CASE STUDY

Immune Repertoire Profiling to Understand the Autoimmune Disease State

INTRODUCTION

Autoimmune diseases impact nearly one in ten individuals and may manifest as any one of 80 different types of autoimmune conditions.[1] These diseases occur when the immune system mistakenly attacks the host's healthy cells, but the reasons for this are not well understood. Several published studies have used immune repertoire sequencing to identify biomarkers indicative of autoimmunity and to explore the underlying mechanisms that contribute to autoimmune disease pathogenesis.[2] However, most studies have been limited in depth and resolution due to restricting the analysis to specific B cell and T cell receptor chains. Immune sequencing technologies can now amplify all 7 chains of the immune repertoire without bias, for an unprecedented, quantitative analysis of B cell receptor (BCR) and T cell receptor (TCR) diversity.

CHANGES IN THE IMMUNE REPERTOIRE CORRELATE WITH AUTOIMMUNE DISEASE

The immune repertoire consists of two cell types, B cells and T cells, that are responsible for both regulating and carrying out the adaptive immune response. Immune repertoire sequencing enables the detection of specific B or T cell clonal populations, even rare populations, that may play a critical role in autoimmune disease pathogenesis. Compared to healthy individuals, patients with autoimmune diseases have demonstrated disrupted B cell receptor (BCR) or T cell receptor (TCR) diversity.[3,4]

Immune repertoire sequencing has enabled a greater understanding of disease pathogenesis in autoimmune diseases, like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). In RA, a large presence of RA-specific autoantibodies produced by B cells has been found, as well as an association to the variation of the gene responsible for determining cell antigen presentation to CD4+ T cells.[5,6] For SLE, previous studies have reported the crucial involvement of the TCR repertoire, and in particular, the CDR3 region found in the variable domain of the TCR β chain that is important for recognizing specific antigens.[7–10]

New studies have used immune sequencing to reveal even more about the role of the immune repertoire in RA and SLE.

CHARACTERIZING THE IMMUNE REPERTOIRE IN RHEUMATOID ARTHRITIS

Rheumatoid Arthritis is caused by a failure of T cell receptors (TCR) and B cell receptors (BCR) of the adaptive immune system to properly differentiate between self and foreign antigens, leading to an attack on the body, resulting in chronic inflammation and joint destruction.[11] However, the precise mechanisms explaining how adaptive immune cells contribute to disease are unknown.

A first-ever, exploratory, in-depth, quantitative analysis of the adaptive immune receptor repertoire (AIRR) of RA patients and healthy controls was conducted by a team led by Dr. Adria Aterido. This study aimed to identify clonal populations important to RA's progression and clinical phenotypes that may not have been characterized in previous studies limited to single receptor-chain analyses.[12] The AIRR is composed of 7 receptor chains, encompassing four TCR (α, β, δ, λ) and three BCR (heavy (H), κ, λ) chains. To capture a maximum number of clonotypes within the expansive AIRR, unbiased, single-assay multiplex amplification and sequencing of all 7 receptor chains was conducted. (**Figure 1**).

The immune repertoire of RA patients displayed distinct profiles. Clone diversity was notably lower in RA patients across all 7 chains compared to healthy individuals, particularly among B cells, with only moderate TCR diversity changes. This decline in diversity was mainly due to expanded BCR H, κ, and λ chains, along with a decreased usage of the λ chain in blood. RA patients exhibited an isotype-specific signature, signifying differing immunoglobulin functionality in RA. Skewed V and J gene segment usage indicated disease-linked V-J pairs. Distinct IGL and IGK clones, meta-clones, and k-mers associated with RA and clinical phenotypes were detected in RA patients. Researchers also identified specific *HLA* alleles linked to RA risk, pointing to altered antigen presentation in T cells. Furthermore, a long CDR3 amino acid sequence in the TCR δ chain was shown to be associated with clinical phenotypes of RA.

Interestingly, after a 12-week course of TNFα

inhibition (TNFi) treatment, a common therapeutic approach for RA, the diversity of BCR H, κ, and λ chains exhibited a notable increase, resembling a similar diversity pattern observed in healthy individuals. Also, responders to TNFi therapy had a different distribution of the CDR3 length within the BCR H chains compared to non-responders. Therefore, AIRR sequencing provides useful data to help stratify patients that may benefit from TNFi therapy.

Dr. Aterido and colleagues successfully developed an accurate AIRR predictor for RA diagnostics and response to TNFi therapy. The classifier achieved an accuracy rate of 95.2% in effectively distinguishing individuals with RA (including those who are seronegative) from those without. This high accuracy was attributed to the incorporation of data from both BCR and TCR clonal types, underscoring the significance of the comprehensive 7-chain analysis. The definitive impact of less diverse κ and λ chains' parameters is shown in the accuracy importance chart (**Figure 2**). The predictor was able to predict about 68.8% of TNFi responders when only patients with extreme clinical responses were assessed. As a result, the AIRR predictor proved to be a potentially useful tool for disease diagnosis in a clinical setting.

Importantly, this study highlights that even the less diverse immune chains contribute signal of diagnostic potential. These chains are often overlooked and not examined historically due to the antigen-specificity conferred by the "DJ" rearrangement in heavy and beta chains. In an exploratory context, it is imperative to consider the entire adaptome, not only the diverse chains, as differences in receptors that have less propensity of diversity—i.e., BCR light chains, TCR alpha and gamma—are typically pronounced. These differences can assist in separating groups by healthy versus disease or responder versus non-responder.

In this study, multiplex 7-chain analysis yielded maximum information from minimal input of precious clinical samples, providing data for a comprehensive analysis of multiple TCR and BCR chains in disease characterization that further advances our understanding of RA pathogenesis.

Figure 1

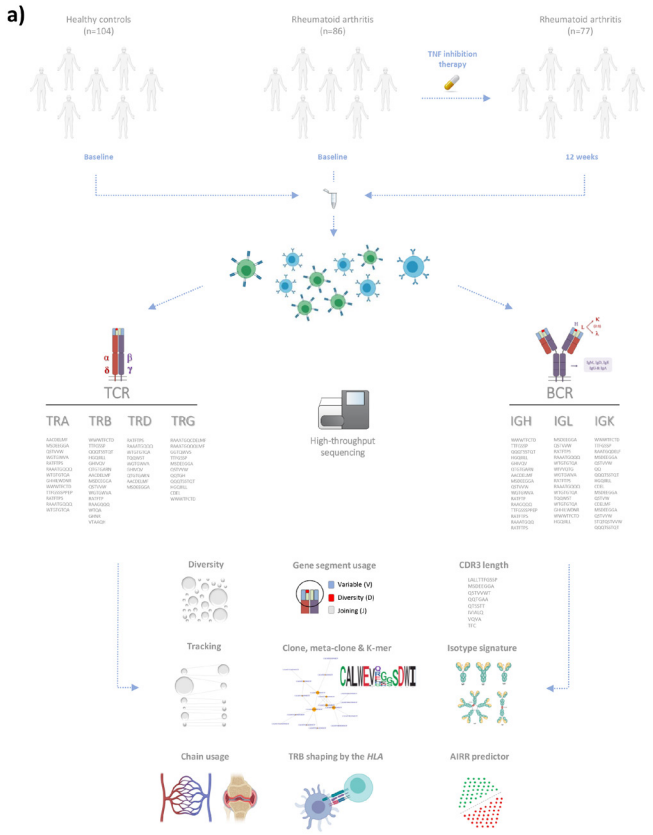

Figure 1. Sequencing the adaptive immune receptor repertoire (AIRR) of RA patients and healthy controls. Comprehensive analyses of four TCR (α, β, δ, λ) and three BCR (heavy (H), κ, λ) chains, which included diversity, gene segment usage, tracking, CDR3 lengths, clone, isotype, chain usage, TCR β shaping, and AIRR predictor analysis. Figure from Aterido, A. et al.[12]

Figure 2

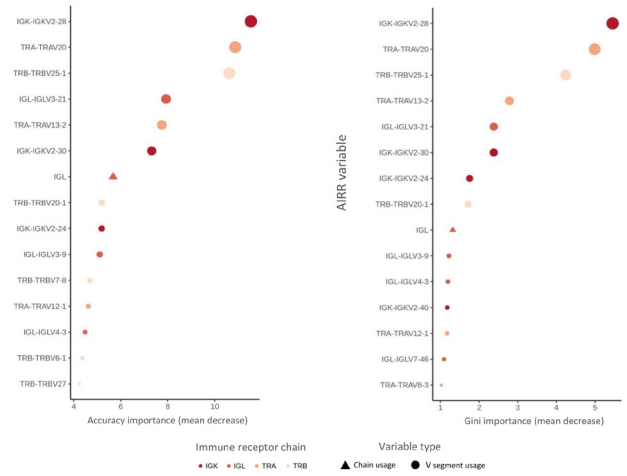

Figure 2. Identification of the 15 AIRR predictor variables that contributed most to the diagnosis of patients with rheumatoid arthritis. Figure from Aterido, A. et al.[12]

CHARACTERIZING THE TCR REPERTOIRE OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS WITH LUPUS NEPHRITIS

For patients with Systemic Lupus Erythematosus (SLE), lupus nephritis (LN) is a severe complication characterized by immune-mediated tissue damage that can lead to kidney failure.[13] Presently, LN is diagnosed by biopsy of the kidney, so there is a high demand for early-detection methods that are less invasive.[14,7–10] Patients with SLE are known to have a TCR repertoire with a lower diversity than healthy individuals, but the TCR diversity in patients that have progressed to LN has not been characterized. Dr. Xiaolan Ye and colleagues characterized the TCR diversity in SLE patients with LN to identify potential biomarkers associated with advanced disease that might serve as a future diagnostic.

The TCR β chain repertoire of SLE patients with LN and healthy controls were characterized using high-throughput RT-PCR multiplex amplification and sequencing of the CDR3 region.[15] Sequences from the V, D, and J genes were compared to those of the TCR β chain germline to determine the quantity and diversity of TCR clonotypes in each group.

Dr. Ye and colleagues reported an impaired TCR diversity in LN patients as a result of partial clonal expansion, with significant differences in V-J combination frequencies between LN patients and healthy individuals.

Using a machine learning model to evaluate V-J combinations, the team was able to clearly distinguish between SLE patients and healthy controls. In addition, specific clones were identified in LN patients that had not be found in previous studies of SLE patients without LN. With further study, these clonotypes may prove useful in developing biomarkers for LN in SLE.

CONCLUSION

Immune repertoire sequencing offers crucial insights into the immune system's role in various autoimmune diseases. By revealing specific TCR and BCR immune profiles associated with clinical phenotype, we may be able to develop new biomarker-based diagnostics, drive the expansion of personalized, targeted treatments, and improve patient outcomes. Recently, advances in immunosequencing technologies have enabled a comprehensive and quantitative analysis of all seven immune chains in a single reaction, enabling researchers to elucidate the molecular mechanisms contributing to disease state and therapy response with unprecedented precision. These technological advances may also help to reveal key immunological information in other mammalian diseases such as multiple sclerosis, primary biliary cholangitis, and autoimmune diabetes.[16–18]

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