To Transplant Or Not To Transplant?

That is the question

Dose Response Features and Parameters to Consider in Serological Evaluation of Transplant Patients

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Abstract

Detecting antibodies against Human Leukocyte Antigens (HLA) is an important procedure in many transplant laboratories because high levels of anti-HLA antibodies are directly associated with higher risks of immediate rejection within the patient. The goal of this study was to use a Luminex-based detection system to implement new titer and affinity parameters that are currently not easily accessible in hopes that the information will be useful for clinical practice. In order to measure antibody HLA interactions, we utilized a unique bead assay that probed the binding of monoclonal antibodies to their HLA targets in antibody titration experiments. Our findings showed that antibodies can be easily titrated, delivering unique S shaped curves. Defined parameters were obtained including Relative Effective Concentration values at 50% saturation (rEC_{50}), as well as maximum response values (MFI). We demonstrated that EC_{50} values can be used to interpret the potency of any given antibody which is influenced by its titer (amount of antibody) and its affinity (binding strength). Overall, the knowledge gained in dose-response studies can be used to establish guidelines for defining strength, affinity, and ultimately tolerance limits of HLA antibodies, allowing to better predict transplant rejections and improve risk management.

Introduction

The testing of HLA antibodies within graft recipients is used to determine the patient's immunological status before and after transplant to identify and reduce potential health risks. High levels of anti-HLA antibodies are directly associated with higher risks of immediate rejection within the patient, which strongly suggests that antibody titer and affinity parameters should be considered in a patient's evaluation. The goal of this study was to use a Luminex-based detection system to implement new performance parameters that are currently not easily accessible in hopes that the information will be useful for clinical practice.

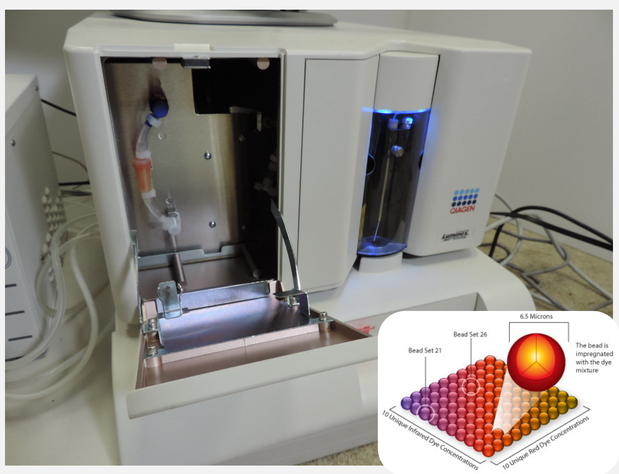
Method

In order to measure antibody HLA interactions, we utilized a unique 120 single antigen bead assay combining soluble HLA molecules at high purity with Luminex beads. The beads have a diameter of 5.4 microns and are colored by two fluorochromes.

In a multi-step process, the assay probed the binding of monoclonal antibodies to their HLA targets through the use of two types lasers that excite the beads:

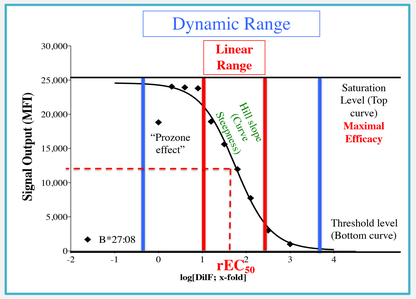
- The red diode laser excites red fluorophores within the beads which enables precise identification of each bead.
- The green laser excites the fluorochrome Cy5 coupled to our reporter molecule, which can detect the precise number of coupled sHLA ligands.

The reflected fluorescence emitted determines the positivity of the reaction according to a threshold fluorescence for each bead. The intensities of red and green fluorescences are both recorded then antibody titrations are created and analyzed using a dose-response model.



Results

The Titration Concept

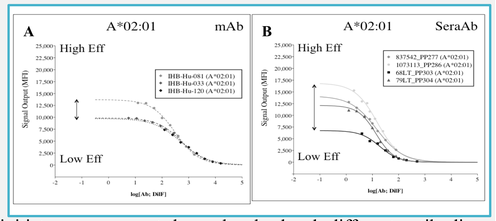


Our findings showed that antibodies can be easily titrated, delivering unique S shaped curves. Representative mAbs and sera were tested applying an 8-point dilution scheme and single titration parameters where calculated using a Sigmoidal dose-response model (variable slope). Hill Slope has no units and describes the steepness of the curve. This variable, if it is positive, the curve increases as X increases. If it is negative, the curve decreases as X increases. A standard sigmoid dose-response curve (previous equation) has a Hill Slope of 1.0.

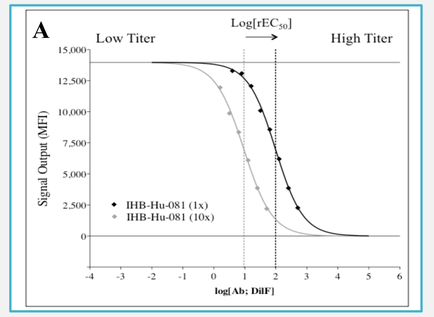
Defined parameters were obtained including Relative Effective Concentration values at 50% saturation (rEC_{50}), as well as maximum response values (MFI). LogrEC_{50} is the X value when the response is halfway between Bottom and Top. We demonstrated that rEC_{50} values can be used to interpret the potency of any given antibody which is influenced by its titer (amount of antibody) and its affinity (binding strength).

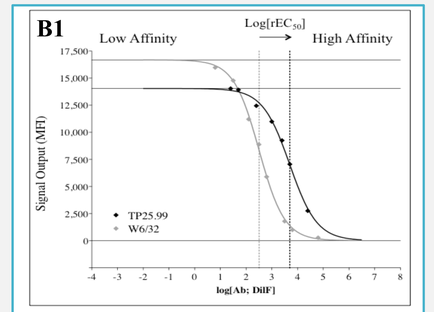
The Efficacy Concept

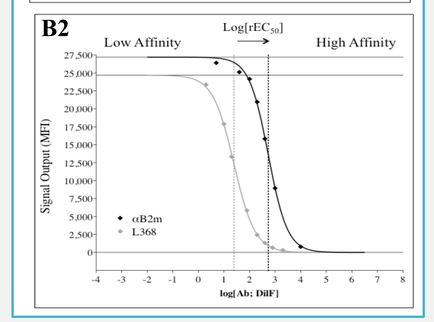
A dose-response curve is determined by two different properties; first how well an antibody binds to the HLA epitope, as seen involving affinity and titer, and secondly how well it causes a response once bound. This property is known as the antibodies maximal efficacy (Eff_{max}). Since efficacy depends on the relationship between receptor occupancy and the ability to initiate a response at the molecular level, different antibodies acting on a single HLA epitope will have different efficacies. Testing of several monoclonal antibodies and serological samples for binding to identical HLA target antigens demonstrates the structure-dependent efficacy variation.



The Potency Concept







Semi-log plots are showing dose-response experiments with sigmoidal functions, probing the interaction of a single HLA-A*02:01 beads with HLA antibodies.

(A) In order to compare titer relationships, the same antibody was tested using a sample at a 10-fold (10x) versus an undiluted (1x) probe. Determining the relative half maximal effective concentration (rEC_{50}) for the 1x and 10x samples delivered values of 98.2 and 9.4, respectively. A quotient of 10.4 was calculated which is very close to the targeted value of 10. Results clearly show that rEC_{50} values and therefore potency is concentration-dependent if antibody affinity is constant.

(B) Alternatively, a second experimental setup was designed to visualize affinity differences of various antibodies under fixed concentration conditions. As seen, antibodies at identical concentrations have different rEC_{50} values suggesting variability in affinities. Conclusively, results indicate that two effects can move rEC_{50} values, titer (A) and affinity (B). Antibodies with higher affinity and/or titer evoke larger responses than antibodies with lower affinity and/or titer. On a log scale (x=log[DilF]), the higher the values the more potent are rEC_{50} values.

Conclusion

Potency analysis seems to have a high potential to deliver clinically relevant information. Deducing dose-response data to rEC_{50} values has the advantage of simplifying data output and allowing comparative analysis on potency rather than MFI. Overall, the knowledge gained in dose-response studies can be used to establish guidelines for defining strength, affinity, and ultimately tolerance limits of HLA antibodies, allowing to better predict transplant rejections and improve risk management.