HLA-specific IgM and IgG antibody competition affects Mean Fluorescence Intensity (MFI) signals in the Single Antigen Assay.

Tri T.M. Vu, Tharindu Abeywardana, Ronald Wang, Marissa Beltran-Lemus, Ning Xu, Ito Wakefield, Rui Pei, and David Lowe
Thermo Fisher Scientific Inc., West Hills, CA, USA

ThermoFisher SCIENTIFIC

Abstract

The presence of anti-HLA antibodies can pose challenges in transplantation. The precise detection of these antibodies by single antigen bead (SAB) assays have helped to greatly improve successful long-term outcomes for transplantation. Currently, the focus of the SAB assays is on the detection of the anti-HLA IgG antibodies. However, IgM is the first immunoglobulin produced and may persist in many patients. IgM and IgG antibodies can recognize the same epitope on HLA molecules. Consequently, anti-HLA IgM can interfere with anti-HLA IgG signals. In this research study, we observed high prevalence of anti-HLA IgM in patient sera, and in some instances, anti-HLA IgM may suppress anti-HLA IgG signals.

Introduction

Anti-HLA IgM is the first immunoglobulin to be produced during de novo donor specific antibodies (DSA) generation. Some anti-HLA IgM producing B-cells undergo isotype switching to produce anti-HLA IgG, while retaining epitope specificity. Anti-HLA IgM antibodies are known to persist in sera and are implicated in interfering with anti-HLA IgG signals. In the current study we have observed significant prevalence of anti-HLA IgM in the sera with high anti-HLA IgG signals. To investigate how the presence of anti-HLA IgM affects anti-HLA IgG signals in SAB assays, we focused on a sample with overlapping signals for both types of antibodies. Additionally, we used One Lambda MagSort™ to separate anti-HLA IgG and IgM antibodies from this same sample to further study their specific binding patterns and eplets.

Materials and methods

Sera screen was performed using single antigen bead (SAB) assays.

Pre-treatment of sera with DTT: 5 µl of 50 mM DTT was added to 45 µl of serum and incubated at 37oC for 30 min. Next, the mixture was centrifuged at 9300 X g for 10 min. 20 µl of the supernatant was used for LABScreen single antigen.

MagSort beads were incubated with Serum 145 at room temperature for 2 hrs with rotation. Post-treatment serum was collected. Beads were washed with MagSort wash buffer twice. Antibodies were eluted with either IgG or IgM elution buffers. Eluants were treated with respective neutralization buffers. Eluted antibodies were tested on SAB assays with anti-IgG or anti-IgM secondary antibodies.

Results

Table 1. Anti-HLA IgM Antibody Signals in Samples with High Anti-HLA IgG Antibody Signals

(A) Anti-HLA class I signals

MFI signal	% of samples with IgG antibody signal	% of samples with IgM antibody signal
0-1,000	2.3	0.3
1,000-5,000	2.3	20.7
5,000-10,000	5.8	38.3
10,000-20,000	76.2	36.1
>20,0000	13.4	4.6

(B) Anti-HLA class II signals

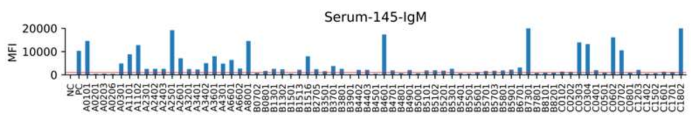
MFI signal	% of samples with IgG antibody signal	% of samples with IgM antibody signal
0-1,000	21.2	2.5
1,000-5,000	30.5	53.4
5,000-10,000	13.4	30.9
10,000-20,000	28.8	13.0
>20,0000	6.1	0.3

First column: MFI signals are grouped into five bins.
Second column: Percentage of samples that have anti-HLA IgG antibody signals falling into those five bins.
Third column: Percentage of samples that have anti-HLA IgM antibody signals falling into those five bins.

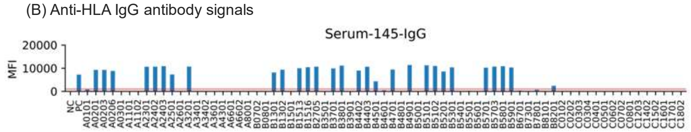
For HLA class I, 89.6% of the samples had anti-HLA IgG signals above 10,000. 79% of these same samples had anti-HLA IgM signals above 5,000 (Table 1A). For HLA class II, 34.9% of the samples had anti-HLA IgG signals above 10,000. 44.2% of these same samples had anti-HLA IgM signals above 5,000 (Table 1A).

Figure 1. IgM and IgG LABScreen profiles for Serum 145

(A) Anti-HLA IgM antibody signals



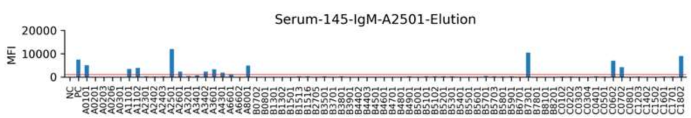
(B) Anti-HLA IgG antibody signals



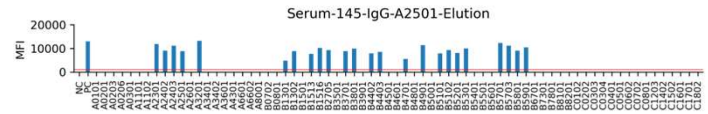
IgM and IgG LABScreen profiles for Serum 145 show both overlapping (eg: A2501, B1516) and non-overlapping (eg: A0101, A0201) HLA specificities.

Figure 2. LABScreen profile for MagSort elutions from Serum 145

(A) MagSort A*25:01 elution with anti-IgM secondary antibodies



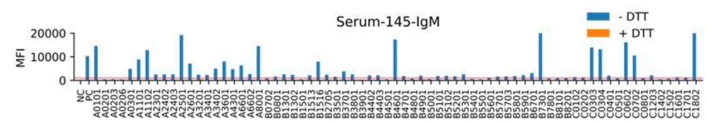
(B) MagSort A*25:01 elution with anti-IgG secondary antibodies



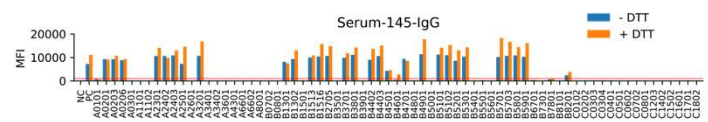
MagSort A2501 was performed for both IgM and IgG on Serum 145. Serum 145 contains anti-HLA IgM antibodies targeting the 90D eplet as confirmed by A2501 IgM elutions (Figure 2A). Serum 145 also contains anti-HLA IgG antibodies targeting the 82LR eplet as confirmed by MagSort A2501 IgG elution (Figure 2B).

Figure 3. LABScreen profile for Serum 145 with DTT treatment

(A) Anti-HLA IgM antibody signals with and without DTT treatment



(B) Anti-HLA IgG antibody signals with and without DTT treatment



Serum 145 was treated with DTT and tested on LABscreen antigen using either anti-human IgG and IgM secondary antibodies. DTT treatment destroyed IgM signal (Figure 3A). The destruction of IgM increased the IgG signals (Figure 3B). The highest increase was observed for A2501 signals, which increased by 2.3-fold. The 90D and 82LR eplets overlap on A2501.

Discussion

We have observed notable persistence of anti-HLA IgM in sera with high anti-HLA IgG (>10,000 MFI). The presence of anti-HLA IgM may be an early indicator of allosensitization, while its prolonged persistence could be due to individual immunological variability. In Serum 145, we observed high MFI signals for IgM targeting overlapping HLA specificities. These IgM signals appeared to mask the corresponding IgG reactivity, as DTT treatment resulted in a marked increase in IgG signal intensity. This finding highlights the importance of research evaluation for anti-HLA IgM specificities, as their presence can interfere with IgG detection and compromise the accuracy of MFI readouts in SAB assays. Furthermore, IgM and IgG MagSort, targeting overlapping HLA specificities can facilitate research characterization of these antibodies.

Conclusion

In conclusion, we observed that many samples with high anti-HLA IgG signals also exhibited significant anti-HLA IgM signals. When IgG and IgM signals overlap, high IgM signals can suppress IgG signals. In such cases, we believe that separating IgM antibodies from IgG antibodies will enable a better characterization of the anti-HLA antibodies.

Trademarks/Licensing