Working towards a malaria vaccine

Dr Giampietro Corradin has spent the last 20 years working on the development of malaria vaccines. From many potential leads, one promising therapy is soon to undergo clinical trials...
Protein synthesis to fight disease

The complicated and lengthy process of vaccine candidate identification is being streamlined by a team at the University of Lausanne, Switzerland. Their focus on malaria has utilised genomics and protein synthesis to efficiently identify a library of potential therapeutics.

MALARIA IS THE most publicised and significant parasitic infection faced today. According to data from the World Health Organization (WHO), globally there are approximately 250 million people afflicted, resulting in more than 600,000 deaths annually, with pregnant women and children the most vulnerable to infection. There are two main species involved in this infection: Plasmodium falciparum and P. vivax. P. falciparum is considered to be the more important of the two as not only does it account for the majority of infections – around 80 per cent – but it is also the more lethal. Although P. vivax is often considered benign, the rising number of associated complications and its relapsing pattern imparts an increasingly significant burden on patients and healthcare systems.

Based at the University of Lausanne, Switzerland, Dr Giampietro Corradin is hoping to utilise solid phase peptide synthesis (SPPS) and high-throughput screening to realise the vision of an efficacious malaria vaccination which would save the lives of millions. Having developed a background in chemistry before moving to more biological studies, it is unsurprising that he is eschewing classical biological techniques in favour of more chemical intensive methodologies.

Whilst there are various strategies to reduce the damage caused by malaria, such as mosquito control, a focus on early diagnosis and the use of effective treatments, these are unlikely to be powerful enough to eliminate malaria.

It is possible to develop immunity to the disease as it has been observed that children in regions where malaria is endemic become progressively protected after repeated exposure; therefore, the development of a cheap and effective immunisation programme appears to be the best solution. However, vaccine discovery is a lengthy and labour-intensive process which never has guaranteed results. This is partly due to the lack of structural, physicochemical or sequence similarity among antigens that have been shown to provide protection, and the large diversity of antigens present in the malaria parasite (> 5,000). This necessitates an identification process which is largely based on trial and error; increasing the speed with which potential candidates can be identified appears to be the clear route to malaria vaccination.

SOLID PHASE PEPTIDE SYNTHESIS

In the search for vaccine candidates, antigens are traditionally inserted in bacteria or other organisms from which the relevant protein(s) is (are) purified before assessing their biological activity. However, the purification processes are lengthy and often leave DNA and other proteins behind as impurities. In addition, the 3D structure of the target antigen is often unknown. As a result, screening the large number of possible antigens in a complex pathogen’s proteome becomes near impossible. This is where SPPS takes centre stage; it can produce protein fragments and small proteins without these impurities, in a much shorter period of time. Due to huge advances in SPPS methodologies in recent years, the technique can now readily produce high yields of peptides/proteins of up to 150-200 amino acids in length. On the other hand, the size limitation of SPPS means that the majority of the proteins in the genome could not be synthesised to assay for activity.

EPITOPES

Vital to finding a solution is an understanding of how the immune system detects antigenic proteins as the antibodies, B cells and T cells only recognise and bind to a particular part of the antigen – the epitope. It is also known that targeting the right epitope among the numerous ones present in an antigen is often sufficient to obtain the desired biological effect as observed for therapeutic monoclonal antibodies. This means that the synthesis of the whole antigenic protein is not required.
This work has the potential to be utilised across the board in vaccine candidate discovery as its general principles can clearly be applied to searching the genomes of any pathogen.

Unfortunately, this approach leads to a number of issues not present when dealing with whole, correctly folded proteins. For the immune system to detect the relevant epitope, it must remain in its native conformation. However, when dealing with a fragment of a protein, folding forces often drive segments into different shapes. This affects its 3D structural organisation so that parts of the protein required for antibody recognition – distant in the primary sequence of the protein but held close together in the protein’s tertiary structure – are no longer functional, leaving epitopes as highly unlikely vaccine contenders.

Species of *Plasmodium* have complicated life cycles, with different proteins being expressed at different stages. Prophylactic vaccinations should be designed to target proteins expressed during the two developmental stages of their life cycle within the human host: the pre-erythrocytic stage and the asexual erythrocytic stage. During the former, the parasites develop in hepatocytes (liver cells) and Corradin’s team believes that the antigens presented by the infected hepatocytes at this stage are vital to activating the T cells required for long-term immunity, a discovery which is supported by the up-regulation of multiple genes within the hepatocytes upon infection. On the other hand, identification of liver stage protective epitopes is a daunting process since T cells recognise the epitope bound to a very polymorphic receptor called the major histocompatibility complex antigen. In addition, the number of parasite specific T cells present in one individual is generally very low, which further limits analysis of the T cell response.

**THE ALPHA HELIX**

All of these factors have led the group to focus its search on segments of proteins presented by *P. falciparum* during the erythrocytic stage. This allows rapid screening of any number of antigens, provided they present the native 3D conformation. To this effect, the team focuses on two common structures which are maintained when isolated from the parent protein: α-helical coiled-coils and unstructured segments – common secondary/tertiary structures of proteins that account for between 2-5 per cent and around 40 per cent of encoded residues in almost all genomes, respectively. They are ideal candidates for this work as they tend to be around 30 to 100 residues in length and water soluble.

Corradin and his colleagues first searched the *P. falciparum* genome for fragments 30 to 40 amino acid residues long which formed α-helical coiled-coils. This is possible as the sequence to structure relationship of these regions is one of the most widely studied and understood associations observed in proteins. These parts of the genome are clearly distinguishable due to the characteristic pattern of hydrophobic (H) and polar (P) amino acid residues observed – HPPHPPPP – known as the heptad repeat. The unstructured domains are also easily identified since they are abundant in hydrophilic amino acids – representing around 80 per cent of the composition.

The group has published a section of its results from a study of 170 fragments, which includes 95 novel α-helical coiled-coils. A large majority of these fragments were recognised by human antibodies from donors living in malaria endemic regions. This agrees with other studies that highlight the large antibody repertoire specific to the malaria parasite and provides hope that an effective vaccine candidate is out there and may even have been identified by Corradin’s research. Selection of unstructured fragments from the corresponding proteins revealed the P27A sequence. Chemical synthesis of this 104 residue-long polypeptide, and subsequent biological and immunological characterisation of P27A-specific human antibodies, indicated its promising vaccine propensity, which will soon be tested in human volunteers.

This work has the potential to be utilised across the board in vaccine candidate discovery as its general principles can clearly be applied to searching the genomes of any pathogen. This ability to rapidly find drug candidates will hopefully lead to many more clinical trials for malaria vaccinations and other infectious diseases.