Quantitative Assays to Measure Therapeutic Antibody Binding to Fc Receptors

BPS Bioscience

Introduction to Fc Receptors

Fc Receptors, expressed on the surface of immune cells, bind the Fc (fragment crystallizable) portion of antibodies and play an essential role in modulating our immune defense system. They also impact the efficacy of therapeutic antibodies and therefore are of great interest in drug development [1]. Indeed, the effectiveness of a therapeutic antibody depends not only on how tightly it binds to the intended target, but also on how long it is present in the patient's blood and how well it engages an appropriate immune response through its interaction with Fc receptors.

Therapeutic antibodies represent a powerful class of drugs used for the treatment of cancer, immune disease, or viral infection. They include neutralizing antibodies targeting cytokines or cytokine receptors, as well as cytotoxic antibodies and antibody-drug conjugates that bind to cancer-specific targets and directly kill the tumor cells. Antibodies against immune targets such as anti-PD-1/PD-L1 or anti-CTLA4 antibodies are designed to enhance immune responses within the tumor microenvironment.

The half-life of an antibody is controlled by binding to the neonatal Fc receptor for IgG (FcRn), which regulates distinct functions in IgG transport and homeostasis. Other Fc receptors trigger immune responses through antibody-dependent cell mediated cytotoxicity (ADCC), a mechanism in which a natural killer (NK) cell is activated by antibodies bound to tumor cells, followed by the lysis of the tumor cell.

These Fc receptors are classified based on the type of immunoglobulin (Ig) that they recognize: Fcγ receptors bind to IgG, Fcα receptors bind to IgA, and Fcε receptors bind to IgE.

The development of new therapeutic antibodies (which are mostly IgGs) may require optimization of their interaction with FcRn or with the appropriate FcγR. Scientists must choose cell models and assays carefully depending on their intended goal.

Antibody Checkpoints

The Fcγ receptors function as antibody checkpoints. They contain multiple extracellular immunoglobulin domains responsible for binding the Fc region of IgG. The FcγR family includes FcγRI (CD64), FcγRIIa (CD32a), FcγRIIb (CD32b), FcγRIIc (CD32c), FcγRIIIa (CD16a), and FcγRIIIb (CD16b), which differ in IgG affinity due to divergences in molecular structure [2, 3]. Five of the receptors are activating and only FcγRIIb is inhibitory (Figure 1).

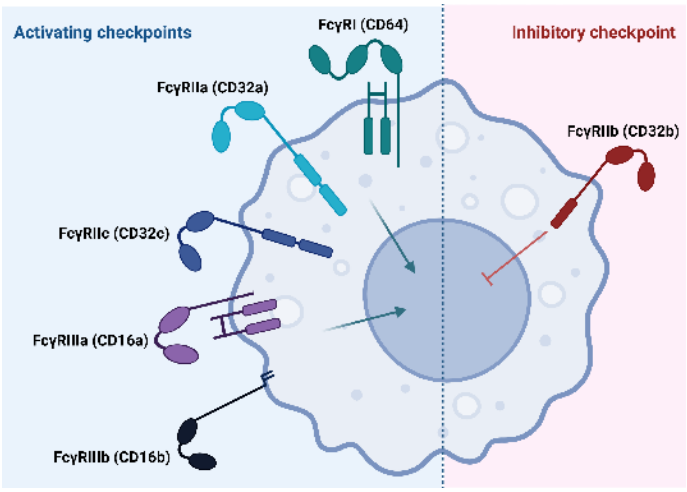

Figure 1. The antibody checkpoint family, expressed on the surface of innate immune cells and B cells, comprises activating and inhibitory Fcγ receptors. Inspired from [4].
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Activating Fcγ receptors contain two ITAMs (immunoreceptor tyrosine-based activation motif) in their cytoplasmic domain, consisting of amino acid sequence

YxxL/Ix(6-8)YxxL/I. FcγRI and FcγRIIIa do not contain an ITAM but signal through another ITAM-containing membrane-anchored subunit.

Upon activation of the receptor by IgG binding, the ITAM is phosphorylated on both tyrosine residues by an intracellular tyrosine kinase of the Src family. It becomes a docking site for SH2 domain-containing signaling proteins, initiating the signal transduction cascade necessary to generate a biological response (Figure 2).

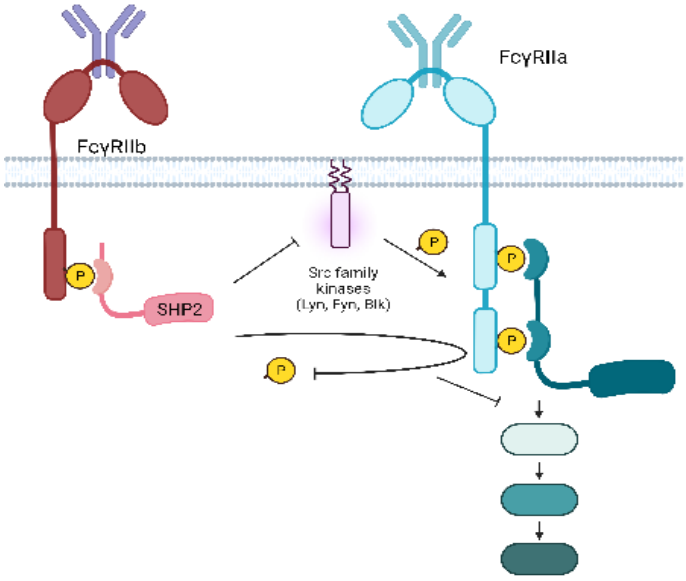

Figure 2: Activating and inhibitory signaling of FcγRIIa (blue) and FcγRIIb (red), respectively.
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Inhibitory FcγRIIb, expressed mostly in B cells, provides a negative feedback loop that controls B cell stimulation. The receptor carries an intracellular ITIM sequence (immunoreceptor tyrosine-based inhibitory motif), which is a single I/VXXYXXL motif. Once phosphorylated, it engages SH2-containing tyrosine phosphatases such as SHP1, SHP2, or SHIP, which antagonize the phosphotyrosine signals [5, 6].

FcγRs participate in various biological functions depending on IgG specificity, cellular expression, and signaling. Receptors present on NK cells bind to antibodies that are attached to infected cells or invading pathogens to promote their lysis. FcγRs on phagocytes bind antibodies attached to invading bacteria to trigger phagocytosis of the bacterium, while FcγRs on eosinophils cause degranulation.

Therapeutic antibodies take advantage of antibody-dependent cell-mediated cytotoxicity (ADCC) activated by FcγRIIIa. Cell-based studies performed during the optimization of a therapeutic antibody will be facilitated by the engineering of target-expressing cells, the design of cell-based assays using reporter target or effector cells, or the design of co-culture assays. These research tools are especially useful in the field of immuno-therapeutics.

Activator of ADCC, FcγRIIIa

The typical ADCC process involves the activation of NK cells, which express mostly FcγRIIIa (CD16a), and the release of cytotoxins that attack the target cells. Human FcγRIIIa exhibits a dimorphism at residue 158, in which variant Val-158 encodes a higher affinity receptor than variant Phe-158. FcγRIIIa potentiates the efficacy of therapeutic antibodies used to treat solid tumors and represents a direct therapeutic target in hematopoietic cancers.

Data shown in Figure 3 illustrate an effective ADCC bioassay using Jurkat cells that overexpress FcγRIIIa-F158. In the example shown here, a conditional luciferase reporter gene under the control of NFAT (Nuclear Factor of Activated T cells) response elements was introduced in the Jurkat cells to allow quantification of NFAT stimulation. The target cells were incubated with an antibody of interest. Upon addition of the Jurkat cells and binding of FcγRIIIa to the antibody, the NFAT signaling pathway was activated, resulting in a dose-dependent

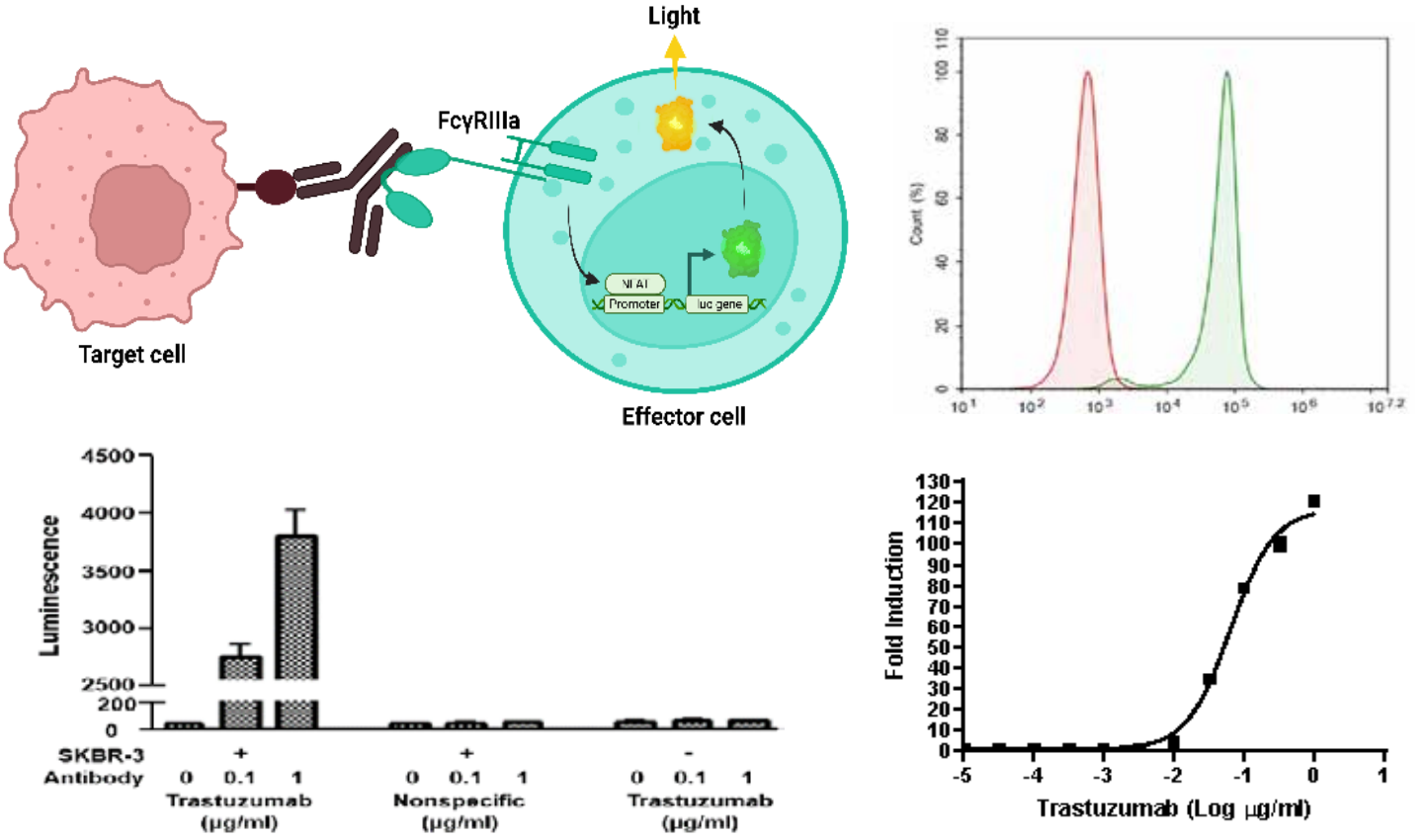

Figure 3. Top left: illustration of the ADCC bioassay principle. Top right: flow cytometry analysis of FcγRIIIa-F158 overexpression in the ADCC Bioassay Effector Jurkat Cell Line (F variant; BPS Bioscience #60540). Bottom left: ADCC response to anti-HER2 antibody drug Trastuzumab in the presence of HER2-expressing SKBR-3 breast cancer cells. Bottom right: ADCC response to increasing doses of Trastuzumab, in a co-culture of SKBR-3 and FcγRIIIa-F158/NFAT luciferase reporter Jurkat cells (EC50 = 28.1 ng/ml).
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increase in luciferase activity. Thus, the ADCC efficacy of different antibodies can be compared directly.

Antibody checkpoint inhibitor FcγRIIb

FcγRIIb (CD32b) operates as a negative regulator of B Cell Receptor (BCR)-induced activation of B cells [7]. The two isoforms FcγRIIb1 and FcγRIIb2, arising from mRNA splicing, differ in expression and function. The presence of exon C1 sequence in FcγRIIb1, which is highly expressed at the surface of B cells, tethers the receptor at the membrane and dramatically increases its half-life at the cell surface. The absence of exon C1 in FcγRIIb 2, expressed in myeloid cells, triggers rapid internalization of the receptor upon ligand binding. FcγRIIb induces the phagocytosis of aggregated immunoglobulins and may function as a "sink" for the removal of IgG immune complexes. Thus, the biological function of FcγRIIb is to tame antibody-dependent responses and to clear the circulation of spent immune complexes. Defects in FcγRIIb1 signaling lead to overt inflammation and are involved in autoimmune diseases.

FcγRIIb is an important therapeutic target for the treatment of B-cell malignancies. Therefore, FcγRIIb-expressing cells can be useful to identify and characterize anti-FcγRIIb antibodies, bi-specific T cell engagers, antibody-drug conjugates, or anti-FcγRIIb CAR (Chimeric Antigen Receptor) cells.

On the other hand, FcγRIIb contributes to the effectiveness of immunotherapy by cross-linking antibodies directed at T cell stimulatory checkpoints such as 4-1BB, OX40, and CD40. Co-culture assays have been designed to characterize the agonist activity of checkpoint antibodies using FcγRIIb-mediated crosslinking.

As shown in Figure 4, FcγRIIb CHO (Chinese Hamster Ovary) cells placed in co-culture with CD137/NF-κB reporter cells validated the activating efficacy of an anti-CD137 antibody.

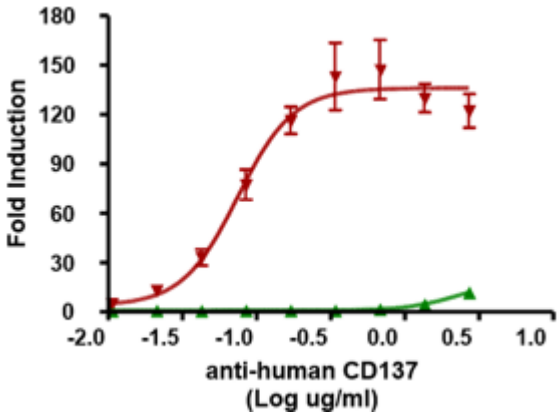

Figure 4. Dose response of anti-CD137 antibody in CD137/NF-κB-reporter HEK293 cells (BPS Bioscience #79289) co-cultured with FcγRIIb CHO cells (BPS Bioscience #79511). Cross-linking of the anti-CD137 antibody by FcγRIIb expressed at the surface of CHO cells potentiated the activation of NF-κB in CD137-expressing HEK293 cells (in red). Control CHO cells are shown in green.

In another experiment, a TCR activator (TCRa) was expressed together with FcγRIIb in CHO cells (BPS Bioscience #78436). This cell line can be used in a co-culture assay to screen for regulators of antibody-mediated signaling and to identify or characterize agonists of FcγRIIb receptor-mediated crosslinking of checkpoint targets. As shown in Figure 5, FcγRIIb amplified the effect of an anti-PD-1 antibody, as indicated by PD-1-mediated inhibition of TCR activity observed in the presence of TCRa/FcγRIIb CHO cells, which was not observed in the presence of control TRCa CHO cells.

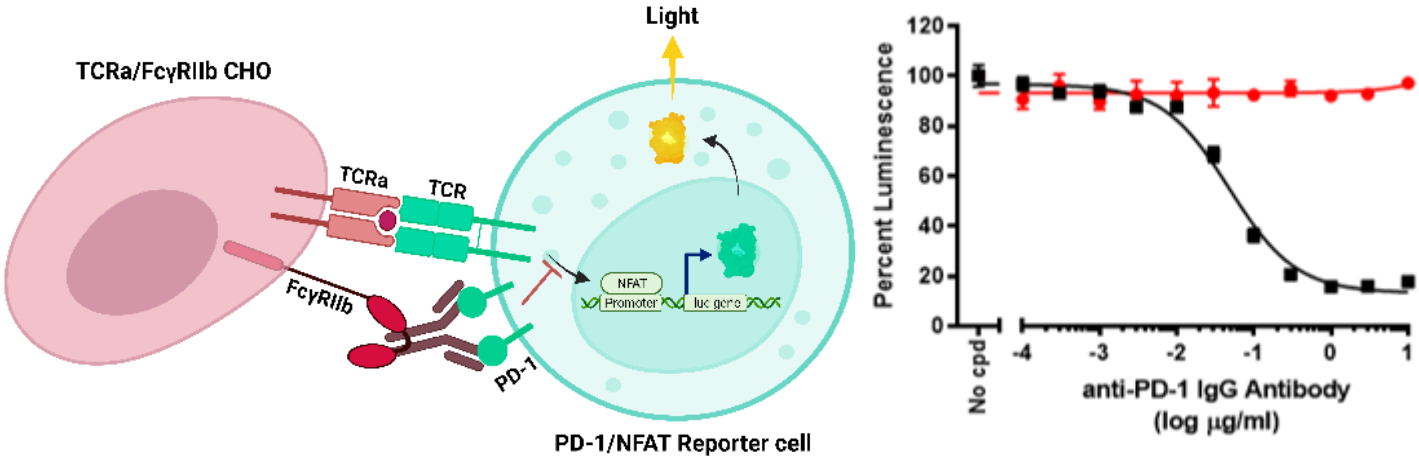

Figure 5. Left: illustration of the co-culture assay. Right: a co-culture assay was performed using the PD-1/NFAT Reporter Jurkat Cell Line (BPS Bioscience #60535) with either the TCRa/FcγRIIb CHO Cell Line (BPS Bioscience #78436) or the TCRa CHO Cell line (BPS Bioscience #60539), in the presence of increasing concentrations of anti-PD-1 antibody (BPS Bioscience #101178).
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FcRn

Neonatal Fc receptor for IgG (FcRn) is a heterodimeric protein similar in structure to MHC class I [8]. It consists of the Fc Gamma Receptor and Transporter, encoded by the FCGRT gene, associated with beta-2-microglobulin. FcRn binds to the Fc region of monomeric IgG and transports the IgG from mother to fetus through the placenta. This receptor contributes to an effective humoral immunity by protecting the IgGs from degradation in the lysosome and recycling them, thereby extending their half-life in circulation. This can be exploited through the engineering of therapeutic antibodies to increase their binding to FcRn, thereby improving their half-life. Evusheld, a cocktail of mutated antibodies with extended half-lives, has been used to treat COVID-19, whereas first-in-class drug Enbrel contains an Fc domain fused to therapeutic protein TNFα to increase the drug's half-life.

Conversely, FcRn itself is a candidate target for autoimmune disease therapy since disrupting the FcRn/IgG interaction is expected to increase IgG clearance, including autoantibodies. The first FDA-approved drug targeting FcRn (efgartigimod), an Fc fragment decoy, provided proof-of-concept and is now used to treat the autoimmune disease myasthenia gravis.

Conclusion

Interactions of antibody drugs with Fc receptors are important factors in therapeutics. We have developed a suite of proteins, assay kits, lentiviruses, and engineered cell lines that enable quantitative Fc receptor activation, ADCC measurement, FcRn binding, and more. BPS Bioscience will continue to accelerate research by developing innovative tools to support optimization of therapeutic antibodies and Fc receptor research.

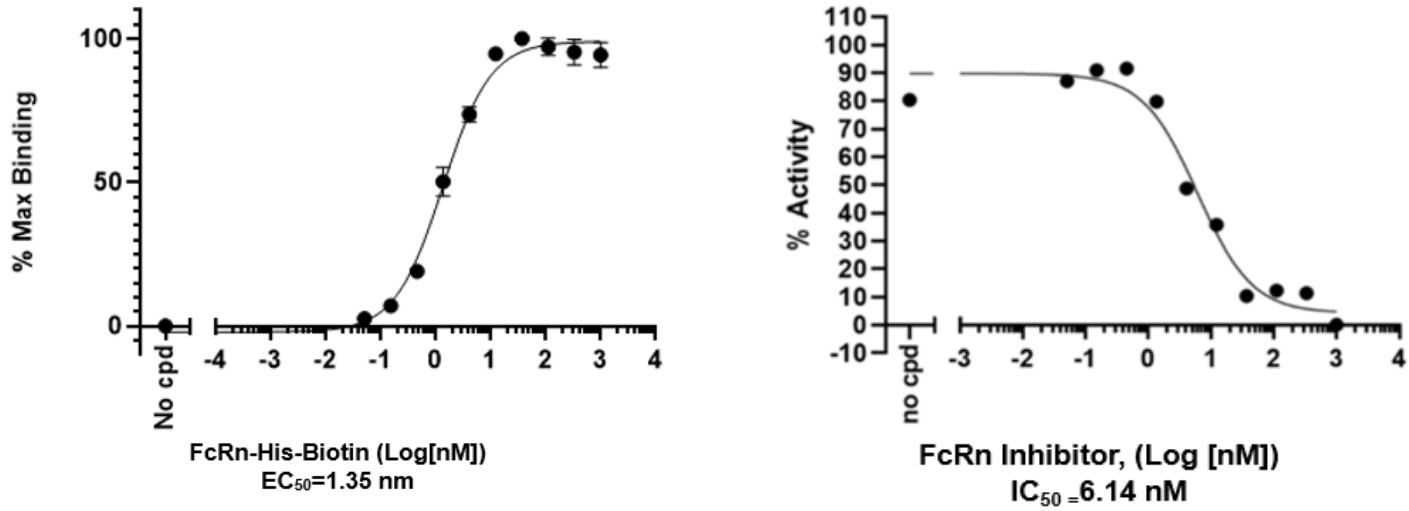

Figure 6. Left: direct binding of a decoy corresponding to an Fc region engineered for high affinity binding to FcRn (EC_{50}=1.35 nM). The decoy was tested using purified, biotinylated human FcRn (BPS Bioscience #71283). Right: inhibition of FcRn binding to IgG1 by FcRn Blocker (BPS Bioscience #101468) using the Fc:FcRn Inhibitor Screening Colorimetric Assay Kit (BPS Bioscience #78501).

Product	Product type	Cat number
ADCC Bioassay Effector Cell F variant Jurkat Cell Line	Cell Line	60540
ADCC Bioassay Effector Cell V variant Jurkat Cell Line	Cell Line	60541
ADCC Bioassay Effector Cell (Mouse) Jurkat Cell Line	Cell Line	79733
ADCP Bioassay Effector Cell FcγRIIa (H variant) NFAT Reporter Jurkat	Cell Line	71273
FcγRIIb CHO Recombinant Cell Line	Cell Line	79511
FcγRIIIa (CD16a) CHO Cell Line	Cell Line	78332
FcγRIIIb (CD16b) CHO Cell Line	Cell Line	78333
FcRL5 HEK293 Cell Line	Cell Line	78374
FcRL5 CHO Cell Line	Cell Line	78375
TCR Activator/FcγRIIb CHO Cell Line	Cell Line	78436
Fc (IgG1):FcRn Inhibitor Screening Colorimetric Assay Kit	Assay Kit	78501
FcγRIIa (Human) CRISPR/Cas9 Lentivirus (Integrating)	Lentivirus	78207
FcγRIIa (Human) CRISPR/Cas9 Lentivirus (Non-Integrating)	Lentivirus	78199
FcγRIIIa (CD16a) Lentivirus	Lentivirus	79876
FcγRIIb (CD32b) Lentivirus	Lentivirus	79877

References

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