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Insights from Fc receptor biology

A route to improved antibody reagents

Jenny M. Woof

Medical Research Institute; University of Dundee Medical School; Ninewells Hospital; Dundee, UK

Fc receptors and their interaction with antibodies will be a major theme at the forthcoming FASEB Science Research Conference on Immunoreceptors to be held in Snowmass this July (details available at www.faseb.org/src/home.aspx, follow the tabs for Immunoreceptors). Since its inception in the mid 1980s, this meeting series has maintained a focus on Fc receptors, and this year's meeting will be no exception.

From a therapeutic viewpoint, there is much to be gained from a detailed understanding of the biology of effector molecules such as Fc receptors and complement. Indeed, knowledge of the interaction of IgG with such molecules has been central to the development of improved mAbs with altered functions and transformed half-lives, tailored for particular therapeutic applications. Examples include mAbs designed to maximize complement recruitment[1] or to enhance Fc receptor engagement and triggering of ADCC,[2-5] or conversely, variants engineered to be unable to engage complement[6] or Fc receptors.[7] Glycoengineering of IgG Fc offers an alternative means to modify effector function capabilities,[8] while development of IgG mutants that display extended or altered serum half-lives has been driven through exhaustive analysis of the interaction with FcRn.[9,10]

Despite the appreciable advances that have been made in unravelling the various facets of Fc receptor biology, new information pertinent to mAb engineering continues to emerge. A flavor of some of these new advances will be given below. They span novel receptors and receptor roles, structure-function relationships, the molecular architecture of signaling complexes, the influence of the membrane lipid environment and scaffolding interactions, isotype considerations, through to technical innovations likely to inform the field.

Remarkably, new receptors that have previously eluded characterization are now being described. These include the IgM receptor, which evidence indicates is a molecule also known as TOPO/Fas apoptotic inhibitory molecule 3 whose gene lies close to other known immunoglobulin receptors on chromosome 1,[11] and a receptor for IgD recently documented on basophils.[12] Moreover, we are seeing an appreciation of new roles for existing Fc receptors. An example is the demonstration in a transgenic study that human FcγRIIa can trigger active and passive anaphylaxis and airway inflammation. Moreover, human mast cells, monocytes and neutrophils were shown to produce anaphylactogenic mediators when FcγRIIA was engaged.[13] Hence IgG may contribute to allergic and anaphylactic reactions in humans by engaging FcγRIIa.

Exciting new structural information on Fc receptors and their ligands is emerging. An important example is the solving of the X-ray crystal structure for human FcγRI.[14] While the structural information supports a ligand binding mode similar to those of FcγRII or FcγRIII, the FG-loop in domain 2 of FcγRI with its conserved one-residue deletion appears critical for high affinity IgG binding. A second example concerns the high responder/low responder (HR/LR) polymorphisms of FcγRIIa, which are linked to susceptibility to infections, autoimmune diseases, and the efficacy of therapeutic Abs. New insights into these differences have been provided by the recent solving of the structure for the complex of the HR allele with IgG Fc.[15] Third, understanding of the human IgE-FcεRI interaction has moved forward significantly through the solving of the X-ray crystal structure of the complex of FcεRI and the entire Fc region of IgE (comprising domains Cε2, Cε3 and Cε4).[16] In a final example, the structural basis for the improved efficacy of nonfucosylated mAbs has been investigated.[17] The X-ray crystal structure of the complex between nonfucosylated IgG Fc and a soluble form of FcγRIIIa carrying two N-linked glycans showed that one of two receptor glycans interacts with nonfucosylated Fc to stabilize the complex. It is proposed that when the Fc glycan is fucosylated this interaction is inhibited due to steric hindrance and, together with the negative effects of Fc fucosylation on the dynamics of the receptor binding site, this provides a rationale for the improved ADCC displayed by nonfucosylated IgG.

A question of interest is precisely how Fc receptors bound to antibody ligands organize themselves within signaling complexes in the cell membrane. Some intriguing clues to this conundrum of molecular architecture are now surfacing. In mast cells, FcεRI molecules loaded with IgE form a synapse when presented with antigen that is mobile within a lipid bilayer, via coalescence into large cholesterol-rich

Correspondence to: Jenny M. Woof; Email: j.m.woof@dundee.ac.uk
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clusters.[18] Of particular relevance to the therapeutic setting, clustering of receptors into immune synapses is also seen with FcγR. For instance, during in vivo ADCC mediated by tumor-specific mAb, clustering of FcγR, actin and phosphotyrosines has been noted at contact zones between tumor cells and macrophages or neutrophils.[19] The theme of the influence of the membrane lipid domain environment on Fc receptor function is taken up elsewhere. It has been shown, for example, that serine phosphorylation of FcγRI influences membrane mobility and function. The cytoplasmic tail of FcγRI interacts with protein 4.1G,[20] and it is proposed that this is mediated via a phosphoserine-dependent mechanism critical for localization of the receptor to lipid rafts.[21] With regard to FcγRIIa, a major role for lipid rafts in the regulation of IgG binding to FcγRIIa has been revealed.[22] Notably, exclusion of FcγRIIa from lipid raft membrane microdomains is able to suppress IgG binding in myeloid cells.

Increased knowledge of the capabilities of Fc receptors specific for other antibody classes is opening up new options for therapy. For example, IgA antibodies may offer a highly useful and efficacious alternative approach of particular relevance to treatment at mucosal sites. Human IgA mAbs have been demonstrated to mediate efficient tumor cell killing[23,24] and to have the capability to control certain infectious diseases.[25,26] The detailed understanding of functional sites in IgA that has resulted from numerous mutagenesis studies,[27] coupled with improved ways to produce and isolate recombinant IgA mAbs[28] should facilitate developments toward therapeutics based on this immunoglobulin class. Similarly, recent studies indicate that IgE may serve as an alternative to the classic IgG backbone for therapeutic antibodies.[29]

Finally, technical innovations seem poised to further inform the field and advances are arriving or may be anticipated from techniques such as solution nuclear magnetic resonance (NMR) spectroscopy,[30] cryo-electron tomography,[31] single particle tracking[32] and ultrasensitive force techniques such as adhesion frequency assays.[33,34]

Interest in Fc receptors continues unabated, and the contribution that the field can make to mAb development and optimisation is unquestionable. The FASEB SRC on Immunoreceptors will serve as a forum for discourse on the above issues and much more, providing invaluable information and networking opportunities for all those interested in ways to maximize the efficacy of mAbs and mAb-based reagents. Registration is open until 24 June 2012.

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