Comprehensive analysis of a liposome library: cytotoxicity and impact on TRIM21 expression

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For years, it was believed that the Fc region of antibodies could only be recognized by membrane receptors: Fc receptors or the neonatal Fc receptor. However this view changed with the discovery of TRIM21 (Tripartite motif-containing 21), a cytosolic Fc receptor. TRIM21 is an E3 ubiquitin ligase ubiquitously expressed across tissues, enabling antibody-mediated immune responses within cells — even in non-immune cells. Owing to its unique properties, TRIM21 recognizes antigen–antibody (Ag–Ab) complexes present in the cytoplasm and catalyzes their polyubiquitination. **Consequently, the Ag–Ab–TRIM21 complex is degraded by the proteasome in a process known as antibody-dependent intracellular neutralization** (ADIN). This mechanism has been confirmed for multiple classes of antibodies. Moreover, ADIN has been proposed as a potential therapeutic strategy for cancer or neurodegenerative diseases by promoting the proteasomal degradation of intracellular targets. Therefore, enabling intracellular transport of monoclonal antibodies (mAb) may allow the targeted clearance of intracellular proteins (Figure 1A) [1].

Lipid-based nanoparticles, such as liposomes, represent one of the safest and most biocompatible nanocarrier systems due to their structural similarity to biological membranes. Several liposomal formulations are already FDA-approved, including those used in cancer therapy and COVID-19 vaccines [2]. Liposomes possess a two-compartment architecture — a hydrophobic lipid bilayer and a hydrophilic aqueous core — allowing the simultaneous encapsulation of chemically distinct molecules. Consequently, liposomes are promising nanocarriers for delivering mAb in ADIN-based therapeutic approaches.

The design of liposomes must be tailored to meet the specific therapeutic requirements, including efficient drug (e.g. mAb) transport to the diseased tissue and controlled intracellular release. To achieve these properties, different lipid compositions are used, which determine liposome biocompatibility and low *in vivo* toxicity. In our study, we constructed a **liposome library** (Figure 1B) that varied in cholesterol content, neutral (DMPC, DPPC or DOPE) or cationic phospholipids (DOTAP or DODAB), and polyethylene glycol (PEG 2 or 5 kDa) modification. Using the film hydration method, we obtained a series of liposomes that were comprehensively characterized in terms of their cytotoxicity and interactions with various cell types. We performed uptake studies using MDA-MB-231 (breast cancer cells), hemolysis assays, and evaluated the immunomodulatory properties of the liposomes. Furthermore, we analyzed their impact on the expression level of the TRIM21 protein, which is crucial for the development of ADIN-based therapies.

Although liposomes are already employed in clinical practice, there remains a significant gap in our understanding of how lipid composition influences cellular interactions. Our findings aim to improve the prediction of liposome performance *in vivo* and support the rational design of safer and more efficient liposomal drug delivery systems.

Acknowledgments

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References

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Figures

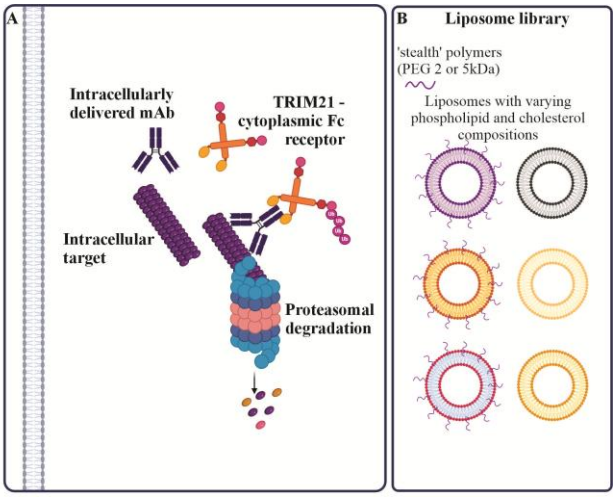

Figure 1. A. Antibody-dependent intracellular neutralization (ADIN). **B.** Liposome library designed for intracellular delivery of monoclonal antibodies. Figure prepared in BioRender.com.