Potential new vaccine against chlamydia

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A chlamydia vaccine will soon enter clinical trials, the first to do so in more than 25 years. Antex Biologics (http://www.antexbiologics.com) reports that vaccination with recombinant subunits of proteins from *Chlamydia trachomatis* protects against both lower reproductive tract infection and chlamydia-induced infertility in mice, and the company plans to begin Phase I human trials by the end of 2002.

Fertility risk

Chlamydia trachomatis is a widespread pathogen that poses serious public health problems worldwide. An obligate intracellular bacterium, it produces a strong inflammatory reaction in mucosal tissues and causes urogenital disease, blindness or pneumonia depending on the site of infection. Chlamydia is now the world's most common sexually transmitted bacterial infection, with an estimated 89 million new cases worldwide in 1995 (World Health Organization; http://www.who.int/home-page/) and four million cases reported each year in the USA. Genital infection is frequently asymptomatic, and can progress undetected to cause pelvic inflammatory disease and female infertility. *C. trachomatis* also causes trachoma, a painful eye condition that is endemic in parts of the developing world and the leading cause of preventable blindness.

Chlamydia infection is easily cured with antibiotics, but permanent damage is often done before the infection is detected. Furthermore, treated individuals often become reinfected, because natural infection confers only short-lived and serovar-specific immunity. Immunization is considered to be the best approach for reducing the burden of chlamydial disease [1], but despite much research no effective vaccine is yet available.

'Chlamydia is a huge challenge,' comments Joseph Igietseme, Chief of the Molecular Pathogenesis Lab at the US Centers for Disease Control (http://www.cdc.gov). 'Using the whole organism for vaccination has proved unattractive, but no single subunit vaccine has yet produced a sterilizing, long-term immunity. Most of the vaccines developed so far only give temporary and partial protection in animals.' Nevertheless, he believes an effective vaccine is possible. 'You need to induce a high level Th-1 response, meaning high frequency of Th-1 cells, and also an IgG and IgA antibody response in the mucosal site of infection. A multi-subunit approach would be one way of doing this; it appears that a single antigen may not be able to deliver enough T-cell epitopes. A highly potent adjuvant might also generate a powerful enough response.'

Vaccine technology

Researchers at Antex Biologics are developing TRACVAX, a vaccine based on recombinant subunits of proteins from *C. trachomatis*. 'We have worked on a number of proteins that have shown various levels of protective efficacy in animal models, both of disease and infection,' says James Jackson, Vice President of Research at Antex. 'Using the antigens in our vaccine we aim to program the immune response to act in a different way than we see after natural infection. We believe this will be effective not only against disease but also against lower reproductive tract infection.'

The vaccine will probably contain a cocktail of recombinant proteins. The exact antigen composition has not yet been disclosed, but it is likely to feature chlamydial proteins from a superfamily known as polymorphic membrane proteins (PMPs). These are believed to be membrane-associated and possibly surface-exposed, although their role in invasion, pathogenesis and/or cell viability is currently unknown. At least two members of the family, pmpG and pmpE, have been found to be highly conserved among the many different chlamydia serovars. Immunization with pmpG protects against *C. trachomatis*-induced infertility in a mouse model [2].

Preclinical studies

This protective effect has recently been confirmed with pmpE [3]. Female mice were given three intranasal doses of 10 μg pmpE plus 5 μg of a modified form of the *Escherichia coli* toxin mLT as an adjuvant. Two control groups were immunized with adjuvant only. Two weeks later, the mice were challenged with a different *C. trachomatis* serovar, which was introduced directly into the uterus. The mice were later mated and their fertility rates monitored over 10 weeks. Of the mice immunized with adjuvant alone, only 9% were reproductively competent. By contrast, 50% of those immunized with pmpE managed to reproduce. However, the protection was only partial: the fertility rate in sham-challenged controls was ~90%. Jackson believes greater protection can be achieved by adjusting the antigen concentration and protocol used.

Immunization produced variable titers of anti-pmpE IgG antibodies, but there was a strong and uniform antigen-specific T-cell proliferative response. 'Based on the antibody responses among individual animals and the resultant cytokine release and antibody isotype profiles, the protective effect apears to correlate with

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both cellular and humoral type immune responses,' Jackson says.

Clinical trials

Human clinical trials will concentrate on female urogenital disease. The route of administration has not been decided, although the researchers have noted that the intranasal route produces a good immune response in the vaginal canal. 'In Phase I trials, as well as testing safety, we need to see if we get a basic immune response comparable to what we've seen in mice,' says Jackson. 'In later-stage trials, we would be looking for a good cellular [immune] response in the lower female reproductive tract. Without a strong cellular [immune] response, you won't get protection.' Antex is also in discussion with potential partners interested in trialing the vaccine against ocular trachoma.

References

1 Igietseme, J.U. *et al.* (2002) Chlamydia vaccines: strategy and status. *BioDrugs* 16, 19–35

2 Maisonneuve, J-F. *et al.* (2001) Immunization with a high molecular weight protein (pmpG) from *Chlamydia trachomatis* confers heterotypic protection against infertility. *American Society for Microbiology 101st General Meeting*, 20–24 May 2001, Orlando, FL, USA (Abstract E-23, p. 197)

3 Jackson, W.J. *et al.* (2002) Mucosal immunization with recombinant pmpE from *Chlamydia trachomatis* serovar L2 confers protection against serovar F-induced infertility. *American Society for Microbiology 102nd General Meeting*, 19–23 May 2002, Salt Lake City, UT, USA (Abstract E-53, p. 182)

News in brief

Targets and mechanisms

'Fountain of youth' receptor identified

Scientists have uncovered evidence of a cellular G-protein-coupled receptor (GPCR) through which the hormone dehydroepiandrosterone (DHEA) acts [1]. DHEA – often referred to as the fountain of youth because of its protective properties – stimulates nitric oxide synthase, which helps to regulate blood pressure, inhibit blood clotting and prevents narrowing of arteries.

Until now, the hormone had no known cellular receptor or identifiable mechanism of action, and this has been a major obstacle to the understanding of how the hormone affects the body. However, researchers at the University of Iowa (UI) Health Center (http://www.uiowa.edu) decided to look in an unusual place for a steroid hormone receptor: 'All steroid hormones have receptors within cells. We found the DHEA receptor on the outside, not inside of cells. We also looked at cells which have not previously been examined. Other investigators have focused their research on immune blood cells or the liver, but we looked at the endothelium, or cells lining the blood vessels,' said the study's lead investigator Joseph Dillon, Assistant Professor of Internal Medicine at UI.

This research shows that there is a receptor for DHEA in the inner lining cells of blood vessels that responds to physiological levels of DHEA and this finding links the hormone to the production of nitric oxide, according to Dillon. This will provide a good starting point for studying the risks and benefits of DHEA as a potential therapy, and could help researchers design clinical trials of the hormone. 'The significance of our study is that it provides a scientific basis for further study of the DHEA action in humans,' said Dillon. The next step in his research will be to define the mechanism by which DHEA produces its effects.

1 Liu, D. and Dillon, J. (2002) Dehydroepiandrosterone activates endothelial cell nitric-oxide synthase by a specific plasma membrane receptor coupled to Gαi2,3. *J. Biol. Chem.* 277, 21379–21388

New cholesterol disorder predicted, then discovered

A team lead by John Kane, Professor of Medicine at the University of California San Francisco (http://www.ucsf.edu) has discovered a new cholesterol disorder that results from a single gene defect [2]. The disorder, which causes severely elevated blood cholesterol levels, is estimated to affect several hundred thousand people in the USA and Europe.

Unusually, the disease was hypothesized before it was discovered. The function of the gene was already known; it codes for the enzyme cholesterol 7-α hydroxylase (CYP7A1), which is essential for the breakdown of cholesterol in the liver. The researchers therefore predicted that mutation of CYP7A1 would block this mechanism and lead to an accumulation of cholesterol in the liver. This, in turn, should reduce the expression of the number of receptors in the liver that can bind to low-density lipoprotein (LDL) from the blood. Hence, it was hypothesized that a mutation in the gene for CYP7A1 should lead to an accumulation of LDL.

To prove this theory, the team searched a genetic database to see if those with a mutated CYP7A1 gene did indeed show signs of increased LDL levels. After screening 12,000 patients, 11 were found who carried the mutation. The family of one patient was studied in depth: of 37 people in this family, nine carried the same mutation. Three siblings who carried two mutant copies of the gene had cholesterol levels above 300 mg dl^{-1} – nearly double the family average, and levels that put them at high risk of coronary heart disease. Even family members with just one copy of the mutated gene had significantly elevated cholesterol levels – equivalent to a heart attack risk more than 50% higher than average.

'We went from a hypothesis to identifying the disorder in patients, rather than the more conventional route of

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