Understanding Antibody Production in Waldenström's Macroglobulinemia

Trevor Chapman and Sherine F. Elsawa

Department of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, Durham, NH 03824

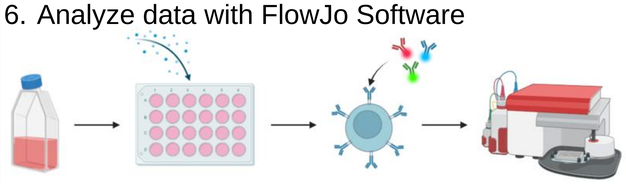
Introduction

- Waldenström's macroglobulinemia (WM) is a low-grade B-cell lymphoma characterized by the overproduction of monoclonal immunoglobin M (IgM).
- A hallmark of WM is the inability of malignant B-cells to undergo class switch recombination (CSR), resulting in the continuous IgM secretion.[3]
- GLI2, a Hedgehog signaling pathway transcription factor, was found to modulate IgM secretion in B-lymphocytes.[1]
- The combination of cytokines can induce CSR in normal B-lymphocytes through distinct signaling pathways [2]

  LPS: activates TLR4-mediated signaling
  CD40L: mimics T-cell help by activating CD40
  BAFF: promotes B-cell survival and differentiation
  TGF-β + IL-4: CSR driving cytokines
- **Goal of study: to investigate the ability of WM cells to undergo class switching when subjected to various cytokine cocktails while simultaneously inhibiting the Hedgehog signaling transcription factor GLI2 in vitro**
- GANT61: GLI1/2 antagonist.
- BCWM.1 and MWCL-1 are cell lines derived from different WM patients.

Methodology

1. Plate starved BCWM.1 and MWCL-1 cells at 1.00x10^{6}cells/ml
2. Generate stimulatory conditions with and without GANT61
3. Incubate for 48hr
4. Stain cells with fluorescent antibodies
5. Analyze with c6 Accuri Flow Cytometer
6. Analyze data with FlowJo Software



Conditions containing GANT61(20uM) or DMSO:

1. No stimulation
2. LPS(25ug/ml)
3. LPS(25ug/ml) + BAFF(0.5ug/ml) + CD40L(100ug/ml)
4. IL-4(10ng/ml) + TGF-β(5ng/ml) + CD40L(100ug/ml)

Gating Strategy

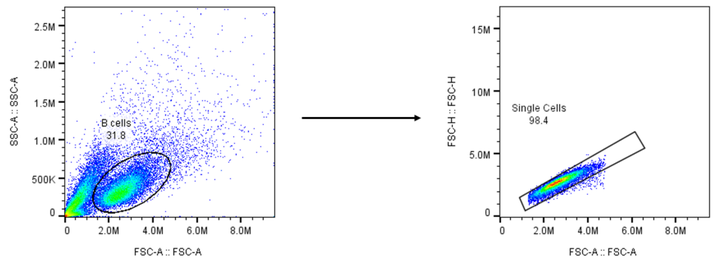


Figure 1. Flow cytometric gating strategy for isolation of WM cells for class switching analysis. Initial selection of B cells (31.8% of total events) based on forward scatter (FSC-A) and side scatter (SSC-A) properties (left panel). Single cells were subsequently isolated (98.4% of gated B cells) using FSC-A versus FSC-H parameters to exclude doublets and cell aggregates (right panel). This purified population was used for downstream analysis of CSR following cytokine stimulation and GANT61 treatment.

Results

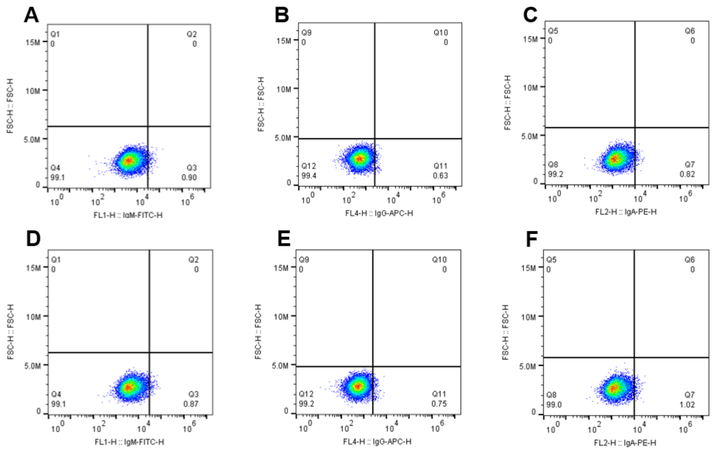


Figure 2. Flow cytometric analysis of immunoglobulin expression in GANT61-treated BCWM.1 cells following cytokine stimulation. Scatter plots showing immunoglobulin isotype distribution in BCWM.1 cells treated with GANT61 and different cytokine conditions. (A-C) Cells treated with GANT61+LPS showing percentages of cells negative for (A) IgM (99.1%), (B) IgG (99.4%), and (C) IgA (99.2%). (D-F) Cells treated with GANT61+LPS+BAFF+CD40L showing percentages of cells negative for (D) IgM (99.1%), (E) IgG (99.2%), and (F) IgA (99.0%).

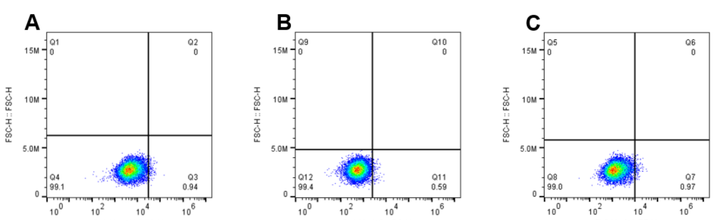


Figure 3. Flow cytometric analysis of immunoglobulin expression in GANT61-treated MWCL-1 cells with TGF-β+IL-4+CD40L stimulation. Representative scatter plots showing immunoglobulin isotype distribution in MWCL-1 cells treated with GANT61 and TGF-β+IL-4+CD40L. Panels show percentages of cells negative for (A) IgM (99.1%), (B) IgG (99.5%), and (C) IgA (99.0%).

Summary

- Flow cytometric analysis failed to detect any immunoglobin surface expression, including the expected IgM that defines WM, indicating a technical issue instead of a biological one.
- The addition of Fc block reagent interfered with antibody-antibody interaction, likely preventing the detection of surface immunoglobin across all samples
- This procedural error prevented us from determining whether class switching occurred as a response to cytokine stimulation and GLI2 inhibition

Next Steps

- Repeat the procedure without the addition of Fc block reagent.
- Repeat the procedure including 24-hour and 72-hour experimental groups.
- In some cases, WM cells can be characterized more closely to antibody secreting plasma cells, thus performing an ELISA to view secreted antibody isotypes would be beneficial.
- Performing CSR assays utilizing end-point PCR viewing the switch (S) regions to analyze immunoglobin class switching.

Acknowledgements

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References

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