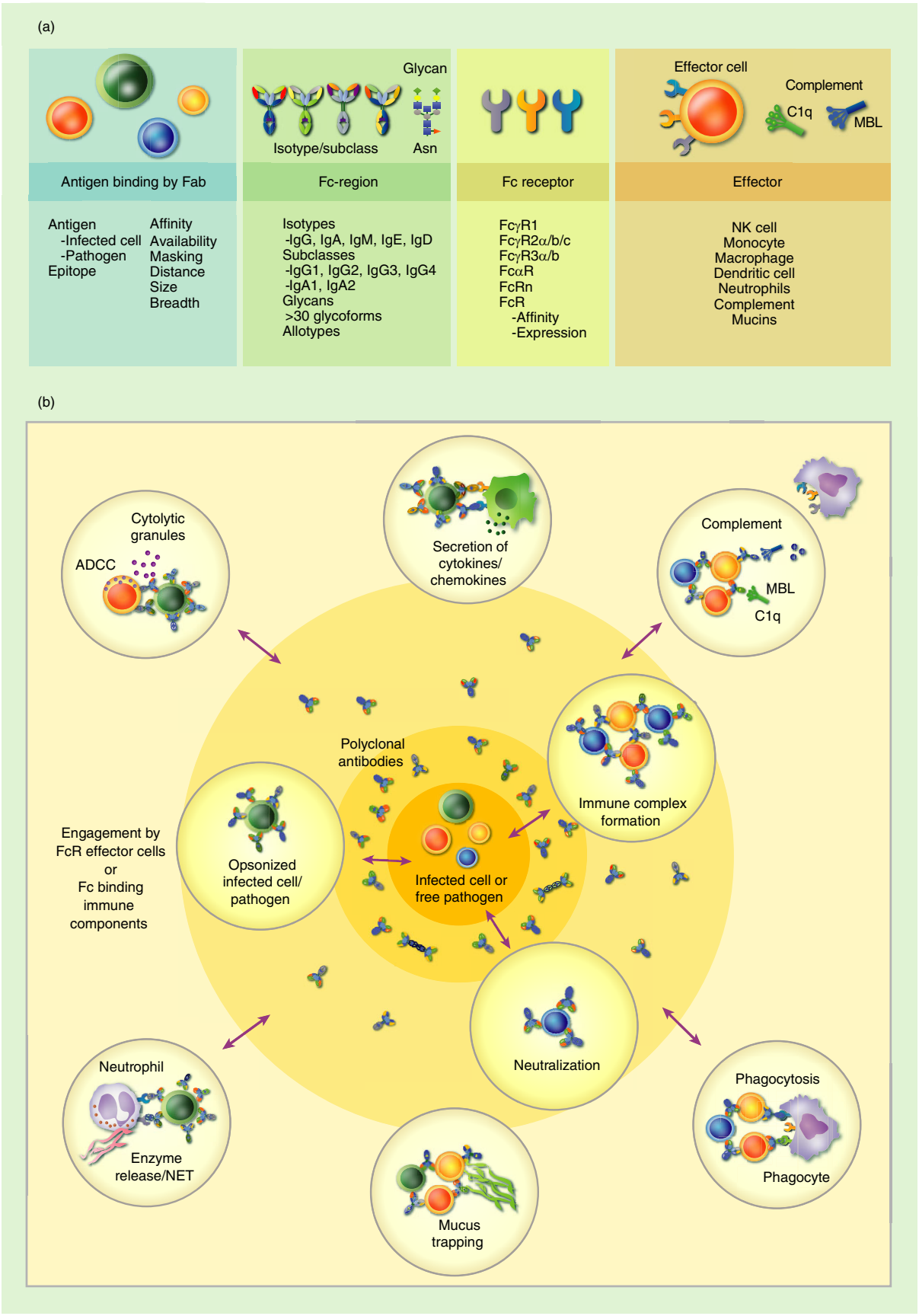
(a)
Glycan
Isotype/subclass
Asn
Effector cell
Complement
C1q
MBL
Antigen binding by Fab
Fc-region
Fc receptor
Effector
Antigen
-Infected cell
-Pathogen
Epitope
Affinity
Availability
Masking
Distance
Size
Breadth
Isotypes
-IgG, IgA, IgM, IgE, IgD
Subclasses
-IgG1, IgG2, IgG3, IgG4
-IgA1, IgA2
Glycans
>30 glycoforms
Allotypes
FcγR1
FcγR2α/b/c
FcγR3α/b
FcαR
FcRn
FcR
-Affinity
-Expression
NK cell
Monocyte
Macrophage
Dendritic cell
Neutrophils
Complement
Mucins
(b)
Cytolytic granules
ADCC
Secretion of cytokines/ chemokines
Complement
MBL
C1q
Polyclonal antibodies
Immune complex formation
Engagement by FcR effector cells or Fc binding immune components
Opsonized infected cell/ pathogen
Infected cell or free pathogen
Neutralization
Neutrophil
Enzyme release/NET
Phagocytosis
Phagocyte
Mucus trapping

Table 1. Examples of functional antibodies involved in the control of infectious viral, bacterial, fungal and parasitic pathogens

Antibody function	Virus	Bacteria	Fungus	Parasite
Antibody-dependent cellular cytotoxicity	Human immunodeficiency virus (HIV)[12,15,18,48–50] Influenza virus,[51–53] Ebola virus,[54,55] Herpes simplex virus[56]	*Salmonella typhi*,[57] *Chlamydia trachomatis*,[58] *Mycobacterium tuberculosis*[33]	*Cryptococcus neoformans*,[59] *Aspergillus*[60]	Schistosomiasis[25] *Strongyloides stercoralis*,[61] *Plasmodium*[62]
Antibody-mediated phagocytosis	HIV,[15,17,45] Influenza virus[63,64]	*Salmonella paratyphi A*,[65] *Clostridium difficile toxin A*,[66] *Mycobacterium tuberculosis*[67]	*Paracoccidioides brasiliensis*,[68] *Aspergillus fumigatus*[69]	*Plasmodium*,[70] *Toxoplasma gondii*[71]
Antibody-mediated complement	Ebola virus,[55] HIV[17,45]	*Pseudomonas aeruginosa*, *Salmonella*,[72] *Borrelia burgdorferi*[73]	*Aspergillus fumigatus*,[69] *Candida albicans*[74]	*Strongyloides stercoralis*,[61] *Plasmodium*[75]
Antibody-mediated enzyme and/or cytokine release	HIV,[15,18,45,48,76] Influenza virus[52,53]	*Mycobacterium tuberculosis*[33]	*Paracoccidioides brasiliensis*[68]	*Schistosoma*,[25] *Leishmania*[77,80] *Plasmodium*[78,79]
Non-neutralizing antibody-mediated pathogen inhibition	HIV[81]	*Coxiella burnetii*,[82] *Chlamydia*[83]		*Plasmodium*[62,84]

These recent human and NHP HIV vaccine studies have highlighted our limited understanding of humoral immune responses and challenges us to shift our analysis of potential humoral immune correlates from being a univariate or 'one component at a time' paradigm (e.g. neutralization or total antibody-binding titres alone) to a multivariate 'many components at once', or systems concept for design of new strategies for more difficult to vaccinate diseases, based on systems-level properties of humoral immunity or as it has been more simply termed 'Systems Serology'.[19,20]

Complexity of functional antibodies

Upon vaccination or infection by a pathogen, the humoral immune response aims to produce diverse, highly polyclonal antibodies to target the foreign pathogens. The functional capacity of the humoral immune response is determined by multiple cumulative factors defined by an antibody's biophysical features that are modulated by genetic, molecular and environmental factors (Fig. 1 and summarized in Table 2). These include the ability of the antibody to effectively recognize the foreign antigen dictated by an antibody's Fab region, along with the capacity of the antibody to engage with surrounding Fc effector cells and immune components (modulated by the antibody Fc portion).

Despite an antibody's Fc region often being referred to as the 'constant' region, the Fc is surprisingly diverse, with subtle modifications having the capacity to significantly alter engagement and affinity to FcRs and/or other Fc-binding immune components, including complement and mucins. These include differences in immunoglobulin isotypes: IgA, IgD, IgE, IgG and IgM, of which IgG is the most predominant immunoglobulin present in healthy human plasma.[21] Although each isotype has its own characteristic properties and functions, IgG is most commonly associated with mediating Fc effector responses, although IgA,[22] IgM[23] and IgE[24] also induce vital roles in protective immunity by activating their respective FcR innate immune cells and/or complement. For example, the importance of IgE and activation of FcεR effector cells for protection against parasitic infections has been well documented.[25] As an additional level of complexity, immunoglobulin isotypes also express different subclasses. For example, IgG consists of four subclasses, IgG1, IgG2, IgG3 and IgG4, each binding with varying affinity to different FcγRs.[26,27]

Beyond subclass, Fc function is also determined by changes in antibody glycosylation, particularly the glycan structure attached at asparagine 297 (Asn297) of the antibody Fc heavy chain,[28,29] which can have important functional consequences by influencing the affinity of IgGs for their respective FcγRs on effector cells and complement proteins. Complete aglycosylation of an antibody abolishes FcγR and complement binding,[30] whereas the presence or absence of particular glycan forms can alternatively inhibit or enhance Fc functionality.[31,32] Table 2 summarizes the many different features of the antigen–Fab antibody and antibody Fc–FcR interactions that can modulate Fc functionality and lists example assays available to allow for the in-depth assessment of these antibody features. Systems Serology aims to use high-throughput assays, to collate a holistic assessment of all antibody features that can potentially modulate Fc functionality, providing us with a detailed portrait, or humoral immune 'signature' associated with protection or control of infection. Although many of these assays have been developed and optimized for use

Table 2. Antibody biophysical features that can modulate Fc functionality

Fab	Examples measurements	Example assays	References
Masking/availability, Antigen density	Abundance of antigen available on pathogen/infected cells		85,86
Size	Smaller pathogen e.g. virus Larger pathogen e.g. parasite, or infected cell	Immune complex assays	87,88
Antigen target	Protein Glycoprotein Glycan Glycolipid	Protein, glycan, glycolipid, glycoprotein screening arrays,	89–92
Epitope	Conformational Linear	Overlapping peptide arrays Protein scaffold arrays Multiplex ELISAs Intracellular Cytokine Staining (ICS)	93–97
Antibody–antigen affinity	Equilibrium constant	Surface plasmon resonance Chaotrope	98–100
Distance	Distance from cell membrane	Assays with variable epitope distances	101
Breadth	Clades, strains, serotypes	Protein arrays Multiplex	102,103

Fc	Examples	Assays	References
Isotype	IgG, IgA, IgM, IgE, IgD	Multiplex ELISAs	95,104
Subclass	IgG1, IgG2, IgG3, IgG4, IgA1, IgA2	Multiplex ELISAs	95,104
Glycosylation	Fucose Galactose Bisecting GlcNAC Sialic acid	Mass spectrometry HPLC CE Multiplex	31,33,104,105
Allotype	IgG1 (six alleles) IgG2 (one allele) IgG3 (13 alleles) IgA (three alleles)	sequencing ELISAs	106–108
FcR/Complement binding	C1q, MBL, FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa, FcRγIIIb, FcaR, FcER (and respective polymorphisms)	ELISA Multiplex	102,104
FcR affinity	FcR binding kinetics	Surface plasmon resonance	109,110

predominately against viruses (especially HIV[18,19]), these assays have the potential to be adapted and optimized for examination of other infectious diseases.[33]

Generating insights into the complexity of the humoral response: Systems Serology

Given the complexity of antibody biophysical features, a quantitative, systems approach will provide new perspective and insight into key quantitative relationships between the features that characterize a vaccine response, confer protection or underpin a desired functional response. A quantitative understanding of relationships between antibody biophysical features, Fc functional responses and clinical outcomes could enable design of new vaccine regimens specifically targeted to enhance or suppress key parts of this system; altering overall network humoral immunity rather than a single component (Fig. 1b). Though advancements in experimental technologies now enable the measurement of large numbers of biophysical antibody features (detailed in Table 2), a major challenge still remains in determining the relative importance of alterations in these antibody features that occur with vaccination, and key quantitative relationships that drive a desired immune response or confer protection. 'Data-driven' modeling (also called 'machine learning') approaches[34] hold great promise for better understanding antibody systems, as they enable integration of high-throughput experimental data to mathematically identify relationships between antibody biophysical features that are associated with important functional outcomes, vaccine regimen, or protection/control of infection (Fig. 2). These approaches can be applied as useful hypothesis-generating tools for new systems-level

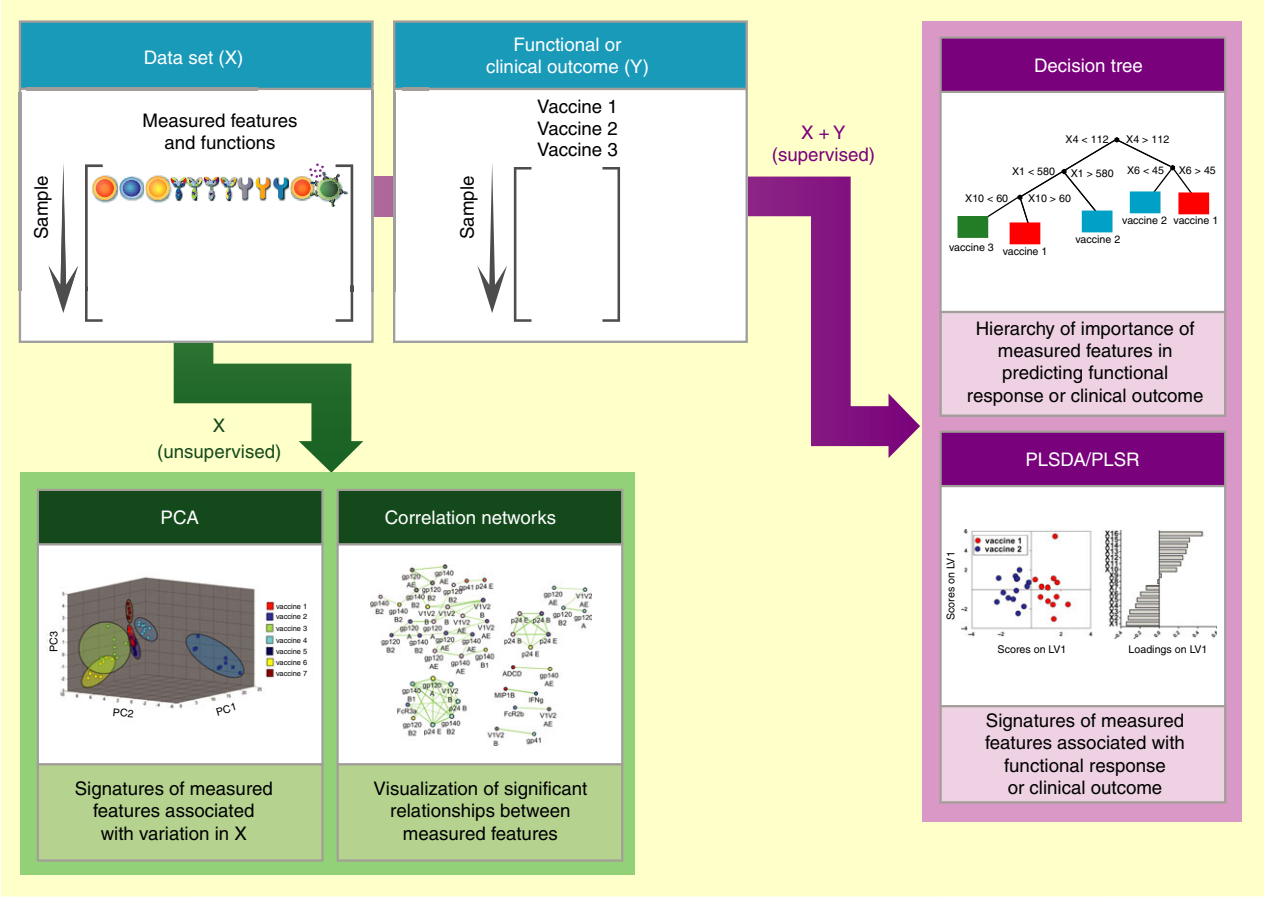

Figure 2. Systems serology data-driven modeling approaches. Systems Serology involves running high-throughput experimental assays that measure antibody biophysical and functional data (X) in parallel with functional or clinical outcomes (Y). Upon collation, the data sets can be interrogated by unsupervised and supervised machine learning computational techniques, including principal component analysis (PCA), correlation networks, partial least square discriminant analysis and regression (PLSDA and PLSR), and decision trees. The correlation network figure was kindly contributed by Manu Kumar and Doug Lauffenburger (MIT).

mechanisms involving multiple antibody features, and have the potential to accelerate our understanding of the humoral immune system by helping to define areas of interest for further experimental testing and additional quantitative models. The value of data-driven approaches in identifying gene and transcriptional signatures correlated with vaccine response has been demonstrated in a wide range of vaccinology applications.[35–37] However, many of these previous studies have specifically focused on identifying genetic and transcriptional correlates of vaccine protection, especially for cellular immunity. In contrast, application of Systems Serology instead aims to focus upon gaining insights to functional humoral immunity.

Data-driven tools: overview and examples

Data-driven models have the potential to provide both better classification of vaccine responses (e.g. between protective and non-protective vaccines) as well as give systems-level insight into networks of antibody biophysical features involved in important functional responses. Altogether, they are able to generate a valuable network 'picture' (Fig. 1b) of key events that may contribute to a specific functional immune response or clinical outcome. In general, all data-driven approaches involve analysis of a large data set ('X': Fig. 2). In the case of Systems Serology, this may include measurements of the antibody's biophysical features (e.g. antibody Fab recognition, antibody isotype, glycosylation, Fc receptor; detailed in Table 2) believed to contribute to a particular outcome (e.g. functional response, vaccine regimen, or protection). A subset of data-driven modelling approaches [including principal component analysis (PCA) and correlation networks] only employ this X data set, searching for significant multivariate relationships between measured features. This subset of approaches is considered 'unsupervised' in that they evaluate relationships between features in X without information about an outcome. The strength of unsupervised approaches lies in the ability to

search for features involved in the differentiation of outcomes in a completely unbiased way. Systemic, unbiased, examination of broad antibody profiles provides us with a more comprehensive understanding of the mechanisms behind specific functions, potentially revealing novel correlates between antibody features and functions that would not normally be identified by traditional approaches.

Other data-driven approaches are considered 'supervised' [including partial least squares discriminant analysis (PLSDA), partial least squares regression (PLSR) and decision trees, Fig. 2], as they identify key relationships in X that are related to an important functional or clinical outcome ('Y'; e.g. functional response, vaccine regimen, or clinical outcome; Fig. 2). Supervised approaches are especially useful for gaining mechanistic insight into networks or systems of immune parameters driving an outcome, because they identify direct relationships between the two. Both unsupervised and supervised approaches are useful in Systems Serology research, depending on the question being asked and the nature of the data. One major advantage of all data-driven approaches is integration, or the ability to merge disparate data sets into a whole. By combining measurements from different sources into the same model, quantitative relationships between biophysical features associated with a clinical or functional outcome can be linked across experimental assays, tissue compartments, and time. Below we give examples of specific data-driven approaches that have been applied in Systems Serology research. In each case, we leave detailed mathematical descriptions to other published work, but highlight applications, advantages and limitations of each in the context of Systems Serology use.

Unsupervised approaches

Perhaps the simplest way to visualize relationships between many different measured parameters is through the construction of *correlation networks* (Fig. 2).[19,38] These diagrams allow for the visualization of significant correlative relationships between paired measured features of interest. These networks can be created by first computing either the Pearson (parametric) or Spearman (non-parametric) correlation coefficient for each pair of measured variables. Relationships across all features can then be visualized through either a web-like structure or a heat map that indicates the direction and strength of each significant correlation. The main advantage of correlation networks is that they are easy to create and interpret, and so often give useful insight into potential mechanistic relationships between features. One drawback is that they are unsupervised, and do not directly relate identified correlative relationships to a clinical or functional outcome of interest (Y). Therefore they have little use as predictive tools. Additionally, only pairwise relationships between measured features are considered; so, true multivariate signatures involving three or more measured features are unattainable. This approach has been used previously to examine antibody network connectivity between antibody biophysical features and functions associated with the humoral response elicited by four different HIV vaccines (VAX003, RV144, HVTN204 and IPCAVD001).[19] Vastly different network topographies or 'humoral signatures' were observed between the different vaccines trials and were able to highlight important mechanisms behind the moderately protective RV144 trial. More specifically, IgG1 and IgG3 where highly connected with multiple antibody Fc effector functions including ADCC, ADCP and antibody-dependent complement deposition, indicating their importance in modulating multiple Fc functions, whereas these interactions were not observed for the other non-efficacious vaccine trials.

Principal component analysis[39] is an unsupervised approach that can be used to determine signatures of measured features that account for the most variation between samples, in a set of measured features. For example, given data set 'X' (Fig. 2) containing measurements of antibody biophysical features, PCA identifies orthogonal, linear combinations ('signatures') of these measured features (termed 'Principal Components') that account for the most variation in the data, without any information about functional or clinical outcomes (Y). Both advantages and disadvantages of PCA arise from the fact that it is an unsupervised approach – the algorithm receives no information about the outcome. This is advantageous, in that response differences can be visualized in an unbiased way, but disadvantageous in that it is not inherently hypothesis-driven. Although the identified principal components represent signatures of measured features that account for the most variation in the data, they are not specifically identified to discriminate between outcomes of interest, as a functional or clinical response (Y) is not included in the model. Hence, they can provide insight into important relationships between measured features, but they cannot directly predict how those features are associated with a functional or clinical outcome. Previously Systems Serology application of PCA applied to *Mycobacterium tuberculosis* serology studies was able to identify the importance of antibody glycosylation in distinguishing latent from active infection.[33]

Supervised approaches

Partial least squares discriminant analysis and partial least squares regression[40,41] are supervised methods that identify signatures of measured features (X) quantitatively related to a functional or clinical outcome (Y) (Fig. 2). Thus, both PLSDA and PLSR require input of both a data set of measured antibody features (X), as well as a measured

outcome (Y). PLSDA and PLSR are differentiated by the fact that in PLSDA, Y contains a discrete class or label information (e.g. vaccine 1, vaccine 2, etc.) for each outcome, whereas Y for PLSR contains continuous numerical data (e.g. ADCC measurements that can range from 0 to 100% cytotoxicity). Y is often a single column of data (e.g. only one outcome variable), but it can also be a matrix with multiple columns in situations for which there are several outcomes of interest. These algorithms determine orthogonal linear combinations ('signatures') of experimentally measured features (X) that best differentiate between outcomes (Y). Each sample can then be scored and plotted on these signatures (termed 'latent variables') to determine model accuracy for predicting clinical outcome based on measured features. Each identified latent variable (signature), contains 'loadings', or specified amounts of each of the measured features. PLSDA and PLSR are especially useful for hypothesis-driven Systems Serology research as they specifically search for signatures directly associated with an outcome [in contrast to PCA, which only evaluates overall variation in the data set (X)]. An important consideration in using PLS algorithms is to ensure that models are not 'overfit',[40] i.e. that the model contains only information about important underlying relationships rather than including random error or noise. This can be avoided by performing cross-validation (reviewed for PLSDA in ref. 40), whereby a smaller portion of the data is reserved to test a model generated by majority of the data. The ability of the model to accurately predict each sample in the test set can then be used to calculate cross-validation error, a measure of the model's predictive ability. If cross-validation error is high, the model can be improved by performing 'feature selection' to remove features that contribute to random error. There are a number of different feature selection algorithms that may be used depending on the nature of the data set, some examples of these include use of variable importance projection (VIP) scores[42] and the least absolute shrinkage and selection operator (LASSO).[43,44] One key advantage of PLS approaches for Systems Serology research is that loadings on latent variables of a feature-selected model can give great insight into co-varying serological features that are most involved in differentiating a functional or clinical outcome. In other words, the 'minimum signature' that best defines a vaccine response can give a picture of key antibody features that would be best used to reconstruct the system (Fig. 1b) for theoretical analysis.

The application of PLSDA/PLSR analysis has been successfully applied in a wide range of Systems Serology settings, including to identify humoral immune correlates of the moderately protective human HIV RV144 vaccine trial, in NHP SIV/SHIV vaccine studies, and to examine the humoral responses induced by topical anti-retrovirals for pre-exposure prophylaxis following HIV infection.[18,19,43,45] In the study of topical anti-retrovirals for pre-exposure prophylaxis following HIV infection,[43] a PLSDA model used with least absolute shrinkage and selection operator feature selection identified a signature of seven measured antibody features that differentiated women in the topical anti-retrovirals and placebo groups with 77% cross-validation accuracy, indicating that topical anti-retroviral application was associated with a specific antibody signature including measurements from different time-points (6 and 12 months) and tissue compartments (plasma and cervicovaginal lavage). Individual antibody measurements did not differentiate between groups. Altogether this illustrates the utility of PLSDA for differentiating functional or clinical outcomes and for integrating antibody measurements to identify new hypotheses for mechanisms that may vary over time or tissue compartments.

Decision trees[38,46] (Fig. 2) provide unique insight into humoral responses in that they are easy to interpret, and can give useful information about the hierarchy of importance and critical ranges (e.g. concentration, binding affinity) of measured antibody features for a particular functional or clinical outcome. For these reasons, they can be especially useful for giving insight into potential mechanistic relationships between measured serological features. A decision tree algorithm works by performing a series of binary tests on the data set of measured antibody features (X), to split samples into groups based on the functional or clinical outcome (Y). The specific binary test performed is selected by the user, and is termed a 'split criterion'.[46] Each split further purifies samples based on functional or clinical outcomes of interest (e.g. vaccine 1 versus vaccine 2 versus vaccine 3, etc.; Fig. 2). The result is a tree-like structure that illustrates the hierarchy of importance of measured features based on outcome, with specific measurement ranges required for each node selected by the algorithm. As with other supervised approaches, an important consideration in using decision tree algorithms is cross-validation to prevent overfitting (described above). If cross-validation determines a decision tree is overfit, 'pruning' may be used to improve the model, whereby peripheral branches of the tree are removed if they contribute little to classification. More detailed information on decision-tree cross-validation and pruning is reviewed in.[46]

Future outlook

Although the data-driven models used in current Systems Serology applications offer the exciting opportunity to integrate high-throughput data to identify key antibody features associated with a protective immune response, insight is still limited to multivariate statistical associations, without quantitative understanding of true cause–

effect relationships that underpin mechanistic function. Although carefully planned experiments based on data-driven models give some insight in this direction, they too are limited. Other quantitative approaches will be needed to truly understand the underlying complexity of these systems; moving beyond statistical associations and towards a quantitative systems-level understanding of mechanism. This will require the use of equation-based methods, also called 'theory-driven' approaches, where mathematical models are constructed based on previous knowledge of a system. Data-driven models can provide the underlying framework for these models – used to decide key parameters that should be included for a given question, boundaries and important input/output. Once constructed, these theory-driven models will provide a valuable hypothesis-testing tool, lending insight into (i) the importance of key antibody parameters in the formation of immune complexes and (ii) the relative importance and synergistic effects of multiple antibody alterations involved in a functional or clinical outcome. These types of approaches have already been employed to optimize the design of antibodies that trap viruses in mucus of the female reproductive tract, determining optimal quantitative ranges of antibody binding affinities that maximize both virion binding and antibody mobility in mucus.[47]

Clearly Systems Serology technologies, both experimental assays and the application of analytical technologies, are still in their infancy. Over time, high-throughput assays to assess biophysical antibody features and functions will continue to be developed and improved, encapsulating a wider range of infectious diseases and allow for the examination of antibody features and functions relevant to different tissue compartments and locations. Furthermore, Systems Serology applications can potentially be expanded to address other diseases associated with humoral immunity, including autoimmune diseases and selective cancers. There is no doubt that Systems Serology will continue to evolve to capture broader applications providing us with an increasingly comprehensive understanding of protective humoral immunity.

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