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# Antigen discovery for the identification of vaccine candidates and biomarkers using a T cell driven approach in combination with positional scanning peptide libraries

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**Abstract**

The prevention and treatment of infectious diseases is highly dependent on the availability of reliable diagnostic tests and protective or therapeutic vaccines. There also exists an urgent need to develop reliable biomarkers to monitor treatment success and to predict disease progression from asymptomatic to symptomatic disease in several disease scenarios. The elucidation of the disease-relevant antigens that elicit the protective immune responses is critical and required for the development of biomarkers, diagnostics, and vaccines. However; one of the main obstacles to the study of antigen specificity in human T cells is their low frequency in PBMC samples. To overcome this problem we have implemented strategies to generate memory T cell libraries and clones specific to the pathogen of interest. Due to the fact that memory T cells represent a repository of the human T cell response to infection, examination of their antigen specificity can efficiently reveal immunogenic and relevant antigens involved in the *in vivo* response to infection or vaccines. To examine the specificity of the memory T cells we use an unbiased collection of antigens together with an *in silico* analysis, namely positional scanning based biometrical analysis. Here we present a summary of our approach and ongoing work on the development of strategies for the culture of memory T cells from patients with Chagas disease. While most studies focus on the identification of vaccine candidates using preselected immunogenic proteins derived from animal models or by or bioinformatics prediction, here we present an innovative approach that directly examines the specificity of the memory response following infection or immunization in humans.


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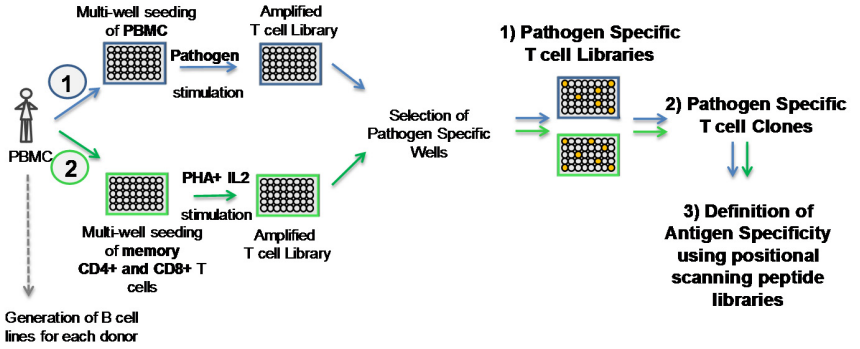
## 1. Introduction

To date, several studies aimed to identify T cell vaccine candidates have used preselected immunogenic proteins derived from experimental analysis on animal models or by bioinformatics prediction of MHC binding. While it is feasible to obtain candidate genes or proteins from bioinformatics analysis, their validation as immunogens is critical and more difficult, requiring further characterization of their immune recognition in humans. Here we describe the advantages and future required optimization steps to a "T cell driven" approach developed by our laboratory that uses human peripheral blood mononuclear cells (PBMC) from vaccinated or infected human patients to generate pathogen specific memory T cell libraries and clones to directly examine their specificity using an unbiased collection of peptides named positional scanning peptide libraries.

## 2. Results

The approach presented here consists of 3 steps: 1) the generation of T cell libraries representing the human memory response to infection or vaccination, 2) the generation of pathogen specific T cell clones and, 3) the screening of the pathogen specific T cell clones with positional scanning peptide libraries to identify *pathogen* immunogenic proteins (Figure 1).

**Figure 1. T cell driven approach.**




The 3 steps of the approach have been validated by our work and the work of others. Specifically, the generation of T cell libraries has been successfully used by Lanzavecchia's group to study the immune response to naturally processed parasite antigens from tetanus toxoid, cytomegalovirus and purified protein derivative[1] and more complex pathogens such as *S. pyogenes, S. aureus and C. albicans*[2,3]. In addition, work from our laboratory in collaboration with the Chagas Laboratory at Instituto de Investigaciones en Ingeniería Genética y Biología Molecular in Buenos Aires, Argentina, has focused on the optimization of several experimental parameters in order to successfully recover the memory response to *T. cruzi* infection in several patients at different stages of the disease (*manuscript in preparation*). In regards to steps 2 and 3, they have been extensively implemented in our laboratory. We have demonstrated the elucidation of T cell specificity using positional scanning peptide libraries in combination with *in silico* analysis with protein databases (biometrical analysis). A number of studies with clones of known specificity has been used to develop the methodology and revealed that the known antigens for most clones rank among the top 50 predicted stimulatory peptides, reviewed in[4]. Furthermore, as shown in table 1, the methodology was successfully utilized for clones of unknown specificity.

**Table 1.** Use of approach with T cell clones of unknown specificity.

| Disease/T cell clone | Generated with | Protein Databases used for Biometrical analysis | Reference |
|---|---|---|---|
| Lyme/CD4 | Borrelia lysate | Borrelia and human | 5 |
| Melanoma/CD8 | PHA | Human | 6 |
| Multiple Sclerosis/CD4 | PHA | Human and virus | 7,8 |
| Diabetes/CD4 | - | Bacteria and virus | 9 |
| Smallpox-Vaccinated donors | Vaccinia virus | Western Reserve vaccinia virus | 10,11 |
| Chronic beryllium Disease | Beryllium | Human | 12 |

One of the great advantages of this approach is that the identified parasite proteins are by definition immunogenic in humans as they are recognized by T cells expanded *in vivo* during infection or vaccination. In the particular case of infections for which vaccines are needed, the identified antigens constitute a database of confirmed immunogenic proteins and therefore can be considered as biomarkers or vaccine candidates. The approach is solely based on the integration of the screening results of an unbiased source of peptides, namely a positional scanning library, with the protein database of the pathogen of interest. The screening of the T cell libraries has been successfully carried out using autologous PBMCs or B-cell lines[13]. The approach neither uses MHC binding prediction algorithms based on HLA haplotype nor requires the *a priori* knowledge of the MHC restriction of the T cell clones to be studied. Furthermore, the information related to the HLA of the target population or the HLA associated to the immune response to the pathogen of interest are not always available. Additionally, being unbiased with respect to the HLA is also advantageous since it is clearly possible that a novel immunoprevalent identified protein binds to an HLA type that was previously not known to be associated to the immune response to the pathogen. Thus, it is possible that by utilizing the presented approach novel proteins and HLA binding associations can be established. Furthermore, in regards to T cell driven vs. MHC driven approaches we have recently published a statistical analysis comparing the efficiency of both methodologies on the identification of immunoprevalent vaccinia antigens. The analysis showed that in general T cell driven approaches are more effective than MHC approaches in identifying predominantly recognized proteins[11].

Another key feature of the presented approach is that depending on what question is to be addressed, the source of human/PBMC samples for the generation of T cell libraries and clones, can be adapted to answer accordingly. For example, in the case of our work with vaccinia virus, the goal of the study was to understand which key proteins were involved in the immune response to vaccinia upon smallpox vaccination with the Dryvax vaccine in humans. This was particularly important in the context of determining if other vaccine candidates such as Modified Vaccinia Ankara would elicit comparable responses to Dryvax[14]. Therefore, in order to help answering this question, T cell clones specific to vaccinia were derived from Dryvax immunized subjects and screened with positional scanning peptide libraries to reveal their specificity[10].

A more complex example is represented with our current collaborative work on Chagas disease. The protozoan parasite *T. cruzi* is the causative agent of Chagas, and is a prominent human health problem in Central and South America. The incidence of *T. cruzi* infection has been reduced by international efforts to control the vector and to improve the safety of blood transfusions. However, it is estimated that up to 10 million people worldwide have chronic infection that may lead to the development of severe Chagas disease, and of those 50,000 will die each year due to different complications caused by the infection. The acute phase of *T. cruzi* infection is short-lived and in some cases accompanied by mild illness. However, on the great majority of the individuals the parasite will persist for 10-30 years as an asymptomatic infection (chronic asymptomatic patients) before it manifests as serious cardiac and/or digestive complications in 20-40% of cases (clinical patients)[15]. Despite its enormous impact on public health, control of *T. cruzi* infection is hampered by the absence of a protective vaccine. Thus, the objective of our work is to identify

immunogenic *T. cruzi* antigens. To do this, our approach directly focuses on generating T cell libraries and clones from chronic asymptomatic patients. The advantage of focusing on this population relies on the fact that most likely these patients have been able to control the infection and therefore, likely to recognize parasite antigens involved in protection. It is also important to remark that in the Chagas field, shortlisting the antigens capable of inducing immunity against *T. cruzi* infection has been very difficult in part due to the fact that *T. cruzi* encodes approximately 10,000 proteins. In this regards, another advantage of the present methodology is that it does not require *a priori* selection of parasite antigens and thus it can be used to identify immunogenic proteins from large pathogens for which another methodologies would be more limiting.

There are some steps on the methodology that could be optimized in order to improve throughput. For example, the cloning of pathogen specific T cells by limiting dilution remains a very time consuming task and therefore decreases the throughput of the approach. In this regards, a recent work by Neller et al.[16] showed the generation of human effector T cell clones by combining an optimized IFN-γ capture assay with the sorting of single effector T cells directly into multi-well plates using a flow cytometer equipped with a single cell deposition unit. This rapid and highly efficient method for cloning effector CD4+ and CD8+ T cells *ex vivo* enables the selection of specific T cell clones without the need for multiple initial rounds of *in vitro* stimulation and cell division. We plan to evaluate the cloning strategy recently presented by Neller et al. for the cloning step included in our approach. This could improve the efficiency on the cloning step and thus on the number of pathogen antigens identified per subject.

Another limiting step of our approach is that clonal cells need to be grown to large numbers (15-30 millions) in order to be screened with the positional scanning peptide libraries. We believe that further efforts on the development of miniature T cell activation assays will be extremely beneficial to reduce the number of clonal T cells required to test combinatorial peptide libraries and to be able to define the spectrum of specificities with higher throughput. Steps towards achieving this goal have already been initiated. For example, we have demonstrated that significant lower antigen concentrations are required for substantial production of GM-CSF by T cells as compared to other cytokines. This makes the detection of GM-CSF a very sensitive readout and may allow decreasing the number of cells needed for the screening. Although this has already been demonstrated for vaccinia virus[10], experiments using *T. cruzi* specific T cells also showed similar results[17].

## 3. Conclusions

Recognized and neglected infectious diseases call for innovative and efficient approaches to identify the candidate pathogen antigens that can be considered for vaccine design and development. In this regards, it is important to highlight that in infectious diseases, such as Chagas disease, in which the causing pathogen encodes for a large number of proteins and the infection causes a chronic asymptomatic state of the disease in a proportion of the patients, the presented approach is expected to be a good tool capable of combining correlates of protection with a methodology that does not need any *a priori* knowledge of candidate immunogenic proteins. By focusing on memory T cell libraries from samples derived from patient populations that have been able to control the infection for a large period of time, it can be hypothesized that the immunoprevalent proteins derived from this study could be involved in the protective immune response to the pathogen and thus will constitute a defined database of vaccine candidates to accelerate the design of a novel preventive and/or therapeutic Chagas vaccine.

## 4. References

1. Geiger, R., T. Duhen, A. Lanzavecchia, and F. Sallusto. Human naive and memory CD4+ T cell repertoires specific for naturally processed antigens analyzed using libraries of amplified T cells. *J Exp. Med* 2009;**206**:1525-1534.
2. Zielinski, C. E., D. Corti, F. Mele, D. Pinto, A. Lanzavecchia, and F. Sallusto. Dissecting the human immunologic memory for pathogens. *Immunol Rev* 2011;**240:**40-51.

3. Corti, D., F. Sallusto, and A. Lanzavecchia. High throughput cellular screens to interrogate the human T and B cell repertoires. *Curr Opin Immunol* 2011;**23:**430-435.
4. Nino-Vasquez, J. J., G. Allicotti, E. Borras, D. B. Wilson, D. Valmori, R. Simon, R. Martin, and C. Pinilla. A powerful combination: the use of positional scanning libraries and biometrical analysis to identify cross-reactive T cell epitopes. *Mol Immunol* 2004;**40:**1063-1074.
5. Hemmer, B., B. Gran, Y. Zhao, A. Marques, J. Pascal, A. Tzou, T. Kondo, I. Cortese, B. Bielekova, S. Straus, H. F. McFarland, R. A. Houghten, R. Simon, C. Pinilla, and R. Martin. Identification of candidate T cell epitopes and molecular mimics in chronic Lyme Disease. *Nature Med.* 1999;**5:**1375-1382.
6. Rubio-Godoy, V., M. Ayyoub, V. Dutoit, C. Servis, A. Schink, D. Rimoldi, P. Romero, J.-C. Cerottini, R. Simon, Y. Zhao, R. A. Houghten, C. Pinilla, and D. Valmori. Combinatorial peptide library based identification of peptide ligands for tumor-reactive cytolytic T lymphocytes of unknown specificity. *Eur. J. Immunol.* 2002;**32:**2292-2299.
7. Sospedra, M., Y. Zhao, H. zur Hausen, P. A. Muraro, C. Hamashin, E. M. de Villiers, C. Pinilla, and R. Martin. Recognition of conserved amino acid motifs of common viruses and its role in autoimmunity. *PLoS. Pathog.* 2005;**1:**e41.
8. Sospedra, M., P. A. Muraro, I. Stefanova, Y. Zhao, K. Chung, Y. Li, M. A. Giulianotti, R. Simon, R. A. Mariuzza, C. Pinilla, and R. Martin. Redundancy in antigen-presenting function of the HLA-DR and -DQ molecules in the multiple sclerosis-associated HLA-DR2 haplotype. *J Immunol* 2006;**176:**1951-1961.
9. Judkowski, V. A., G. M. Allicotti, N. Sarvetnick, and C. Pinilla. Peptides from common viral and bacterial pathogens can efficiently activate diabetogenic T-cells. *Diabetes* 2004; **53:**2301-2309.
10. Judkowski, V., A. Bunying, F. Ge, J. R. Appel, K. Law, A. Sharma, C. Raja-Gabaglia, P. Norori, R. G. Santos, M. A. Giulianotti, M. K. Slifka, D. C. Douek, B. S. Graham, and C. Pinilla. GM-CSF Production Allows the Identification of Immunoprevalent Antigens Recognized by Human CD4+ T Cells Following Smallpox Vaccination. *PLoS. ONE.*2011*;* 6:e24091.
11. Judkowski, V. A., R. G. Santos, A. Bunying, M. A. Giulianotti, J. R. Appel, and C. Pinilla. Novel T cell driven approach leads to the identification of immunoprevalent antigens. *Procedia in Vaccinology* 2012;**6:**14-20.
12. Falta, M. T., C. Pinilla, D. G. Mack, A. N. Tinega, F. Crawford, M. Giulianotti, R. Santos, G. M. Clayton, Y. Wang, X. Zhang, L. A. Maier, P. Marrack, J. W. Kappler, and A. P. Fontenot. Identification of beryllium-dependent peptides recognized by CD4+ T cells in chronic beryllium disease. *J Exp. Med* 2013;**210:**1403-1418.
13. Pinilla, C., J. R. Appel, V. Judkowski, and R. A. Houghten. Identification of B cell and T cell epitopes using synthetic peptide combinatorial libraries. *Curr Protoc. Immunol* 2012;Chapter 9:Unit9.
14. Parrino, J., L. H. McCurdy, B. D. Larkin, I. J. Gordon, S. E. Rucker, M. E. Enama, R. A. Koup, M. Roederer, R. T. Bailer, Z. Moodie, L. Gu, L. Yan, and B. S. Graham. Safety, immunogenicity and efficacy of modified vaccinia Ankara (MVA) against Dryvax challenge in vaccinia-naive and vaccinia-immune individuals. *Vaccine* 2007;**25:**1513-1525.
15. Rassi, A., Jr., A. Rassi, and J. A. Marin-Neto. Chagas disease. *Lancet* 2010;**375:**1388-1402.
16. Neller, M. A., M. H. Lai, C. M. Lanagan, O. C. LE, A. L. Pritchard, N. R. Martinez, and C. W. Schmidt. High efficiency ex vivo cloning of antigen-specific human effector T cells. *PLoS One.2014;* **9**:e110741.
17. Longhi, S. A., A. Atienza, P. G. Perez, A. Buying, V. Balouz, C. A. Buscaglia, R. Santos, L. M. Tasso, R. Bonato, P. Chiale, C. Pinilla, V. A. Judkowski, and K. A. Gomez. Cytokine production but lack of proliferation in peripheral blood mononuclear cells from chronic Chagas' disease cardiomyopathy patients in response to T. cruzi ribosomal P proteins. *PLoS Negl. Trop. Dis* 2014; **8:**e2906.