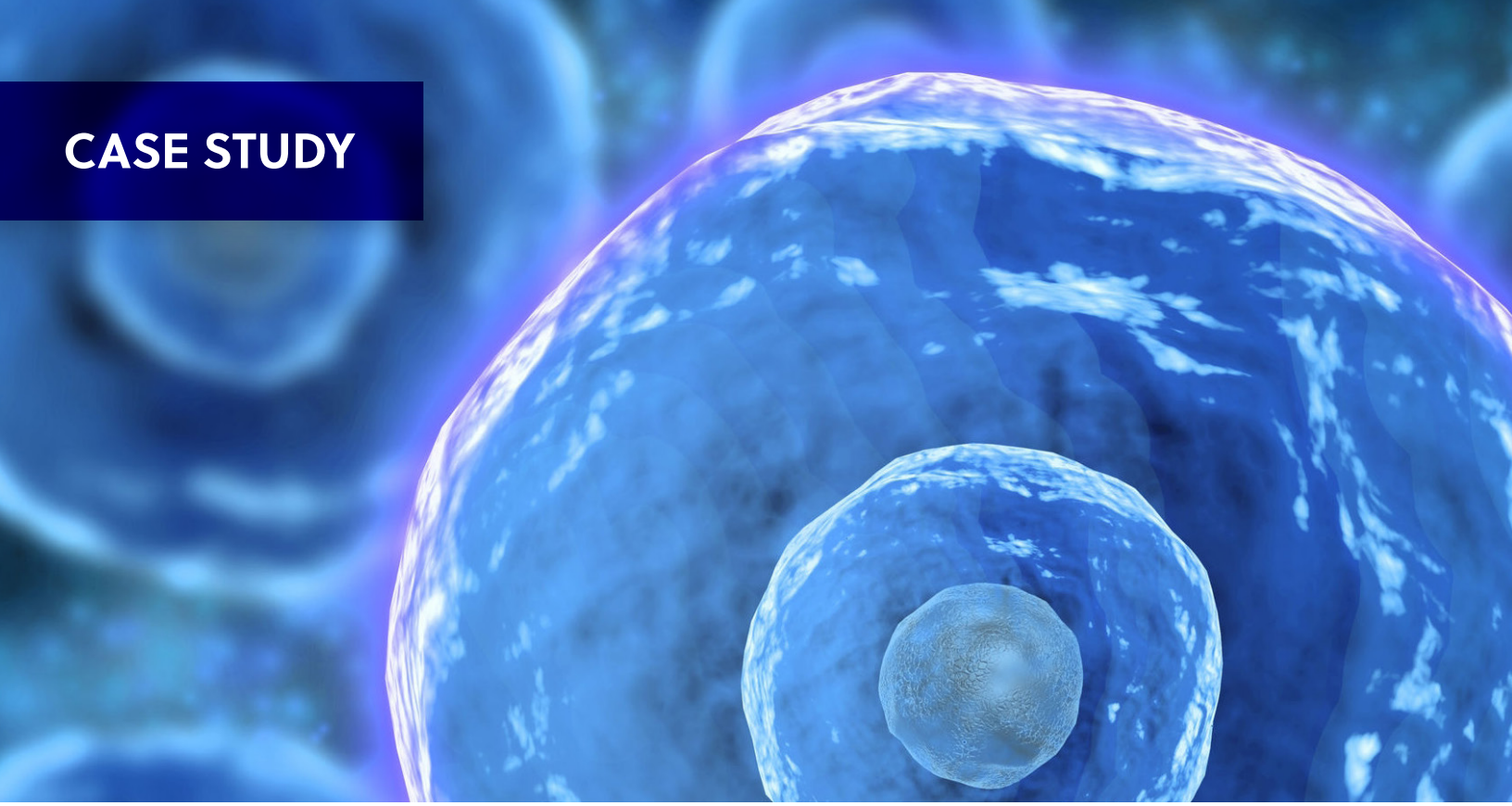

CASE STUDY

The evolution of antibody-based treatments for infectious disease

From convalescent plasma to single cell immune sequencing

INTRODUCTION

Long before the individual components of the adaptive immune system were characterized, doctors vaguely understood that the ability to fight off infection can be transferred between patients. This process, which began with transferring convalescent plasma from recovered patients to recently infected patients in the early 1900s, eventually evolved into modern antibody-based therapies. Now, a new technology, known as single cell immune sequencing, is being used to shortcut this process such that neutralizing antibodies can be rapidly characterized and synthesized.

CONVALESCENT PLASMA: AN EFFECTIVE BUT RISKY SHOTGUN APPROACH

The first use of serum to treat viral infections dates back to 1901, when a Nobel Prize was awarded for the use of animal-derived serum against diptheria[1]. Since then, this approach has been used to address numerous infectious disease outbreaks, including

Spanish Flu[2], H5N1[3], H1N1[4], and Ebola[5], despite the fact that the reason for treatment efficacy—neutralizing antibodies—would not be fully characterized until the 1950s.

Although these so-called convalescent plasma therapies can be effective—they are credited with reducing Spanish Flu mortalities by as much as 50 percent—serum treatments are not without risk. Importantly, serum treatments consist of polyclonal antibodies, which may not target a single epitope, and are prone to inducing anaphylactic reactions[1]. Furthermore, there is also a risk that Antibody Dependent Enhancement (ADE) can occur. With ADE, if the donor sera is not neutralizing, the virus can use the non-neutralizing antibodies to hitch a ride into cells, thereby worsening the infection. There has thus been substantial interest over the last 50 years in developing safer, more reliable antibody-based treatments.

THE TRANSITION TO ANTIBODY ISOLATION AND IMMUNE RECEPTOR SEQUENCING

In the late 1970's methods for isolating monoclonal antibodies (mAB) from immunized mice and then humans began to be described[6]. Typical techniques involved culturing B lymphocytes, initially producing a mixture of polyclonal antibodies, and then diluting and screening until a monoclonal population against the antigen of interest was identified[7].

The ability to sort B cells according to their antigen-affinity and cloning and heterologous expression of heavy and light chains reduced the time required to characterize neutralizing antibodies from years to weeks[1]. Antigen-specific populations of B cells could then be sequenced in order to identify the genetic blueprint of the expressed B cell receptor (BCR) genes. As a result, a 2018 review reported that while only one clinical antiviral antibody (targeting RSV) is commercially available, 26 more are in development[8].

MOVING TOWARDS A MORE TARGETED APPROACH WITH SINGLE-CELL SEQUENCING

Despite significant advances since the early days of serum therapy, several technical challenges remain that have limited the effectiveness of antibody-based treatments for infectious disease[8], including:

1. Loss of rare clones during B cell library generation: preparing a BCR library for sequencing requires amplification, which is biased towards more abundant nucleic acids. Rare clonotypes are easily lost during bulk BCR sequencing unless techniques catered towards rare clonotype discovery are used.
2. The loss of cognate pairing between heavy and light chains that occurs during bulk BCR sequencing: when DNA/RNA is extracted from bulk samples of B cells, it becomes unclear which heavy chains and which light chains went together to produce the target antibody.

By addressing these challenges, single cell immune sequencing has the potential to further reduce timelines for mAB production from weeks to days. In single cell immune sequencing, sorted cells are plated into individual wells, and sequenced. Single cell sequencing has four main advantages when it comes to identifying mAbs. First, each individual cell is isolated, so each sample is inherently monoclonal. Second, the cognate pairing of heavy and light chains is maintained. Third, the nucleic acids from each cell occupy their own amplification reaction during library preparation, so rare clonotypes are maintained in the

final library. Fourth, if desired, specific-B cells can be co-stained with antigen and sorted from a complex mixture of cells, allowing a direct route from primary cells to responder variable regions.

CASE STUDIES IN MALARIA AND COVID-19

Recently, a single cell sequencing approach was used to identify the antibodies that are responsible for preventing malaria transmission in vaccinated patients. Researchers at the National Institute of Allergy and Infectious Disease (NIAID) teamed up with iRepertoire to sequence individual B cells from patients that had been treated with a transmission blocking vaccine[9]. The study took advantage of iPair, the first commercial single cell sequencing technology, which is available as a service from iRepertoire. Using iPair, they identified B cell clones that appeared more than once in a patient sample indicating that the immune system was likely selecting these clones. They generated 10 full length, fully human antibodies from the data and identified one potent neutralizing antibody from these expanded clones. This antibody was further characterized and is likely broadly neutralizing based upon its interaction with a conserved epitope.

The NIAID team's malaria study demonstrated that iPair can be used to pull out antigen-specific antibodies from patient populations. Shortly after this work was completed, iRepertoire went to work applying a similar approach to COVID-19. Within two days of receiving the first COVID-19 positive sample, researchers at iRepertoire identified B cell populations that were expanding in response to the virus. Scientists at iRepertoire and collaborators are now working to determine if the antibody is neutralizing and can be produced at scale.

CONCLUSION

For over 100 years, physicians and scientists have been scrambling to respond to pandemics. Antibodies produced in the immune system of survivors are still the most potent protectant against waves of infectious disease. With the advent of single cell sequencing, we now have a technology that can enable the detection of antibodies against infection in a matter of days. Single cell sequencing has only been applied to infectious disease research in a small number of cases to date, leaving a wealth of opportunities for expanding our understanding of the adaptive immune system's response to infectious disease and exploring new options for antibody-based treatments.

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