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Editorial

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Experimental Determination of Antibody Affinity and Avidity: Guidance and Considerations

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Monoclonal antibodies (mAbs) have emerged as a tremendously important class of biologics. MAbs, when used as passive immunotherapy drugs, offer a dizzying array of possible mechanisms of action ranging from: immune effector functions, delivery of cytotoxic/radioactive agents, to modulating cell signaling. Regardless of the specific mechanism of action, the crucial event that lies at the heart of their therapeutic function is mAb binding to its cognate antigen. Quantification of the interaction involves two interrelated, but distinct concepts: *affinity and avidity*. The antibody literature is rife with misconceptions and misapplications of these terms. Here, we provide some context, clarity, and guidance for both authors and reviewers of manuscripts for publication in *Monoclonal Antibodies in Immunodiagnosis and Immunotherapy* with regards to experimentally determined affinity values.

An antibody's *affinity* refers to the interaction strength between a single antibody binding site (i.e., one Fab) to antigen. The strength of the interaction is given by the equilibrium rate constant (K_D). Because affinity is a monovalent process, a straightforward and direct approach to its determination is to employ monovalent Fab or ScFv fragments for binding analysis. Fab fragments can readily be prepared from IgG by proteolytic digestion with papain[1] or recombinantly produced by transient transfection in mammalian cells.[2] Affinity measurement can be carried out using numerous methods but a convenient and commonly used approach is surface plasmon resonance (SPR), which provides label-free, real-time kinetic data. While IgG can be used for affinity determination, resultant avidity effects (discussed below) can complicate analysis. For SPR experiments to measure antibody affinity, there are two possible configurations which can be used to minimize bivalent or multivalent antibody-antigen interactions. (*i*) If monovalent Fab or ScFv fragments are used, either the antigen or the Fab could be immobilized and used as ligands in the SPR experiment. (*ii*) However, if IgG is going to be used for SPR affinity measurement, the only configuration available to minimize bivalent binding is to immobilize the IgG on the sensor chip (ligand), and flow the antigen over the surface (analyte).

In some cases, laboratories may not have ready access to SPR instrumentation, and instead must rely on more traditional equilibrium-based approaches to report antibody affinity, such as competitive ELISA or flow cytometry. Both of these approaches typically use IgG. For affinity determination this may be problematic as sorting out monovalent affinity *vs* avidity may not be straight forward (see below). Outside of avidity considerations, equilibrium-based approaches require that equilibrium actually be reached for accurate measurement of K_D. Incubation times may exceed several hours in the case of very high affinity interactions in order to reach equilibrium (for an excellent review on the topic the readers are referred to Jarmoskaite et al.[3]) If equilibrium based assays are used to measure antibody affinity and avidity (apparent affinity) the experimenter must take care to demonstrate that equilibrium has actually been reached.[3] Moreover, caution must be taken when using these approaches to determine the apparent affinity of an antibody.

Antibody *avidity* on the other hand refers to the *cumulative* binding strength from multiple (two in the case of an IgG) antibody binding sites interacting with antigen. Avidity is a paramount characteristic to the therapeutic activity of antibodies and is important in a variety of effector functions (for an excellent review see Oostindie et al.[4]). These authors also highlight the importance of avidity in autoimmune diseases, and state that the avidity of autoantibodies against self-antigens can enhance their pathogenicity, as observed in myasthenia gravis in monkeys, or may also ameliorate some autoimmune diseases.[4] Avidity is sometimes called apparent affinity, and is frequently what is being measured if IgG is employed in commonly used binding experiments. The effect of avidity can be quite pronounced in multivalent situations, such as the antigen being densely packed on a surface like a cell membrane, or if the antigen itself has multiple binding sites. In these cases, a low affinity antibody (K_D μM-mM range) may display an avidity order of

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magnitude stronger in the nM-pM range.[5] The presence of two binding sites on an IgG molecule means that once one antibody arm has engaged with antigen, the second antibody binding site will display an accelerated association rate (k_{on}) owing to an increased local concentration. Additionally, dissociation of one antibody arm from its antigen does not change the local concentration, as the 2nd antibody arm remains bound to antigen. This means that the antibody may effectively irreversibly bind antigen, as each antibody arm can bind and rebind without the entire complex dissociating. If this is the case, then discussions of antibody avidity as a reversible, equilibrium process or "apparent affinity" are irrelevant.

When reporting or evaluating antibody affinity we recommend to the readership of *Monoclonal Antibodies in Immunodiagnosis and Immunotherapy* that (*i*) regardless of experimental approach, when possible monovalent antibody fragments should be used in lieu of IgG for affinity determination; (*ii*) kinetic methods like SPR and BLI are the preferred approaches for affinity determination; (*iii)* if equilibrium methods are used to measure affinity or avidity, equilibrium should be demonstrated; (*iv*) the term affinity should be used in reference to the monovalent K_D value and avidity used when referring to the relative binding strength of intact IgG

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