COMPARATIVE STUDY OF FC RECEPTOR BINDING AND IMMUNE MODULATION: NANOBODY-FC VS IgG IMMUNE COMPLEXES

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Nanobodies (Nbs) are single-domain antibodies that are gaining traction in therapeutics and biosensing because they are small, robust, and can access cryptic epitopes[1]. When formatted as Fc fusions, Nbs can act as IgGs and trigger Fc receptor-driven responses to immune complexes (ICs). Here, we paired spectroscopic ellipsometry (SE) with quartz crystal microbalance with dissipation monitoring (QCM-D) to quantify binding kinetics and affinity, calculate dry mass, hydration, and map epitope relationships for Nb-Fc and SARS-CoV-2 spike (S) ICs engaging with FcγRI.

SE measured nanomolar IC-FcγRI dissociation constants, with Sb15/S exhibiting the strongest binding, comparable to IgGCR/S. QCM-D likewise showed pronounced FcγRI recognition of Sb/S complexes. Optical modeling of SE data indicated an FcγRI layer thickness of 3.3 nm and thinner IC layers for Nb complexes (18–22 nm) than for IgGCR/S (30 nm). The highest dry surface mass density was observed for Sb14/S (54.14 ng/cm^2), followed by CR3022/S (45.1 ng/cm^2), Sb15/S (39.06 ng/cm^2), and Sb68/S (30.68 ng/cm^2). Spike alone generated only a negligible QCM-D response and no dependable SE signal, consistent with FcγRI binding requiring pre-assembled ICs. Competitive QCM-D measurements further suggested that Sb14 and Sb15 share an epitope, whereas Sb68 targets a distinct region with partial overlap to human IgG CR3022.

In macrophage assays, Nb/S ICs were phagocytosed without induction of inflammatory cytokines, while spike alone entered predominantly via passive endocytosis. Only Sb15/S and Sb68/S increased CD83 (linked to inflammation resolution) and showed a trend toward elevated CCR2, whereas CD86, CCL5/CCR5, and CCL2 remained unchanged[2]. Notably, the largest/highest-affinity complexes did not increase CD83, implying that Fc-mediated signaling depends on IC architecture, not affinity alone.

Overall, Nb-based ICs engage FcγRI with IgG-like strength yet form thinner surface assemblies. SE/QCM-D readouts of IC structure provide a basis for selecting nanobodies to tune Fc-effector outcomes and deepen the knowledge on surface-assay design.

[1] M. I. Mustafa and A. Mohammed, “Nanobodies: A Game-Changer in Cell-Mediated Immunotherapy for Cancer,” SLAS DISCOVERY, vol. 28, no. 8, pp. 358–364, Aug. 2023, doi: 10.1016/j.slasd.2023.08.008.
[2] M. Luo et al., “CD83 mediates the inhibitory effect of the S1PR1 agonist CYM5442 on LPS-induced M1 polarization of macrophages through the ERK-STAT-1 signaling pathway,” International Immunopharmacology, vol. 143, no. Pt 3, p. 113526, Nov. 2024, doi: 10.1016/j.intimp.2024.113526.