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Highlight

Struggling to get a universal meningococcal vaccine and novel uses for bacterial toxins in cancer treatment

Craig Daniels,[1] Tino Krell,[2] Carmen Michán[3] and Juan L. Ramos[2]*

[1]*Structural Proteomics in Toronto, UHN and University of Toronto, Banting and Best Department of Medical Research; C.H. Best Institute 112 College Street, M5G 1L6, Toronto, Ontario, Canada.*

[2]*Consejo Superior de Investigaciones Científicas, Estación Experimental del Zaidín, Dept. of Environmental Protection, C/ Prof. Albareda, 1, E-18008 Granada, Spain.*

[3]*Universidad de Córdoba, Campus de Rabanales, Dept. of Biochemistry and Molecular Biology, Edificio Severo Ochoa C-6, 2ª Planta, 14071, Córdoba, Spain.*

In the Early View section of *Microbial Biotechnology* online Engedal and colleagues (2010) provide an extensive review of the Shiga toxin and its use in targeted cancer therapy and imaging. Shiga toxins are produced by *Shigella dysenteriae* and some strains of *Escherichia coli*. The toxins are composed of two non-covalently linked, modular parts: the A moiety (StxA) which contains the enzymatically active A1 fragment, and the non-toxic, pentameric binding moiety (StxB). A unique property of Stx is that it binds specifically to the glycosphingolipid globotriaosylceramide (Gb3) located on the surface of target cells and is then internalized by endocytosis. Consequently, in toxin-sensitive cells, the Stx/Gb3 complex is transported to the endoplasmic reticulum through the Golgi apparatus, the StxA is then translocated to the cytosol, where it irreversibly inhibits protein synthesis by means of modification of ribosomal 28S RNA. Interestingly, Gb3 shows a relatively restricted expression in normal human tissues, and has been reported to be highly expressed in many types of cancers.

Engedal and colleagues cover the introduction of Stx and its intracellular transport, a description of Gb3 and the current methods being utilized to detect its cellular expression, and a synopsis of previously published reports detailing Gb3 overexpression in human cancers. Surprisingly, enhanced expression of Gb3 has been reported in cancers of diverse tissues including but not limited to colorectal carcinoma, primary human testicular cancer tissues and breast and ovarian cancers (Nudelman *et al.*, 1983; Ohyama *et al.*, 1990; LaCasse *et al.*, 1999). These findings along with the relatively restricted expression of Gb3 in normal human tissues raise the prospect that Stx derivatives, which specifically bind to Gb3, could be used for targeted therapy and imaging of tumours. However, because the viability of such medical applications depends on several factors further research is warranted into the potential application of Stx or StxB derivatives in cancer medicine, questions that are required to be addressed are: to what degree Gb3 is expressed at the cell surface of normal cells, and to what degree and how Stx binds to and is taken up and transported inside both normal and malignant cells. These and other aspects related to the use of Stx for imaging and targeted cancer therapy of Gb3-expressing cancers are extensively discussed in this current review.

Neisseria meningitidis is a virulent human pathogen and continues to be a major cause of mortality throughout the world with children and adolescents forming the primary risk group. The incidence of invasive meningococcal disease is low but infections can lead to death within several hours if untreated. *Neisseria meningitidis* can be classified into five serogroups, namely A, B, C, W-135 and Y. The capsular polysaccharides of serogroup A, C, W-135 and Y are highly immunogenic and consequently several polysaccharide-based vaccines covering these serotypes have been licensed. However, the capsular polysaccharide of serogroup B (MenB) resembles structures present on human cells which consequently results in a poor immunogenicity of the bacterial polysaccharide and does not permit its use as vaccine antigen. In their review in *Microbial Biotechnology* titled 'Vaccine development against *Neisseria meningitidis*' Ulrich Vogel and Heike Claus summarize the challenges in the development and optimization of vaccines against this pathogen (Vogel and Claus, 2010).

The major challenge consists in the development of a universal *N. meningitidis* vaccine which includes MenB. The need for such a vaccine is underscored by data that

*For correspondence. E-mail juanluis.ramos@eez.csic.es; Tel. (+34) 958 181608.

show that MenB infections amount to around two-thirds of all meningococcal infections in Germany. Since a vaccine approach using unmodified polysaccharide is not feasible for MenB, the authors summarize alternative strategies which include the use of protein subunit antigens, mimotope antigens or the use chemically modified polysaccharide antigens. Due to the resemblance of the capsular polysaccharides with structures present on human cells the induction of autoantibodies using modified polysaccharide as antigens cannot be ruled out. In addition, the lack of success in generating a MenB vaccine using traditional approaches has led to the exploration of ways which are novel in vaccinology, such as the use of inactivated lipopolysaccharide (LPS) as antigen. LPS is a highly toxic compound and its detoxification is a delicate issue and future studies are required to reveal the efficiency of this approach.

On of the main challenges in vaccine development is the evaluation of the efficiency of new vaccine candidates the testing is seriously hampered by the low incidence of the meningococcal disease and implies that vaccine efficiency cannot be determined in clinical trials. Therefore, other surrogates of protection need to be used, such as bactericidal activity of the induced antibodies, to estimate the efficiency of a vaccine candidate.

Another major difficulty is related to the fact that the human nasopharynx is the natural habitat of *N. meningitidis* and immune selection drives an extensive evolution of surface antigens. As a result the meningococcal population is diverse and dynamic. As a consequence the issue of strain coverage of any vaccine prototype needs to be addressed, i.e. the vaccine efficiency towards a panel of strains has to be monitored. The extent of strain coverage is likely to be an important criterion for vaccine licensing. Humans are also host to non-pathogenic meningococci, which were shown to contribute to natural immunity against disease. Therefore, care needs to be taken in defining the broadness of the vaccine. The vaccine should strongly impact on a wide range of different pathogenic meningococci but should preferentially not react with the beneficial, non-pathogenic strains.

A number of MenB vaccine prototypes are currently under investigation. Many questions concerning these vaccines will only be answered following licensure, such as their efficiency, impact on non-pathogenic strains and the frequency of vaccine-induced immune escape of the bacteria. Therefore, a first generation MenB vaccine will only be a starting point for the continuous development and optimization of such a vaccine. The speed by which investigation advances will determine whether children and adolescents of the generations to come will be protected from this dreadful disease.

Continuing on the vaccine development topic is the review written by Kroll and colleagues; plasmids serve as important tools for the construction of recombinant microorganisms with multiple biotechnological potential. The authors present an extensive review dealing with different ways to create stable plasmids while avoiding antibiotic resistances (Kroll *et al*., 2010). The article includes a clear description of the diverse plasmid addiction systems (PASs) based on toxin/antitoxin (TA), metabolic requirements or operator repressor titration. Following a brief description of the latter two mechanisms of plasmid stability, the authors provide a substantial narrative of multiple applications which utilize the TA system. All of the applications combine a stable toxin with an unstable (or less stable) antitoxin, either of a proteinaceous or of a nucleotidic nature. Since the initial description of the first TA system based on the *hok* gene for programmed cell death (PCD) by S. Molin and colleagues (1987) these systems have been widely used in bioremediation. Of particular interest are the suggestions of PAS applications in the design of live vaccine therapies capable of responding to environmental signals and on the construction of antibiotic-free genetic tool/vectors for the study of highly pathogenic organisms. The authors anticipate that the use of new PASs, both natural and artificial, for the construction of heterologous expression systems will reduce not only their environmental risk but also their production costs. Clearly further research in this exciting field will provide us with many potential biotechnologically useful tools.

Pathogens pose a significant threat to human and animal health and are an important facet of robust agricultural practices; naturally, dealing with these pathogens is of utmost importance. This subject has been dealt with before in *Microbial Biotechnology* by Di Stefano and colleagues (2009) who reported that biofilms formed by *Staphylococcus aureus* and *S. epidermidis* (two reference strains and two ocular isolates) were very relevant to the overall virulence of the bacteria. Based on this article and others Daniels and colleagues (2010) proposed that reliable detection tools for rapid and sensitive analysis and typing of infectious pathogens are needed, a topic that is presently undertaken by Kostic and colleagues (2010) in *Microbial Biotechnology*. The authors present a diagnostic microarray for the detection of relevant bacterial food- and water-borne pathogens. Microarray technology offers several advantages in comparison with conventional microbiological culture-based techniques and other molecular methods, including the possibility of parallel, specific and rapid detection of many different organisms in one single assay. The current microarray platform is based on sequence-specific end labelling of oligonucleotides. The extended probe set consists of 63 oligonucleotides targeting the *gyrB* gene of the 24 most common food- and water-borne pathogens. The gene *gyrB* is a robust phylogenetic marker (Yamamoto and Harayama,

1996). Another relevant feature of this detection method is sensitivity. The sensitivity of microbial diagnostic microarrays is defined as the lowest amount of nucleic acid (cells) needed for successful detection (absolute sensitivity). The microarray presented by Kostic and colleagues allowed detection of as few as 10^4 cfu. Another key issue in the use of array technology is validation; Kostic and colleagues (2010) approached this by using a set of reference strains and a set of spiked environmental samples. The reliability of the obtained data was additionally verified by independent analysis of the samples using fluorescence *in situ* hybridization and conventional microbiological reference methods. Last but not least the microarray-based pathogen detection microarray was compared with the current microbiological reference methods and provided comparable results. Thus, the advantage of microarray technology for the detection of multiple pathogenic strains is clear; hopefully this technology will be functionally implemented in the near future.

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