Isolating HLA-specific IgM and IgG from serum using different MagSort elution buffers

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Abstract

Presence of donor-specific antibodies (DSAs) against HLA antigens poses numerous challenges to organ transplantation. Therefore, characterization of DSAs has become increasingly utilized in pre- and post-transplant risk-stratification. One Lambda MagSort facilitates the characterization of IgG DSAs from complex sera. To extend the utilization of the MagSort product, we have developed IgM elution buffer to isolate HLA specific IgM antibodies. MagSort with IgM elution buffer successfully isolated HLA specific IgM antibodies from patient sera. Thus, MagSort with IgG and IgM specific elution buffers can be utilized to further dissect the complex sera at antibody isotype level.

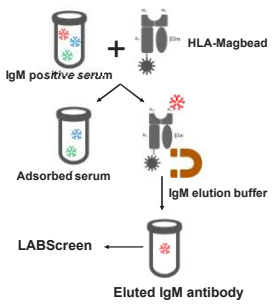
Introduction

Patient sera can contain multiple donor specific antibodies (DSAs) with different isotypes due to various sensitizing events. IgG and IgM antibody isotypes are associated with antibody mediated rejection (ABMR) and graft loss (1). Additionally, the presence of different HLA specific antibody isotypes can add to the complexity of some allosera. Therefore, characterization of DSAs at antibody isotype level can be of importance in pre- and post-transplant risk-assessment. One Lambda MagSort was developed and optimized to isolate IgG antibodies from patient sera. Current IgG elution buffer (MagSort MAGELBUF) cannot efficiently elute HLA specific IgM. To overcome this challenge and further explore the utility of the MagSort product, we developed buffers that allow the isolation of IgM antibodies from patient sera. IgM elution buffer can efficiently isolate IgM antibodies targeting both Class I and Class II HLA. Further, MagSort with IgG and IgM specific elution buffers can isolate DSAs with different HLA binding patterns from the same serum.

Materials and methods

IgG elution buffer (MagSort MAGELBUF) and IgM elution buffer were developed. Sera were screened to obtain IgM/IgG positive sera. We performed serum treatment using indicated MagSorts on respective Sera. MagSort beads were incubated with respective sera 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 LABScreen Single Antigen (One Lambda, LS1A04) with anti-IgG or anti-IgM secondary antibodies **(Figure 1**).

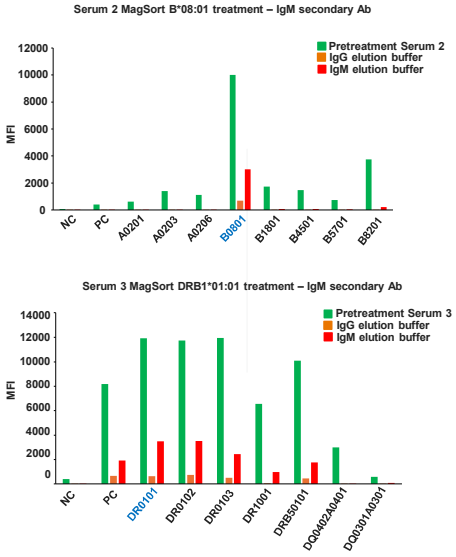
Figure 1. HLA specific IgM isolation using MagSort



Results

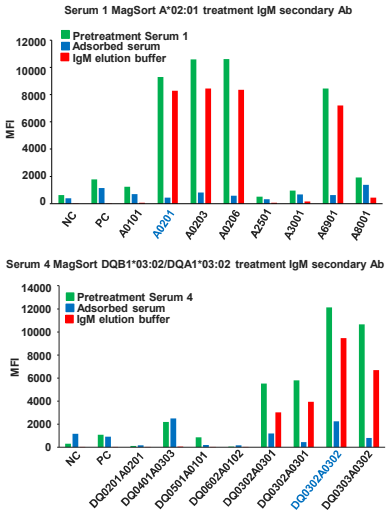
Given the importance of characterizing HLA specific IgM in patient sera, we used One Lambda MagSort to isolate IgM antibodies from IgM positive sera. Flow cytometry analysis of the magnetic beads after serum treatment with anti-human IgM-PE clearly showed that HLA magnetic beads captured IgM antibodies successfully (data not shown). However, the eluted antibodies using MAGELBUF yielded only a weak signal on LABScreen (**Figure 2**). Next, we tested different elution conditions for optimal IgM antibody isolation from sera and developed IgM elution buffer. IgM elution buffer efficiently elutes both Class I and Class II HLA specific IgM antibodies (**Figure 3**). We also tested the potential of using MagSort with IgG and IgM elution buffers to detect isotype specific reactivity patterns of anti-HLA antibodies in specific sera. Correspondingly, treatment of Serum 2 with MagSort B*08:01 and Serum 3 with MagSort DRB1*01:01 yielded distinct reactivity patterns on LABScreen for elutions with IgG and IgM buffers (**Figure 4**) .

Figure 2. MagSort IgG elution buffer does not efficiently elute Class I and Class II specific IgM antibodies



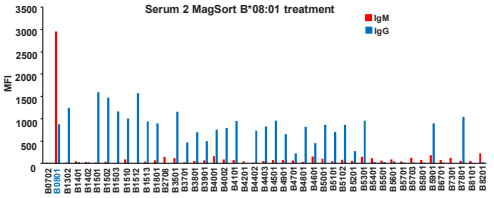
Top panel - Serum 2 was treated with MagSort B*08:01 and captured antibodies were eluted with either IgG or IgM elution buffer. Untreated Serum 2 and eluted antibodies were tested on LABScreen Single Antigen (One Lambda, LS1A04) with anti-IgM secondary antibodies. Bottom panel - Serum 3 was treated with MagSort DRB1*01:01 and captured antibodies were eluted with either IgG or IgM elution buffer. Untreated Serum 3 and eluted antibodies were tested on LABScreen Single Antigen (One Lambda, LS2A01) with anti-IgM secondary antibodies.

Figure 3. IgM elution buffer efficiently elutes both Class I and Class II specific IgM antibodies

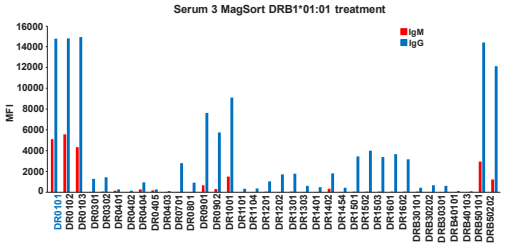


Top panel - Serum 1 was treated with MagSort A*02:01 and captured antibodies were eluted with IgM elution buffer. Untreated Serum 1, post-treatment serum (Adsorbed serum) and eluted antibodies were tested on LABScreen Single Antigen (One Lambda, LS1A04) with anti-IgM secondary antibodies. Bottom panel - Serum 4 was treated with MagSort DQB1*0302/DQA1*03:02 and captured antibodies were eluted with IgM elution buffer. Untreated Serum 4, post-treatment serum (Adsorbed serum) and eluted antibodies were tested on LABScreen Single Antigen (One Lambda, LS2A01) with anti-IgM secondary antibodies.

Figure 4. MagSort with IgM and IgG elutions can detect different reactivity patterns in a given serum



Serum 2 was treated with MagSort B*08:01 and captured antibodies were eluted with either IgM elution buffer or IgG elution buffer. Antibodies eluted with IgG elution buffer were tested on LABScreen Single Antigen (One Lambda, LS1A04) with anti-IgG secondary antibodies and antibodies eluted with IgM elution buffer were tested on LABScreen Single Antigen (One Lambda, LS1A04) with anti-IgM secondary antibodies.



Serum 3 was treated with MagSort DRB1*01:01 and captured antibodies were eluted with either IgM elution buffer or IgG elution buffer. Antibodies eluted with IgG elution buffer were tested on LABScreen Single Antigen (One Lambda, LS2A01) with anti-IgG secondary antibodies and antibodies eluted with IgM elution buffer were tested on LABScreen Single Antigen (One Lambda, LS2A04) with anti-IgM secondary antibodies.

Discussion

Routinely, HLA laboratories test for IgG HLA specific antibodies but it has been reported that IgM HLA antibodies, particularly if formed post-transplant can have a direct impact upon rate of graft failure (ref 2). The development of a specific buffer which enables the direct elution of IgM HLA antibodies can assist in further investigating the clinical relevance of these antibodies. Other potential applications of adaptation of the MagSort reagents to allow for IgM/IgG antibody differentiation may include studies into antibody binding kinetics or the exploration into whether a diverse HLA specific isotype composition in serum impacts upon MFI readouts.

Conclusion

Newly developed IgM elution buffer efficiently isolate HLA-specific IgM antibodies from sera. One Lamba MagSort with IgG elution buffer and IgM elution buffer can be utilized to identify isotype specific reactivity patterns in complex sera.

References

1. R. M. McKenna, S. K. Takemoto, P. I. Terasaki, Anti-HLA antibodies after solid organ transplantation. Transplantation 69, 319–326 (2000).
2. Babu et al. Clinical Relevance of Donor-Specific IgM Antibodies in HLA incompatible Renal Transplantation: A Retrospective Single-Center Study. Clin Transpl. 2016:32:173-179

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