sequences. The resulting tree, generated using a maximum likelihood method, divided the enzymes into two clear groups with the *E. coli* and *A. aquifex* (such as *Helicobacter pylori*) sequences in different classes[3]. When other enzymes were characterized, those that were grouped with *A. aeolicus* were found to require metal for activity, whereas those (like *Salmonella typhi*) that were grouped with *E. coli*, were not. There was no other common feature between the enzymes in either class[3]. Furthermore, PD404182 was discovered to be a potent inhibitor of all enzymes in both classes apart from the hyperthermophiles. It is therefore hoped that analogues of this compound could prove to be active against a wide range of gram-negative bacteria.

Future prospects

Robert Kretsinger (University of Virginia, VA, USA) says 'Specific KDO 8-P synthase inhibitors could well be viable drugs, but it is important to remember that only one in a hundred promising drugs ever reaches the market.' Woodard agrees with this cautious assessment: 'Our inhibitor does not kill bacterial cells as well as we would like.' However, it will be difficult to improve its binding through chemical modification until a crystal structure of the enzyme–inhibitor complex has been solved. Woodard's group is initially concentrating on trying to improve the solubility of PD404182 and its ability to cross the cell wall but, so far, they have not had much success.

However, if these problems can be overcome, PD404182 would have two important advantages as a lead compound. First, it has previously been patented as a non-steroidal anti-inflammatory drug, so some data on its toxicity and metabolic properties is available in the literature. Also, compounds targeting the LPS pathway could be useful as drugs even if they are not cytotoxic. The lipopolysaccharide layer prevents many antibiotics that are active against gram-positive bacteria from entering gram-negative cells. It is possible that a compound like PD404182, given as adjuvant therapy with one of these antibiotics, could break down this layer enough to allow the antibiotic entry into the cells.

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A sticky end for pathogens?

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Novel flow-based assay technologies designed to study the interaction between pathogens and host cells could lead directly to new drug discovery targets. LigoCyte Pharmaceuticals (Bozeman, MT, USA) has developed two promising anti-infective technology platforms: a Proteoflow™ system to develop new targets for anti-infective therapeutics and vaccines, and nanoparticle technology that can be used to provoke an immune response without the risk of infection.

ProteoFlow™ assays

The flow-based assays originally developed by LigoCyte were inspired by the cell–cell interactions involved in inflammation, where white blood cells are recruited and transported by shear forces in the blood and are required to stop instantaneously at the site of inflammation. This ability to attach to inflamed tissue has been subsequently studied using a flow-based assay consisting of a glass tube lined with endothelial cells through which blood components are flowed, and observing and quantifying the characteristics of the fluorescently labelled white blood cells by video microscopy. By altering the expression of proteins, such as receptors, on the cells and adding various purified proteins, antibodies or inhibitors, the interactions of the immune cells with the endothelial cells can be characterized, and compounds that inhibit inflammation can be studied (Fig. 1). Similar models using epithelial cells simulate the lining of the intestines and airways.

LigoCyte has now applied this technology to the development of assays to study the interaction of pathogens such as *Escherichia coli* and *Candida albicans,* both with host cells and homotypic binding between the pathogens themselves. For example, *E. coli* form colonies by adhering to other *E. coli* cells that have already attached to mucosal epithelium, which results in the upregulation of virulence factors. Inhibition of the initial attachment to the host cell would, therefore, reduce the likelihood of colonization and further infection at existing sites of inflammation, such as a surgical

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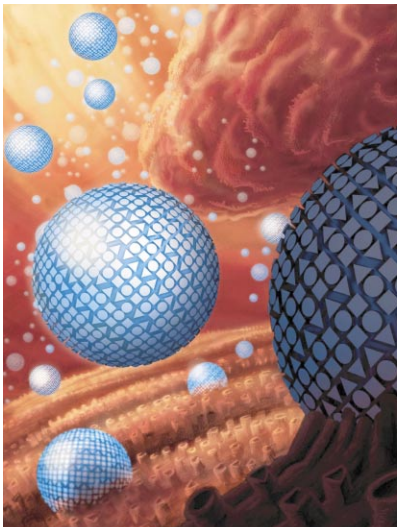
wound. Using the ProteoFlow assay, pathogens can be passed through artificial blood vessels or along epithelial cells in the presence of various inhibitors, to reveal the mechanisms used to adhere to the host cells. Robert Bargatze, Vice President of R&D, LigoCyte, believes that the identification of adhesins is a very exciting area of research: 'Adhesins represent virulence factors in terms of the organism or its toxin's ability to adhere and colonize and are the key players in pathogenicity.'

Novel targets

The adhesion of pathogens via protein and carbohydrate ligands expressed on the cell surface is difficult to study accurately in a static assay, such as a petri dish, because some cells cannot bind in this environment, and yet are able to bind when studied in a flow-based assay. In fact, some cell-binding interactions occur only under flow conditions. The identification of ligands involved in pathogen–cell adhesion present novel groups of targets for therapeutics and vaccines and has great potential for use in drug and vaccine targeting. For example, if a protein responsible for pathogen attachment to epithelial cells in the gastrointestinal mucosa is identified, this protein could theoretically be used to target drugs to the epithelial mucosa. Indeed, LigoCyte has used an adhesin from reovirus, which infects immune-uptake sites known as M-cells in mucosal tissue, to target a vaccine (orally or nasally administered) to these cells, and they have achieved a high mucosal IgA immune response. This is of particular interest because it has previously been difficult to raise IgA responses from intramuscular injections. Further, Bargatze revealed that LigoCyte's partnering of a therapeutic antibody and vaccine has resulted from the identification of a hydrophobic protein involved in the pathogenesis of *C. albicans*. 'In an *in vitro* human cell model, an excellent correlation between the adhesion of *C. albicans* to cells and invasion of the vascular endothelial was observed, which was subsequently blocked by a monoclonal antibody raised to the protein.'

Nanoparticle technology

LigoCyte has also developed a synthetically engineered virus-sized particle, consisting of a polymerized liposome on which antigens can be presented and oriented and used for adhesion, targeting and to provoke an immune response. Complex antigens can, therefore, be mimicked by using a combination of simple ligands presented at different heights and densities, which could result in a similar immune response to that observed after exposure to the pathogen, but without the virulence. The nanoparticles can also be used to target large amounts of antigen to, for example, an M-cell, to increase the 'payload' of antigen delivered to an immune uptake site. LigoCyte is currently carrying out research funded by the National Institutes of Health (Bethesda, MD, USA) to develop this technology for the inhibition of eosinophil recruitment to the lungs of asthma patients.



Schematic representation of nanoparticle technology.

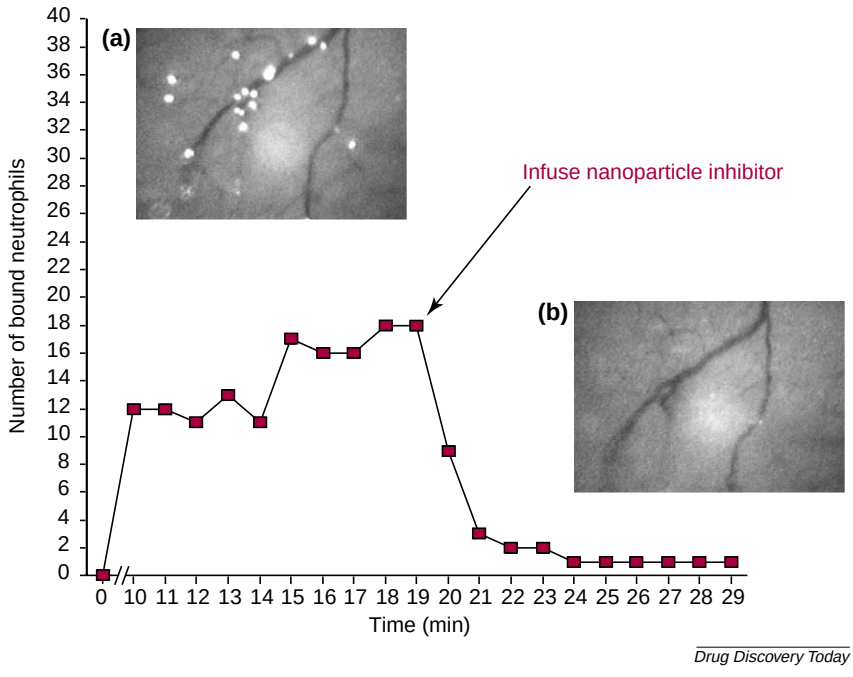


Figure 1. ProteoFlow™ flow-based assay showing (a) fluorescently labelled neutrophil recruitment in the presence of a cytokine, tumour necrosis factor-α (TNF-α). (b) The addition of a nanoparticle inhibitor (developed by LigoCyte) after 19 min results in a rapid decrease in neutrophil attachment to the vascular endothelium.

Ongoing R&D

LigoCyte is involved in several partnerships with other companies that are using these technologies in drug and vaccine development. Corixa (Seattle, WA, USA) has licensed both an antibody and a vaccine based on LigoCyte's work, and Merck (Rahway, NJ, USA) is evaluating compounds developed for inflammatory bowel disease, differentiating lead candidates using LigoCyte's flow-based assays. Bargatze says that the next step for LigoCyte is: 'to take advantage of the targeting to the M-cell site using both antigens and DNA constructs that encode antigens, for the development of highly effective vaccines.'

New opportunities for enhanced ovarian cancer prevention

Martina Habeck, Freelance writer

Researchers might have found the mechanism by which oral contraceptives (OCs) help prevent ovarian cancers. They think it is mainly a direct effect of the hormone progestin on the ovarian epithelium, an effect that is unrelated to ovulation inhibition. This knowledge is hoped to enable the development of OCs that provide enhanced ovarian cancer prevention benefits.

Ovarian cancer is the sixth most common cancer among women worldwide and causes more than 100,000 deaths per year[1]. However, routine OC use has been shown to protect against the disease. Data from large cohort and case-control studies show that the risk of ovarian cancer is decreased by 40% in past/present users of OCs and by >50% in long-term users (>5 years)[2].

Mechanism of OC protection

Why OC use reduces ovarian cancer risk is not known with certainty, although the general assumption has been that these drugs reduce the lifetime number of ovulations. Repeated cycles of ovulation can cause recurrent damage to ovarian epithelium, and can eventually result in genetic mutations, triggering cancer.

However, this explanation is not conclusive. Little information is available on progestin-only contraceptives, but it appears that this type of contraceptive is also associated with a reduced risk of ovarian cancer, while often having no effect on ovulatory cycles. These progestins (i.e. synthetic formulations of the female hormone progesterone) are included in OCs because they thicken the cervical mucus, thus making it difficult for the sperm to reach the uterus or fallopian tube.

Furthermore, long-term OC use has been shown to have a disproportionately greater protective effect than can be attributed solely to ovulation suppression. This realization prompted researchers at the Duke University Medical Center (Durham, NC, USA) to look for other biological effects of OCs that could be responsible for their protective properties.

Table 1. Apoptotic effect of hormone treatment on the ovarian epithelium of cynomolgus monkeys

Hormone treatment	Apoptotic cell counts (median percentage)
No hormones (control)	3.9%
Oestrogen component of Triphasil (ethinyl estradiol)	1.8%
Triphasil	14.5%
Progestin component of Triphasil (levonorgestrel)	24.9%

Role of progestin?

Gustavo Rodriguez and his team examined the ovaries of monkeys that had been treated for three years with OCs. Four groups of cynomolgus macaques (75 in total) were randomized to receive no hormones, the OC Triphasil (a combination of an oestrogen and a progestin component), the oestrogen component of Triphasil, or the progestin component of Triphasil. Results showed that treatment with Triphasil or its progestin component, unlike the other two groups tested, resulted in an increased rate of apoptosis in the ovarian epithelium[3] (Table 1).

This finding that the hormone could activate one of the key *in vivo* mechanisms to eliminate cells that are prone to malignant transformation, was supported

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