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Promiscuous antibodies characterised by their physico-chemical properties: From sequence to structure and back

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ABSTRACT

Human B cells produce antibodies, which bind to their cognate antigen based on distinct molecular properties of the antibody CDR loop. We have analysed a set of 10 antibodies showing a clear difference in their binding properties to a panel of antigens, resulting in two subsets of antibodies with a distinct binding phenotype. We call the observed binding multiplicity 'promiscuous' and selected physico-chemical CDRH3 characteristics and conformational preferences may characterise these promiscuous antibodies. To classify CDRH3 physico-chemical properties playing a role in their binding properties, we used statistical analyses of the sequences annotated by Kidera factors. To characterise structure-function requirements for antigen binding multiplicity we employed Molecular Modelling and Monte Carlo based coarse-grained simulations. The ability to predict the molecular causes of promiscuous, multi-binding behaviour would greatly improve the efficiency of the therapeutic antibody discovery process.

Contents

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1. Introduction

Immunoglobulins (Ig) are a crucial component of the adaptive immune response. Adaptive immunity is distinct from innate immunity in that it confers a highly specific defense against invading pathogens and is capable of creating memory against foreign molecules (antigens), enabling a rapid response upon repeated exposure to the same antigen. Immunoglobulins are produced by B cells and are either displayed on the cell surface, as B cell receptors, or are secreted into the extracellular environment and circulate as antibodies in the blood.

Antigen recognition is mediated by the antibody variable regions, which are located at each of the two apical sites on the 'Y' arms of the antibody. A huge diversity of specificities in the antibody repertoire is achieved by gene rearrangement processes, whereby variable (V), diversity (D) and Joining (J) genes recombine to produce a complete heavy chain (VDJ) or light chain (VJ) variable gene. The rearranged gene is expressed in conjunction with a constant region that confers the functional attributes of the antibody. The heavy chain and the light chain variable regions of the immunoglobulin protein fold to form a conserved β-sheet framework interspersed by six hypervariable loops or "complementarity determining regions" (CDRs), so-called because they come together to form the antigen-binding site. Amongst the CDRs (there are three from each chain), the third loop on the heavy chain (CDR-H3) is the most diverse because it is encoded by a stretch of nucleotides spanning all three IGHV-D-J gene segments. Similarly, the equivalent light chain region (CDR-L3) is also diverse although not to the same extent. Site-directed mutagenesis and loop grafting studies have shown that the H3 loop can be sufficient to define antibody specificity (Xu and Davis, 2000). Crystal structure analyses have also identified the CDR-H3 region as being centrally positioned in the antigen-binding site, always in contact with antigen and, in some cases, able to change conformation upon binding (Stanfield and Wilson, 1994). In fact, CDR-H3 is the only exception to the canonical structure model, which has identified all remaining CDRs as belonging to one of a few discrete conformations on the basis of sequence length and composition (Shirai et al., 1996; Chothia and Lesk, 1987; Chothia et al., 1989; Shirai et al., 1999).

A consequence of the random nature of the Ig gene rearrangement process is that a significant proportion of antibodies produced in the bone marrow may be autoreactive (Wardemann et al., 2003). Potentially dangerous autoreactive antibodies must be removed from the repertoire at tolerance checkpoints in the development process in order to avoid autoimmune diseases such as systemic lupus erythematosus (Yurasov et al., 2005) and rheumatoid arthritis (Samuels et al., 2005). Some antibodies are capable of binding multiple chemically and structurally diverse antigens, which means that although an antibody may be produced with the potential to usefully bind to exogenous antigens, it may result in binding to self-antigens. Thus previous literature on tolerance and autoimmunity will quite often refer to "polyreactivity" of an antibody. Polyreactive antibodies occur in normal human sera and are thought to act as a first line of defense against foreign antigens (Guilbert et al., 1982). They have been shown to cause bacterial lysis (Zhou et al., 2007a, 2007b), induce complement and clear apoptotic cells (Zhou et al., 2013). So in the case of polyreactive antibodies a trade-off balance between potentially useful initial activity and potentially harmful anti-self effects has to be maintained.

In the literature people refer indiscriminately to polyreactive and/or polyspecific antibodies and the definition intrinsic in the prefix 'poly' is matter of debate (Dimitrov et al., 2013). It has been recently clarified that this term does not refer to the case of multiple binding due to some stickiness of the antibody chemicophysical properties and that a large screen over a panel of putative antigens is needed before extracting antibodies that clearly react specifically to a subset of these target antigens. Another term that is frequently used when referring to antibodies multi-binding behaviour is promiscuity (James and Tawfik, 2003a, 2003b). This term has long been investigated in the field of enzyme binding (Nobeli et al., 2009), and has been referred to as functional promiscuity, again implying some specific functional behaviour resulting from the particular type of binding mode. It is claimed by Favia at al. (Nobeli et al. 2009) that the term promiscuous may imply at times what they refer to as 'invisible' phenotypes, observable only under certain conditions. In the past our laboratory has adopted the term promiscuity as generally applicable to proteins that show multiple partners but may use different strategies to bind in such a polyvalent way (Fornili et al., 2013). We use herewith the term promiscuous in referring to a subset of antibodies where binding to a number of tested antigens in the same experimental conditions were detected, as compared to others that do not show binding.

Despite its importance in human health and disease, the molecular basis of antibody multi-binding behaviour remains largely obscure. Towards this goal, it has been noted that some polyreactive antibodies have particularly high frequencies of aromatic amino acids in the CDR-H3 region (Droupadi et al., 1994) and high isoelectric points in heavy chain Fv regions. It has also been hypothesised that exposed hydrophobic patches are associated with so-called antibody promiscuity (Barbas et al., 1997). Somewhat paradoxically however, a role for specific hydrogen bonding has also been proposed (James and Tawfik, 2003a). Comparisons of germline versus antigen-induced antibodies have shown that the former are more likely to be polyreactive (Chen et al., 1991) and more flexible (Manivel et al., 2000), which may suggest that one of the features of polyreactive antibodies is enhanced flexibility. In support of this, a structural analysis of a promiscuous antibody in the free and bound states has demonstrated that it adopts a different conformation when bound (James et al., 2003). In contrast however, Sethi et al. (2006) have more recently shown that structurally diverse epitopes (the precise binding site on the antigen) bind differentially to a structurally common paratope (the precise binding site on the antibody), implying that paratope flexibility can be limited.

Here, we compare the structural properties of ten antibodies,