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8   **Prospects from Systems Serology Research**

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32   **Key words: Antibody, Fc, Fab, Fc Receptors, Vaccine**

33   **Abstract:**

34   Antibodies are highly functional glycoproteins capable of providing immune protection

35   through multiple mechanisms, including direct pathogen neutralization and the engagement

36   of their Fc-portions with surrounding effector immune cells and immune components that

37   induce anti-pathogenic responses. Small modifications to multiple antibody biophysical

38   features induced by vaccines and other therapeutic regimens can significantly alter functional

39   immune outcomes, though it is difficult to predict which combinations confer protective

40   immunity. In order to give insight into the highly complex and dynamic processes that drive

41   an effective humoral immune response, here we discuss recent applications of "Systems

42   Serology", a new approach that uses data-driven (also called 'machine learning')

43   computational analysis and high-throughput experimental data to infer networks of important

44   antibody features associated with protective humoral immunity and/or Fc functional activity**.**

45   This approach offers the ability to understand humoral immunity beyond single correlates of

46   protection, assessing the relative importance of multiple biophysical modifications to

47   antibody features with multivariate computational approaches. Systems Serology has the

48   exciting potential to help identify novel correlates of protection from infection and may

49   generate a more comprehensive understanding of the mechanisms behind protection,

50   including key relationships between specific Fc functions and antibody biophysical features

51   (e.g. antigen recognition, isotype, subclass and/or glycosylation events). Reviewed here are

52   some of the experimental and computational technologies available for Systems Serology

53   research and evidence that the application has broad relevance to multiple different infectious

54   diseases including viruses, bacteria, fungi and parasites.

55

56   **Word count:** 3955 words excluding abstract, references, tables and figure legends

57   **Introduction:**

58   In 1776, Edward Jenner inoculated a child with matter from a cowpox sore on a milkmaid's

59   hand, and noted that the child was then protected against smallpox infection [1]. This event

60   was the beginning of modern-day vaccines, which have transformed society and saved

61   millions of lives. As the success of vaccines has been wonderfully beneficial, it has

62   influenced our approach to the study and treatment of infectious diseases. Vaccination

63   methods today remain largely based on broad single-target approaches, similar to those first

64   employed by Jenner more than 200 years ago [2]. More specifically, many of the currently

65   licensed vaccines focus on inducing a single immune correlate, with the detection of total

66 binding antigen-specific antibodies or neutralizing antibodies being the most common
67 assessment for protection against pathogens including polio, influenza, yellow fever,
68 hepatitis, HPV, pertussis and pneumococcus [3, 4]. However, for many of the world's deadliest
69 pathogens, including ebola, malaria and HIV, the development of an effective vaccine has
70 been hindered largely due to our inability to elucidate the immune correlates of protection by
71 traditional approaches.

72

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

75 Antibodies (Abs) are highly functional glycoproteins that are a vital immune component for
76 protection and control of infectious diseases. For a number of vaccines (e.g., polio, influenza,
77 tetanus, etc.) neutralizing Abs against the pathogen or toxins have been identified as the
78 correlates of protection. Interestingly, for many other vaccines (eg., Hepatitis A), total
79 pathogen-specific binding Abs have been identified as correlates of protection, yet the
80 specific mechanisms behind these pathogen-specific binding Abs remain unclear [4]. Beyond
81 neutralization, Abs are capable of providing immune protection through multiple additional
82 mechanisms, via engagement of their Fc (Fragment crystalizable) portions. To date, only one
83 licensed human vaccine (Pneumococcus) has identified Fc-mediated functional Abs as a
84 correlate of protection [5]. However, there is growing evidence that supports the role for Fc
85 functional Abs in the control of a wide range of pathogens including bacterial, viral, fungal
86 and parasitic infections. These Abs have the unique capacity to bridge the gap between innate
87 and adaptive immunity, by harnessing both the specificity of the humoral adaptive immune
88 response provided by the Ab's Fab (Fragment antigen-binding) region, which recognizes the
89 pathogen, as well as by rapidly activating Fc Receptor (FcR) innate immune effector cell
90 responses (e.g., complement) via the Ab's Fc region. Activation can induce a range of anti-
91 pathogenic immune responses including but not limited to Ab dependent cellular cytotoxicity
92 (ADCC), Ab dependent cellular phagocytosis (ADCP), Ab dependent complement activity
93 and Ab dependent cytokine, chemokine and/or enzyme release (Figure 1). Importantly, FcR
94 innate immune effector cells are abundantly located throughout the body and can be recruited
95 by these non-neutralizing Abs without any need for prior antigen sensitization [6, 7].

96

97 Emerging evidence from multiple infectious disease models strongly suggest that functional
98 Abs are important for mediating control and/or protection against viral, bacterial, fungal and
99 parasitic pathogens. Moreover, the fact that several bacterial (e.g., Streptococcus [8]) and viral

100 (e.g., HSV [9]) pathogens have evolved to encode proteins that specifically protect them from

101 Fc mediated Ab functions [10], further supports the notion that these non-neutralizing anti-

102 microbial properties of Abs play a vital role in protection from infectious diseases. Examples

103 of the importance of Fc functional Abs in the control and/or protection of different pathogens

104 are summarized in Table 1.

105

106 **Lessons learned from HIV Vaccines Trials**

107 Despite three decades of intense research, the development of an effective vaccine against

108 HIV continues to produce lackluster results. To date, only one human Phase III HIV vaccine

109 trial has shown a modest, but significant level of efficacy (31.2%) [11]. Surprisingly, this

110 RV144 vaccine trial did not induce CD8+ T cell cellular immunity, broadly neutralizing Ab

111 responses or high binding antigen-specific Ab levels [11, 12]. Instead immune correlates analysis

112 identified the importance of Abs targeting the V1V2 region of the HIV envelope and Ab

113 dependent cellular cytotoxicity (ADCC) activity, in the absence of high levels of IgA [12, 13].

114 Follow-up analyses discovered additional features of the humoral immune response

115 associated with protection, including the preferential induction of IgG3 responses [14, 15], which

116 were able to mediated multiple Ab effector functions including ADCC, Ab mediated cytokine

117 and chemokine production from NK cells and Ab mediated cellular phagocytosis (ADCP) in

118 a coordinated manner, otherwise known as polyfunctional Ab immunity [15].

119

120 Furthermore, multiple non-human primate (NHP) SIV/SHIV vaccine studies have recently

121 been conducted highlighting the complexity of potential correlates of protection.

122 Administration of an adenovirus vector 26 (AD26) prime followed by an envelope protein

123 boost in NHP was able to provide 50% protection against repetitive SIV challenges [16].

124 Interestingly, protective efficacy was not associated with a neutralization, but instead

125 polyfunctional Ab immune responses (incorporating six different Ab Fc functions) were

126 associated with protection [16]. Similarly, other NHP studies have correlated both ADCP and

127 Ab dependent complement deposition (ADCD) with protective efficacy [17]. More recently,

128 partial protection from SHIV infection was observed in NHP when administered with a

129 canary pox prime (ALVAC)/ recombinant pentavalent envelope protein vaccine [18]. Multiple

130 humoral immune correlates were associated with decreased risk of infection, including

131 plasma Ab binding to HIV-infected cells, ADCC Ab titers, NK cell-mediated ADCC and Ab

132 mediated activation of MIP-1ß [18].

133

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

141

Complexity of functional antibodies:

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

151

152 Despite an Ab's Fc region often being referred to as the 'constant' region, the Fc is
153 surprisingly diverse, with subtle modifications having the capacity to significantly alter
154 engagement and affinity to FcRs and/or other Fc binding immune components, including
155 complement and mucins. These include differences in immunoglobulin isotypes: IgA, IgD,
156 IgE, IgG and IgM, of which IgG is the most predominant immunoglobulin present in healthy
157 human plasma [21]. While each isotype has their own characteristic properties and functions,
158 IgG is most commonly associated with mediating Fc effector responses, although IgA [22], IgM
159 [23] and IgE [24] also induce vital roles in protective immunity by activating their respective FcR
160 innate immune cells and/or complement e.g. the importance of IgE and activation of Fc-
161 epsilon R effector cells for protection against parasitic infections has been well documented
162 [25]. As an additional level of complexity, immunoglobulin isotypes also express different
163 subclasses for example: IgG consists of four subclasses, IgG1, IgG2, IgG3 and IgG4, each
164 binding with varying affinity to different FcγRs [26, 27].

165

166 Beyond subclass, Fc function is also determined by changes in Ab glycosylation, particularly
167 the glycan structure attached at asparagine 297 (Asn297) of the Ab Fc heavy chain [28, 29],

168   which can have important functional consequences by influencing the affinity of IgG's for

169   their respective FcγRs on effector cells and complement proteins. Complete aglycosylation of

170   an Ab abolishes FcγR and complement binding [30], whereas the presence or absence of

171   particular glycan forms can alternatively inhibit or enhance Fc functionality [31, 32]. **Table 2**

172   summarizes the many different features of the antigen-Fab Ab and Ab Fc-FcR interactions

173   that can modulate Fc functionality and lists example assays available to allow for the in-depth

174   assessment of these Ab features. Systems Serology therefore aims to use high throughput

175   assays, to collate a holistic assessment of all Ab features that can potentially modulate Fc

176   functionality, providing us with a detailed portrait, or humoral immune "signature" associated

177   with protection or control of infection. While many of these assays have been developed and

178   optimized for use predominately against viruses (especially HIV [18, 19]), these assays have the

179   potential to be adapted and optimized for examination of other infectious diseases [33].

180

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

182   Given the complexity of Ab biophysical features, a quantitative, systems approach will

183   provide new perspective and insight into key quantitative relationships between the features

184   that characterize a vaccine response, confer protection, or underpin a desired functional

185   response. A quantitative understanding of relationships between Ab biophysical features, Fc

186   functional responses and clinical outcomes could enable design of new vaccine regimens

187   specifically targeted to enhance or suppress key parts of this system; altering overall network

188   humoral immunity rather than a single component (Figure 1B). Though advancements in

189   experimental technologies now enable the measurement of large numbers of biophysical Ab

190   features (detailed in Table 2), a major challenge still remains in determining the relative

191   importance of alterations in these Ab features that occur with vaccination, and key

192   quantitative relationships that drive a desired immune response or confer protection. "Data-

193   driven" modelling approaches [34] (also called "machine learning" approaches) hold great

194   promise for better understanding Ab systems, as they enable integration of high-throughput

195   experimental data to mathematically identify relationships between Ab biophysical features

196   that are associated with important functional outcomes, vaccine regimen, or

197   protection/control of infection (Figure 2). These approaches can be applied as useful

198   hypothesis-generating tools for new systems-level mechanisms involving multiple Ab

199   features, and have the potential to accelerate our understanding of the humoral immune

200   system by helping to define areas of interest for further experimental testing and additional

201   quantitative models. The value of data-driven approaches in identifying gene and

202　transcriptional signatures correlated with vaccine response has been demonstrated in a wide

203　range of vaccinology applications [35-37]. However many of these previous studies have

204　specifically focused on identifying genetic and transcriptional correlates of vaccine

205　protection, especially for cellular immunity. In contrast, application of Systems Serology

206　instead aims to focus upon gaining insights to functional humoral immunity.

207

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209

210　**Data-driven tools: overview and examples**

211　Data-driven models have the potential to provide both better classification of vaccine

212　responses (e.g., between protective and non-protective vaccines) as well as give systems-

213　level insight into networks of Ab biophysical features involved in important functional

214　responses. Altogether, they are able to generate a valuable network "picture" (Figure 1B) of

215　key events that may contribute to a specific functional immune response or clinical outcome.

216　In general, all data-driven approaches involve analysis of a large data set ('**X**': Figure 2). In

217　the case of Systems Serology, this may include measurements of the Ab's biophysical

218　features (e.g., Ab Fab recognition, Ab isotype, glycosylation, Fc receptor etc.; detailed in

219　Table 2) believed to contribute to a particular outcome (i.e. functional response, vaccine

220　regimen, or protection). A subset of data-driven modelling approaches (including principal

221　component analysis (PCA) and correlation networks) only employ this X data set, searching

222　for significant multivariate relationships between measured features. This subset of

223　approaches is considered "**unsupervised**" in that they evaluate relationships between features

224　in X without information about an outcome. The strength of unsupervised approaches lies in

225　the ability to search for features involved in the differentiation of outcomes in a completely

226　unbiased way. Systemic, unbiased, examination of broad Ab profiles provides us with a more

227　comprehensive understanding of the mechanisms behind specific functions, potentially

228　revealing novel correlates between Ab features and functions that would not normally be

229　identified by traditional approaches.

230

231　Other data-driven approaches are considered "**supervised**" (including partial least squares

232　discriminant analysis (PLSDA), partial least squares regression (PLSR) and decision trees,

233　Figure 2), as they identify key relationships in X that are related to an important functional or

234　clinical outcome ("**Y**"; e.g., functional response, vaccine regimen, or clinical outcome)

235　(Figure 2). Supervised approaches are especially useful for gaining mechanistic insight into

236 networks or systems of immune parameters driving a response, because they identify direct
237 relationships between the two. Both unsupervised and supervised approaches are useful in
238 Systems Serology research, depending on the question being asked and nature of the data.
239 One major advantage of all data-driven approaches is integration, or the ability to merge
240 disparate data sets into a whole. By combining measurements from different sources into the
241 same model, quantitative relationships between biophysical features associated with a clinical
242 or functional outcome can be linked across experimental assays, tissue compartments, and
243 time. Below we give examples of specific data-driven approaches that have been applied in
244 Systems Serology research. In each case, we leave detailed mathematical descriptions to
245 other published work, but highlight applications, advantages and limitations of each in the
246 context of Systems Serology use.

247

248 *Unsupervised approaches:*
249 Perhaps the simplest way to visualize relationships between many different measured
250 parameters is via the construction of ***correlation networks*** (Figure 2) [19, 38]. These diagrams
251 allow for the visualization of significant correlative relationships between paired measured
252 features of interest. These networks can be created by first computing either the Pearson
253 (parametric) or Spearman (non-parametric) correlation coefficient for each pair of measured
254 variables. Relationships across all features can then be visualized via either a web-like
255 structure or a heat map that indicates the direction and strength of each significant
256 correlation. The main advantage of correlation networks is that they are easy to create and
257 interpret, and thus often give useful insight into potential mechanistic relationships between
258 features. One drawback is that they are unsupervised, and do not directly relate identified
259 correlative relationships to a clinical or functional outcome of interest (Y). Therefore they
260 have little use as predictive tools. Additionally, only pairwise relationships between measured
261 features are considered; thus, true multivariate signatures involving three or more measured
262 features are unattainable. This approach has been used previously to examine Ab network
263 connectivity between Ab biophysical features and functions associated with the humoral
264 response elicited by four different HIV vaccines (VAX003, RV144, HVTN204, and
265 IPCAVD001) [19]. Vastly different network topographies or 'humoral signatures' were
266 observed between the different vaccines trials and were able to highlight important
267 mechanisms behind the moderately protective RV144 trial. More specifically, IgG1 and IgG3
268 where highly connected with multiple Ab Fc effector functions including ADCC, ADCP and

269   ADCD indicating their importance in modulating multiple Fc functions, while these
270   interactions were not observed for the other non-efficacious vaccine trials.

271

272

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

290

291   ***Supervised approaches:***

292   ***Partial least squares discriminant analysis (PLSDA) and partial least squares regression***
293   ***(PLSR)*** [40, 41] are supervised methods that identify signatures of measured features (X)
294   quantitatively related to a functional or clinical outcome (Y) (Figure 2). Thus, both PLSDA
295   and PLSR require input of both a data set of measured Ab features (X), as well as a measured
296   outcome (Y). PLSDA and PLSR are differentiated by the fact that in PLSDA, Y contains a
297   discrete class or label information (e.g.,vaccine 1, vaccine 2, etc.) for each outcome, while Y
298   for PLSR contains continuous numeric data (e.g., ADCC measurements that can range from
299   0-100% cytotoxicity). Y is often a single column of data (e.g., only 1 outcome variable), but
300   it can also be a matrix with multiple columns in situations for which there are several
301   outcomes of interest. These algorithms determine orthogonal linear combinations
302   ('signatures') of experimentally measured features (X) that best differentiate between

303   outcomes (Y). Each sample can then be scored and plotted on these signatures (termed 'latent

304   variables') to determine model accuracy for predicting clinical outcome based on measured

305   features. Each identified latent variable (signature), contains 'loadings', or specified amounts

306   of each of the measured features. PLSDA and PLSR are especially useful for hypothesis-

307   driven Systems Serology research as they specifically search for signatures directly

308   associated with an outcome (in contrast to PCA, which only evaluates overall variation in the

309   data set (X)). An important consideration in using PLS algorithms is to ensure models are not

310   'overfit' [40], i.e. that the model contains only information about important underlying

311   relationships rather than including random error or noise. This can be avoided by performing

312   cross-validation (reviewed for PLSDA in [40]), whereby a smaller portion of the data is

313   reserved to test a model generated by majority of the data. The ability of the model to

314   accurately predict each sample in the test set can then be used to calculate cross-validation

315   error, a measure of the model's predictive ability. If cross-validation error is high, the model

316   can be improved by performing 'feature selection' to remove features that contribute to

317   random error. There are a number of different feature selection algorithms that may be used

318   depending on the nature of the data set, some examples of these include use of variable

319   importance projection (VIP) scores [42] and the least absolute shrinkage and selection operator

320   (LASSO) [43, 44]. One key advantage of PLS approaches for Systems Serology research is that

321   loadings on latent variables of a feature-selected model can give great insight into co-varying

322   serological features that are most involved in differentiating a functional or clinical outcome.

323   In other words, the "minimum signature" that best defines a vaccine response can give a

324   picture of key Ab features that would be best used to reconstruct the system (Figure 1B) for

325   theoretical analysis.

326

327   The application of PLSDA/PLSR analysis has been successfully applied in a wide range of

328   Systems Serology settings, including to identify humoral immune correlates of the

329   moderately protective human HIV RV144 vaccine trial, in non-human primate SIV/SHIV

330   vaccine studies, and to examine the humoral responses induced by topical anti-retrovirals for

331   pre-exposure prophylaxis following HIV infection [18, 19, 43, 45]. In the study of topical anti-

332   retrovirals for pre-exposure prophylaxis following HIV infection [43], a PLSDA model used

333   with LASSO feature selection identified a signature of 7 measured Ab features that

334   differentiated women in the topical anti-retrovirals and placebo groups with 77% cross-

335   validation accuracy, indicating that topical anti-retroviral application was associated with a

336   specific Ab signature including measurements from different time points (6 and 12 months)
337   and tissue compartments (plasma and cervicovaginal lavage). Individual Ab measurements
338   we unable to differentiate between groups. Altogether this illustrates the utility of PLSDA for
339   differentiating functional or clinical outcomes and for integrating Ab measurements to
340   identify new hypotheses for mechanisms that may vary over time or tissue compartments.

341

342

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

360

361

362   **Future Outlook**
363   While the data-driven models used in current Systems Serology applications offer the
364   exciting opportunity to integrate high-throughput data to identify key Ab features associated
365   with a protective immune response, insight is still limited to multivariate statistical
366   associations, without quantitative understanding of true cause-effect relationships that
367   underpin mechanistic function. While carefully planned experiments based on data-driven
368   models give some insight in this direction, they too are limited. Other quantitative approaches
369   will be needed to truly understand the underlying complexity of these systems; moving

370 beyond statistical associations and towards a quantitative systems-level understanding of
371 mechanism. This will require use of equation-based methods, also called "theory-driven"
372 approaches, where mathematical models are constructed based on prior knowledge of a
373 system. Data-driven models can provide the underlying framework for these models – used to
374 decide key parameters that should be included for a given question, boundaries and important
375 input/output. Once constructed, these theory-driven models will provide a valuable
376 hypothesis-testing tool, lending insight into 1) the importance of key Ab parameters in the
377 formation of immune complexes and 2) the relative importance and synergistic effects of
378 multiple Ab alterations involved in a functional or clinical outcome. These types of
379 approaches have already been employed to optimize the design of Abs that trap viruses in
380 mucus of the female reproductive tract, determining optimal quantitative ranges of Ab
381 binding affinities that maximize both virion binding and Ab mobility in mucus [47].

382

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

393

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402

407

408 **Figure 1: Dynamic complexity of the humoral immune response**

409 A). The functional capacity of the humoral immune response is determined by complex

410 biophysical antibody features including i). the pathogen being targeted and the ability of the

411 antibody's Fab to recognize different antigens, ii). an antibody's Fc region's diversity which

412 in turn can modulate the antibodies capacity to engage with iii). Fc Receptor/immune

413 molecules and iv). availability of the Fc Receptors on different effector cells/immune

414 molecules in the surrounding environment. B). The combination of the pathogen targeted

415 (e.g. infected cell versus small infectious particles) and binding by an Ab's Fab determines

416 opsonisation, neutralization and immune complex formation. The composition of the Fc-

417 regions of these Abs can in turn modulate the functional immune response by surrounding

418 effector cells/immune molecules potentially inducing a range of functions including but not

419 limited to ADCC, Ab mediated secretion of cytokines, Ab mediated enzyme release/NET

420 (neutrophil extracellular trap) formation, Ab dependent phagocytosis, Ab mediated

421 complement activity, mucus trapping etc. dependent on the cellular Fc receptor expression or

422 immune components available.

423

424

425 **Figure 2: Systems serology data-driven modelling approaches**

426 Systems Serology involves running high-throughput experimental assays that measure Ab

427 biophysical and functional data (X) in parallel with a functional or clinical outcomes (Y).

428 Upon collation, the datasets can be interrogated by unsupervised and supervised machine

429 learning computational techniques, including Principal Component Analysis (PCA),

430 correlation networks, Partial Least Square Discriminant Analysis and Regression (PLSDA

431 and PLSR), and decision trees. Correlation network figure was kindly contributed by Manu

432 Kumar and Doug Lauffenburger (MIT).

433

434

435 **Table 1: Examples of functional antibodies in the control of infectious viral, bacterial,**

436 **fungal and parasitic pathogens**

Antibody Function	Virus	Bacteria	Fungus	Parasite
ADCC	HIV [12, 15, 18, 48, 49, 50], Influenza [51-53], Ebola [54, 55], HSV[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 [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 [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 [52, 53]	*Mycobacterium tuberculosis* [33]	Paracoccidioides Brasiliensis [68]	Schistosomiasis [25], Leishmania [77, 80] Plasmodium [78, 79]
Non-neutralizing Antibody mediated Pathogen inhibition	HIV [81]	*Coxiella burnetii* [82], Chlamydia [83]		Plasmodium [62, 84]

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440

441 **Table 2: Antibody Biophysical Features that can modulate Fc functionality**

442

Fab	Examples measurements	Example Assays	References
Masking/ Availability, Antigen Density	Abundance of antigen available on pathogen/infected cells		[85, 86]
Size	Smaller pathogen eg virus Larger pathogen eg 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 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**	**Reference**

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 Spec HPLC CE Multiplex	[31, 33, 104, 105]
Allotype	IgG1 (6 alleles) IgG2 (1 allele) IgG3 (13 alleles) IgA (3 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]

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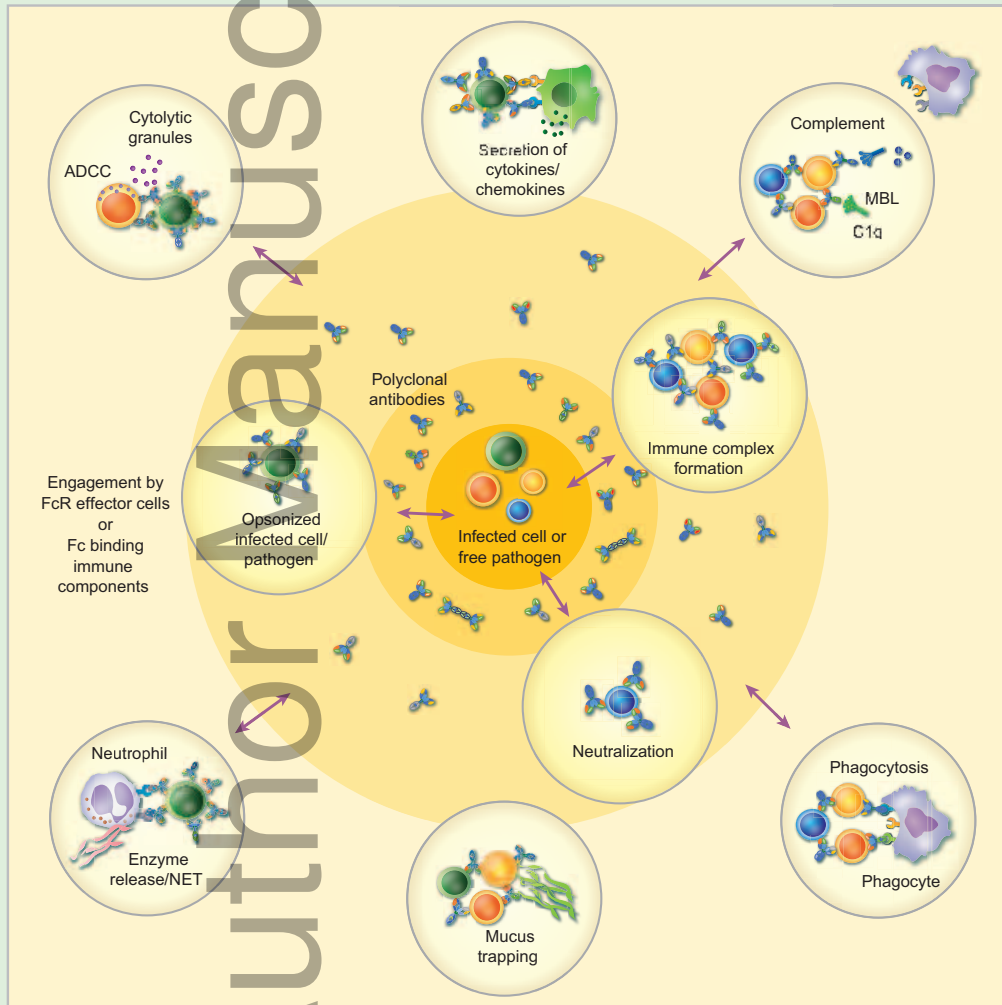
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A

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

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