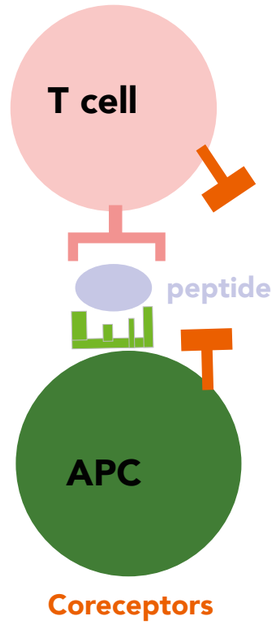
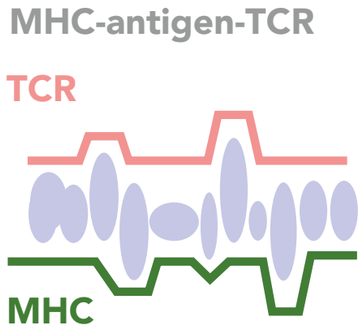
Unveiling the human immune response

Dr Clemencia Pinilla is a pioneer in the field of combinatorial chemistry, which involves the chemical synthesis of up to millions of compounds in a single process to produce libraries of compounds. For the past two decades Dr Pinilla and her team have been leading the way in developing and utilising positional scanning combinatorial libraries for T-lymphocyte antigen discovery. Her research has had a ground-breaking impact on our understanding of the human immune response in a wide array of diseases including infections, autoimmune disorders and cancer, with far reaching implications for medical progress.

At the Torrey Pines Institute for Molecular Studies (TPIMS), California, Dr Clemencia Pinilla is an Associate Member leading research that investigates antigens recognised by T lymphocytes (T cells). T cells play crucial roles in both pathological and physiological immune responses. As well as defending against infectious organisms, T cells respond against cancerous cells and are involved in autoimmune responses. Therefore, the identification of the specific antigens that T cells recognise during an immune response is of vital importance for gaining insight into a vast range of human diseases, as well as for the development of effective vaccines.

T cells recognise antigens on antigen presenting cells (APC) by interaction with the T-cell receptor. The antigens are short peptides; the APC processes proteins into fragments to create the peptides, which are then presented on the APC surface. It is these antigens and the associated epitopes (the part of the antigen recognised by the T cell) that Dr Pinilla's research aims to identify.





TCR:
- Recombinant proteins
- Overlapping peptides
- Positional Scanning Libraries
- Immunogenic

MHC:
- Prediction and binding
- Elution
- Immunogenicity needs to be determined

DEVELOPING THE TOOLKIT

Dr Pinilla's research in the field of immunology began with her investigating the potential of positional scanning libraries for the study of T-cell specificity and devising the concept for the use of the system for this application. These compound libraries are comprised of up to trillions of peptides and allow unbiased identification of T-cell antigens that stimulate the T-cell clones analysed. She cites the importance of collaboration to her work and, in particular, the contributions of Drs Roland Martin and Mireia Sospedra from Universitatsspital Zurich.

Following successful early studies demonstrating that the method is highly effective in identifying antigens recognised by T cells, Dr Pinilla and her team went on to work on further expanding the method's capacity for antigen discovery by combining the technique with biometrical analysis. This brought together the results obtained using positional scanning libraries to identify T-cell antigens from protein sequence databases. The techniques have proved to be extremely powerful when combined. Positional scanning-based biometrical analysis can systematically integrate the results of the positional scanning libraries composed of trillions of peptides with protein databases and predict and identify with high accuracy peptides and their corresponding antigens recognised by disease relevant T cells. Through this unique and innovative approach, both native and cross-reactive sequences for the T cells can be elucidated, for clones of both known and unknown specificities. This is important because specific T-cell responses for many diseases, ranging from infections to cancer, remain largely unknown due to a lack of identified antigens.

A NEW ERA FOR VACCINE DISCOVERY

Historically, vaccines typically consisted of mixtures of attenuated or inactivated causative agents. In recent decades, molecular techniques have enabled increasingly refined vaccine design by using immunogenic (causing a response from the immune system) protein antigens in recombinant vaccines. These vaccines use the antigens from a protein to stimulate an immune response, instead of the causative agent itself; knowledge of antigen epitopes recognised by the immune system during disease is therefore of critical importance. The discovery of candidate antigens to inform vaccine development had been limited by the fact that approaches have relied on pre-selection of pathogen proteins or peptide antigens, followed by assessment of whether they elicit a positive T cell response. Dr Pinilla and her team have developed a novel "T-cell driven" approach to investigating antigens recognised by T cells, which they have recently refined, focusing on the immune response involved in Chagas diseases.

INVESTIGATING IMMUNE RESPONSE TO EXOGENOUS AGENTS

Chagas disease results from infection by the protozoan *Trypanosoma cruzi* and is a major health problem across the globe, resulting in a larger healthcare burden than malaria due to a lack of therapeutic and protective vaccines. However, through Dr Pinilla's research in collaboration with Dr Karina Gomez at the Institute of Genetic Engineering and Molecular Biology, INGEBI in Buenos Aires, the identification of the immunogenic antigens recognised by the human immune response to infection with the parasite is helping to open doors towards the discovery of effective Chagas vaccines.

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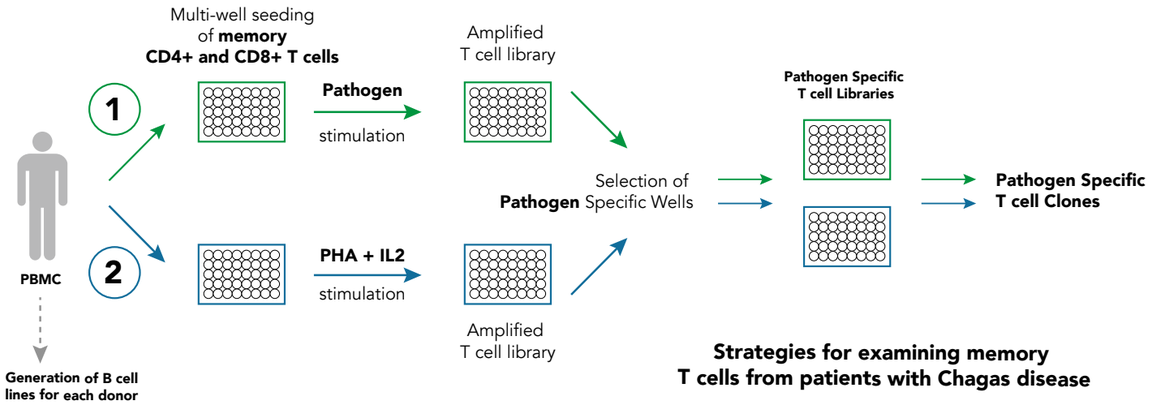

Strategies for examining memory T cells from patients with Chagas disease

Prior to her research focus on Chagas disease, Dr Pinilla worked on another virus that is used in the vaccine for smallpox, vaccinia virus (VACV). She identified vaccinia-specific T-cell antigens from immunised humans, contributing to our knowledge of the immune response to immunisation with vaccinia, which could lead to the development of new improved smallpox vaccines. Another of her earlier projects employing positional scanning-based biometrical analysis led to the identification of the previously unknown peptide specificity of CD4+ T cells that occur during Lyme disease. Dr Pinilla has also worked on projects investigating the antigen specificity of T cells involved in the human immune response to cytomegalovirus (CMV), which is responsible for more congenital birth defects than any other virus, and HIV-1.

It is not only infections that can elicit an immune response. Alongside Dr Andrew Fontenot from University of Colorado Anschutz Medical Campus, Pinilla has also worked on a project that led to the first ever discovery of an antigen involved in metal-induced hypersensitivity. Chronic beryllium disease (CBD) results from a genetic predisposition to the hyper-sensitive reaction on exposure to beryllium metal (Be). A metal-induced hyper-sensitive immune response occurs, causing an influx of CD4+ T cells specific to beryllium (Be) into the lungs. Pinilla's research found that there is an interplay between antigenic peptides and Be in the generation of the immune response that occurs in these cases.

FURTHERING OUR UNDERSTANDING OF AUTOIMMUNE DISORDERS

In addition to this Dr Pinilla has also focused her efforts on several diseases involving an immune response that are not known to result from a specific exogenous agent. Her research has discovered the epitopes recognised by T cells in numerous cancers and she has carried out extensive work unravelling the destructive immune response that occurs during multiple sclerosis (MS).

MS is an autoimmune disease and neurological condition that affects the central nervous system (CNS) resulting in damage to the coating surrounding nerve fibres, called myelin. The condition is thought to be due to a CD4+ T-cell-mediated autoimmune response, with the disease developing in genetically susceptible individuals in combination with environmental triggers. Relapses often occur following viral infections and it is suspected that viruses play a role in the disease.

Dr Pinilla, in collaboration with Drs Roland Martin and Mireia Sospedra, has investigated CD4+ T cells from the cerebrospinal fluid (CSF) of MS patients and used positional scanning-based biometrical analysis to investigate the samples. They found that several of the T cells exhibited high levels of cross reactivity with different peptides, as well as a lack of specificity for variants of APCs associated with T cell interaction. Overall, these findings amounted to a lower degree of specificity than had ever before been identified for these cells, which could account for some of the pathology of MS in patients.

Looking to the future, the team's work developing the positional scanning combinatorial libraries can be used to help create and refine vaccines against multiple diseases. In particular, Pinilla and her team are planning to generate libraries and clones of memory CD4+ T cells from patients infected with *Trypanosoma cruzi*. These can then be used to identify the antigen specificities using positional scanning libraries, which will further contribute to the design of novel vaccines against Chagas disease. With a health burden greater than that of malaria, a vaccine for this disease could have positive health benefits for millions across the globe.

Q&A

How did your background in microbiology lead to you developing combinatorial chemistry techniques for discovering antigen specificity?

In 1986, I was working in Colombia with Dr Manuel E Patarroyo in the identification of antigens recognised by antibodies from patients with malaria and tuberculosis and I had the opportunity to meet Dr Richard A Houghten, at the time an investigator at Scripps Clinic. Dr Houghten had recently developed the simultaneous multiple peptide synthesis method, and he invited me to be a postdoctoral fellow in his laboratory. I joined in 1987 to work on a project that at the time was named 64 million peptides and resulted in our *Nature* 1991 publication, in which we described the synthesis and use of a hexapeptide synthetic combinatorial library for basic research and drug discovery. In other words, since my early days in science I have been interested in the identification of the antigens that are recognised by the immune system during infectious disease. The later development of the decapeptide combinatorial libraries in 1994 and the understanding that T cells' 'unique identifier', the T-cell receptor, recognises a linear peptide led to our efforts to understand how combinatorial libraries could be used for the identification of peptides recognised by T cells. Our first collaborator was Dr Roland Martin at NINDS, NIH. He had generated a number of CD4 clones that became the model systems that we used for the development of the biometrical analysis in collaboration with Drs Richard Simon and Yindong Zhao at NCI, NIH.

What early challenges did you face in applying the positional scanning libraries technique for identifying T-cell antigens?

The early challenges remain the same. They are, firstly, to have a clear rationale and sufficient evidence to be sure that the T cell clones to be studied are relevant to the disease being investigated and secondly, to expand clonal cells in sufficient numbers (at least 30–40 million) for the screening of the positional scanning libraries and the identified peptides.

What aspects of your research have you found the most exciting in your career?

The collaborations with investigators with different scientific expertise and interest on the elucidation of T-cell receptor specificities are very rewarding and exciting to me. The elucidation of peptides that activate each of the T-cell receptors that we have studied has taught us something new. Every time that we identified individual peptides that activate a T cell as a result of the testing of mixtures of billions of peptides and the deconvolution of their activities, I am delighted at how well the process works. Finally, the increase in the efficiency of the methodologies of T-cell culture and screening and therefore the knowledge of T-cell specificity has encouraged us to continue our work that will lead to a better understanding of the immune response, the development of diagnostics, the modulation and intervention in autoimmune diseases, and the development of vaccines.

How important has team work and collaboration been to your work so far?

Team work has been essential to all our studies; I find that it is when we really try to understand a scientific problem from different points of view that we are able to develop solutions. I have worked closely with the chemists at TPIMS (Drs Richard A Houghten and Marc A Giulianotti) on devising procedures to improve the analysis and reproducibility of the synthesis of the complex mixtures that comprise the positional scanning libraries. Currently, I work with Dr Radleigh Santos, a mathematician by training, on continuing to improve the biometrical analysis and to develop analysis platforms for the analysis of immunomonitoring results of immunotherapy clinical studies. Also, I very much value all of our collaborators and their interests in T-cell specificity in different diseases. We apply what we have learned from our previous studies and continue to let the screening data and the most unbiased methodologies of deconvolution guide the peptide and antigen identification process.

Are there any other potential applications of combinatorial chemistry techniques within the field of immunology?

For any molecular interaction where a ligand is needed, TPIMS combinatorial libraries would be useful. If the interest or need is to identify naturally occurring peptides, the L-amino acid peptide positional scanning libraries in combination with the biometrical analysis would be useful tools. We think that studying the specificity of the T cell response to large pathogens using positional scanning libraries is a clear application of TPIMS combinatorial libraries, since other T-cell driven approaches would be more difficult to use. Similarly in cancer, the identification of neo antigens would be another potential application. On the other hand, if a small molecule was desired to block or activate a molecular interaction, TPIMS small molecule libraries in positional scanning format would be the libraries that I would suggest.

Detail

RESEARCH OBJECTIVES

Dr Pinilla's pioneering work in the field of positional scanning combinatorial libraries focuses on elucidating the human immune response to pathogens. The method allows for the discovery of antigens recognised by T cells which can then be used in the development of vaccines. Her work has spanned multiple diseases and disorders and she is now focused on the identification of antigens using a T cell driven approach that could contribute to the development of a vaccine for human Chagas disease.

FUNDING

NIH: NIAID

COLLABORATORS

- Jon R Appel
- Andrew Fontenot
- Richard A Houghten
- Valeria Judkowski
- Marc A Giulianotti
- Roland Martin
- Radleigh G Santos
- Mireia Sospedra

BIO

Dr Pinilla is an Associate Member at TPIMS. She is recognised as a pioneer in the field of combinatorial chemistry with over 25 years of experience, particularly in the use of positional scanning libraries for the identification of ligands for a wide range of targets. These studies have involved collaborative projects with multiple investigators at TPIMS and other institutions worldwide resulting in more than 130 peer-reviewed publications.

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