Fc Receptor Testing – a Useful Tool for Assessment of Monoclonal Antibodies

Role of Fc receptors

Fc receptors (FcR) are widely distributed cell-surface proteins. They are found for example on phagocytes like macrophages and monocytes, granulocytes like neutrophils and eosinophils, as well as lymphocytes of the innate immune system (natural killer cells) and adaptive immune system (B-cells). FcRs are cellular receptors for immunoglobulins (Igs) and function as central mediators of antibody-triggered effector functions. The binding of the antibody (immune complex) to FcRs results in a variety of reactions such as the release of inflammatory mediators, antibody dependent cellular cytotoxicity (ADCC) and phagocytosis of an immune complex. The most general function attributed to FcRs is that upon antibody attachment to the surface of microbes or microbe infected cells, microbial pathogens are eliminated.

Types of Fc receptors

There are several types of FcRs with different functions. All these receptors bind to the back end (Fc portion) of IgG1, IgG2, or IgG4 antibodies. The cellular receptors for IgG, responsible for mediating the connection between the humoral response and cellular effector functions, belong to the Fcγ receptor (FcγR) family. To date, four classes of Fcγ receptors have been identified. Functionally, the Fcγ receptors can be divided into activating and inhibitory receptors. FcRs also differ in their antibody affinities due to their different molecular structure. Likewise, the different IgG subclasses have unique affinities for each of the Fcγ receptors.

FcγRI (CD64)

FcγRI, also cluster of differentiation (CD) 64 is responsible for phagocytosis and the activation of macrophages, neutrophils, eosinophils, and dendritic cells. FcγRI has an extracellular portion composed of three Ig-like domains, one more domain than FcγRII or FcγRIII. FcγRI binds to IgG more strongly than FcγRII or FcγRIII. Main functions are phagocytosis, cell activation, activation of respiratory burst, and induction of microbe killing. After binding IgG, FcγRI interacts with an accessory chain, i.e. the common γ chain, which possesses an immunoreceptor tyrosine-based activation motif (ITAM) that is necessary for triggering cellular activation.

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FcγRIIA (CD32)

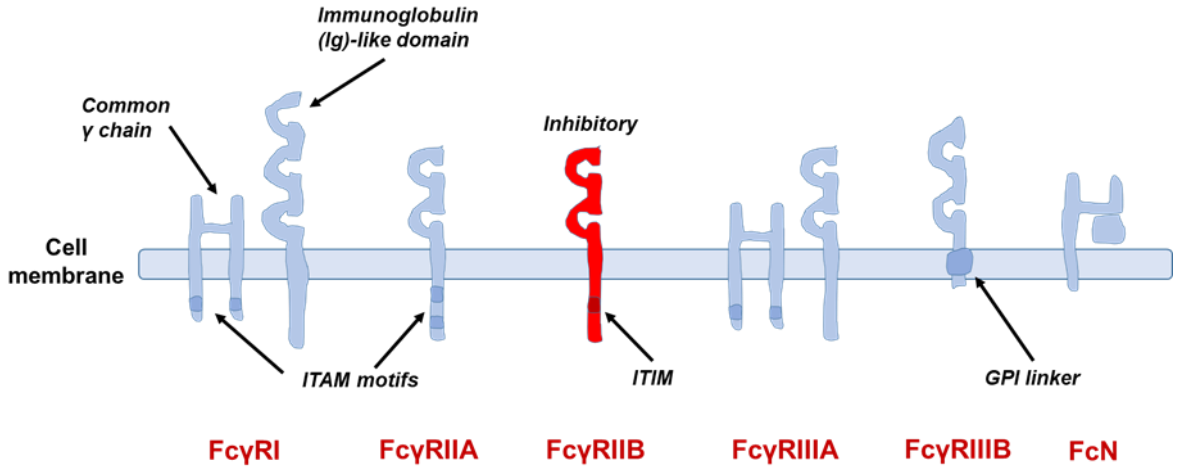
FcγRIIA and FcγRIIB result from genetic polymorphisms. FcγRIIA is activating cellular and humoral immune responses. It is mainly responsible for antibody-dependent cellular phagocytosis (ADCP).

FcγRIIB (CD32)

FcγRIIB is a key immune checkpoint that prevents dendritic cell maturation and does not promote immune activation. The inhibitory function is mediated by the immunoreceptor tyrosine-based inhibition motif (ITIM).

FcγRIIIA (CD16a)

FcγRIIIA is expressed on mast cells, macrophages, and natural killer cells. It is a medium affinity receptor mediating ADCC by NK cells and T cells, phagocytosis by macrophages, cytokine production by NK cells and lymphocytes, and regulation of immunoglobulin production. FcγRIIIA also interacts with the common γ chain.



FcγRIIIB (CD16b)

FcγRIIIB is expressed on human polymorphonuclear leukocytes (neutrophils) and eosinophils. It is the only Fc receptor anchored to the cell membrane by a glycosyl-phosphatidylinositol (GPI) linker and is triggering calcium mobilization and neutrophil degranulation.

Neonatal Fc receptor (FcRn)

FcRn is similar in structure to MHC class I and is also frequently analyzed for its interaction with antibodies. Because it is involved in transferring IgG from a mother via placenta or milk to her fetus or suckling infant, it is called the neonatal Fc receptor. It is widely distributed across epithelial and endothelial cells. This receptor also binds IgG, is involved in preservation of the antibody, and thus has a major impact on the pharmacokinetics of antibodies.

Assessment antibody-FcR interactions

As receptor binding of antibodies is linked to the safety and efficacy of the final therapeutic drug, assessment of antibody-FcR interactions during development and manufacturing of biotherapeutics is essential.

In vitro analysis of antibody-FcR interactions is the most important tool to determine the effector mechanisms, binding characteristics and affinity parameters that will subsequently predict antibody activity in vivo.

For example, FcR panel analyses can be used for originators / new biological entities for release and stability studies. The way the antibodies interact with the effector targets upon degradation may then be changed during development of the drug.

When developing biosimilar molecules, it must be confirmed that the interactions of the biosimilar with the Fc receptors are equivalent to that of the originator.

Assays for analyzing antibody-FcR interactions

To study antibody-FcR interactions, the methods mainly used are based on enzyme-linked immunosorbent assays (ELISAs), fluorescence-activated cell sorting (FACS), and surface plasmon resonance (SPR) assays (e.g. via Biacore). Furthermore, ADCC, ADCP, CDC and / or surrogate binding assays are often cell based.

Strategies for analysis

Strategies for the assessment of antibody-FcR interactions may vary depending on the type of antibody. For example, with neutralizing antibodies like IgG4, binding activity to FcRs like CD16, CD32, or CD64 is relatively low. Neutralizing antibodies may have low interactions with the Fc receptor panel, but on the other hand have strong interactions with FcRn, especially at

low pH. Effector antibodies such as ADCC antibodies or ADCP show strong, reproducible interactions with the FcRs. Buffer and background conditions must be optimized for each antibody to minimize nonspecific interactions. Association and dissociation times for receptors with the antibodies have to be optimized as well. Depending on quantitative or qualitative questions, the methods must be validated with different parameters.

All these issues have to be considered for the set-up of an analytical method and VelaLabs can support you for choice of the optimal strategy. VelaLabs provides all types of FcR binding assays for getting detailed information about antibody - receptor interaction. Our service includes for example SPR (Biacore) methods involving capture of antibody or Fc receptor using suitable coupling strategies, and qualitative and quantitative kinetic analysis of FcγR / FcRn interaction providing rate constants for association and dissociation and affinities (ka, kd, KD).

If you are interested in the study of antibody-FcR interactions and like to get more information, as well as a work plan and quotation, please contact us via email at velabd@vela-labs.at or give us a phone call at +43 189 059 7911.

We are happy to talk with you about your project soon.