Hapten engineering

Raising antibodies against the smallest of small molecules

In recent years, antibody engineering has become one of the most important and most productive routes to drug discovery. It is now widely accepted that this approach can reduce development times and increase potential success, compared with classical drug development. Until recently, the isolation and production of therapeutic-antibody products has been concentrated on larger, protein targets. A research team at Haptogen has now overcome the significant technical difficulties that have been associated with raising antibodies to the smallest of small molecules — bioactive haptens — and, for the first time, anti-hapten therapeutics can be realized. Here, we outline our pioneering approach to antibody engineering and present the results of early work in the important field of anti-infective therapy.

The human immune system is primed to react to infection events, typically initiated by bacteria, viruses or larger-molecular-mass proteins derived from these organisms. Evolution has honed the body’s defences to counter these relatively large intruders, but smaller molecules are overlooked and circulate freely. Indeed, many current drugs function in vivo because they are small enough to evade the immune response. As a consequence of this evolutionary process, the raising of antibodies to proteins (even human antibodies) is now relatively straightforward. In contrast, the isolation of antibodies that recognize smaller-molecular-mass antigens (typically below 1500 Da, and often referred to as haptens) has remained a significant technical challenge.

The role and therapeutic potential of haptens

Approximately 10 million different molecules could be regarded as haptens. Of these, at least 200000 are man-made, and include drugs, pesticides and industrial chemicals. Biological organisms also synthesize and make abundant use of haptens for a host of activities, for example, as antibiotics, toxins and hormones, and as part of a plethora of related signalling systems. As with all antigens, the specificity and sensitivity of anti-hapten antibodies can be a significant issue, particularly when considering their administration as therapeutics. Depending on the application, cross-reactivity with related molecules could be either of benefit or a distinct disadvantage. Through the careful design of antigens and the use of appropriate antibody-isolation strategies, human antibodies that are fully cross-reactive or do not cross-react at all with related molecules, can be selected and isolated. This is often possible, even when molecules differ by as little as a single methyl group.

However, the approach is not limited to the isolation of antibodies to small-molecular-mass targets (haptens). Recently, the isolation of human antibodies specific for the molecular signature (haptenic structures) of larger-molecular-mass targets, such as polymers, peptides, modification-state proteins, glycoproteins, bio-toxins and a number of cell-surface antigens, has been undertaken. In general, the strategy (termed Haptomics™) works well for targets considered difficult or beyond the reach of current antibody approaches.

One of Haptogen’s current research interests is a group of haptens involved in the complex process of cell-to-cell communication. This occurs in all organisms, both single-celled and multicellular and the transfer of information through the movement of haptens, as illustrated by ligand–receptor binding, is one of the most frequently used routes by which cells communicate with each other. Researchers have long targeted these pathways in an attempt to gain therapeutic benefit. Competing concentrations of mimetic drugs are administered to interfere or block ligand–receptor binding. In drug discovery terms,
This new and exciting approach is currently being developed for exploitation in a number of therapeutic areas including anti-infectives (described below), the central nervous system and oncology.

A new approach to a well-known problem

Infectious diseases are major killers. Second only to cardiovascular diseases, they are responsible for a quarter of all deaths worldwide. The continuing development of resistance to current drug therapies is a significant issue, with the World Health Organization (WHO) recently reporting that almost 35% of Staphylococcus aureus isolates from the UK’s hospitalized population are methicillin-resistant. Last year, the Public Health Laboratory Service (PHLS) confirmed the isolation of a vancomycin-resistant strain from a 46-year-old man in England and leading scientists, such as Professor Hugh McGavock of Ulster University and others, predict an impending crisis if the use of existing antibiotics is not restricted and new anti-infective compounds are not developed.

A hapten-based antibody approach, combined with recent advances in understanding the significance of cell-to-cell signalling and quorum sensing (the necessary build up of a critical mass of bacteria that triggers the switch from benign to pathogenic), offers a powerful alternative to traditional drug-discovery programmes.

By directing potential drugs towards bacterial signalling molecules, rather than the bacterial cells themselves, it is reasonable to expect that there will be significantly less selection pressure exerted on bacterial populations to develop resistance to the new treatments. It seems likely that a new class of anti-infective therapies with applications against a broad range of Gram-positive and Gram-negative bacteria will result from this work. To illustrate the potential advantages of a hapten-based drug, consider the specific therapy for Pseudomonas aeruginosa and some recent work that has been carried out at Haptogen.

Ps. aeruginosa

Ps. aeruginosa accounts for almost 80% of opportunistic infections by...
It is clear from the high-mortality rates that large amounts of antibiotic therapy units around the world due to *Pseudomonas* infections. To put this into context, 1500 people die every day in intensive care from sepsis. To put this into context, 1500 people die every day in intensive care therapy units around the world due to *Ps. aeruginosa* infections.

Current approaches to treating acute infections involve dosing with large amounts of antibiotic. It is clear from the high-mortality rates that *Ps. aeruginosa* strains show intrinsic resistance to many structurally unrelated antibiotics. Importantly, a number of *Ps. aeruginosa* vaccines are now entering phase III clinical trials, but, whereas these vaccines may have value in reducing nosocomial *Ps. aeruginosa* infections, they are unlikely to show efficacy in the critically ill, where patient immune systems have collapsed.

As we have seen, the hapten-based approach relies on interfering with cell-to-cell signalling. In the case of *Ps. aeruginosa*, the aim is to ‘switch-off’ the production of extracellular virulence factors (Figure 1). Blocking this signalling system effectively keeps the bacteria in a benign state, unable to cause disease. These signalling molecules are very small (<200 Da) and are a class of compounds common to all Gram-negative bacteria. Haptogen has recently been able to show for the first time that immuno-capture of bacterial cell-signalling molecules can indeed interfere with bacterial communication with a resulting loss of pathogenic potency (Figure 2). A secondary biological role of these signalling molecules is to act directly as pathogenesis-related factors, down-regulating the immune system and therefore making the patient less able to fight the disease. So, the administration of anti-hapten antibodies may have the advantages of both reducing the infective potency of *Ps. aeruginosa*, and, at the same time, increasing the ability of the patient to clear the infection. Importantly, because these signalling molecules are vital to the biology of the bacterium, it is almost inconceivable that *Ps. aeruginosa* could develop drug resistance to this antibody-based approach.

**Conclusion**

Immunotechnology is ‘coming of age’, with antibodies showing interesting potential as pharmaceuticals. They can provide a ‘natural’ therapy for the treatment of disease that is safe, long-lasting, deliverable and efficacious. Today, the technology tool-kit available to antibody engineers allows the selection of antibodies that show both high affinity and exquisite specificity for the target antigen. Furthermore, the ability to tailor-make antibodies for each specific application has generated a growing number of therapeutic opportunities, ranging from simple blocking of function to sophisticated modulation of cell signalling, cell stimulation and cell killing.

The hapten-based approach to antibody drug discovery, illustrated here by our work on anti-infectives, looks set to generate novel drugs that show utility against all quorum-sensing bacterial organisms and are less likely to lead to the development of resistant bacterial strains.