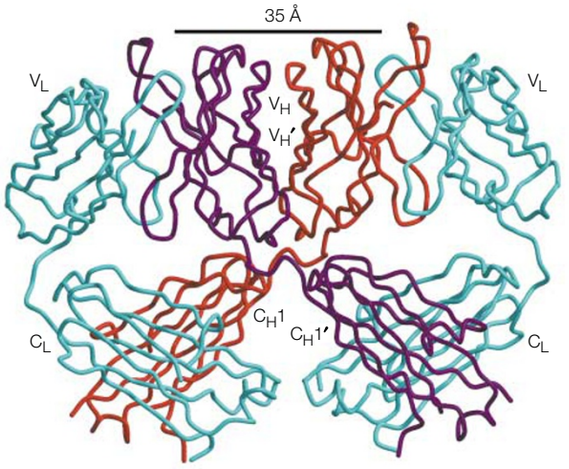
ANTIVIRAL IMMUNITY

Antibodies lock arms against HIV



Structure of two domain-swapped Fab molecules. Light chains are shown in blue and the heavy chains from Fab1 and Fab2 are shown in red and purple. Reprinted with permission from Calarese *et al.* © American Association for the Advancement of Science (2003).

A rapid mutation rate and a protective coating of host-derived sugars seem to have made HIV antibody proof, and in recent years, the neutralizing-antibody approach to HIV vaccine design has taken a back seat. But, neutralizing antibodies are now back in the spotlight, as a recent report in *Science* shows.

One of the rare neutralizing HIV-specific antibodies that has been isolated from infected patients is the monoclonal antibody 2G12, which binds with high affinity to oligomannose residues on gp120. To find out exactly how 2G12 binds to gp120, two groups, led by Dennis Burton and Ian Wilson at the Scripps Research Institute, La Jolla, USA, determined the crystal structure of the antigen-binding fragment (Fab) of 2G12 together with its carbohydrate ligand. What they discovered was a completely new and unanticipated mode of antigen binding.

Rather than binding the antigen as a monomer, two 2G12 Fabs interlock, forming a dimer in which their heavy-chain variable (V_H) domains are 'swapped'. The result is an extended multivalent antigen-binding site that can recognize the close repeating pattern of sugars that mask the surface of gp120 (see diagram). A conventional antibody could never

INNATE IMMUNITY

DCs: the immune system's flexible friends

The idea of dendritic-cell (DC) plasticity is gaining momentum, but the ability to secrete large quantities of the type 1 interferons (IFNs), IFN-α and IFN-β, in response to infection with viruses has long been considered a special feature of plasmacytoid DCs. These cytokines are important for linking innate and adaptive immune responses. But now, Diebold and colleagues report a further level of DC plasticity — other types of DC can also produce large quantities of type 1 IFNs.

Because some studies indicated that plasmacytoid DCs are not essential for the production of type 1 IFNs following infection with the Armstrong strain of lymphocytic choriomeningitis virus (LCMV), Diebold and colleagues decided to investigate which other cell types can produce type 1 IFNs. Non-plasmacytoid CD11c^{hi} DCs isolated from mice that were infected with the DC-tropic Armstrong variant clone 13 could produce large quantities of type 1 IFNs after *in vitro* culture.

The authors next investigated the link between virus infection and the production of type 1 IFNs. They used the synthetic double-stranded RNA (dsRNA) mimic polyI:C to stimulate non-plasmacytoid DCs. Mimicking virus infection by cytosolic delivery (by electroporation or delivery with lipofectamine) of polyI:C or virus dsRNA to splenic DCs or bone-marrow-derived DCs enabled them to produce as much type 1 IFN as did plasmacytoid DCs. This was found to be independent of signals delivered through the receptor for dsRNA, Toll-like receptor 3 (TLR3), as the production of IFN-α by bone-marrow-derived DCs from TLR3-deficient mice was unimpaired. Mice deficient in the TLR adaptor MyD88 could also produce IFN-α, indicating that production of IFN-α by these DCs is TLR independent.

So, if the TLR pathway isn't important, how is dsRNA recognized by non-plasmacytoid DCs? Protein kinase R (PKR) can bind dsRNA and has been implicated in IFN responses to virus infection. Using PKR-deficient DCs or inhibiting PKR activity led to a reduction in the level of type 1 IFNs, but an increase in the level of interleukin-12, in response to stimulation with polyI:C plus lipofectamine and CD40 ligand.

Many viruses can evade PKR-mediated responses — for example, influenza virus encodes the NS1 protein, which can bind and sequester dsRNA. Does this allow the virus to block the production of type 1 IFNs by non-plasmacytoid DCs? Non-plasmacytoid bone-marrow-derived DCs produce low levels of IFN-α when infected with influenza virus, but infection with an NS1-deficient virus variant resulted in the production of high levels of IFN-α, even though this variant infects only a small fraction of bone-marrow-derived DCs. Interestingly, influenza virus is often used to stimulate the production of type 1 IFNs by plasmacytoid DCs.

These results show that non-plasmacytoid DCs can produce large amounts of type 1 IFNs during infection with virus and, once again, studying viruses has indicated something new about the immune system — a TLR-independent pathway for the activation of DCs.

Elaine Bell

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WEB SITE

Caetano Reis e Sousa's lab: http://science.cancerresearchuk.org/research/loc/london/lifch/sousac/

achieve this due to steric constraints. As Dennis Burton told *Nature*, "It's an ideal molecular solution to recognizing a tight cluster of repeating patterns".

Whereas the sugars on the surface of gp120 are closely clustered, on host proteins the same sugars tend to be widely spaced, so the unusual antibody structure allows the recognition of a unique feature of the virus.

Researchers can now start thinking about how to exploit this chink in the armour of HIV. The authors of the paper point out that one possibility is to engineer carbohydrate vaccines that mimic the close array of sugars on gp120.

Jennifer Bell

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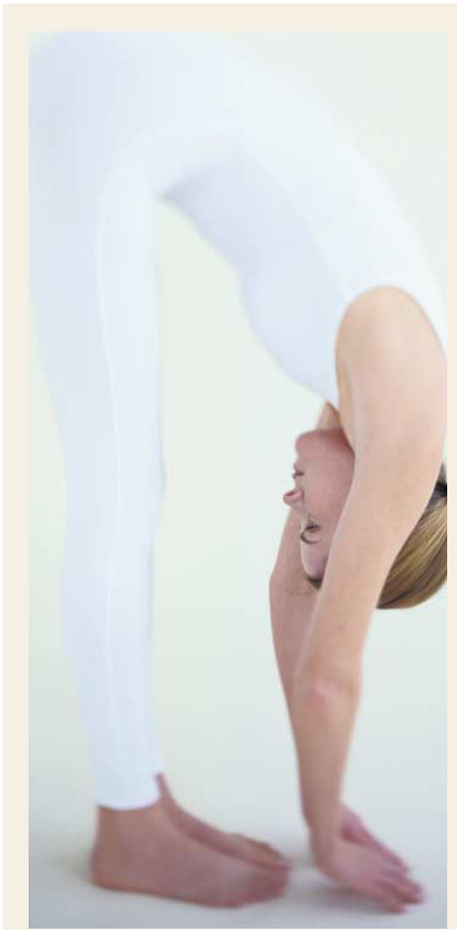
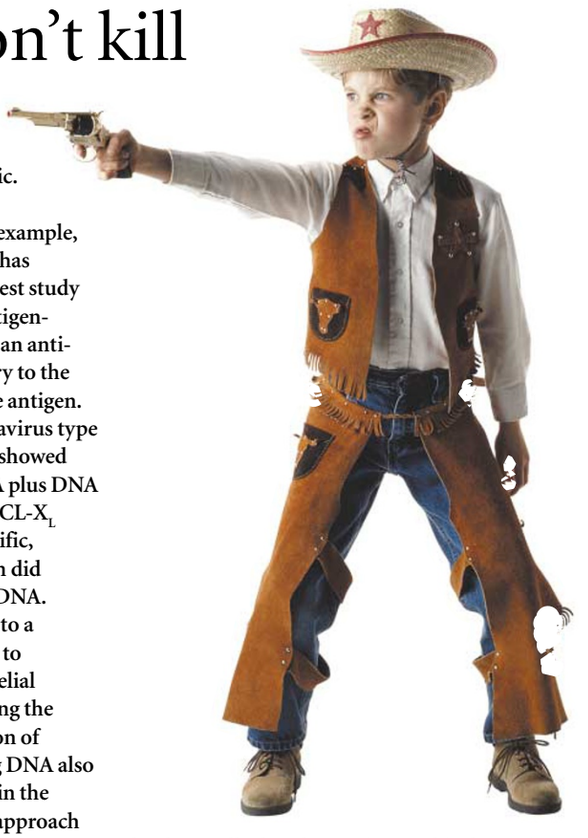


VACCINES

Shoot but don't kill



DNA vaccines are quick and easy to produce, and have few side effects, but they are often only weakly immunogenic. However, work on improving the immunogenicity of DNA vaccines (for example, through the use of cytokine adjuvants) has renewed hope in this approach. This latest study shows that the co-administration of antigen-encoding DNA with DNA that encodes an anti-apoptotic molecule by gene-gun delivery to the skin can enhance T-cell responses to the antigen.

Kim *et al.* used the human papillomavirus type 16 protein E7 as a model antigen. They showed that vaccination with E7-encoding DNA plus DNA encoding the anti-apoptotic molecule BCL-X_L resulted in a greater number of E7-specific, interferon-γ-secreting $CD8^+$ T cells than did vaccination with E7 DNA plus control DNA. This effect on T-cell number translated to a positive effect on the immune response to challenge with E7-encoding TC-1 epithelial tumour cells in mice. As well as inhibiting the formation of TC-1 tumours, the addition of BCL-X_L-encoding DNA to E7-encoding DNA also enhanced inhibition of tumour spread in the blood to the lungs, indicating that this approach has both protective and therapeutic effects.

The authors then showed that this anti-apoptotic vaccine strategy could be further enhanced by targeting antigen to the MHC class I and class II antigen-presentation pathway. They achieved this by using DNA encoding E7 antigen linked to the sorting signal of lysosome-associated membrane protein type 1 (LAMP1), which targets E7 to endosomal/lysosomal compartments. This strategy enhanced memory responses to the antigen as shown by analysing E7-specific $CD8^+$ T-cell responses 14 weeks after DNA vaccination.

By looking at the effects of minimal mutations that abrogate the anti-apoptotic activity of BCL-X_L, the enhanced immunogenicity that results from adding BCL-X_L-encoding DNA was shown to be due to these anti-apoptotic actions. Also, other anti-apoptotic proteins, such as BCL-2 and X-linked inhibitor of apoptosis protein (XIAP), and dominant-negative caspase mutants had similar, although less marked, effects.

The authors explain these results in terms of prolonging dendritic-cell (DC) survival. They suggest that gene-gun delivery to the skin can target antigen-presenting cells (APCs), known as Langerhan's cells, in the skin. Expression of BCL-X_L by these cells increases the life span over which they can express and present antigen to $CD8^+$ T cells and it might protect the APCs from killing by the T cells that they activate. This theory is supported by the observation that addition of BCL-X_L-encoding DNA increases the number of E7-expressing DCs in the lymph nodes five days after vaccination and decreases the number of apoptotic cells.

These results are in contrast to previous studies showing enhanced immunogenicity with the use of pro-apoptotic strategies such as FAS (CD95). However, these studies have mainly used intramuscular immunization, which targets DNA to non-professional APCs (myocytes), which are more susceptible than DCs to pro-apoptotic stimuli. It is probable that myocyte apoptosis enhances antigen uptake and presentation by APCs. A combination of these two approaches — using tissue-specific promoters to target anti-apoptotic proteins to professional APCs and pro-apoptotic proteins to non-professional APCs — could further improve vaccine efficacy.

Kirsty Minton

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WEB SITE
T.-C. Wu's lab: http://pathology2.jhu.edu/TCLab/