Overcoming Interference from Anti-CD38 in Flow Cytometric Crossmatch Testing

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Objective

Daratumumab is a CD38-directed cytolytic antibody used for the treatment of multiple myeloma. It is a human immunoglobulin G (IgG) kappa monoclonal antibody that binds to CD38 surface expressing cells, including circulating T and B lymphocytes, and therefore may interfere with crossmatch tests. Daratumumab-mediated positive reactivity may persist for up to 6 months after the last administration. The aim of this study was to circumvent this interference allowing interpretable flow cytometric crossmatch (FCXM) results for patients in need of transplantation.

Methods

Lymphocytes were isolated from peripheral blood with a Ficoll density gradient and enriched with immunomagnetic negative selection using HLA WB Total Lymphocyte Enrichment Kits and RoboSep-S (StemCell Technologies) per manufacturer's instructions.

Lymphocytes pretreated with either 50 mM DTT (Sigma-Aldrich) for 10 minutes at 37˚C or 1 µg of purified mouse anti-human CD38 (BD Biosciences, clone HIT2) for 10 minutes at room temperature and washed once were compared to untreated lymphocytes in FCXM tests.

Anti-CD38 was diluted in sera to final concentrations of 0, 40 and 400 µg/ml to mimic therapeutic levels. Dosed sera were compared in three color flow cytometric crossmatches performed on a BD FACSCanto II flow cytometer and analyzed with FACSDiva software. Mouse monoclonal antibodies used for staining include PE-CD19 (clone Leu-12), APC-CD3 (clone SK7) and FITC-conjugated anti-Human IgG (clone G18-145) obtained from BD Biosciences.

Cell surface expression of CD38 was measured using FITC-conjugated anti-CD38 (clone HB7) and MHC expression was assessed using anti Human HLA-A/B/C PE (clone G46-2.6) and anti-Human HLA-DR/DQ/DP FITC (clone Tu39) from BD Biosciences

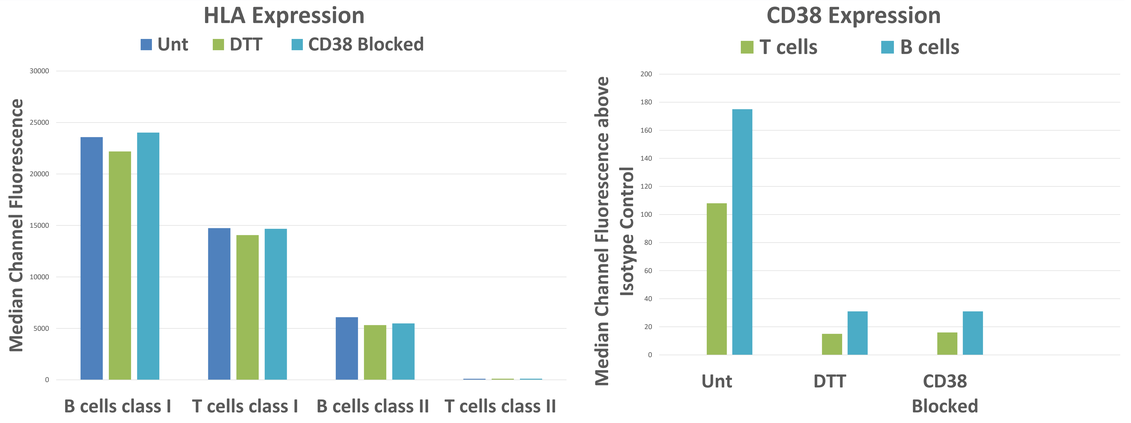
Results

Table I. Effect of Lymphocyte Treatment in FCXM with Daratumumab-containing sera

Comparison of Median channel fluorescence values and Ratio to negative control (in parentheses) in surrogate donor crossmatches. Sera dosed with 40 (data not shown) and 400µg/mL reacted in a dose-dependent fashion.

	Untreated		DTT treated		CD38 blocked	
	T cells	B cells	T cells	B cells	T cells	B cells
Neg Ctl	113	198	110	149	107	154
Pos Ctl	700 (6.2)	1042 (5.3)	706 (5.5)	996 (6.7)	588 (5.5)	956 (6.2)
Neg Ctl 0µg/mL Dara	110 (1.0)	197 (1.0)	109 (1.0)	147 (1.0)	110 (1.0)	152 (1.0)
Neg Ctl 400µg/mL Dara	234 (2.1)	453 (2.3)	111 (1.0)	174 (1.2)	111 (1.0)	164 (1.1)
Pt #1 No DSA 0µg/mL Dara	123 (1.1)	198 (1.0)	112 (1.0)	145 (1.0)	114 (1.1)	141 (0.9)
Pt #1 No DSA 400µg/mL Dara	221 (2.0)	760 (3.8)	112 (1.0)	440 (3.0)	114 (1.1)	159 (1.0)
Pt #2 High DSA 0µg/mL Dara	1999 (18.3)	2734 (14.6)	1864 (16.9)	2639 (13.0)	1771 (16.1)	2653 (13.3)
Pt #2 High DSA 400µg/mL Dara	1716 (15.7)	2693 (14.4)	1793 (16.2)	2659 (13.1)	1595 (14.5)	2583 (13.0)

Figure I. Effect Treatment on HLA and CD38 Cell Surface Expression



Results

- Both DTT and blocking with anti-CD38 reduced interference from Daratumumab without affecting the sensitivity or specificity of the FCXM test
- HLA expression was not significantly impacted by treatments
- Anti-CD38 blocking was more effective than DTT treatment at blocking the drug's target

Discussion

Interference in RBC antibody testing from Daratumumab has been shown to be negated by treating the RBCs with DTT, The efficacy of this treatment with lymphocytes in FCXM tests has been reported.

DTT treatment of RBCs is known to denature Kell blood group antigens and it's effect on other antigens is not known although cell surface of HLA was not significantly impacted in this study.

A limiting factor to this study was the lack of patient serum samples pre and post Daratumumab treatment

Conclusions

- Anti-CD38 interference in FCXM can be reduced using 0.05M DTT which cleaves most of the cell surface CD38 target
- Pretreatment of cells by blocking the CD38 with monoclonal antibody eliminates the interference by Daratumumab
- Interference from Anti-CD38 in the presence of strong DSA does not appear to cause significant interference
- More studies are needed to determine the best method of treatment