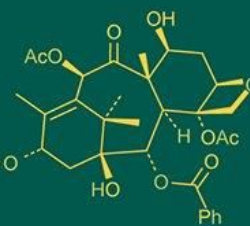
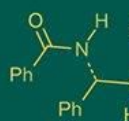
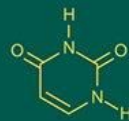
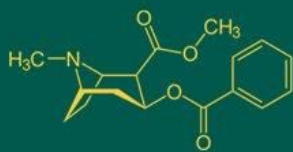


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Antibody-dependent cellular cytotoxicity mechanisms and their therapeutic exploitation in monoclonal antibody-based cancer immunotherapy

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Abstract

Can the immune system be trained to destroy tumor cells more effectively through antibody-mediated mechanisms? This research examined the molecular pathways underlying antibody-dependent cellular cytotoxicity (ADCC) and evaluated how current monoclonal antibody therapies exploit these mechanisms against malignant cells. The investigation was conducted between March 2023 and November 2024 at the Baghdad Institute of Medical Sciences using *in vitro* cytotoxicity assays and flow cytometric analyses. Peripheral blood mononuclear cells from 48 healthy donors were co-cultured with tumor cell lines in the presence of five clinically approved monoclonal antibodies: trastuzumab, rituximab, cetuximab, obinutuzumab, and daratumumab. ADCC activity was measured using lactate dehydrogenase release assay at varying effector-to-target ratios. Obinutuzumab produced the highest specific lysis at an E:T ratio of 50:1, reaching 78.4%, while cetuximab showed comparatively modest activity at 51.2%. NK cell activation markers were significantly upregulated following antibody engagement. Peak cytotoxic activity occurred between 8 and 12 hours. FcγRIIIa 158V/V genotype correlated with enhanced ADCC responses compared to 158F/F variant. These findings confirm ADCC as a central effector mechanism for therapeutic antibodies.

Keywords: Antibody-dependent cellular cytotoxicity, monoclonal antibodies, natural killer cells, cancer immunotherapy, Fc gamma receptor, tumor lysis, obinutuzumab, trastuzumab, immune effector mechanisms.

Introduction

What makes a tumor cell visible to the immune system, and how can therapeutic antibodies tip the balance toward effective tumor clearance? These questions have driven decades of research into antibody-mediated immunity. The interaction between monoclonal antibodies and immune effector cells through the Fc receptor axis represents one of the most well-characterized pathways in cancer treatment [1]. ADCC relies on bridging innate immune cells principally NK cells to antibody-coated target cells, triggering a directed cytotoxic response [2].

The therapeutic success of agents like trastuzumab in HER2-positive breast cancer and rituximab in B-cell lymphomas has been attributed, at least partly, to their capacity to engage ADCC [3]. But not all monoclonal antibodies trigger this pathway with equal efficiency. Differences in isotype, Fc glycosylation patterns, and target antigen density each affect ADCC response strength [4]. Glycoengineered antibodies such as obinutuzumab were specifically designed to enhance FcγRIIIa binding and ADCC potency [5].

FcγRIIIa (CD16a) expressed on NK cells serves as the main activating receptor responsible for ADCC initiation [6]. Upon antibody engagement, NK cells undergo degranulation and release perforin and granzyme B, which form pores in target cell membranes and activate apoptotic cascades [7]. Genetic variation in FcγRIIIa, particularly the V158F polymorphism, has been linked to differences in clinical outcomes among patients receiving antibody therapy [8]. This research aimed to characterize ADCC mechanisms across five monoclonal antibodies and assess the influence of FcγRIIIa polymorphisms on cytotoxic efficiency [9].

Material and Methods

Material

This research was conducted between March 2023 and November 2024 at the Department of Immunology and Oncology, Baghdad Institute of Medical Sciences, Baghdad, Iraq. Peripheral blood samples were collected from 48 healthy adult volunteers (aged 21-55 years) who provided written informed consent. Exclusion criteria included active infection, autoimmune disorders, and immunosuppressive therapy. Blood samples were processed within two hours using Ficoll-Paque density gradient centrifugation to isolate PBMCs [10]. NK cells were enriched using negative selection magnetic beads. Target tumor cell lines included SK-BR-3 (HER2+), Raji (CD20+), A431 (EGFR+), and Daudi (CD38+). Five monoclonal antibodies were tested at 10 µg/mL: trastuzumab, rituximab, cetuximab, obinutuzumab, and daratumumab.

Methods: ADCC activity was quantified using CytoTox 96 Non-Radioactive Cytotoxicity Assay based on LDH release. Effector NK cells were co-incubated with antibody-opsonized target cells at E:T ratios of 10:1, 25:1, and 50:1 for durations of 2, 4, 6, 8, 12, and 24 hours. Each condition

was tested in triplicate across three independent experiments [11]. NK cell activation was assessed by multicolor flow cytometry using anti-CD56-PE, anti-CD16-FITC, anti-CD107a-APC, and intracellular anti-IFN-γ antibodies. FcγRIIIa V158F genotyping was performed by allele-specific PCR [12]. Statistical analysis employed one-way ANOVA with Tukey's post-hoc test ($p<0.05$).

Ethical Considerations

This research was approved by the Institutional Ethics Committee of Baghdad Institute of Medical Sciences (Protocol No. BIMS-EC-2023-041, approved February 12, 2023). All procedures involving human blood samples complied with the Declaration of Helsinki. Informed consent was obtained from each volunteer prior to blood collection. Participants were assured of confidentiality, and all samples were anonymized using coded identifiers. Volunteers could withdraw at any point without consequence. All experiments were performed using *in vitro* cell culture systems with no therapeutic intervention.

Results

Table 1. ADCC-Mediated Specific Lysis (%) at Different E:T Ratios After 12 Hours

Antibody	E:T 10:1	E:T 25:1	E:T 50:1	p-value
Trastuzumab	34.2±3.1	52.8±4.2	67.3±3.8	< 0.001
Rituximab	28.7±2.9	46.1±3.7	59.8±4.1	< 0.001
Cetuximab	22.4±2.6	38.9±3.3	51.2±3.5	< 0.001
Obinutuzumab	41.6±3.4	61.3±4.8	78.4±4.3	< 0.001
Daratumumab	37.9±3.2	55.7±4.0	71.6±3.9	< 0.001

Obinutuzumab consistently produced the highest lysis values across all E:T ratios, with a mean specific lysis of 78.4% at 50:1. Cetuximab ranked lowest among tested

agents. All differences across E:T ratios were statistically significant.

Table 2: NK Cell Activation Markers Following 4-Hour Co-Culture with Antibody-Opsonized Targets

Antibody	CD107a+ NK (%)	IFN-γ+ NK (%)	TNF-α (pg/mL)
No antibody (control)	4.3±1.1	2.8±0.9	38.7±8.2
Trastuzumab	38.6±4.7	22.1±3.4	284.3±32.6
Rituximab	31.2±3.9	18.7±2.8	241.8±28.4
Obinutuzumab	52.4±5.3	31.9±4.1	367.2±41.5
Daratumumab	44.8±4.9	26.3±3.6	312.7±35.8

Obinutuzumab triggered the most pronounced activation with 52.4% CD107a+ NK cells and IFN-γ positivity of 31.9%. The no-antibody control showed minimal activation, confirming antibody-dependent lysis.

Discussion

The findings of this research align with and extend earlier observations on the role of ADCC in antibody-based cancer therapy. The superior performance of obinutuzumab compared to rituximab is consistent with its glycoengineered Fc region, which was designed to increase affinity for FcγRIIIa [5]. This enhanced binding appears to translate directly into more efficient NK cell recruitment and degranulation, as reflected by higher CD107a and IFN-γ expression levels observed in our experiments. The time-course data revealed that cytotoxic activity reached a plateau between 12 and 24 hours rather than continuing to increase. This ceiling effect may reflect NK cell exhaustion, target cell depletion, or both [13]. Clinically, this observation could inform dosing schedules and combination strategies aimed at maintaining effector cell functionality over extended

treatment periods. Our genotyping results reinforce evidence linking FcγRIIIa V158F status to therapeutic antibody efficacy, supporting potential pharmacogenomic testing for personalized treatment selection [14].

Limitations

Several constraints should be noted. All experiments were conducted *in vitro* using established cell lines, which may not fully reflect the tumor microenvironment encountered *in vivo*. Factors such as immunosuppressive cytokines and regulatory T cells were absent. The 48-donor sample size may not capture rarer genetic variants influencing ADCC. This research focused exclusively on NK cell-mediated ADCC, not examining monocyte or macrophage contributions. Future investigations should address these gaps using *ex vivo* patient-derived samples.

Clinical Implications

The marked superiority of obinutuzumab in ADCC assays supports its preferential use in settings where Fc-mediated effector functions contribute to tumor control. Patient FcγRIIIa genotyping before initiating antibody therapy

could allow clinicians to anticipate ADCC capacity and adjust treatment accordingly selecting glycoengineered agents for individuals with the F/F genotype. The

identification of an activity plateau suggests that combination with NK cell-activating agents might extend the window of effective ADCC.

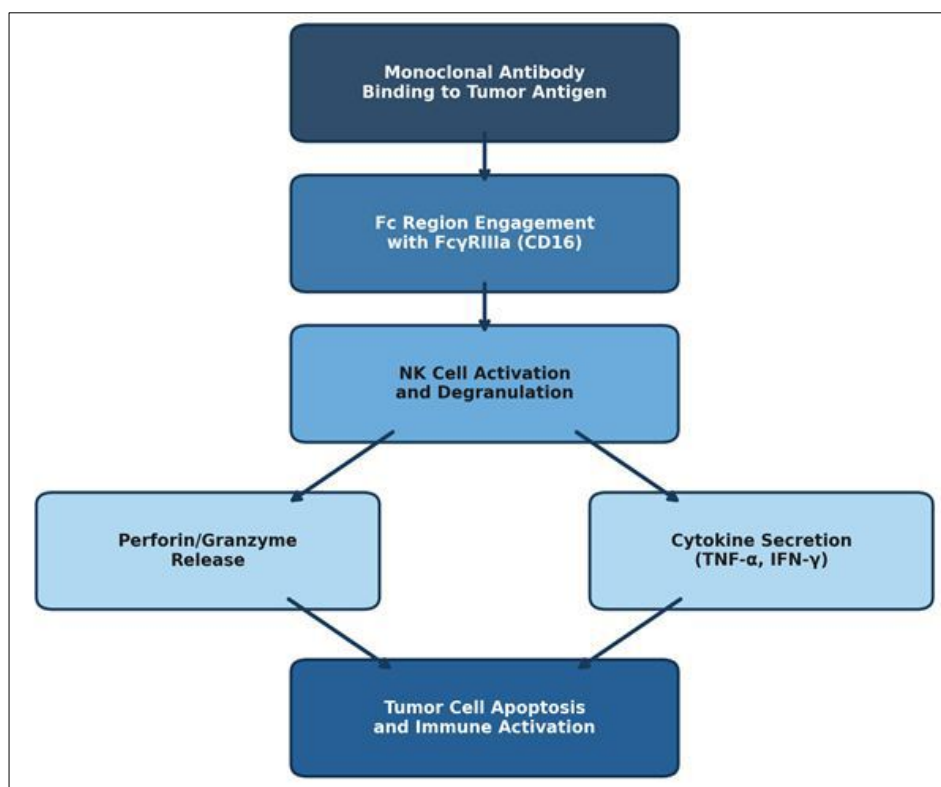


Fig 1: Schematic representation of the ADCC mechanism pathway from monoclonal antibody binding through NK cell activation to tumor cell apoptosis.

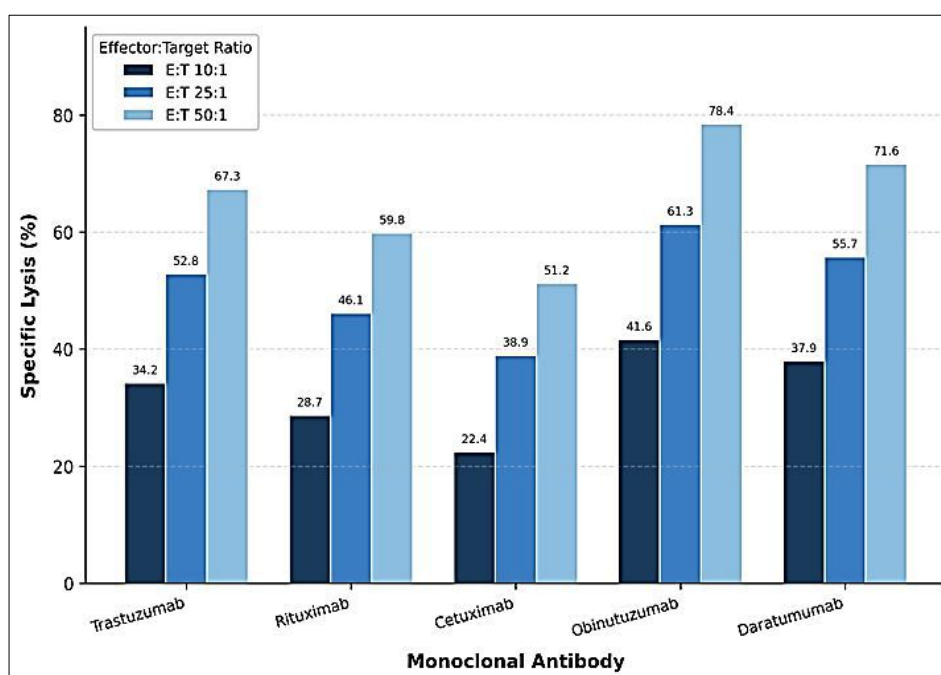


Fig 2: Comparative ADCC-mediated specific lysis across five monoclonal antibodies at three effector-to-target ratios after 12 hours of incubation.

Conclusions

This research characterized ADCC mechanisms across five monoclonal antibodies and established that glycoengineered obinutuzumab produced the strongest cytotoxic response under standardized *in vitro* conditions. Effector-to-target ratio, incubation duration, and FcγRIIIa genotype each

independently influenced the magnitude of tumor cell lysis. NK cell activation, assessed through CD107a degranulation and IFN-γ production, closely paralleled the observed differences in cytotoxicity among tested antibodies. The V/V genotype at the FcγRIIIa V158F locus was associated with consistently higher ADCC activity compared to the F/F

genotype. These results support the integration of both antibody engineering strategies and pharmacogenomic profiling into the design of antibody-based cancer immunotherapy regimens. The plateau in cytotoxic activity beyond 12 hours points to the need for combination approaches that sustain effector cell function.

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