LigandTracer

Application Note

Fc-mediated effector functions of antibodies

LigandTracer® is a device designed to monitor biological interactions in real-time and is most commonly used to study the binding of antibodies to target receptors on live cells. Monoclonal antibodies (mAbs) can mediate their function directly through their bound antigen but, in addition, they can also trigger certain immune responses via their Fc-terminus. Therapeutic mAbs are almost exclusively of the IgG isotype that can interact with Fcγ-receptors on immune cells, leading to antibody-dependent cellular cytotoxicity (ADCC). The Fc-terminus of IgG mAbs also possesses a binding site for the subunit C1q of the first component of the complement system and cell-bound IgG can thereby trigger complement-dependent cytotoxicity (CDC).

Experimental details

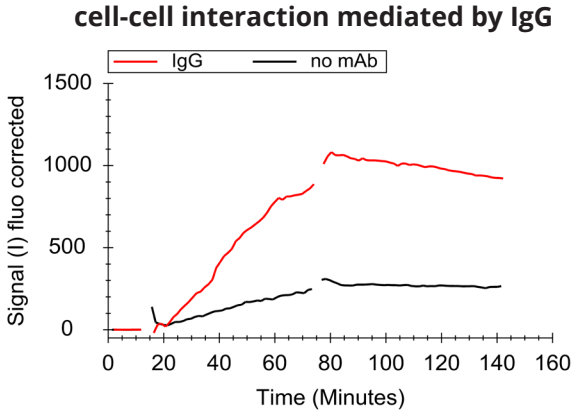
mAb mediated cell-cell interactions

The interaction between the B-cell line Daudi and primary neutrophils isolated from the blood of a healthy donor was followed in the presence and absence of an anti-CD20 IgG mAb. For this purpose, Daudi cells were immobilized with the help of a biomolecular anchor molecule (BAM) in each compartment of LigandTracer MultiDish 2×2 *for coatings*[1,2]. One Daudi cell spot was pre-incubated at room temperature with 50 nM unlabeled anti-CD20 IgG mAb for 1 h whereas the other spot served as a no-mAb control. The dish was placed in LigandTracer Green and after a 15 min baseline reading, 3 million calcein-labeled neutrophils were added to each target cell spot. The formation of cell-cell complexes was observed for 1 h, followed by replacement of the neutrophil solution with cell culture media (Fig. A). This enabled assessment of the stability of the formed cell-cell interactions. The experiment was also performed with an anti-CD20 IgA mAb (Fig. B). To compare how the different mAbs mediate cell-cell interactions, the slope of the binding was normalized to their respective no-mAb controls and then compared to each other (Fig. C). The stability of the mAb mediated cell-cell interactions was calculated by fitting a single exponential decay to the dissociation phase (starting at ca. 80 min) (Fig. C). In this example, neutrophil binding was more stable for IgA opsonized cells.[3]

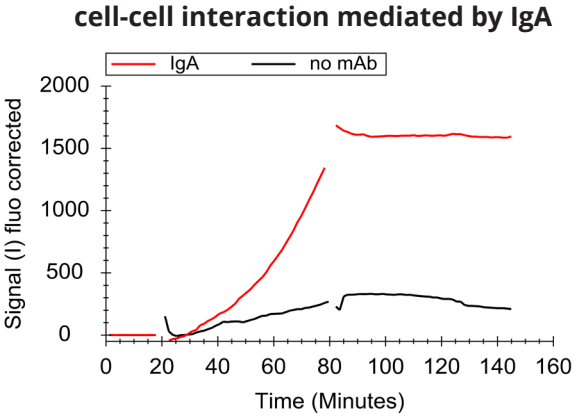
C1q binding to mAb coated cells

The binding of fluorescent C1q to cell-bound anti-CD20 mAb Ofatumumab (OFA) was recorded in real-time with LigandTracer Green. The CD20 expressing B-cell line Ramos and the CD20 negative cell line K562 were immobilized in each compartment of a MultiDish 2×2 *for coatings* with the help of BAM[1,2]. One compartment of the MultiDish was pre-incubated with 60 nM unlabeled OFA for 1 h at room temperature whereas the other half served as a no-mAb

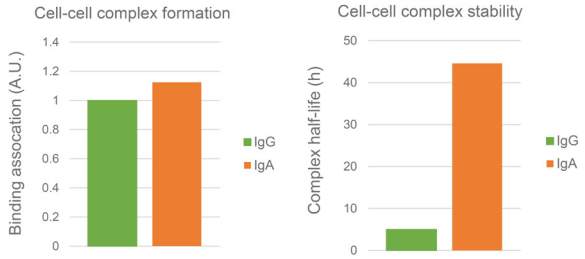
A



B

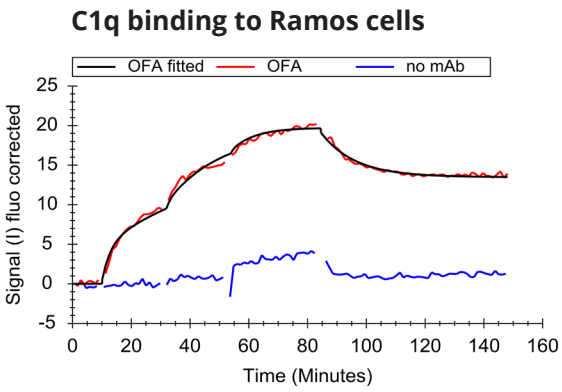


C

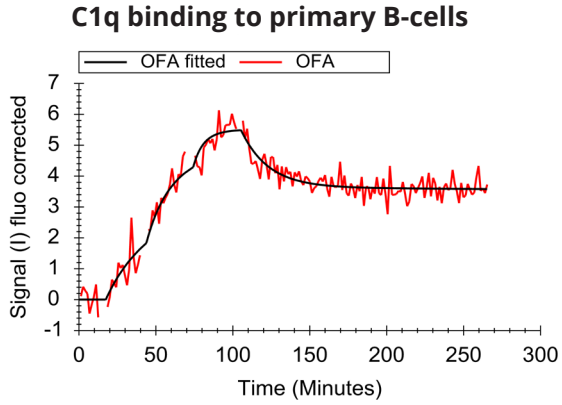


control. The prepared dish was placed in LigandTracer Green and fluorescent C1q was added in three increasing concentrations, followed by dissociation in cell culture media containing unlabeled OFA at equal concentration as in the incubation solution (60 or 0 nM; Fig. D). Clear binding to OFA-opsonized cells was observed, whereas control cells not coated with antibodies gave little signal. The experiment was also performed with primary B-cells isolated from the blood of healthy donors (Fig. E) (data courtesy of Dr. Anja Lux, University Erlangen-Nürnberg). Binding traces from both experiments displayed a biphasic dissociation pattern with a fraction of C1q molecules releasing rapidly and a second fraction of C1q remaining stably bound. Due to the observed biphasic dissociation, a 1:2 model that assumes two independent binding processes was fitted to the curves, resulting in kinetic rate constants and affinity values as listed below.[4]

D



E



	Stronger interaction			Weaker interaction		
Cell type	**k_{a1} (1/(M*s))**	**k_{d1} (1/s)**	**K_{D1} (M)**	**k_{a2} (1/(M*s))**	**k_{d2} (1/s)**	**K_{D2} (M)**
Ramos	2.2E+05	1.0E-06	4.5E-12	3.2E+06	1.4E-03	4.5E-10
Primary B-cells	1.8E+05	1.6E-06	8.5E-12	2.6E+05	9.4E-04	3.7E-09

Conclusions

With LigandTracer it is possible to set-up complex binding assays with (highly) multivalent ligands in a relevant cellular context. This enables the next level of antibody characterization, where binding studies are employed to investigate effector functions. Understanding how antibody binding influences functional efficacy can provide valuable knowledge for designing next-generation therapeutics.

References

1. Technology Note: MultiDish 2x2.
2. Protocol: Attaching suspension cells for LigandTracer measurements.
3. Brandsma, A. M. *et al.* Potent Fc Receptor Signaling by IgA Leads to Superior Killing of Cancer Cells by Neutrophils Compared to IgG. *Front. Immunol.* **10**, (2019).
4. Bondza, S. *et al.* Complement-dependent activity of CD20-specific IgG correlates with bivalent antigen binding and C1q binding strength. *Front. Immunol.* **11**, (2020).