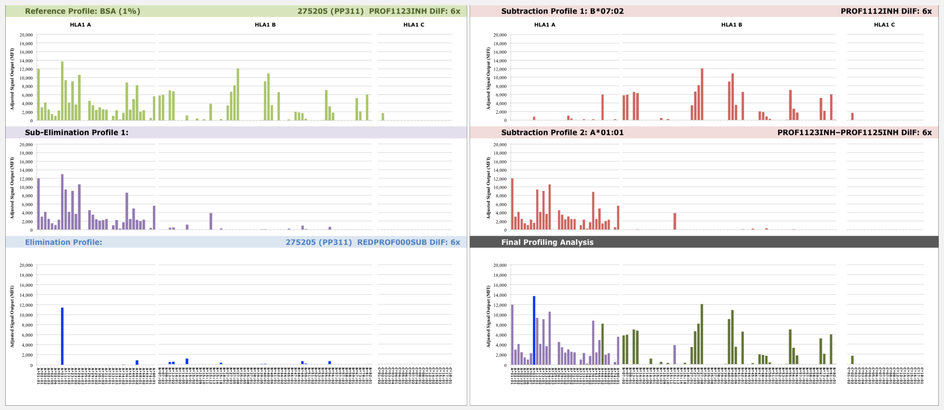
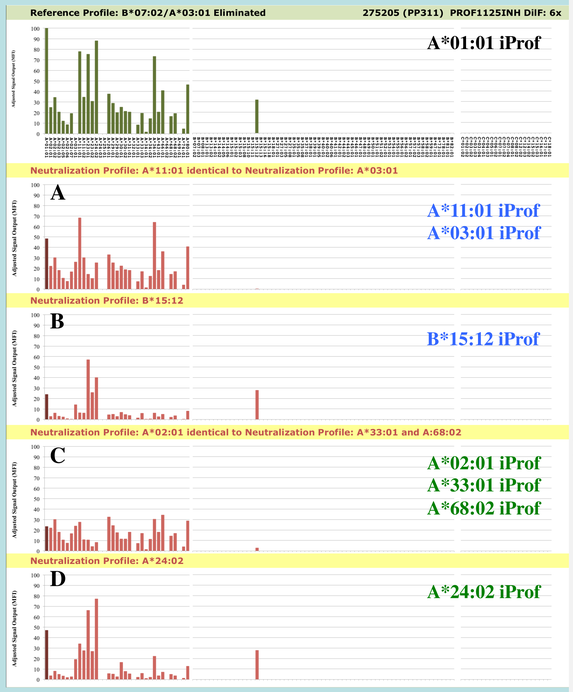
The Complexity of Anti-HLA Antibodies in Immunological Response Patterns of Sensitized Transplant Patients

Rico Buchli[1], Arend Mulder[2], Annette Jackson[3], Anat R. Tambur[4], René J. Duquesnoy[5], Rebecca D. McAdams[1], Rick Eggers[6], Georgina Lopez Padilla[6], Daniel Zehnder[7], David P. Lowe[8], David C. Briggs[9], Robert Higgins[10], Frans H.J. Claas[11], Mike Hogan[6], William H. Hildebrand[1,12]

[1]Pure Protein LLC, Oklahoma City, OK; [2]Leiden University Medical Center, Leiden, Netherlands; [3]Johns Hopkins University, Baltimore, MD; [4]Feinberg School of Medicine, Northwestern University, Chicago, IL; [5]University of Pittsburgh Medical Center, Pittsburgh, PA; [6]GMSBiotech, Tucson, AZ; [7]University Hospitals of Coventry & Warwickshire NHS Trust, Coventry, United Kingdom; [8]Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, United Kingdom; [9]NHSBT Birmingham, Birmingham, United Kingdom; [10]University Hospitals of Coventry & Warwickshire, Coventry, United Kingdom; [11]Leiden University Medical Center, Leiden, Netherlands; [12]Oklahoma University Health Sciences Center, Oklahoma City, OK



Case Study 1: Demonstration of a Tier 1 analysis, deconvoluting a complex human sera into 3 distinguishable patterns that was created by immunization events involving B*07:02, A*01:01 and A*03:01. Neutralization Profiles (red) subtracted from the Reference Profile (green) show individual antigen-related Ab patterns. Identification of the three immunizing alleles and usage of their sHLA counter-part as neutralizer ultimately can eliminate/cancel all specific responses. In many cases, more than one antigen is involved contributing to a higher complexity level not readily visual without specific allele inhibition experiments.



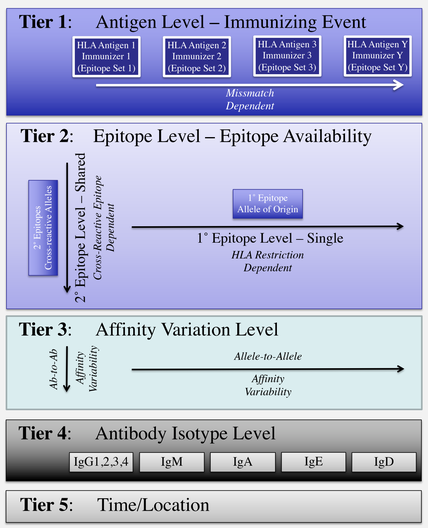
Case Study 2 (iProfs): Sera complexity of an A*01:01 immunized individual showed strong epitope sharing with A*11:01/A*03:01 allele. Another shared group was identified related to A*02:01/A*33:01/A*68:02 with antibodies of weaker epitope recognition potency. In contrast, B*15:12 and A*24:02 epitope sharing is much more limited. These iProfs show still a great complexity and suggest superposition of Ab patterns towards multiple epitopes which cannot readily be resolved. Collection of iProfs showing the summary of all epitope interaction of a patients Ab pool directed against a specific HLA molecule can greatly assist in confirming eligibility or ineligibility to receive a particular organ (physical crossmatch).

Immunologic Profile (iProf):
undefined single HLA pattern generated by polyclonal Abs recognizing multiple epitopes on a single HLA allele.

Epitope Profile (eProf):
defined single HLA epitope pattern generated by a monoclonal Ab recognizing a single epitope.

Abstract

Due to the increasing demand for more accurate and meaningful data, the determination of MFI values alone is not sufficient anymore to evaluate a patient's risk potential. In this study, we aimed to demonstrate the enormous complexity of Ab patterns in serological specimen applying additional investigative tools. Our results show that generally all response profiles found in transplant patients are superpositions of more simple Ab responses which can be deconvoluted into individual sub-patterns. Single alleles can be identified as primary cause of one or more immunizing events. Comparative analysis using known monoclonal Ab patterns is required to conclude on individual epitope assignments.



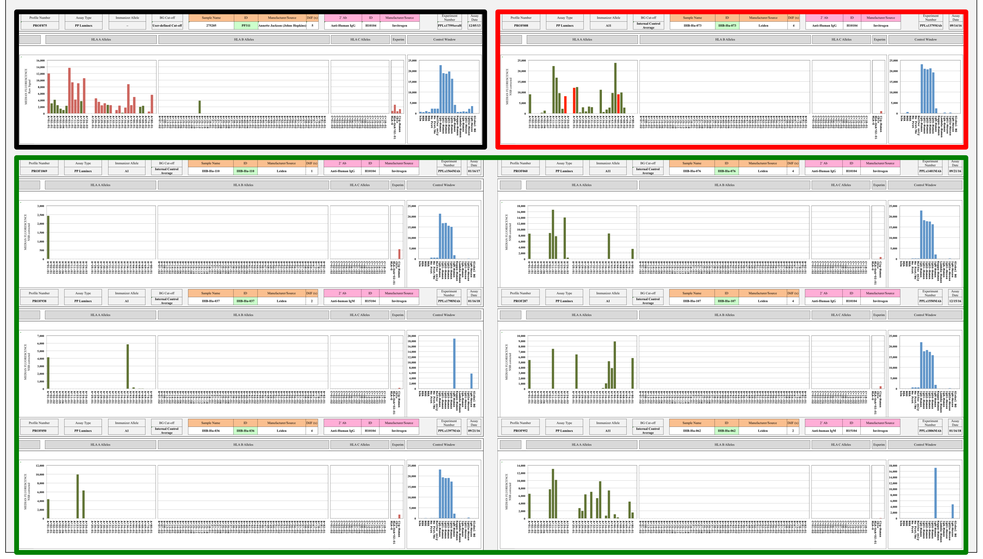
Introduction

Rejection-causing antibodies in organ transplant patients are directed against the received graft and polyclonal in nature. They are generated by random combinations of gene sets that encode different antigen binding sites, followed by random mutations, which create further diversity. In addition, antibody genes also re-organize in a class switching process creating different isotypes. Gene recombination, somatic hypermutation, and affinity maturation serve to increase the diversity of the antibody pool and impact the antibody's antigen-binding affinity. Considering HLA being the largest polymorphic system with thousands of different proteins known, it is not surprising to find an enormous Ab complexity even within simple sera specimen. Furthermore, Ab composition changes occur over time, never delivering a constant view of the response pattern. Within this study, we attempted to capture and showcase some of the complexity of serological samples categorized within different tier categories.

Conclusion

We believe that the understanding of the complicated nature of transplant-related Ab profiles can greatly enhance our knowledge about the composition of individual patient sera. The knowledge gained about the apparent complexity of specific immune patterns will help us to reach the next chapter of patient care and contribute to a better risk management in Ab-related graft rejection.

Case Study 3 (eProfs): Experimental procedures become rather involved to resolve a complex serological Ab mixture (iProf) to the epitope level. Utilizing known monoclonal Ab patterns (eProfs) in an algorythm-based recognition process can suggest or exclude the binding of sera Abs to specific epitopes. As seen in the original iProf (**black box**) all dark red labeled alleles could be connected to existing eProfs (**green box**). Additional epitopes seem to exist on A*01:01 (**black box**) for which no eProf pattern could be defined yet (green labeled alleles). Additionally, epitopes can be excluded where an eProf (**red box**) contains alleles (red) not included within the original pattern (**black box**).



Case Study 4: Analysis of a serological specimen obtained from a multiparous woman (top) immunized with A*02:01. Subsequent immortalization of peripheral blood cells of the same woman has produced stable hybridomas secreting monoclonal antibodies. Resulting patterns show that the simple serological profile is composed of superimposed signal patterns of different antibodies recognizing different epitopes. It can also be assumed that the Ab level of IHB-Hu-081 (SN607D8) is lower than the IHB-Hu-033 (SN230G6). In general, our studies showed that no sera, even simple in its recognition pattern can be assumed monoclonal.