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Progress toward improved understanding of antibody maturation

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Abstract

Upon encountering an antigen, antibodies mature through various rounds of somatic mutations, resulting in higher affinities and specificities to the particular antigen. We review recent progress in four areas of antibody maturation studies. (1) Next-generation and single-cell sequencing have revolutionized the analysis of antibody repertoires by dramatically increasing the sequences available to study the state and evolution of the immune system. Computational methods, including machine learning tools, have been developed for reconstituting antibody clonal lineages and for general repertoire analysis. (2) The availability of X-ray structures, thermodynamic and kinetic data, and molecular dynamics simulations provide information on the biophysical mechanisms responsible for improved affinity. (3) In addition to improved binding to a specific antigen, providing affinity-independent diversity and self/nonself discrimination are fundamental functions of the immune system. Recent studies, including X-ray structures, yield improved understanding of both mechanisms. (4) Results from in vivo maturation help to develop methods of in vitro maturation to improve antibody properties for therapeutic applications, frequently combining computational and experimental approaches.

Introduction

After exposure to an antigen, antibodies specific to that antigen will be enriched through the process of antibody maturation, which involves clonal selection, expansion and somatic hypermutation [1]. Immunoglobulin (Ig) genes are mutated and any resulting B cell receptors (BCRs) which have acquired higher affinity are favored for survival; the humoral response will become dominated by these mutated receptors, which confer protection in subsequent antigen exposures. Such a rapid cycle of mutation and selection bolsters the host defense, with antibody affinity improving 10 to 5,000 fold during the immune response [2]. This complex process raises a number of interesting questions. Where do the mutations occur? What is the effect of the mutations on antibody structure and flexibility? How do the mutations change antibody properties, primarily affinity, on and off rates, specificity, and

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stability? How does the immune system provide self/nonself discrimination? How deterministic are the developmental pathways (lineages)? Are these similar in different individuals? While interpreting experimental data to answer these questions provides necessary insight, the major test of understanding is whether the changes associated with antibody maturation can be predicted with any reasonable accuracy, and whether there is sufficient information for developing therapeutic antibodies. As shown in this short review focusing on aspects of antibody maturation (Figure 1), during the last two or three years a number of important discoveries substantially improved our understanding of the immune system, and large scale collection of data and the development of novel methods predict further progress.

Analysis of antibody repertoires

The collection, or repertoire, of antibodies within an organism convey its immune status, describes its innate ability to deal with invading or harmful substances, and acts as a history of how the organism has previously responded to similar challenges [1]. Recent advances in next-generation sequencing (NGS) have revolutionized strategies for antibody repertoire analysis by dramatically increasing sample depth compared to previous low-throughput methods [2]. New methods have also been developed for single-cell sequencing, which allow large-scale determination of paired light (L) and heavy (H) chains. In addition to computational tools for reconstituting antibody clonal lineages [3], these advances can provide valuable insights into how the immune system works, including how it is initially capable of protecting against diverse threats, but produces higher affinity antibodies after antigen exposure [1]. Researchers now have easy access to a vast number of sequences. For example, the Observed Antibody Space (OAS) database, contains over 1 billion sequences [4]. A number of specialized sequence analysis tools are also available [5], and have enabled accurate models of somatic hypermutation to be established [6], leading to the creation of software that simulates the repertoires [3,7]. In particular, the analyses were employed to study the effect of disease on the immune system [8] and to monitor the impact of organ transplant [9]. Machine learning was also used to predict vaccination status or the presence of disease [10], and in view of the availability of sequence data it is expected to become a major tool to study the repertoires.

The impact of mutations on antibody structure, flexibility, and binding affinity

While sequences alone provide valuable information regarding the immune response, 3D structures are the best to determine how an antibody governs its binding properties and interacts with an antigen [1,11]. One of the mechanisms to achieve increased affinity in mature antibodies has been shown to be mutations to the residues in the complementarity-determining regions (CDRs) of the variable chains. The mutations in CDRs may drive affinity maturation through two main mechanisms and their combinations. On one extreme, mutations that increase shape complementarity of the interface, improve electrostatic interactions, hydrogen bonding, and promote increased burial of hydrophobic regions in the interface all improve binding by enthalpic means. The alternative and even better studied

mechanism involves decreasing entropic penalties associated with complex formation due to the rigidification of some CDRs. The CDR H3 loop has proven to be of particular importance in both mechanism, as it has been shown to form the most contacts on average with the antigen, while also demonstrating highest structural variation even without direct mutations [12]. A well-studied example of the entropy-driven increase of affinity is a B-cell lineage expressing broadly neutralizing influenza virus antibodies, as discussed in Schmidt et al. [13]. The lineage was derived from a subject immunized with a trivalent vaccine and was comprised of three mature antibodies, the unmutated common ancestor, and a common intermediate (Figure 2), all with the CDR H3 inserting into the conserved receptor-binding pocket of influenza hemagglutinin. Mutations that almost exclusively occur in non-H3 CDR and framework regions rigidify the conformation of the H3 loop very close to its bound conformation, as demonstrated by the analysis of structures and binding kinetics. Long time-scale molecular dynamics simulations revealed that the maturation increases the probability of the H3 loop being close to its conformation in the antigen-bound structure [13]. Rigidification of the H3 loop by remote mutations was also reported for an anti-HIV neutralizing antibody [14]. In another recent study, Fernández-Quintero et al. [15,16] analyzed pairs of antibody fragments which differed in specificity and stage of affinity maturation. Using a combination of metadynamics and molecular dynamics (MD) simulations, they observed substantial rigidification in flexibility and plasticity as reflected by a decrease of conformational diversity. However, a large scale study by Jeliazkov et al. [17] focusing on CDR H3 loops did not find substantial differences in the flexibility of naïve and antigen-experienced antibodies. Molecular dynamics simulations revealed a spectrum of changes in flexibility, indicating that while rigidification may be important, it is not the only biophysical mechanism leading to improved affinity.

Changes in conformation and flexibility also determine the kinetics of antibody-antigen binding. The already mentioned study of influenza antibody maturation by Schmidt et al. [13] reported two orders of magnitude increase in the on-rate and one order of magnitude decrease in the off-rate values, in good agreement with the observation that the major change is the preorganization of the CDR H3 region. In contrast, Rosenfeld et al. [18] found that improved antibody-based ricin neutralization by affinity maturation was correlated with slower off-rate values. We think that this variation is due to the difference in the shape of the antibody epitopes. The H3 loop of neutralizing antibodies targeting influenza HA must find the fairly narrow sialic acid binding site [13], which is the major binding energy hot spot [19,20]. This suggests that appropriate preorganization and rigidity of the H3 loop increases the k_{on} values, whereas the k_{off} values are less affected due the scarcity of mutations in H3. In contrast, modeling of the ricin-binding antibody suggests that the mutations may increase this variant's conformational flexibility, which may improve its ability to bind ricin [18].

Maturation for improved specificity

The selection of antibody variants and somatic hypermutation play at least two main roles in generating a robust B cell immune response [21]. The first is the classical process of affinity maturation, in which the antibody adapts to fit more perfectly to the antigen structure. The second role is the generation of affinity-independent diversity and the ability to adapt to changes in the antigen. The latter outcome may be particularly important for protection

against pathogens such as influenza virus that mutate rapidly enough to reinfect a previously exposed individual. McCarthy et al. [21] described an extensive structural and biophysical analysis of a lineage of B cell antigen receptors (BCRs) directed against the receptor binding site of subtype H1 influenza virus hemagglutinin (HA). The antibodies were obtained from a donor who was born in 1989 and in 2008 received a trivalent influenza vaccine. The lineage included 8 antibodies, three in one principal branch and five in the other. As described previously [13], the CDR H3 was found to fit with an invariant pose into the small sialic acid binding site of HA, but in each of the two branches the rest of the Fab reoriented specifically from its position in the unmutated common ancestor (UCA). The reorientation generated new contacts, which compensated for contacts lost as the HA itself mutated during the time between the donor's initial exposure and his vaccination. The presence of cells producing antibodies from divergent branches like these thus offers broader protection when compared to cells from only a single, linear evolutionary trajectory [21]. In a large scale study, Shehata et al. [22] analyzed biophysical properties of human antibodies derived from multiple B cell subsets, and found that somatic hypermutation was associated with increased antibody specificity. However, they observed that maturation reduced both hydrophobicity and thermal stability compared with naive B cell-derived mAbs. In agreement with this finding, Julian et al. [23] reported that co-selection of compensatory mutations to maintain thermodynamic stability was required for efficient affinity maturation of antibody variable domains.

An important question is how antibodies develop the specificity to differentiate foreign antigens that mimic self-antigens. Burnett et al. [24] generated B cells in a mouse model displaying an antibody that cross-reacted with two related protein antigens expressed on self versus foreign cells. They found that the concentration of B cells remained low until challenged with a high-density foreign antigen, which initiated germinal center recruitment and antibody gene hypermutation. The mutations primarily decreased self-affinity, and increased foreign affinity at a slower rate. Crystal structures revealed that these mutations exploit subtle structural differences in order to achieve 5000-fold preferential binding to foreign over self-epitopes. The interesting conclusion was that antibody mutation away from self-reactivity deferred the need to acquire stringent self-tolerance until after an infection. However, retaining self-reactive clones in the naïve antibody repertoire as substrates for protective antibody responses was required to retain the ability to detect all foreign antigens.

Watanabe et al. [25] used single-cell cultures to determine the repertoires of human B cell antigen receptors before and after the second B cell tolerance checkpoint in both healthy donors and in patients with systemic lupus erythematosus (SLE). Among healthy donors, roughly 70% of transitional B cells before the second checkpoint recognizing foreign antigens also bound human self-antigens, but peripheral tolerance halved the frequency of the self-reactive mature B cells. However, in SLE patients who are defective in the second tolerance checkpoint, frequencies self-reactive B cells remained unchanged during maturation. The authors concluded that cross-reactivity between foreign and self-epitopes may be more common than previously believed [25]. This agrees with the observations of Burnett et al. [24] that such cell are needed in the native repertoire, but their concentration is low and are increasingly eliminated upon mutations to respond to an infection.

In vitro maturation of therapeutic antibodies

Antibodies have become very important therapeutics, as evidenced by an increasing number of FDA-approved monoclonal antibodies [26–28]. Antibody drugs have many advantages over small-molecule drugs, including superior specificity, prolonged serum half-life, and high druggability [26,29]. Antibody discovery platforms use either a display-based library approach (phage, yeast, ribosome, mammalian, or other systems) or an immunization and hybridoma screening strategy for antibody isolation [30]. In vitro affinity is needed when the affinity of antibodies generated by these methods does not meet the requirement for drug development. Moreover, to reduce their antigenicity, humanization of antibodies generated from non-humanized animals frequently results in reduction of antibody affinity, which has to be restored [31]. The display methods mentioned above can be used for in vitro affinity maturation, and successful applications have been reported [32]. Other tools are random mutagenesis by error-prone PCR, and combinatorial mutagenesis limited to the CDRs [33]. Reprogramming the antigen specificity of B cells using CRISPR-Cas9 genome-editing technologies is a more recent and very innovative approach [34]. However, these methods of in vitro affinity maturation can be laborious and time consuming, and hence a variety of computational approaches have been developed [28,30,35]. Although the methods of in silico antibody maturation and methods of de novo antibody design partially overlap, here we focus only on the first application, and refer to recent reviews [11,36] for the design tools.

Computational antibody maturation generally requires a high-quality antibody-antigen co-crystal structure as the starting point, and an algorithm which calculates the energy change ocurring upon mutation. As an example, Purisima and co-workers developed the ADAPT (Assisted Design of Antibody and Protein Therapeutics) platform for improving and modulating antibody affinity [37,38]. The method uses a combination of three scoring functions, and tests the impact of mutating residues one-by-one without changing the initial conformation of the backbone. In spite of these simplifying assumptions, the platform provided triple mutants that exhibited over 30-fold improvements in binding affinity. Kuroda and Tsumoto [35] and Cannon et al. [30] also provided examples of successful application, although in the latter the computational method was guided by experimental alanine scanning.

In vitro maturation generally attempts to optimize several properties, including affinity, specificity, stability, and solubility. A common challenge is that an improvement in one property (e.g., affinity) can lead to a deficits in another (e.g., stability). Rabia et al. [29] studied potential trade-offs and the possibility of co-optimizing multiple antibody properties [29]. An additional but very important goal of antibody maturation is avoiding “developability issues” such as poor stability or high levels of aggregation. Raybould et al. [27] provided guideline values for five metrics implicated in poor developability, including the total length of CDRs, the extent and magnitude of surface hydrophobicity, positive charge and negative charge in the CDRs, and asymmetry in the net heavy- and light-chain surface charges. The guideline cutoffs for each property were derived from the values seen in clinical-stage antibody therapeutics.

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** of outstanding interest

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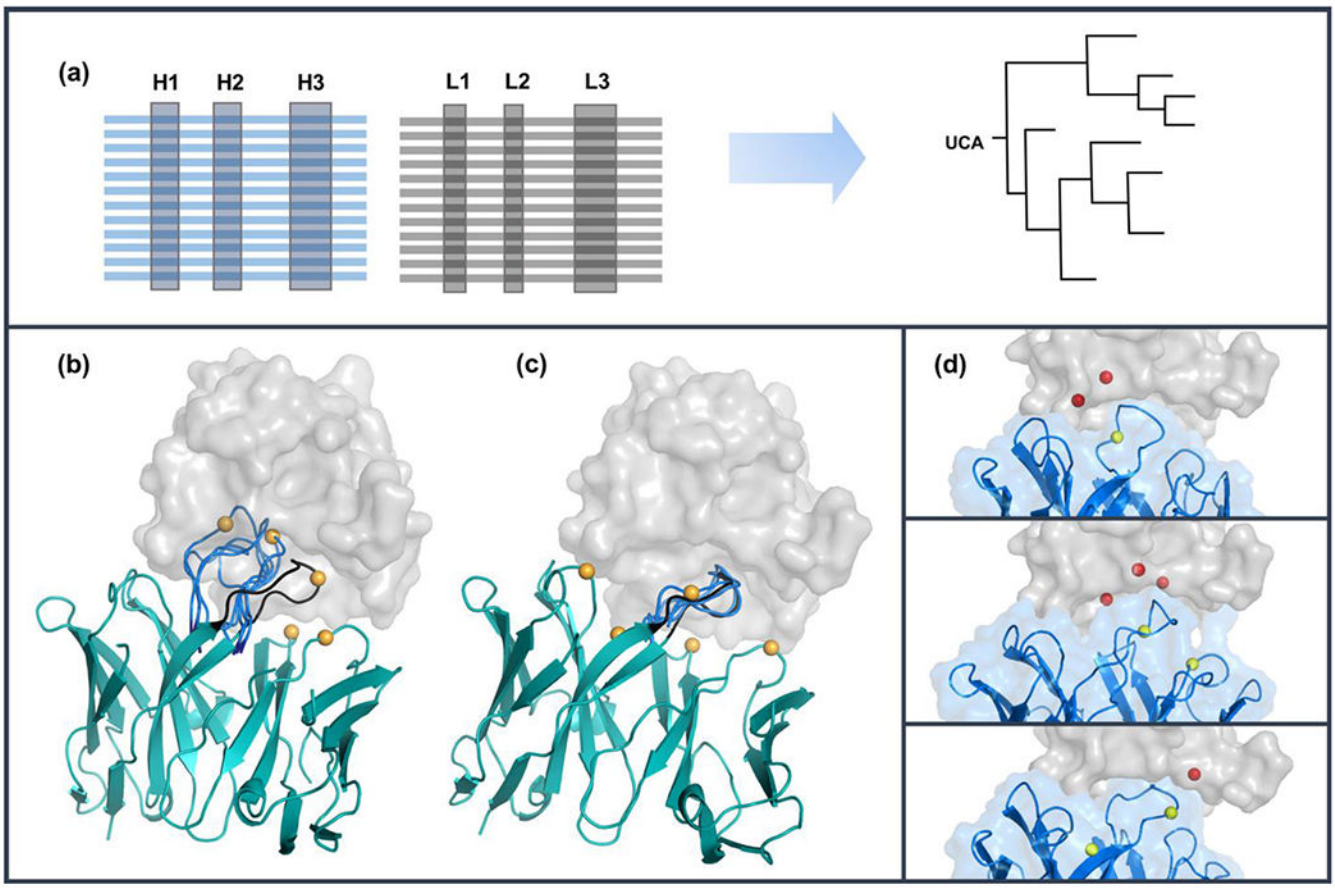

Figure 1.

Focus areas in antibody maturation. **(a)** Large collections of sequences representing entire antibody repertoires are now available to establish models of somatic hypermutation and to reconstruct antibody clonal lineages. **(b)** Enthalpy-driven improvement of affinity is frequently caused by mutations of contact residues in the CDRs that increase shape complementarity interface, improve electrostatic interactions, hydrogen bonding, and promote increased burial of hydrophobic regions in the interface. **(c)** Alternatively, the binding affinity can be improved by the rigidification of some interacting loops, in most cases H3, thereby reducing the entropy loss upon binding. Binding can be improved by mutations outside the loops directly contacting the antigen. **(d)** The panels represent mutations on the antibody (*yellow* spheres) to compensate for the naturally occurring mutations of the antigen (shown as *red* spheres) in frequently mutating viruses such as influenza.

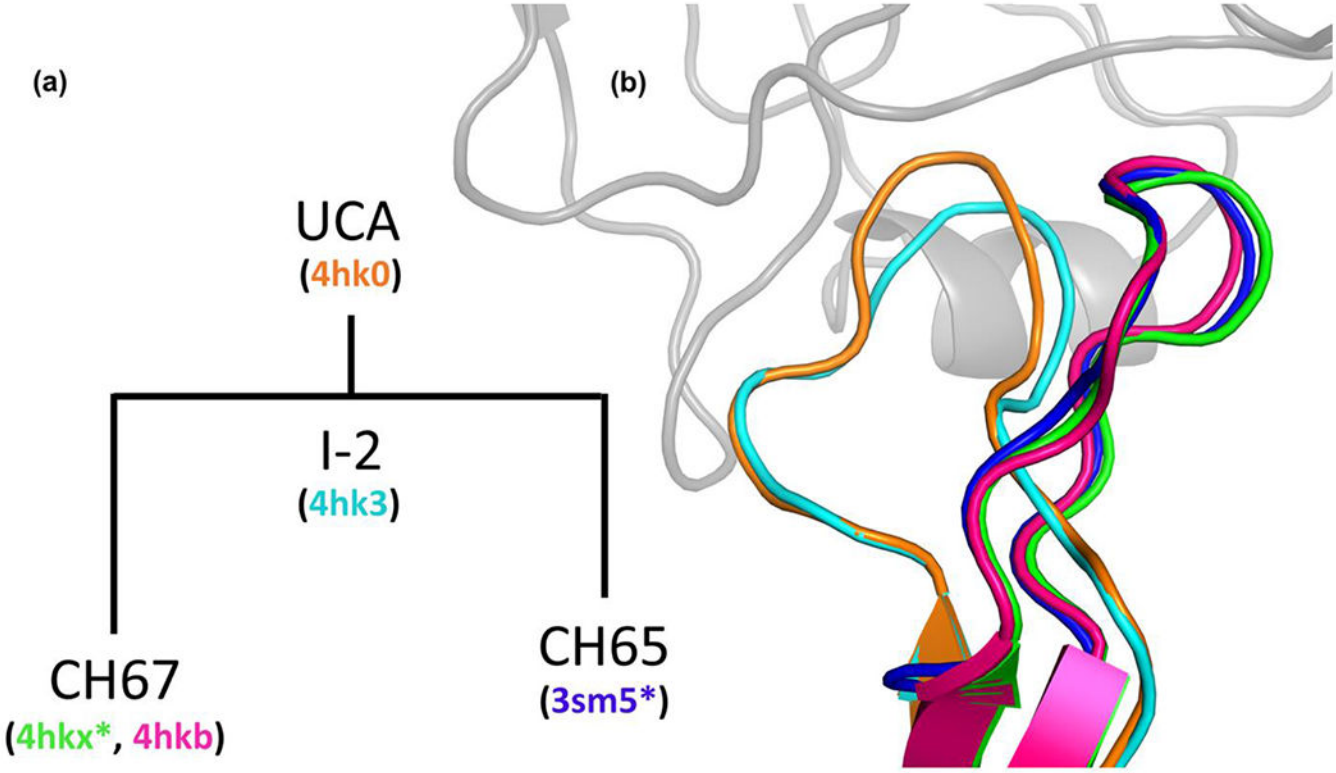

Figure 2.

Example of preconfigured H3 loop in entropy-driven improvement of binding affinity. **(a)** B-cell lineage expressing broadly neutralizing influenza virus antibodies that bind to the sialic acid receptor region of the hemagglutinin (HA), including the UCA (PDB ID 4hk0), a common intermediate I-2 (4hk3) and two of the mature antibodies. X-ray structures are available for both the unbound and HA-bound variant CH67 (4hkb and 4hkx, respectively). Only the HA-bound structure is solved for the variant CH65. **(b)** The conformations of the H3 loop in the five structures shown on the left reveal that the unbound structure in the mature antibody CH67 (4hkb, *purple*) comes very close to the conformations of the loop in the HA-bound structures (4hkx and 3sm5, colored *green* and *blue,* respectively). The star indicates HA-bound structures.