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Leonard Moise

University of Rhode Island, lmoise@uri.edu

Steven F. Moss

Anne S. De Groot

University of Rhode Island, annied@uri.edu

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Invited editorial for “Expert Review of Vaccines”

Moving *H. pylori* Vaccine Development Forward with Bioinformatics and Immunomics

Leonard Moise^{1,2*}, Steven F. Moss³ and Anne S. De Groot^{1,2}

¹ EpiVax, Inc., Providence, Rhode Island, USA

² Institute for Immunology and Informatics, University of Rhode Island, Providence, Rhode Island, USA

³ Department of Medicine, Division of Gastroenterology, Rhode Island Hospital & Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

***Correspondence to:** Leonard Moise, Ph.D., Institute for Immunology and Informatics, University of Rhode Island, 80 Washington Street, Providence, RI 02903. Tel: (401) 277-5245; Fax: (401) 277-5154. Email: lmoise@mail.uri.edu

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The problem

Helicobacter pylori infects the gastric mucosa of half the human population, leading to chronic gastric inflammation in all and clinically important adverse outcomes in a sizable minority. While most infections with this gram-negative bacterium are not associated with symptoms, gastric or duodenal ulcers ultimately develop in approximately 10% of colonized individuals, with gastric adenocarcinoma or mucosal-associated lymphoid tissue lymphoma occurring in about 1%, decades after the initial infection. *H. pylori* infection is unevenly distributed, being most prevalent in resource-poor countries (in the range of 70-90%) and in as few as 10% or less of some Western populations [1]. Excitement about developing a vaccine based on the genome of *H. pylori* abated soon after the sequence was published, when effective antibiotic therapy was discovered. Unfortunately, the multidrug regimens that are required to eradicate infection currently remain out of reach for most of the affected patients, they do not prevent re-infection, and antibiotic-resistant strains are increasingly common [2].

The recent emergence of resistance to antimicrobial therapy has stirred new interest in the development of an anti-*H. pylori* vaccine [3], but the complex immunopathogenesis of *H. pylori* has frustrated successful vaccine development. *H. pylori* elicits an intense inflammatory response to infection, involving the recruitment of multiple innate and adaptive immune cell populations to the stomach. However, persistent infection for the lifetime of the host usually follows, demonstrating that this bacterium successfully evades natural host defenses. Most evidence points to an initial pro-inflammatory response mounted by type 1 T helper CD4⁺ cells (Th1) producing interferon- γ that is eventually dampened by CD4⁺CD25^{high} regulatory T cells (Treg) expressing FoxP3 [4]. As a result, the pathogen is not eradicated, and persistent infection develops, contributing to the development of chronic gastritis. A successful vaccine, be it prophylactic or therapeutic, must be able to tip the immune system balance in favor of an immune Th1 response that will overcome the immunosuppressive properties of Tregs naturally

induced by *H. pylori*. Neither whole inactivated *H. pylori*, bacterial vectored vaccines expressing *H. pylori* proteins, nor recombinant subunit vaccines have demonstrated protective immunity in clinical trials despite their recognition by humoral and cell-mediated arms of the immune system. The time is ripe for a novel approach to vaccine design that overcomes the limitations of these conventional approaches.

A promising solution

Advances in the fields of genomics and computation have led to the emergence of a new approach to vaccine development, better known as reverse vaccinology or genome-to-vaccine design. The field is only about 10 years old, yet the first reverse vaccinology product, Novartis' Bexsero, a vaccine against meningococcus B, is about to be licensed in Europe. One of the major hurdles facing reverse vaccinologists is choosing the best candidate immunogens from the wealth of targets provided by whole-genome sequencing. The number of antigens comprised by bacterial pathogens can be quite large. The genetic diversity of hypermutating viruses with much smaller genomes can also pose a significant problem. Moreover, the genomic landscape is amplified by diversity within a single species [5].

Bioinformatics tools developed to harness the emerging genomic sequence "haystack" can be used to identify potentially immunogenic proteins by their predicted subcellular localization (e.g. membrane proteins or secreted proteins) or homology to virulence factors of related pathogens. Together with proteomics data describing antigen expression abundance and kinetics, these tools narrow down 'antigen-space' considerably but they themselves do not provide *immunological* direction to vaccine antigen selection. Perhaps the most utilitarian tool, in this regard, is the T-cell epitope predictor. A T-cell epitope peptide derived from an invading pathogen interfaces with a major histocompatibility complex (MHC) molecule at an antigen presentation cell surface and a T cell receptor at the T cell surface. This interaction is essential

for activation of protective adaptive immune responses. CD8⁺ and CD4⁺ T cells play important roles in containing infections and protection after re-exposure. CD4⁺ T cell responses are critical for robust CD8⁺ T cell proliferation and function and for their differentiation into memory cells [6,7]. Moreover, CD4⁺ T cell responses help B cells to produce antibodies that are the mediators of protection in all currently approved human vaccines [8]. Thus, a T-cell epitope predictor may identify pathogen-derived sequences that are good vaccine antigens and/or identify key T cell epitopes that are important adjuncts for effective humoral immune response. Furthermore, as cognate T cell help improves the quality and kinetics of antibody responses, T-cell epitope predictors, when used to identify proteins that have the greatest number of T cell epitopes, may be particularly useful for fishing out good candidates for whole antigen vaccines.

We have developed a genome-to-vaccine strategy, with a central role for T-cell epitope predictors, that identifies immunogenic and protective T-cell epitopes. While it can be specifically tailored to a microbe's particular immunopathogenic profile, this approach can be generally applied to any pathogen, according to the following steps: 1) Genomes are computationally mined to identify genes that encode proteins with promising antigenic properties such as secretion, up-regulated expression, reported immunogenicity and virulence [9,10]. Alternatively, with no experimental bias introduced, complete open reading frame datasets can be analyzed. 2) Immunoinformatics tools are then used to discover sequences likely to bind to MHC proteins for presentation to T cells. To cover a large swath of MHC polymorphisms, 'supertype' alleles are screened. For personalized vaccine design, the particular MHC type of an individual would be considered. Depending on the correlate of protective immunity that is desired, proteins can be triaged by epitope content (those with more T cell epitopes may be better immunogens), or epitopes can be selected and used as the vaccine "payload." 3) Where epitopes are being used to vaccinate, the next step is to synthesize selected sequences as peptides and evaluate for MHC binding and antigenicity in infection survivors or vaccinees. 4)

Finally, prototype vaccines are designed, constructed and evaluated for immunogenicity and efficacy in humanized mice [11]. If epitope-driven vaccines are the objective, the resulting epitopes may be formulated as multiple peptides, as concatamers in the form of DNA or delivered as multi-epitope protein vaccines. Clearly, this strategy strips a pathogen down to the minimal essential sequence information needed to generate immunity, thereby removing the pathogen's inherent adjuvant and delivery properties that make it naturally immunogenic. Building these features back into the final product is necessary, of course, but a key advantage of epitope-based vaccine design is that it allows for the subsequent selection of best-in-class adjuvant and delivery technologies.

We have applied this genomes-to-vaccine approach to develop an *H. pylori* therapeutic vaccine that was evaluated in the p27 knockout mouse model of gastric cancer induced by chronic *H. pylori* infection [12,13]. The *H. pylori* J99 and 26695 genomes were screened for CD4⁺ T-cell epitopes that bind both human and mouse MHC, using the predictor algorithm EpiMatrix. To identify epitopes with potential to recognize multiple MHC types for broad coverage of the MHC-diverse human population, we used the ClustiMer algorithm, which finds regions of a protein with high epitope density. Epitope cluster predictions were experimentally validated for MHC binding and T cell reactivity in p27 knockout mice infected with the mouse-adapted *H. pylori* strain SS1. Immunoreactive epitopes that bound MHC and that were also present in strain SS1 were assembled into a multi-epitope vaccine in DNA and peptide formats using a heterologous prime-boost immunization strategy. The multi-epitope vaccine administered intranasally to SS1-infected mice induced a broad immune response as determined by interferon- γ production in ELISpot assays; much broader, in fact (when examining immune responses to the epitopes), than the response elicited by a whole bacterial lysate which would have included the proteins from which the epitopes were derived. This result was paralleled in *H. pylori* burden in mice immunized intranasally, which exhibited a significant reduction compared with mice vaccinated

with whole bacterial lysate. Histopathological indices of inflammation and gastric cellular damage were low at the distal timepoint that was evaluated, and not significantly different among the experimental groups.

Emerging issues in epitope selection

We have long considered T-cell epitopes that are conserved between self and infectious pathogens to potentially drive cross-reactive T cells toward immune disequilibrium under the inflammatory conditions of vaccination. These sequences have therefore been excluded from our vaccines. Our current thinking about T-cell epitope selection is evolving as new data demonstrate that the human microbiome actively shapes immune responses. Post-thymic educated regulatory T cells play a critical role in orchestrating this interaction via antigen presentation [14], and thus we believe careful consideration must be given to T-cell epitope sequences conserved between commensals and infectious pathogens, much in the same way that we do for self. As the gut microbiome is estimated to contain approximately 33 million genes, the amount of sequence data to sort through to identify the molecular specificities of these T cells is staggering. We are now beginning to design a new class of computational tools and data analysis methods to handle this enormous task.

Conclusion

The utility of the genome-to-vaccine approach for developing vaccines for pathogens has been emerging from our work and others [15,16,17,18]. The convergence of genomics, bioinformatics and immunology has expanded our vaccine design toolkit and the results generated thus far suggest that there is new hope for tackling the many pathogens against which we currently lack effective vaccines. Proof-of-concept studies for a T-cell epitope-driven *H. pylori* vaccine demonstrate this potential, and we expect that fine-tuning the epitope selection criteria will help epitope-based vaccines take their place amongst conventional vaccines that have already had

so great an impact on human health.

Financial & competing interests disclosure

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