Methods 65 (2014) 95–104

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Understanding the role of cross-arm binding efficiency in the activity of monoclonal and multispecific therapeutic antibodies

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ARTICLE INFO

Article history:
Available online 18 July 2013

Keywords:
Immunoglobulin
Bispecific antibody
Multispecific antibodies
Cross-arm binding
Avidity
Computational modeling

ABSTRACT

Antibodies are essential components of the adaptive immune system that provide protection from extracellular pathogens and aberrant cells in the host. Immunoglobulins G, which have been adapted for therapeutic use due to their exquisite specificity of target recognition, are bivalent homodimers composed of two antigen binding Fab arms and an immune cell recruiting Fc module. In recent years significant progress has been made in optimizing properties of both Fab and Fc components to derive antibodies with improved affinity, stability, and effector function. However, systematic analyses of the efficiency with which antibodies crosslink their targets have lagged, despite the well-recognized importance of this cross-arm binding for optimal antigen engagement. Such an understanding is particularly relevant given the variety of next-generation multispecific antibody scaffolds under development. In this manuscript we attempt to fill this gap by presenting a framework for analysis and optimization of antibody cross-arm engagement. We illustrate the power of this integrated approach by presenting case studies for rational multispecific antibody design based on quantitative assessment of the interplay between antibody valency, target expression, and cross-arm binding efficiency. We conclude that optimal design parameters for cross-arm binding strongly depend on the biological context of the disease, and that cross-arm binding efficiency needs to be considered for successful application of multispecific antibodies.

1. Introduction

Monoclonal antibodies and antibody-like molecules have emerged as the leading class of protein therapies [1–3]. They are designed to mimic the function of natural immunoglobulin G molecules, i.e., to bind, neutralize and clear undesirable entities from human bodies [4]. Monoclonal antibodies are bivalent modular molecules that comprise three functional arms: two identical Fab arms and an Fc module [5–8]. These modules enable different components of an antibody's mechanism of action: the Fab arms perform high affinity and specificity recognition of the target antigen, whereas the Fc module engages immune cells, yields stable association of heavy-chain pairs, and provides favorable pharmacokinetic behavior [9–12].

The advances in molecular biology techniques over the last twenty years have allowed researchers to conduct careful exploration of antibody structure–function relationships. *In vitro* evolution techniques, such as targeted mutagenesis [13,14], phage display [15–17], yeast display [18,19], ribosomal display [20], CIS display [21] and *Escherichiacoli* display [22] have been helpful as they provided means for high-throughput exploration of large numbers of defined antibody variants. *Invitro* selection techniques have been combined with fully human libraries to yield Fab or Fv antibody fragments that do not require chimerization or humanization [23,24] and target specific epitopes of interest [25]. They also have been used for affinity maturation [26,27] and stability engineering [28–30] of antibody leads.

Engineering of the Fc module has also attracted significant attention. Directed evolution techniques were used to derive Fc variants that bind to Fc receptors with higher affinity and specificity [31–33], show altered engagement of complement [34,35], and possess longer circulation half-life in non-human primates [36–38]. Additionally, targeted engineering has led to the discovery of Fc variants that are monovalent or heteromeric [39–41] or that can perform independent antigen recognition [42,43].

Given that the antibody sequence space is vastly larger than the maximal sequence complexity that these techniques can interrogate, the above research efforts have focused on optimization of Fab arms and Fc modules separately. There are, however, properties

Abbreviations: HER2, human epidermal growth factor receptor 2; HER3, human epidermal growth factor receptor 3; HRG, heregulin; IGF-1R, insulin-like growth factor-1 receptor; IGF-1, insulin-like growth factor-1; pAKT, phosphorylated AKT; dsIgG, dual-specific IgG.

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1046-2023/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ymeth.2013.07.017

of antibodies that depend on the interplay between Fab and Fc. These include antibody-induced target internalization and clearance [44–46] and binding avidity [47], both of which derive from multivalent Fab binding to target in the context of Fc-mediated dimerization. In addition to affecting functional potency of monoclonal antibodies, multivalent binding to target is fundamentally important for the emerging therapeutic class of multispecific antibodies [48–50]. For the purposes of this manuscript, we have termed this multivalent binding ability of antibodies or antibody-like molecules "cross-arm binding".

Cross-arm binding is difficult to interrogate and engineer for several reasons: first, the sequence space of intact antibody is enormous and cannot be adequately sampled with available experimental techniques; second, monitoring and interpretation of multiple high-affinity binding events on the surface of one or multiple cells require quantitative assays and sophisticated analysis; third, these events are usually time-dependent; and fourth, they are typically linked [51]. It is, therefore, not surprising that comparatively to Fab and Fc engineering little work has been done to purposefully modulate this inter-arm property.

Computational models of antibody–target interactions, utilizing the mathematics of differential equations, are well suited for addressing this gap in rational antibody design. Mathematical descriptions of both antibody–target interactions can integrate parameters describing both intrinsic, monovalent binding affinity, and multivalent binding avidity resulting from antibody-mediated crosslinking [52,53]. Early models of antibody binding were used to describe activation of the allergy response due to clustering of Fcε receptors mediated by IgE–antigen complexes [54–56]. More recent approaches, from the field of oncology, have linked models of antibody binding to cell surface receptors to kinetic models of receptor-mediated signal transduction. By doing so, the joint model can describe the relationship between antibody properties and the potency of cellular signaling inhibition, thereby facilitating antibody design decisions [57].

Previously, we have used this approach to introduce an integrated computational and experimental method for assessing the role of cross-arm binding in driving antibody potency [58]. Because that work focused solely on bivalent IgG antibodies, in the current work we extend this approach to exploring the importance of cross-arm binding in multispecific antibody design. We show that cross-arm binding results from the interplay of three parameters: antibody valency, target receptor levels, and the intrinsic crosslinking efficiency of the antibody. We describe methods that can be used to determine these parameters from readily available experimental data. Additionally, we demonstrate that it is possible to simulate how these properties affect functional activity of an antibody-like molecule, by presenting examples for rational therapeutic engineering of antibody-like molecules for oncology use that are based on modulation of cross-arm binding.

2. Theory

The assessment of cross-arm binding derives from computational models that describe the interaction of antibodies or antibody-like molecules with cell surface target(s). In these models, differential equations use the mass-action kinetic formalism in order to describe the time evolution of antibody–target binding. The simplest, bivalent antibody case has been described previously [58] and is summarized here. This model has two reactions, one characterized by monovalent binding of antibody to target (Fig. 1, left), and the second reflecting cross-arm, multivalent binding (Fig. 1, right). Translating these reactions into ordinary differential equation results in the following model:

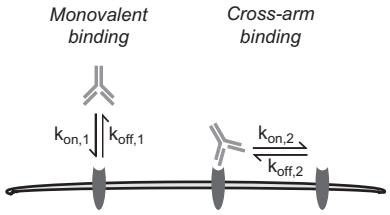

Fig. 1. Monovalent and cross-arm binding reactions for a bivalent IgG antibody. The binding of bivalent antibodies to cell surface antigens is characterized by an initial, monovalent binding event that localizes the antibody to the cell membrane, followed by cross-arm binding of the free antibody arm to another cell surface antigen. The strength of cross-arm binding relative to monovalent binding (characterized by the parameter χ) incorporates both a high local concentration of antibody due to restriction of diffusion to the cell membrane, and epitope and format-specific steric variability.

$$\frac{d}{dt}[\mathrm{Ab}] = -k_{on,1}[\mathrm{Ab}][\mathrm{R}] + k_{off,1}[\mathrm{Ab:R}]$$
$$\frac{d}{dt}[\mathrm{R}] = -k_{on,1}[\mathrm{Ab}][\mathrm{R}] + k_{off,1}[\mathrm{Ab:R}] - k_{on,2}[\mathrm{Ab:R}][\mathrm{R}] + k_{off,2}[\mathrm{R:Ab:R}]$$
$$\frac{d}{dt}[\mathrm{Ab:R}] = k_{on,1}[\mathrm{Ab}][\mathrm{R}] - k_{off,1}[\mathrm{Ab:R}] - k_{on,2}[\mathrm{Ab:R}][\mathrm{R}] + k_{off,2}[\mathrm{R:Ab:R}]$$
$$\frac{d}{dt}[\mathrm{R:Ab:R}] = k_{on,2}[\mathrm{Ab:R}][\mathrm{R}] - k_{off,2}[\mathrm{R:Ab:R}]$$

Monovalent antibody–target binding rates k_{on} and k_{off} are incorporated into the model parameters as follows:

$$k_{on,1} = 2k_{on}$$
$$k_{off,1} = k_{off}$$
$$k_{on,2} = \chi k_{on}$$
$$k_{off,2} = 2k_{off}$$

Parameters $k_{on,1}$ and $k_{off,2}$ include stoichiometric corrections due to antibody bivalency. The parameter χ represents the intrinsic cross-arm binding efficiency between antibody and receptor and incorporates two phenomena. First, an antibody bound to cell-surface receptor is restricted to a narrow, quasi-two-dimensional space above the cell membrane that greatly increases the local concentration of antibody [59]. The magnitude of this "reduction of dimensionality" effect depends on the geometric reach of a cell-surface tethered antibody, but in general significantly increases the apparent affinity of the cross-arm binding event. Second, cross-arm binding incorporates a restriction of rotational, torsional, and bending freedom that is inherent in the geometry required for crosslinking. This restriction will tend to reduce the apparent affinity of cross-arm binding. This phenomenon is epitope- and format-dependent, as demonstrated by available data suggesting that different antibodies exhibit a varying ability to cross-link adjacent receptors on the same cell surface [60,61]. Recent simulations of heterobivalent ligand binding by Vauquelin and Charlton describe the opposing effects incorporated in χ using separate parameters [62], but find that they are linked mathematically, motivating the simpler treatment given here.

Simulation of the computational binding model (using software such as MATLAB® SimBiology®- http://www.mathworks.com/products/SimBiology) requires specification of values for antibody concentration, target expression level, monovalent antibody–target binding kinetics, and cross-arm binding efficiency. Of these parameters, antibody concentration and target expression level reflect the choice of experimental conditions, while antibody monovalent binding parameters can be measured directly using cell-free methods such as surface plasmon resonance or kinetic exclusion assay [63,64].