Protein design to new vaccines development
Where is the limit?

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Introduction

Abstract. There is no broadly effective vaccine available for *Neisseria meningitidis* serotype B (MenB) since the capsular polysaccharide, on which vaccines against the other pathogenic serotypes are based, is structurally identical to human fetal brain-cell adhesion molecules. Thus, immune tolerance renders the MenB capsule poorly immunogenic, and the fear of generating autoimmunity hampers its use in a vaccine[1].

State of the art. The current efforts are focused on surface-exposed protein antigens and a new strategy called Reverse Vaccinology (RV) has allowed the identification of novel candidates. The present review hypothesizes that the optimization of those candidates by protein engineering may lead to the first universal vaccine against meningitis B[2].

Results

The main approach is a multicomponent vaccine (5CMV) which includes 5 protein antigens identified by RV and selected based on bactericidal assays *(Table1)*. Though, as none of them elicited an antibody response strong enough when individually tested, they were formulated as three recombinant proteins with the aim of inducing better and broader protection: GNA1870 was fused with GNA2091, and GNA2132 was fused with GNA1030, using a 20 Glycines linker in both cases *(fig1A)*. GNA1994 was included alone in the vaccine, as it's an homotrimer that loses the quaternary structure when combined with others, and its membrane-anchor domain was deleted (Δ351-403) *(fig1B)*. An Outer Membrane Vesicle (OMV) of the bacterium was also added in the vaccine, after demonstrating a 10 fold increase in the titer of antibodies elicited in its presence (*fig1C*). 5CMV is currently developed by Novartis, and has overcome Phase III clinical trials with success[3,4].

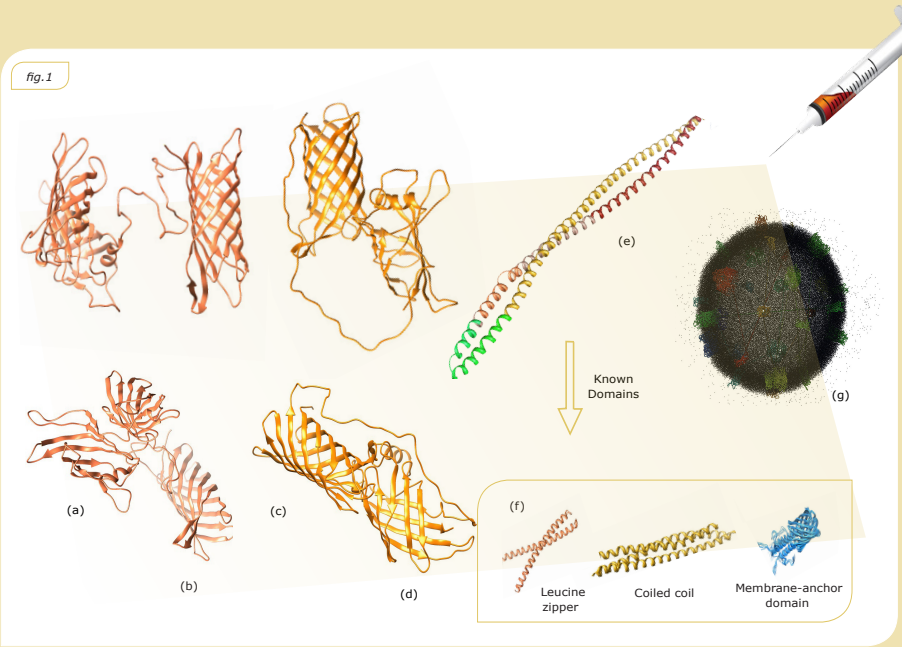

table1. Characteristics of the five antigens included in 5CMV, which were called as GNA (Genome Neisseria Antigen) plus a digit.

	Antigen names	Strengths and weakness
(a)	Neisseria surface protein (NspA o GNA2091)	Highly conserved, but not immunogenic in the native form because of poor epitope exposition
(b)	Factor H binding protein (fHbp o GNA1870)	Highly immunogenic but extremely variable, without crossed protection
(c)	Neisseria Heparin Binding Antigen (NHBA o GNA2132)	Only elicits protective antibodies when high expressed and in cooperation with antibodies induced by fHbp
(d)	Putative periplasmic protein (GNA1030)	The immunogenicity source is unknown, but induces bactericidal antibodies in combination with the rest
(e)	Neisseria adhesin A (NadA o GNA1994)	Highly immunogenic, but only present in 50-75% of the isolated strains

fig1. A possible structure of the components included in the 5CMV was proposed, as it is not present in any database. It required a previous homology modeling of GNA1994 and GNA1030, due to the fact that their structures have not been solved yet. Both GNA1030 model and the fusions shown from two different sights (a-d) were built using the Modeller program[6], and a possible structure of a GNA1994 monomer was obtained from iTasser server[7](e), as any good template for Modeller program was found. The known domains of GNA1994 and an example of OMV[8] are shown (f,g). The quality of the images was improved using informatics[8].

Discussion

- 5CMV was formulated by combining RV and protein engineering *(fig2)*.
- The same procedure could be applicable to other vaccines with similar limitations: high variability and low distribution of the antigens.
- The limits of the strategy are still unknown, but it opens "The Structural Vaccinology Era", where the eradication of diseases that have challenged the scientific community for years could cease to be a utopia[5].

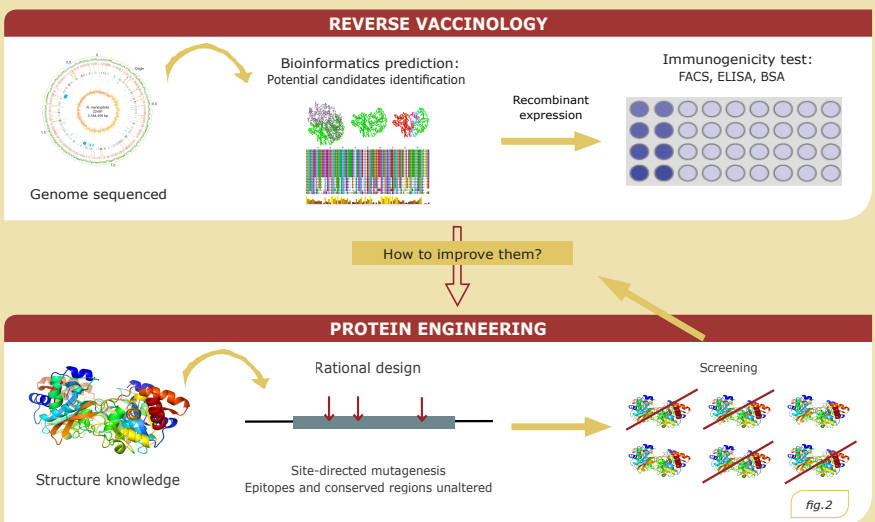

fig2. Suggested strategy for novel vaccines design if the pathogen genome and the antigenic structures are known. (A) RV is a computer-based procedure which, starting from the meningococcal genome, identifies novel antigens following criteria of (1) minimum variability, (2) surface exposed localization and (3) broadly distribution between the population[4]. (B) Rational protein designs based on the structure are able to optimize the identified antigens.

References. **(1)** *Tan, Carlone, and Borrow 2010;* **(2)** *M Pizza, V Scarlato, V Masignani, M M Giuliani et al. 2000;* **(3)** *Giuliani et al. 2006;* **(4)** *Gorringe and Pajon 2012;* **(5)** *Dormitzer, Grandi, and Rappuoli 2012; (6) N. Eswar, M. A. Marti-Renom, B. Webb, M. S. Madhusudhan, D. Eramian, M. Shen, U. Pieper, A. Sali. Comparative Protein Structure Modeling With MODELLER. Current Protocols in Bioinformatics, John Wiley & Sons, Inc., Supplement 15, 5.6.1-5.6.30, 2006; (7) http://zhanglab.ccmb.med.umich.edu/I-TASSER/ (8) Courtesy from Dr. Khalid, S. from University of Southampton (9) Chimera progam*