Antigen synthesis opens the door to a broad-spectrum AIDS vaccine

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Twenty years after the discovery of HIV, scientists are a step closer to the development of a vaccine. Using a technique called one-pot synthesis, scientists from The Scripps Research Institute (http://www.scripps.edu/) have designed synthetic antigen-like compounds that mimic the cluster of sugars recognized by the 2G12 antibody, which neutralizes HIV [1].

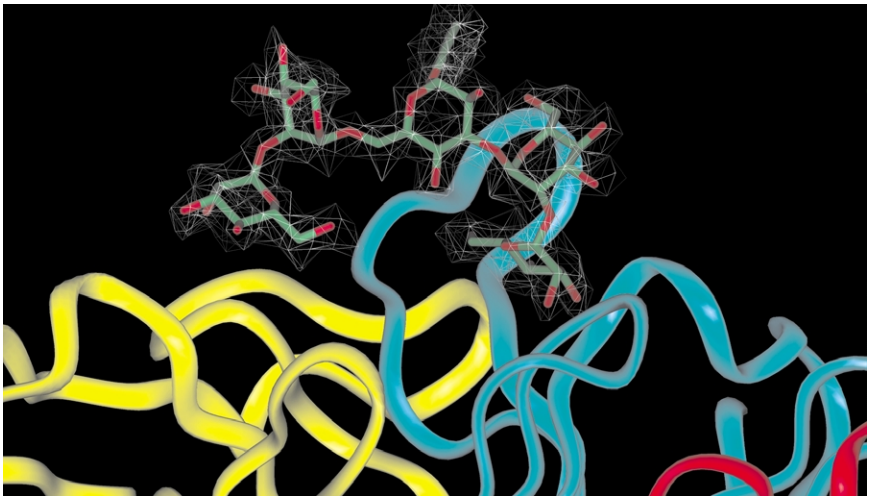

Figure 1. A computer model of a synthetic antigen interacting with the 2G12 antibody. Courtesy of The Scripps Research Institute (http://www.scripps.edu/).

Using sugars as antigens

'The HIV particle is fully covered with sugars and easily escapes from the immune system. Using sugars as antigens may thus provide a new opportunity and new direction in vaccine development, if the sugar antigen can be attached to an appropriate carrier to activate the immune system', said Chi-Huey Wong, a co-author on the paper.

With the numbers of those infected with HIV increasing annually – the WHO estimates that there are 40 million people living with HIV – developing a vaccine for HIV is of crucial importance. 'The major difficulty has been that conventional means of producing antibodies that are effective against HIV have failed, and many of the initial efforts to generate cellular responses generated responses that were too weak to provide confidence that they might protect', said Paul Spearman, from Vanderbilt University Medical Center (http://www.mc.vanderbilt.edu/), summarizing the problems of developing an effective vaccine. He added that, although we can generate stronger cellular responses, 'we are still waiting for the right vaccine that can provide effective neutralization.'

The silent face of gp120

'Natural infection does not produce many broadly neutralizing antibodies (antibodies that will neutralize different HIV variants). So the straight forward mimicry-of-natural-infection approach has not worked out and we have been forced to come up with new strategies', said Dennis Burton, from Scripps and Director of the Neutralizing Antibody Consortium, a co-author on the paper.

Designing immunogens that are capable of eliciting broadly neutralizing antibodies is a major goal of HIV vaccine research. The team from Scripps focused on an antibody, 2G12, which recognizes a conserved (invariant) cluster of oligomannose sugars on the 'silent face' of gp120 – the envelope protein of HIV – and neutralizes the virus. 2G12 was isolated from one of the rare HIV-positive individuals whose immune system is able to combat the virus.

Describing why the mannose cluster is a good target for vaccine development Burton said, 'The mannose cluster is not found, as far as we can tell, on any other self protein. Therefore, it is 'foreign' and recognized by the antibody system. Individual mannose residues are found on many self proteins, they are not foreign and not recognized by the antibody system to any great degree.' He added 'in a sense the virus has gone too far in cloaking itself with sugars, it has produced this abnormal cluster.'

The team hope to use the structural information obtained from the complexes of 2G12 with oligomannose chains to design antigens that will elicit a 2G12-like antibody response. In this

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study, using a one-pot synthesis method, they designed several synthetic compounds that bind to 2G12. One-pot synthesis, developed by Wong, enables researchers to assemble carbohydrate structures quickly. 'The one-pot reaction was conducted in such a way that the building blocks were added to the reaction flask in sequence according to their reactivity, with the most reactive one being added first, so the saccharide was assembled with the structure as expected. All the protecting groups were then removed and the product was purified', said Wong. The technique can be automated and programmed. 'All other methods of oligosaccharide syntheses require protection and deprotection in every glycosidic bond formation and thus the process is slow, tedious and expensive', he added.

Retrovaccinology

The synthesis of these antigen-like compounds is the first step in a process called retrovaccinology. Explaining the concept, Burton said 'simply, in vaccinology one typically has a vaccine and puts that in to people to produce neutralizing antibodies. We have the antibodies, but not the vaccine, so we are trying to work in reverse, from antibodies to vaccine'. Spearman is enthusiastic about the approach taken by the Scripps team, 'I think this type of approach is on the cutting edge of vaccinology, and that this is essential because of the marked failure of conventional approaches. gp120 protein vaccines have been a failure, at least as currently constructed. This approach takes the intricate molecular detail of the binding of a neutralizing antibody and tries to re-create the immune response to get there. This is a very clever approach'.

There are many challenges ahead in the road to a HIV vaccine including the variability of HIV, developing immunogens that elicit protective T-cell responses and understanding how attenuated SIV vaccines protect in animal models. Meanwhile, Burton is part of the Neutralizing Antibody Consortium, which supports scientists working to generate immunogens that elicit broadly neutralizing antibodies to HIV. They are exploring the structural interactions between broadly neutralizing monoclonal antibodies and HIV to aid more effective vaccine design.

Next, Wong and his colleagues plan to test their compounds as antigens in mice to see if they can elicit strong antibody production and measure the antibodies binding to HIV. 'We will then develop the good antigens as potential vaccines', he said.

Reference

1 Lee H.K. *et al.* (2004) Reactivity-based one-pot synthesis of oligomannoses: defining antigens recognized by 2G12, a broadly neutralizing anti-HIV-1 antibody. *Angew. Chem. Int. Ed. Engl.* 43, 1000–1003

Parkinson's disease in the PINK

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A new gene has joined the list of genes mutated in hereditary forms of Parkinson's disease (PD). The discovery by European researchers of PD-associated mutations in *PINK1*, which encodes a mitochondrial kinase, provides a direct link between mitochondria and PD pathogenesis and could lead to new treatments for PD.

An unexpected culprit

PD is a common neurodegenerative disorder caused by the progressive loss of dopaminergic neurons. Patients are usually treated with levodopa but, although this initially improves motor symptoms, many patients later develop dyskinesias.

Most cases of PD are sporadic but researchers believe that identifying the genes mutated in rare familial forms of PD will provide insights into how sporadic PD develops. Past genetic studies have identified mutations in parkin, α-synuclein and DJ1. Now, Nicholas Wood, head of the Department of Molecular Neuroscience at the Institute of Neurology, London, UK (http://www.ion.ucl.ac.uk) and co-workers have discovered a fourth mutated gene.

'In 2001, we identified an Italian family with autosomal recessive hereditary early-onset PD,' explains Wood, 'and mapped the affected gene to chromosome 1.' The discovery of a Spanish family and a second Italian family allowed the researchers to home in on a region of chromosome 1 containing 40 genes. 'We ended up sequencing most of these,' says Wood, to find the gene associated with PD.

As the researchers report in *Science* [1], affected individuals in the Spanish family had a homozygous mutation in the putative kinase domain of PINK1 (PTEN-induced putative kinase 1); in both Italian families the kinase domain was truncated. 'We hadn't put *PINK1* at the top of our list

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